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Analysis of target genes of the transcription factor CREM during mouse spermatogenesis

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1. SUMMARY

CREM (cAMP Responsive Element Modulator) belongs to the CREB family of transcription factors. The CREM τ , an activator splice isoform, is highly expressed after meiosis in round spermatids. CREM τ seems to be the major trigger of the expression of many genes at the late stages of spermatogenesis. CREM protein deficient males are sterile due to an arrest of spermatogenesis at stages 2-5 of round spermatids. It results in the absence of cells of all further stages including spermatozoa. Thus, CREM τ is absolutely required for further development of these cells.

The main task of present thesis was to determine the CREM τ downstream target genes, i.e. the genes which are down regulated or not expressed in CREM knockout mice.

By use of subtractive suppression hybridisation we have cloned mRNAs expressed in wild type but not in a CREM $-/-$ mutant mouse (CREM-dependant mRNAs). 12000 clones were analysed by sequencing and hybridisation. Redundancy of this library has been reduced by high-density filter hybridisation with the most abundant clones. 950 obtained clusters represent 161 known mouse genes, 119 homologous to known genes, 226 mouse ESTs and 48 ESTs from other species and 199 novel sequences (last update - 7.12.2000).

Expression of these clones studied by the high-density filter hybridisation with total testicular cDNA. Most of these clones are shown to be expressed in wild type but down regulated in knockout. The spermatogenic stage specific expression profiles were determined by the hybridisation with the cDNA from prepubertal mice at certain stages of spermatogenesis. Several important functional groups of genes like transcription factors, signal transduction proteins, metabolic enzymes and others are coexpressed at the latest stages of spermatogenesis. The mRNAs down regulated in CREM knockout shown to be expressed postmeiotically at the same time as the CREM τ protein. These mRNAs may be defined as the CREM τ target genes (direct or secondary targets).

These data contain new information about the gene expression during spermatogenesis. In addition, these data provide preliminary selection of genes to search for direct CREM target genes. These data may be applied for diagnostic and therapeutic intervention in infertile patients with spermatogenic abnormalities.

2. INTRODUCTION

2.1. Spermatogenesis

2.1.1. Cell differentiation during spermatogenesis

Spermatogenesis is the sequence of cytological events that results in the formation of haploid spermatozoa from precursor stem cells (Fig. 1, p. 10). This process begins by the mitotic division of germ cell spermatogonia to give rise to diploid spermatocytes, which themselves replicate their DNA content before undergoing the two successive meiotic divisions, which results in the production of haploid round spermatids. The latter germ cells develop to mature spermatozoa in the process of spermiogenesis, which involves an extensive biochemical and morphological restructuring. The process occurs in a precise and co-ordinated manner within the seminiferous tubules. During the entire developmental process the germ cells are encapsulated within the Sertoli cells, and in this way they are supplied with growth factors and nutrients. Cellular debris generated during spermiogenesis are also processed by the Sertoli cells. As the spermatogonia mature, they move from the periphery towards the lumen of the tubule until the mature spermatozoa are conducted from the lumen to the collecting ducts (Browder, Erckson et al. 1991).

Precursor germ cells in a defined segment of the tubule differentiate in synchrony, and these zones of differentiation progress along the length of the tubules as a wave (Russel, Ettlín et al. 1990) (Fig. 1, p. 10). Cross section through a given point of the tubule thus reveals that the proportion of cells at the various stages changes according to its position relative to the waves of differentiation. The cycle of the seminiferous epithelium has been defined as the series of changes occurring in a given area of the seminiferous epithelium between two successive appearances of the same cellular association (Fig. 2, p. 11). There are 12 different cellular associations (I-XII) within the seminiferous epithelium in the mouse (Russel, Ettlín et al. 1990). During prepubertal development of the testis the germ cells involved in the first round of spermatogenesis are synchronised in their development.

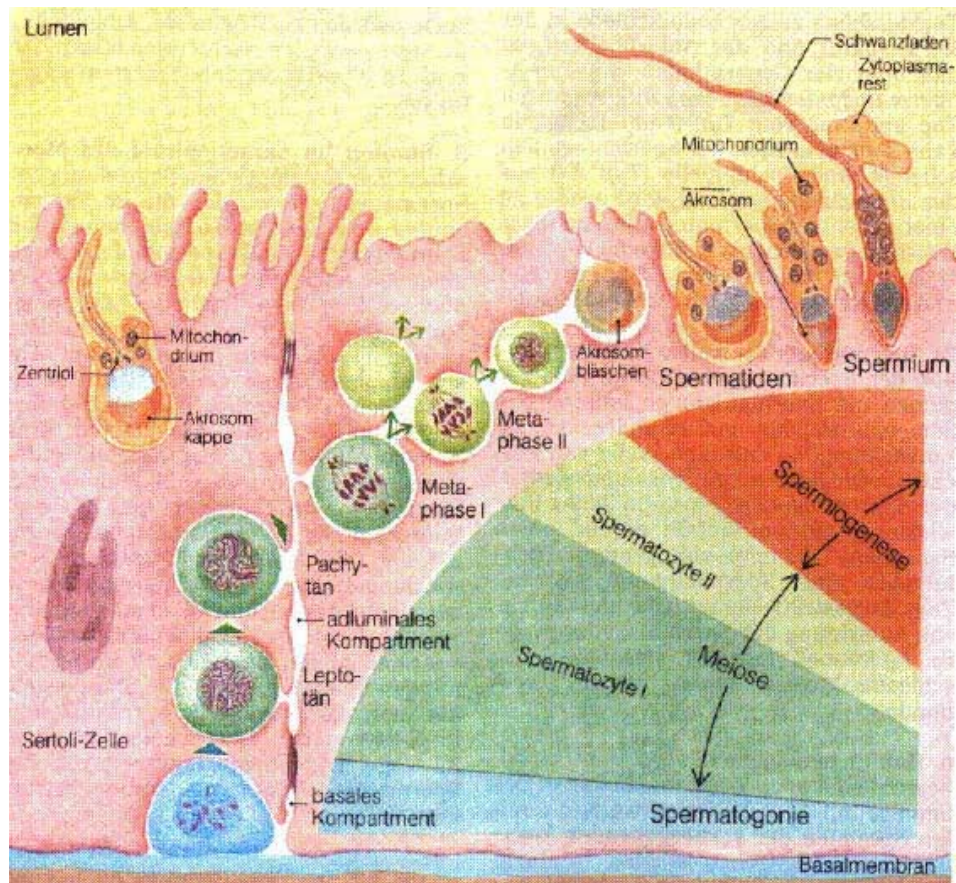


Figure 1. Spermatogenesis. Spermatogenetic cells develop from spermatogonia to spermatozoa. They move stepwise from the basal membrane to the lumen surrounded by the membrane of Sertoli cells, which nourish and control germ cells development.

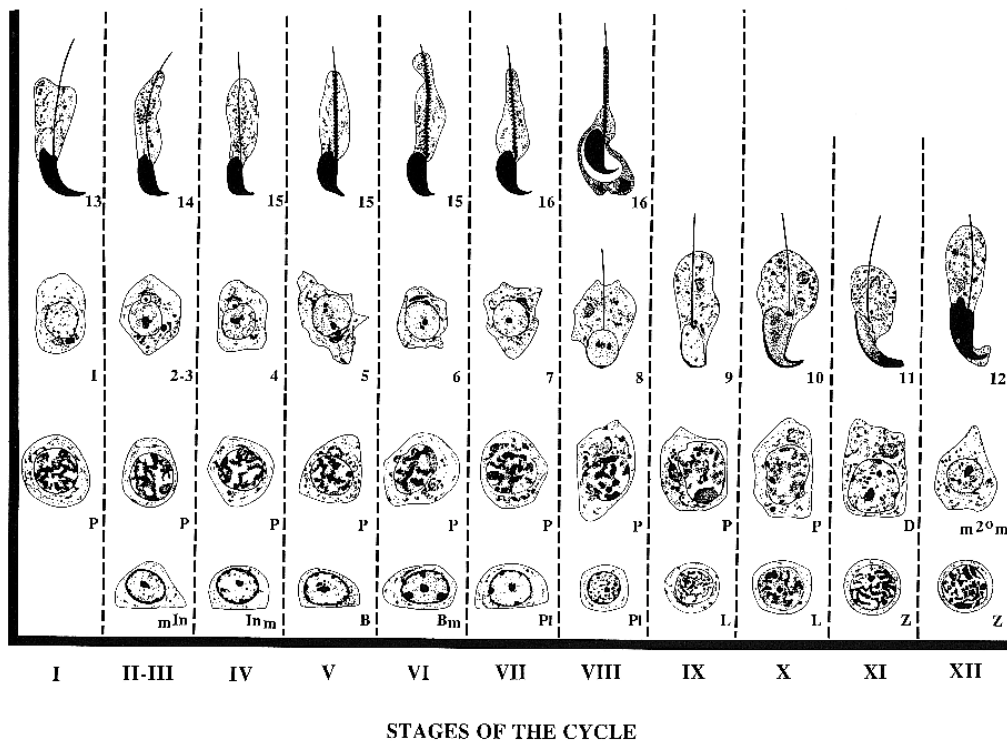


Figure 2. The cellular associations during the mouse spermatogenic cycle. Each particular part of seminiferous tubule gradually undergoes 12 spermatogenic stage cycle and contain the set of spermatogenic cells of several corresponding stages. For example, the seminiferous tubule at stage V contain spermatogenic cells of four stages: 1) spermatogonia B; 2) pachytene spermatocytes; 3) round spermatids at stage 5; 4) elongated spermatids at stage 15 (figure from (Browder, Erickson et al. 1991)).

Thus, 3-5 days after birth the spermatogonial progenitor cells start to proliferate mitotically; by 9–10 days the first wave of cells have differentiated into preleptotene spermatocytes; on days 13–14 these cells differentiate into pachytene spermatocytes; by day 18 meiosis is complete and the cells constitute spermatids; finally, by 30–32 days the cells form condensing spermatids (Bellve, Cavicchia et al. 1977). The somatic testis cells, namely the Sertoli and Leydig cells, are present at all stages of testicular development; however, the proportion of the total cell population that they constitute decreases as the germ cell population proliferates. Thus the day of appearance of a gene product during this early stage of development can be used to ascertain the cell type in which it is expressed. It was established that CREM τ constitutes an abundant transcript from the pachytene spermatocyte stage onwards (Foulkes, Mellstrom et al. 1992).

2.1.2. Gene expression during spermatogenesis

Mammalian spermatogenesis provides an excellent model system to study gene expression during differentiation of a defined cell lineage. The cytology of spermatogenesis is very well established. Cells at every particular stage have a specific structure and may be easily discriminated from each other. The alterations of gene expression during sperm development are dramatic. Strict correlation is found amongst the cell type and the expression pattern. Many genes exhibit stage-specific expression during spermatogenesis. For example, c-fos and c-myc are expressed in spermatogonia, c-jun, c-kit and pgk-1 in spermatogonia and spermatocytes (Wolfes, Kogawa et al. 1989; Sorrentino, Giorgi et al. 1991; McCarrey, Berg et al. 1992), HSP70.2 and CCK in spermatocytes only (Persson, Rehfeld et al. 1989; Erickson 1990; Allen, Dix et al. 1996).

Most dramatic changes happen during differentiation of round spermatids to mature sperm cells. Almost all proteins and structures become substituted by new proteins and structures characteristic for sperm. The protein degradation and synthesis machineries are very active in round spermatids to realise these changes. For example histones become ubiquitinated and then degraded in round spermatids (Baarends, Hoogerbrugge et al. 1999) and protamines and transition proteins are synthesised to substitute the histones and thereby compact the chromatin (Kistler, Sassone et al. 1994; Ha, van et al. 1997).

The general transcriptional machinery is very active in round spermatids. For example, the TATA binding protein (TBP) accumulates in early haploid germ

cells at much higher levels than in any other somatic cell type. Indeed, adult spleen and liver cells contain 0.7 and 2.3 molecules of TBP mRNA per haploid genome equivalent, respectively, while adult testis contain 80–200 molecules of TBP transcript per haploid genome equivalent (Sassone-Corsi 1997). In addition to TBP, TFIIB and RNA polymerase II are also overexpressed in testis (Sassone-Corsi 1997).

A highly specialised transcriptional mechanisms ensure stringent stage-specific gene expression in the germ cells. What can be identified as the specific checkpoints correspond to the activation of transcription factors; these regulate gene promoters with a restricted pattern of activity in a germ cell specific fashion (Sassone-Corsi 1997). Importantly, there is also evidence that general transcription factors are differentially regulated in germ cells. It is very likely that groups of genes are coregulated in a stage specific fashion. It may be possible that some special transcription factors may trigger the expression of multiple genes after meiosis.

The set of genes encoding proteins required for spermiogenesis are upregulated in round spermatids. Possibly most of them are coregulated by the few particular transcription factors. CREM τ may be one of them as it is expressed strictly in round spermatids at very high abundance.

2.2. Transcription factor CREM

CREM belongs to the CREB family of transcription factors. This family consists of three members: CREB - cAMP responsive element binding protein, ATF - activating transcription factor, and CREM - cAMP responsive element modulator. All these proteins share high homology with each other. In response to different signals these transcription factors may be phosphorylated by protein kinase A, PKC or Cam kinases, bind to CRE (cAMP responsive element) site in promoters and activate or repress the expression of corresponding direct target genes (Sassone-Corsi 1995).

CREM τ may be activated without phosphorylation by binding to ACT protein (Fimia, De Cesare et al. 1999). The CREM protein has multiple splice isoforms acting in different specific ways (Sassone-Corsi 1995; Sanborn, Millan et al. 1997).

2.2.1. Splice isoforms of CREM and their transcription activities

Recent studies have now firmly established that differential transcript processing is central to the regulation of CREM expression. This control seems to be exerted at three different levels: alternative splicing, alternative polyadenylation and alternative translation initiation (Foulkes and Sassone 1996). The importance of these mechanisms is reinforced by the fact that all the CREM isoforms which incorporate the P-box exons (CREM α , β , γ , $\alpha\tau$, τ , τ_1 and τ_2 ; Fig. 3, p. 15) are generated from a GC-rich promoter (P 1) which has been shown to behave as a housekeeping promoter directing a non-inducible pattern of expression (Molina, Foulkes et al. 1993; Stehle, Foulkes et al. 1993).

Characterisation of the genomic organisation of the CREM gene has revealed the molecular basis for this extensive family of isoforms. The gene is multiexonic with the coding region divided into 9 exons which are distributed over more than 80 kb of DNA (Laoide, Foulkes et al. 1993). Exons accurately define functional domains (Fig. 3, p. 15) (de Groot and Sassone-Corsi 1993; Laoide, Foulkes et al. 1993).

The two glutamine-rich domains are encoded by two distinct exons. There is some evidence that these domains determine the interaction with basal transcription machinery such as polymerase II cofactors (Sassone-Corsi 1995).

The phosphorylation box (P box, also known as the Kinase Inducible Domain, KID), localised between two glutamine rich domains, contains the phosphorylation site at serine 117. PKA, PKC and CamK kinases able to phosphorylate CREM at this site. CBP/p300 bind to the phosphorylated domain and transcription activation occurs (De Cesare and Sassone-Corsi 2000).

The DNA-binding domains DBDI and DBDII form different variants of the bZip domain (basic and leucine zipper domain) by alternative splicing. The basic part is positively charged and bears the DNA-binding function. The leucine zipper is rich in leucine residues which necessary for dimerisation based on hydrophobic protein-protein interactions.

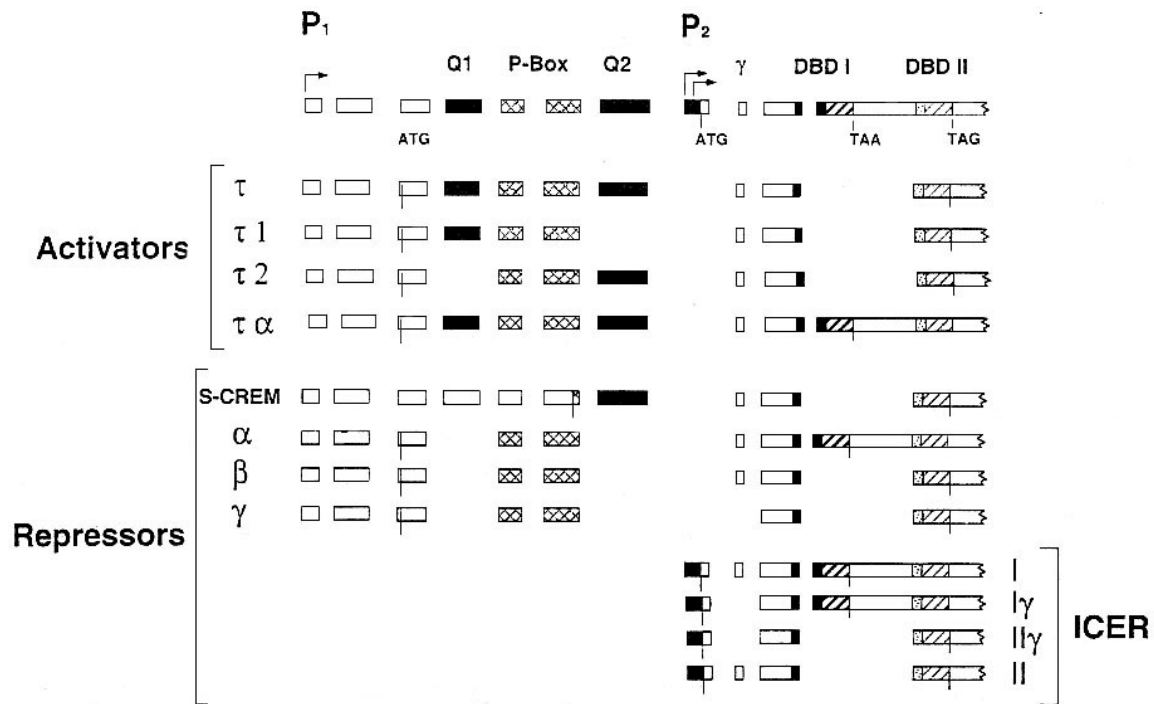


Figure 3. Splice isoforms of CREM. Activators and repressors from the same gene. Top section: schematic representation of the CREM gene. Exons encoding the glutamine-rich domains (Q1 and Q2), the P-Box, the γ domain (γ) and the two alternative DNA binding domains (DBDI and DBDII) are shown. The bottom part represents the various activator and repressor isoforms which have been described to date. The activator and repressor isoforms τ , $\tau 1$, $\tau 2$, $\tau \alpha$, α , β and γ are all derived from the P1 promoter which is GC-rich and directs a non-inducible pattern of expression. The repressor S-CREM is generated from the CREM τ transcript by the use of alternative AUG translation initiation codon. The intronic, cAMP-inducible P2 promoter directs expression of the ICER family of repressors. A family of four types of ICER transcript is generated by alternative splicing of the DBD and γ -domain exons: ICER-I, ICER-I γ , ICER-II, ICER-2 γ (figure from Foulkes and Sassonne-Corsi 1996).

Activating isoforms of CREM τ contain all the domains mentioned: DBD domain serving for DNA binding; P-box domain - for phosphorylation dependent binding with CBP/p300 and one of Q domains - for interaction with basal transcription machinery. The P-box domain and at least one of the Q domains are absolutely required for activating function of the CREM protein.

All inhibitory isoforms of CREM possess one of the splice variants of the DNA binding domain DBD but miss Q or P-box domains. For instance, CREM α , β and γ isoforms do not contain both Q domains excised out by splicing. S-CREM protein translated from alternative internal downstream ATG translation initiation site thereby misses upstream part including Q1 and P-box domains. The ICER (for inducible cAMP early repressor) transcribed from alternative promoter located closer to 3' does not contain the P-box and both Q domains.

Additional versatility is obtained by the possibility to generate transcripts with different 3' untranslated regions (Foulkes, Schlotter et al. 1993). Ten copies of the sequence AUUUA are distributed throughout the 3' untranslated region. This element has been demonstrated to confer mRNA instability in other genes. By the use of alternative polyadenylation sites the CREM gene can generate transcripts bearing different numbers of these elements and thus having different stability. During spermatogenesis the use of the most 5' polyadenylation site is hormonally regulated: transcripts polyadenylated at this site are much more stable because nine of the AUUUA elements are absent (Foulkes, Schlotter et al. 1993). It leads to very high amount of CREM τ protein in spermatids.

2.2.2. Two alternative ways of CREM activity regulation

The CREM transcription factor may be activated in two ways: i) by phosphorylation by PKA, PKC and Cam kinases; ii) phosphorylation independent activation by the interaction with the ACT protein. Since only one publication about the ACT protein is available (Fimia, De Cesare et al. 1999) and the cAMP dependant activation of CREM in testis is possible, both types of CREM activation are reviewed here.

2.2.2.1. cAMP dependent regulation of CREM

Various endocrine and neuronal functions are governed by the cAMP-dependent signalling pathway. In eucaryotes, transcriptional regulation upon stimulation of the adenylyl cyclase signalling pathway is mediated by a family of cAMP-responsive nuclear factors (Fig. 4, p. 18). This family consists of CREB, CREM and ATF transcription factors which may act as activators or repressors. These factors contain the basic domain/leucine zipper motif and bind as dimers to cAMP-response elements (CRE). The function of CRE-binding proteins is modulated by phosphorylation by several kinases. Direct activation of gene expression by CREB requires phosphorylation by the cAMP-dependent protein kinase A of serine-133. In the case of CREM it is serine-117 (De Cesare, Fimia et al. 1999).

The intracellular levels of cAMP are regulated primarily by adenylyl cyclase. This enzyme is modulated by various extracellular stimuli mediated by receptors and their interaction with G proteins (McKnight, Clegg et al. 1988). The binding of a specific ligand to receptor results in the activation or inhibition of the cAMP-dependent pathway, ultimately affecting the transcriptional regulation of various genes through distinct promoter responsive sites. Increased cAMP levels directly affect the function of the tetrameric protein kinase A (PKA) complex (McKnight, Clegg et al. 1988). Binding of cAMP to PKA regulatory subunits releases the catalytic subunits. Catalytic subunits translocate from cytoplasmic and Golgi complex anchoring sites and phosphorylate a number of cytoplasmic and nuclear target proteins on serines in the context X-Arg-Arg-X-Ser-X (McKnight, Clegg et al. 1988; Sassone-Corsi 1995). A number of isoforms for both the regulatory and catalytic subunits have been identified, suggesting a further level of complexity in this response (McKnight, Clegg et al. 1988). In the nucleus the phosphorylation state of transcription factors and related proteins appears to directly modulate their function and thus the expression of cAMP-inducible genes (Sassone-Corsi 1995). Thus, there is a direct link between the activation of G-coupled membrane receptors and CRE-mediated gene expression (Montmayeur and Borrelli 1991) (Fig. 4, p. 18).

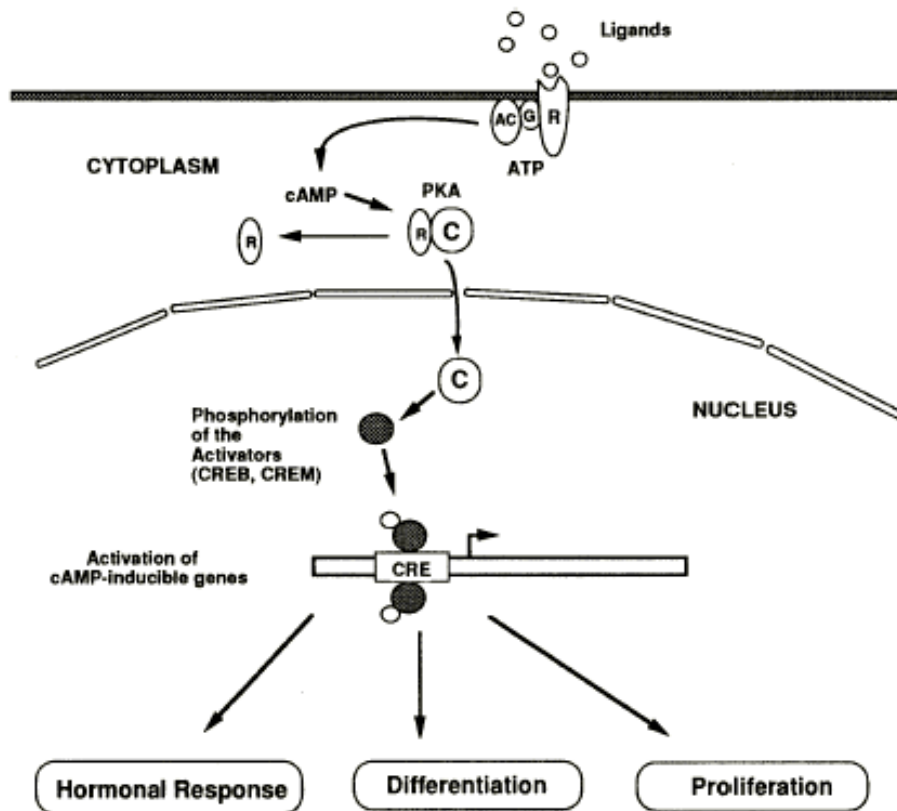


Fig. 4 The cAMP dependent signal transduction pathway. Schematic representation of the route whereby ligands at the cell surface interact with membrane receptors (R) and result in altered gene expression. Ligand binding activates G-proteins (G) which in turn stimulate the activity of the membrane-associated adenylyl cyclase (AC). This converts ATP to cAMP which causes the dissociation of the inactive tetrameric PKA complex into the active catalytic subunits and the regulatory subunits. Catalytic subunits migrate into the nucleus where they phosphorylate and thereby activate transcriptional activators such as CREB and CREM. These factors bind to CREs found in the promoters of cAMP-responsive genes to activate transcription. This event leads to the regulation of key physiological functions.

Analysis of regulatory sequences of several genes allowed the identification of promoter elements which mediate the transcriptional response to increased levels of intracellular cAMP (Sassone-Corsi 1995). A consensus CRE site constitutes an 8-bp palindromic sequence (TGACGTCA) (Sassone-Corsi 1995). Several genes which are regulated by a variety of endocrine stimuli contain similar sequences in their promoter regions although at different positions.

2.2.2.2. Phosphorylation and CBP independent activation of CREM by binding with ACT protein

Transactivation by CREB and CREM not always depend on CBP. CRE-binding proteins are relatively ubiquitous and uninducible (Sassone-Corsi 1995; Montminy 1997). However, in adult male germ cells, CREM is expressed at levels that are hundreds of times higher than those in other tissues (Foulkes, Mellstrom et al. 1992). Surprisingly, although it activates transcription of postmeiotic genes, CREM is unphosphorylated in male germ cells.

Thus, activation by CREM must occur independently of Ser117 phosphorylation and, therefore, of the binding of CBP. By the yeast two-hybrid screen of a testis-derived cDNA library, using the CREM activation domain as a bait, a clone was identified that encodes the protein ACT (for activator of CREM in testis) (Fimia, De Cesare et al. 1999). The distinctive feature of the ACT is the presence of four complete LIM motifs and another half motif at the N-terminus. The LIM domain comprises a conserved cysteine and histidine-rich structure that forms two adjacent zinc fingers (Dawid, Breen et al. 1998). This structural motif was first identified in the protein products of three genes, Lin-11, Isl-1 and Mec-3. The LIM domain functions is a protein-protein interaction domain (Dawid, Breen et al. 1998). LIM domains can be present with other functional protein motifs, such as homeobox and kinase domains, but ACT belongs to the LIM-only class of proteins (LMO) and contains no other structural motif (Dawid, Breen et al. 1998). Several lines of evidence point to the co-ordinated expression of CREM and ACT. ACT is abundantly and exclusively expressed in testis; ACT colocalises with CREM in spermatids; and ACT and CREM exhibit the same expression pattern during testis development (Fimia, De Cesare et al. 1999). CREM and ACT efficiently associate; the biological significance of this is that ACT has an intrinsic transactivation capacity and can convert CREM into a powerful transcriptional activator (Fimia, De Cesare et al. 1999) (Fig. 5, p. 21). Most

importantly, coactivation through ACT can also occur in yeast, which lacks CBP and TAF130 homologs. Thus, ACT can bypass the need for CREM phosphorylation. Indeed, ACT converts the inactive, Ser117-Ala CREM mutant into a transcriptionally active molecule both in yeast and in mammalian cells (Fimia, De Cesare et al. 1999). Thus, in male germ cells ACT provides a novel, tissue specific, phosphorylation-independent route for transactivation by members of the CREB family (Fig. 5, p. 21).

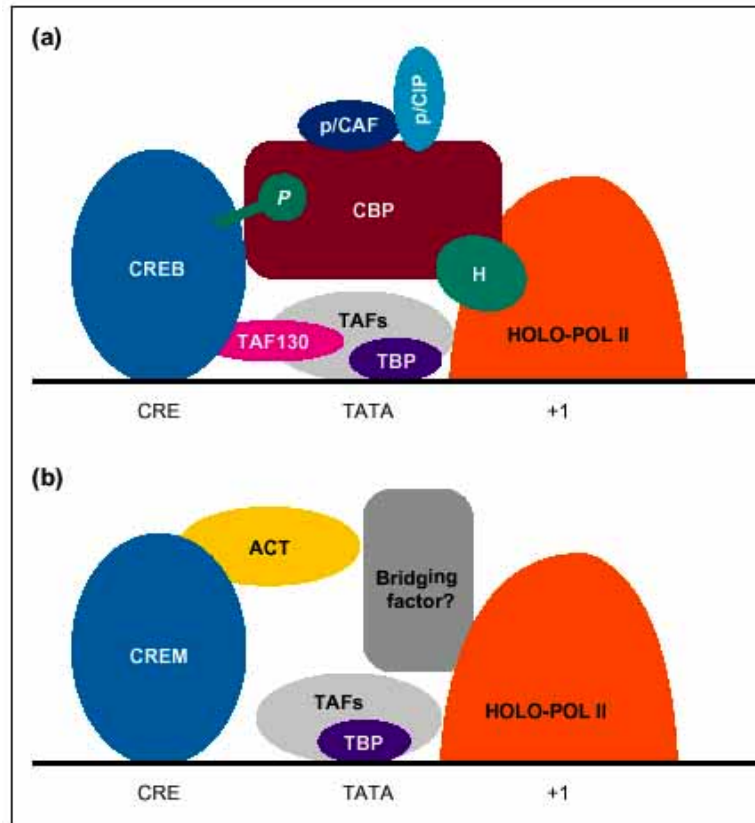


Figure 5. CREB and CREM mediated transcription through different coactivators.

(a) Classical view: Phosphorylation of Ser133 of CREB (or Ser117 of CREM) promotes binding to CREB-binding protein (CBP) and subsequent transcriptional activation. Interaction with TAF130 is constitutive and is mediated by the Q2 domain of CREB.

(b) Model for coactivation by ACT in testis. ACT exerts its function independently of Ser117 phosphorylation and in the absence of TAF130. This model provides an alternative activation pathway. A hypothetical bridging factor links ACT to the basal transcription machinery. CRE, cyclic-AMP-responsive element; H, RNA helicase A; HOLO-POL II, RNA polymerase II holoenzyme; p/CAF, p300/CBP-associated factor; p/CIP, p300/CBP cointegrator associate protein; TAFs, TBP-associated factors; TBP, TATA-box-binding protein (figure from De Cesare et al., 1999).

2.2.3. The CREM functions in different tissues

The CREM gene encodes various transcription factors which play key physiological and developmental roles within different tissues. The specific roles of CREM have been addressed using CREM knockout mice generated by gene targeting. So far three abnormalities were found in CREM knockout mice: altered circadian cycle, delayed liver regeneration and impairment of spermatogenesis.

2.2.3.1. CREM knockout mice: Altered circadian cycle and delay of liver regeneration

CREM proteins are thought to play important roles within the hypothalamic–pituitary axis and in the control of rhythmic functions in the pineal gland. CREM-null mice show a drastic increase in locomotion. In contrast to normal mice, the CREM-deficient mice display no circadian cycle of locomotion activity. The anatomy of the hypothalamic suprachiasmatic nuclei, the centre of the endogenous pace-maker, is normal in mutant mice. Remarkably, CREM mutant mice also elicit a different emotional state, revealed by a lower anxiety in two different behavioural models, but they preserve the conditioned reactivity to stress (Maldonado, Smadja et al. 1999).

The lack of CREM causes a 10-hr delay in the post-PH (partial hepatectomy) proliferation wave and deregulation in the expression of cyclins A, B, D1, E, and cdc2, as well as of c-fos and tyrosine aminotransferase (TAT). Thus, CREM appears to co-ordinate the timing of hepatocyte proliferation during the process of liver regeneration (Servillo, Della Fazio et al. 1998).

2.2.3.2. CREM knockout mice: Impairment of spermatogenesis

It has been previously shown that the transcriptional activator CREM τ is highly expressed in postmeiotic cells (round spermatids) (Fig. 6, p. 24).

CREM knockout males are unable to reproduce despite the normal mating behaviour. In contrast, CREM knockout females are fertile. Heterozygous mice display reduced fertility. The testes of the CREM-deficient mice displayed a reduction of 20–25% in weight. Analysis of the seminal fluid of heterozygous mice compared to normal littermates demonstrated a 46% reduction in the

overall number of spermatozoa, a 35% decrease in the ratio of motile spermatozoa, and a twofold increase in the number of spermatozoa with aberrant structures. Most of the aberrant spermatozoa were characterised by a kink and bubble like structure midway along the tail. Strikingly, analysis of the seminal fluid from homozygous CREM-deficient mice revealed a complete absence of spermatozoa. This result demonstrates a dramatic impairment of spermatogenesis in the CREM knockout mice (Blendy, Kaestner et al. 1996; Nantel, Monaco et al. 1996).

To determine the nature of the sperm deficiency in the CREM knockout mice detailed anatomical analysis of the seminiferous epithelium was performed. Tubules from the CREM-knockout mice display a 20–30% reduced diameter and completely lack the normal spermatogenic wave and the corresponding dark sections. Squash preparations from consecutive segments of the seminiferous epithelium demonstrate that spermatogenesis in the CREM-deficient mice is interrupted at the stage of very early spermatids (Fig. 7, p 25). Late spermatids are completely absent while there is a significant increase in apoptotic germ cells. Neither elongating spermatids nor spermatozoa are observed, while somatic Sertoli cells appear to be normal (Fig.7, p. 25) (Blendy, Kaestner et al. 1996; Nantel, Monaco et al. 1996).

The homozygous males are sterile, demonstrating the necessity of a functional CREM transcription factor for male fertility. The homozygous female mice were fertile and displayed apparently normal ovary structure.

A series of postmeiotic germ cell-specific genes are not expressed in the testis of CREM knockout mice : protamine 1 and 2, Tp-1, MCS, RT7, Krox-20, Krox-24 and cal spermin (Blendy, Kaestner et al. 1996; Nantel, Monaco et al. 1996).

This phenotype is reminiscent of cases of human infertility as there is no CREM expression in humans affected by arrest at the spermatocyte stage.

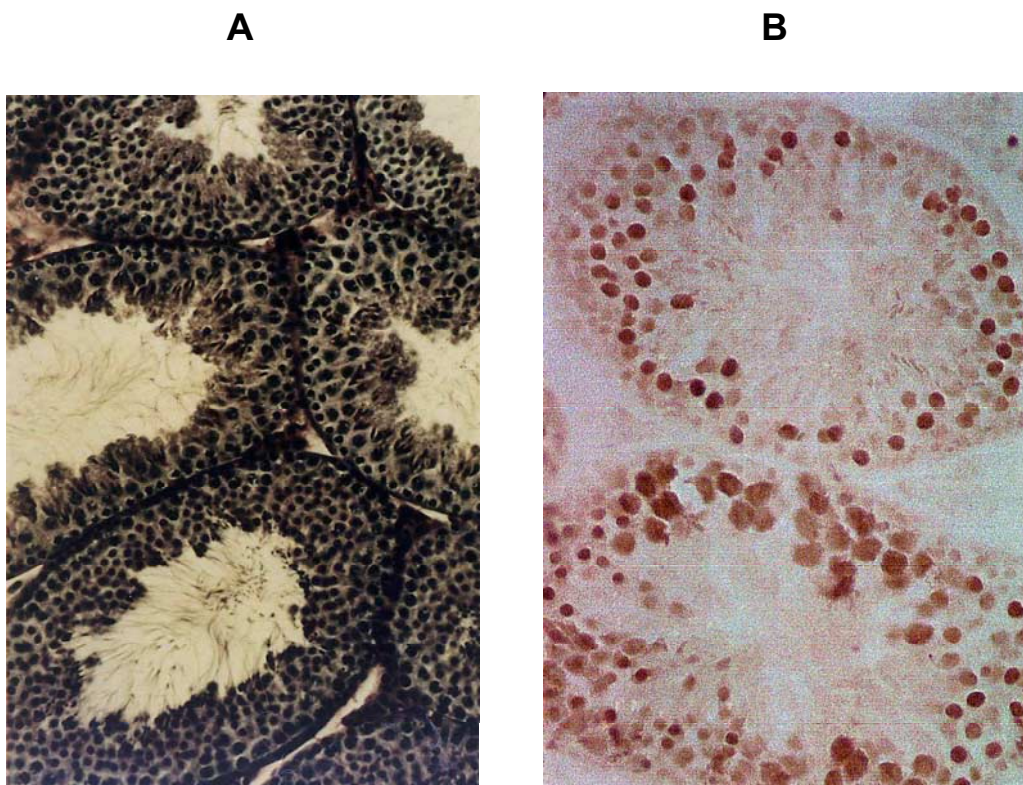


Figure 6. CREMt protein is expressed in round spermatids. A. Testicular sections in adult 5-week-old wild type mice stained with periodic acid Schiff's base and haematoxylin. B. Immunohistochemistry of testicular sections stained with anti-CREMt antibody (Photos from Blendy et al, 1996).

A. Wild type



B. CREM knockout

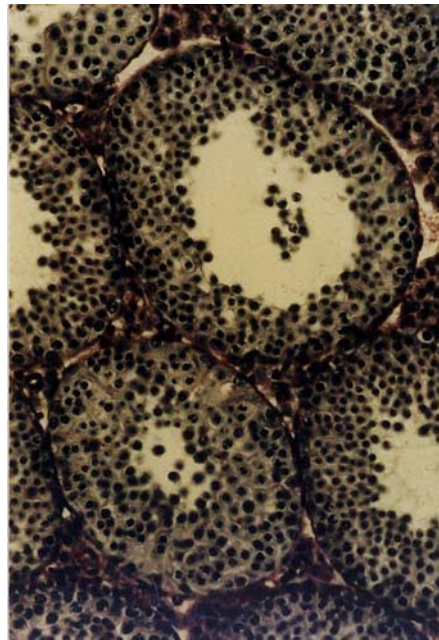


Figure 7. In CREM knockout spermatogenesis is arrested at stage of round spermatids. Stages later than 3 including spermatozoa are absent in CREM knockout. While spermatozoa are visible in the seminiferous lumen of wild type, the lumen of CREM knockout is empty (Photos from Blendy et al, 1996).

2.2.3.3. Possible role of CREM τ in spermatogenesis

Spermiogenesis is a complex process by which postmeiotic male germ cells differentiate into mature spermatozoa. This process involves remarkable structural and biochemical changes which are under the hormonal control of the hypothalamic-pituitary axis.

The pattern of CREM expression in the testis constitutes the first indication of its crucial role. In testis CREM is the subject of a developmental switch in expression (Bartsch, Casadio et al. 1998). Characterisation of the CREM isoform expressed in the adult testis reveals that it encodes exclusively the CREM τ activator, while in prepubertal testis only the repressor forms are detected at low levels. Thus, importantly, the developmental switch of CREM expression also constitutes a reversal of function (Bartsch, Casadio et al. 1998). To address the precise role played by CREM in the testis the expression patterns of the RNA and protein have been defined in relation to spermatogenesis (Galliot, Welschof et al. 1995).

The CREM τ protein is not detected in pachytene spermatocytes but in more mature germ cells which have undergone meiosis. Specifically, CREM τ protein is restricted to round spermatids, mainly at stages VII–VIII of seminiferous tubule differentiation (Fig. 6, p. 24). In the mouse, overall transcription ceases at about stage IX, when transition proteins and protamines replace the histones in order to compact and condense the chromatin (Laoide, Foulkes et al. 1993). Thus, since CREM protein is not detectable in spermatozoa, CREM transactivator function must be restricted to the late phase of transcription before the compaction of the DNA. The absence of CREM protein in the spermatocytes is due to a translational delay of CREM mRNA. Translational control is an important regulatory mechanism of gene expression during spermatogenesis.

The abundance of CREM τ protein suggests an important role it plays in haploid germ cells. Several genes have been identified which are transcribed at the time of appearance of the CREM protein, and which have CRE-like sequences in their promoter regions (Montminy 1997).

To date at least four genes, RT7 (Galliot, Welschof et al. 1995), transition protein-1 (Molina, Foulkes et al. 1993), angiotensin-converting enzyme (Radhakrishnan, Perez-Alvarado et al. 1997), and caldesmon (Sun and Means 1995) have been shown to be targets of CREM-mediated transactivation in germ cells. In all these cases there

are several lines of evidence directly implicating CREM τ as a tissue- and time-specific regulator.

CREM binds to the CRE-related sequences in the promoter of these genes and is able to activate their expression in transient transfection assays. In addition, in an *in vitro* transcription system using a nuclear extract of seminiferous tubules both the addition of a CREM-specific antibody and the excess of CRE competitor decrease RT7 transcription (Galliot, Welschhof et al. 1995). These results suggest that by recognising various CRE sequences CREM directs the testis-specific activation of numerous haploid-expressed genes. Interestingly, most of the genes activated at the same time as the appearance of CREM encode structural proteins. For example, transition protein and protamine are detectable around day 22 during mouse spermatogenesis, exactly when CREM protein is synthesised during spermiogenesis (Galliot, Welschhof et al. 1995).

It remains unknown how many direct target genes of CREM τ are expressed in round spermatids. Expression profiling experiments may provide more new candidates for direct target genes of CREM τ .

2.3. Modern approaches to study gene expression

CREM is a member of the CREB family of transcription factors. To study CREM-dependant gene expression. CREM knockout mice were generated and used for differential expression analysis in mice.

2.3.1. The mouse as a model organism. Genetic manipulations on mouse

One of the most important tasks of modern biology is to provide the knowledge applicable to medicine. Since most experimental work is impossible to carry out in humans, special organisms useful for experiments are needed. Such an organism should be easy handled in the laboratory and close enough to human in order to allow the extrapolation of data to humans. Mice are very suitable for these purposes.

The mouse genome is very homologous to human. Most of translated sequences are of high homology to human (Bentley 2000). Genetic manipulations on mouse genome achieved high specificity and sophistication. The mouse *Mus musculus* is the model system on which most new technologies have been developed (Muller 1999).

Formerly the quantitative genetics and genetic analysis of domestic animals have dependent entirely upon the exploitation either of alternative alleles or of pre-existing polymorphisms, which were segregated in the stock by selection and breeding. The rate of improvement of livestock was therefore limited by natural variation. But now it become possible to modify genes deliberately, and to study the phenotypic effect of such mutations in the whole animal or particular tissue (Muller 1999).

Gene targeting allows inserting different kinds of DNA fragments into defined site of the mouse genome. By substitution of particular parts of the gene by the artificial DNA one can manipulate genes in a large range. Various kinds of mutations may be introduced into the gene of interest: specific point mutations, deletions or insertions. Some of these mutations will represent loss or gain of specific function of the mutated domain (Muller 1999).

It is possible to govern deliberately the expression level of a given gene in a given tissue at a given time. Currently the most useful is the introduction of the open reading frame shift leading to complete absence of the protein, thereby generating so called genetic knockout, i.e. mouse deficient for a particular protein. This approach was used for generation of CREM-deficient mice studied in the present thesis (Blendy, Kaestner et al. 1996).

The most important advantage of genetic manipulations is that by this way we study the *in vivo* situation. It highly improves the reliability of data obtained from experiments.

Indeed, because of these extensive opportunities the mouse is considered as one of very important objects of biological study.

2.3.2. Modern methods to study gene transcription

The northern blot hybridisation, RNase protection and RT-PCR became routine methods for the detection of expression levels of particular mRNAs. All these three methods provide quite reliable quantitative results but each experiment give the information only about few mRNAs represent the one gene - one experiment approach.

One of the conclusions from the Human Genome Project is that there are about 35.000-120.000 of genes in mammalian genome (Ewing and Green 2000; Liang, Holt et al. 2000). This makes impossible to study gene expression by the one gene - one experiment approach. For example, two groups were working on the CREM

knockout and found 8 differentially expressed genes only (Blendy, Kaestner et al. 1996; Nantel, Monaco et al. 1996). It reflects very low efficiency of common methods of expression study.

Due to the invention of cDNA array approach into the gene expression explorations last years become crucial point of qualitative switch from the one-experiment - one-gene approach to the one-experiment - thousand-genes approach which is gradually developing to the one - experiment - genome approach (Rafalski, Hanafey et al. 1998; Ramsay 1998).

There are several kinds of the DNA arrays: the cDNA arrays on a nylon support (the high-density filters), the cDNA or oligonucleotide arrays on a glass support (the glass-chips), and oligonucleotide bead-based fiber-optic arrays (Rafalski, Hanafey et al. 1998). In our experiments we used self-made cDNA arrays on a nylon membrane support. The conventional northern blot hybridisation was used to confirm the high-density filter hybridisation results.

2.4. Goals.

The general goal of the present thesis is the study of the CREM dependent gene expression during mouse spermatogenesis. It may be subdivided to two more particular tasks:

1. The comparison of the gene expression in wild-type versus CREM-knockout testis.
2. The study of expression during mouse spermatogenesis.

2.5. Experimental approaches.

It was shown that the CREM knockout males display the loss of expression of at least 8 genes expressed in wild-type round spermatids (Blendy, Kaestner et al. 1996; Nantel, Monaco et al. 1996). According to the literature the loss of several hundreds of genes in CREM knockout was expected. This pool of differentially expressed mRNAs is the combination of direct CREM target gene products in aggregate with the indirect target genes expressed in the spermatogenic cells of the latter stages, which are missing in the mutant. To address the CREM dependent expression in mouse testis we used the following approaches (Fig. 8, 9, pp. 31-32):

1. To clone differentially expressed mRNAs we used the Subtractive Suppression Hybridisation (SSH). From the wild-type testis cDNA the mutant

testis cDNA was subtracted. These cDNAs were ligated into pBKS vector resulting in cremSL library of 12000 clones.

2. To determine the sequence of all clones we used the automatic PCR-based sequencing in combination with the redundancy reduction by the hybridisation of labelled redundant clones with spotted on the nylon membranes the complete set of clones from cremSL library.

3. To study the expression profiles we used the high density filter hybridisation with the total cDNA from adult wild-type and mutant testes and from testis from prepubertal mice of different ages.

The stages of explorations of CREM dependent expression in testis are demonstrated on Fig. 8 (p. 31) and in more details on Fig. 9 (p. 32).

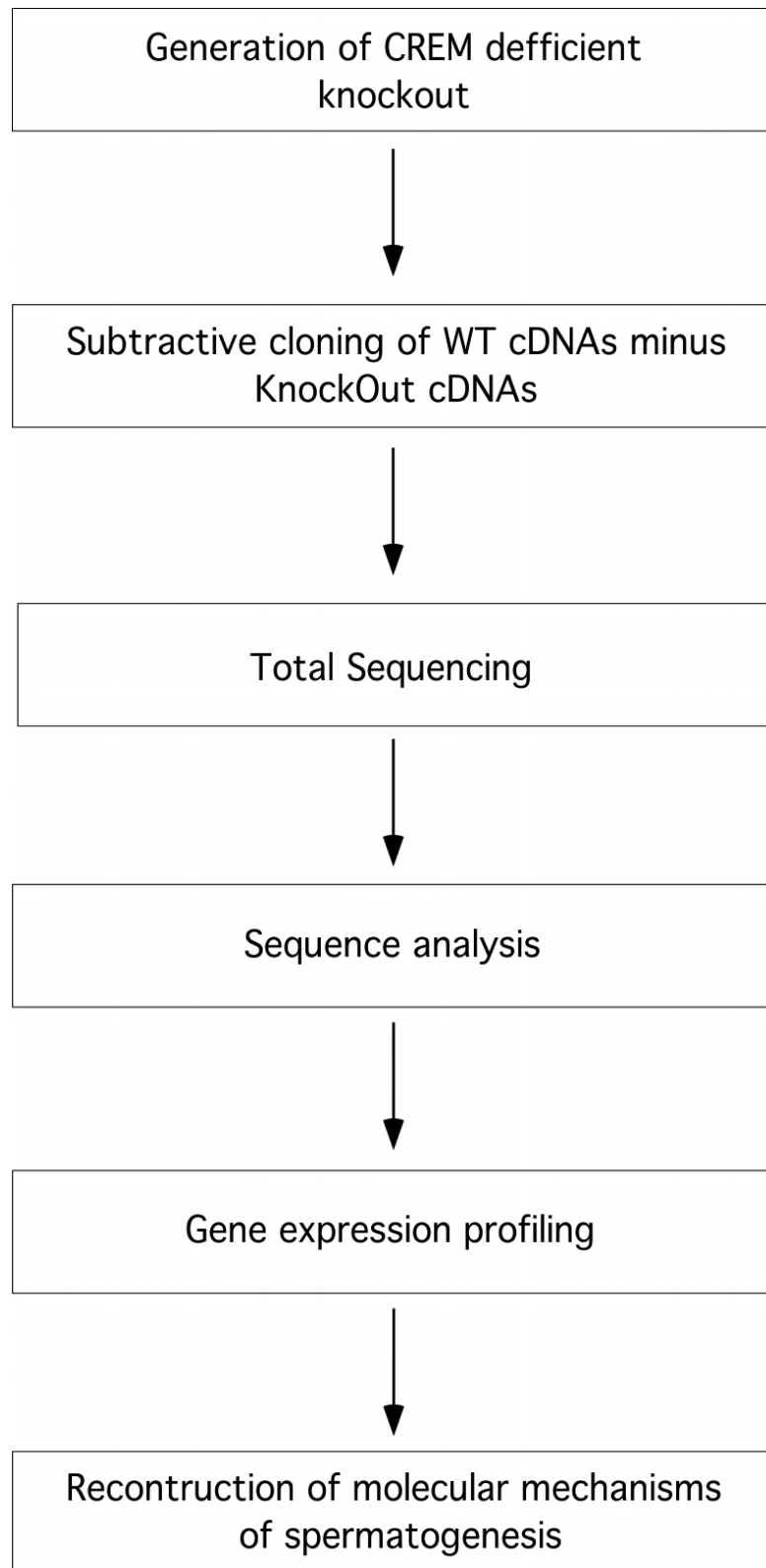


Figure 8. General approaches to study CREM τ dependent gene expression in testis.

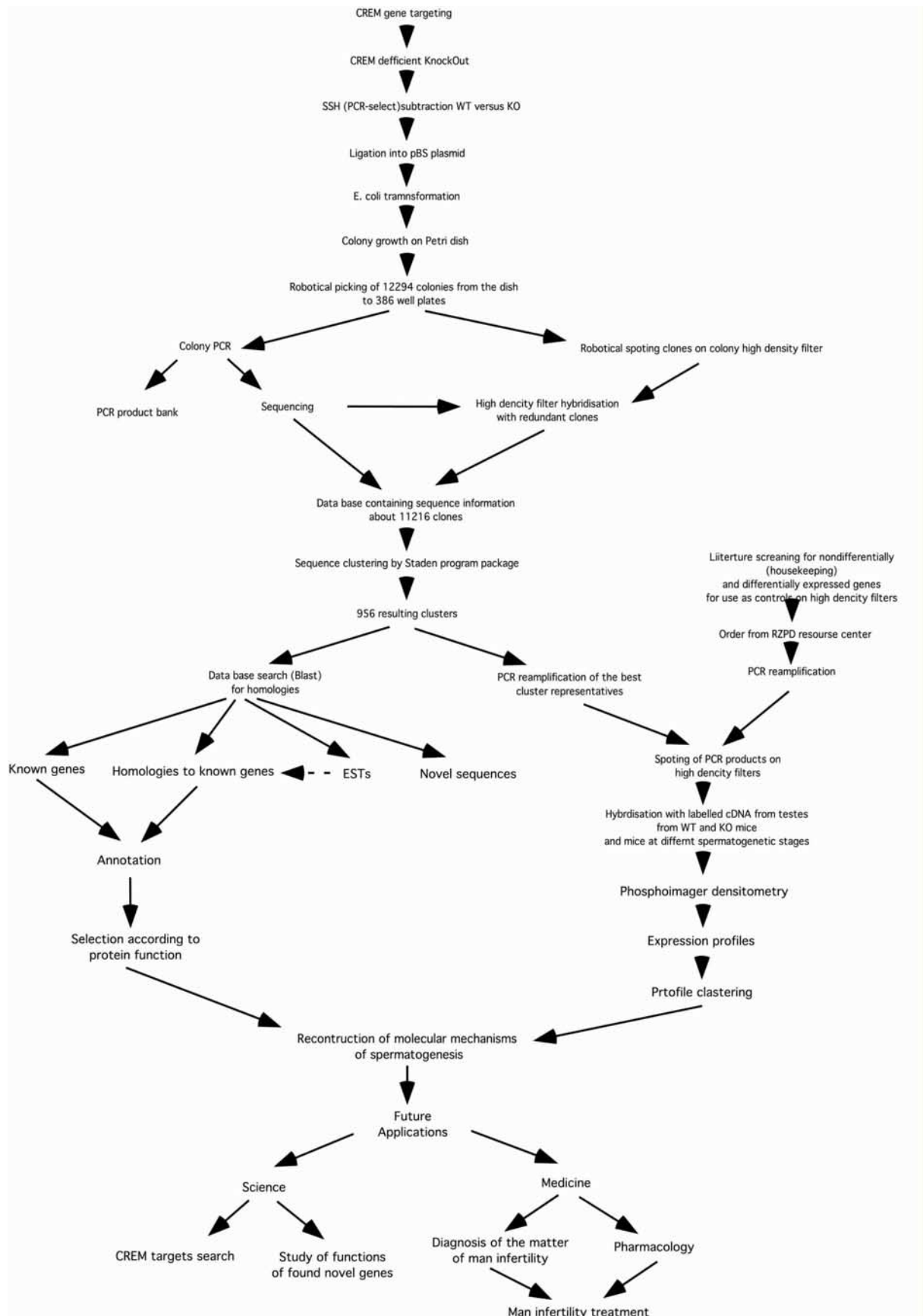


Figure 9. The steps of study of CREM τ dependent transcription .

3. RESULTS

3.1. Subtractive cloning of mRNAs downregulated in testis of CREM knockout mice

In order to identify the genes differentially expressed in CREM deficient mice we constructed a subtracted and normalised library using the SSH technology (Subtractive Suppression Hybridisation, (Diatchenko, Lukyanov et al. 1999)). We have chosen this approach for two reasons. First, several scientific groups had positive results using SSH for differential cloning. Second, SSH is easy and fast to begin with due to the availability as PCR-select Kit (Clontech, USA) (von Stein, Thies et al. 1997; Tchernitsa, Zuber et al. 1999).

The pool of mRNAs from testis of CREM knockout mice has been subtracted from mRNAs of wild-type mice (Fig. 10, p. 34). According to the procedures the subtracted cDNA should be enriched with sequences which are downregulated or missing in the CREM deficient testis.

The standard approach to evaluate the subtraction efficiency is the Southern blot hybridisation of particular housekeeping genes with the subtracted and common cDNAs immobilised on the membrane. Usually the GAPDH gene provided by Clontech in the PCR-select kit should be subtracted by SSH. It should hybridise as very solid band with common cDNA but not with subtracted cDNA. In our case this control did not work because there is the GAPDS specific testis splice isoform of GAPDH which cross hybridise with the GAPDH probe revealing a solid band of hybridisation with subtracted cDNA (data not shown). Indeed, we could not evaluate the subtraction efficiency by Southern blot.

The aliquot of the PCR product obtained after SSH was separated in the agarose gel. It looked like a smear without obvious bands indicating that this PCR product is very complex, i.e. it consists of many different DNA fragments of different length.

3.2. CremSL library construction

According to the complexity of SSH PCR product and our capacities we decided to make a library of about 12000 clones.

3.3. Total sequence determination of all cremSL clones

All clones from the cremSL-subtracted library were totally analysed by a combination of automatic PCR sequencing and redundancy reduction by the high-density filter hybridisation. Data were collected in a data base.

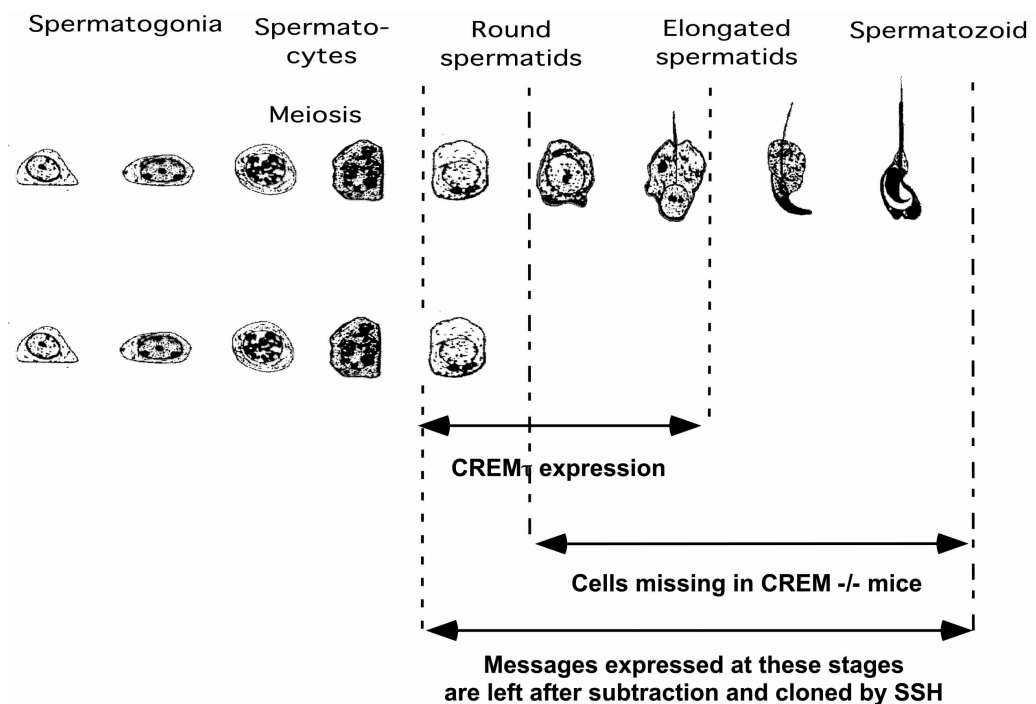


Figure 10. The differential cloning of CREM targets. By use of SSH the pool of mRNAs expressed in knockout testis was subtracted from the pool of mRNAs expressed in wild-type testis. As the result the CREM τ target mRNAs were cloned.

3.3.1. Reduction of library redundancy by high density filter hybridisation

By the use of a robot the high-density colony filters with the spotted cremSL library were produced.

Clones found to be redundant according to sequencing were hybridised by Southern with high-density filters with spotted cremSL library. The hybridised clones were identified and excluded from the further sequencing. Clones displayed different redundancy. The redundant clone 2-L19 is represented in the library in 603 copies (Fig. 11, p. 36).

In addition to the clones from cremSL library, the longer RT-PCR cloned products which cover several RsaI fragments of particular cDNA were used. For instance, GAPD-S cDNA clone covering all three RsaI fragments hybridises with 751 cremSL clones.

Sequencing revealed many clones representing empty vectors. To identify empty vectors a special oligonucleotide was designed. In appropriate stringency of hybridisation this primer hybridises with empty vectors only. 490 clones were found to be the empty vectors. It reflects the vector self-ligation due to the incomplete vector's ends dephosphorylation during the preparation of the pBS vector for the cloning.

Such an approach allowed us to determine all 11216 sequences in 3400 sequencing reactions and 127 high-density filter hybridisations.

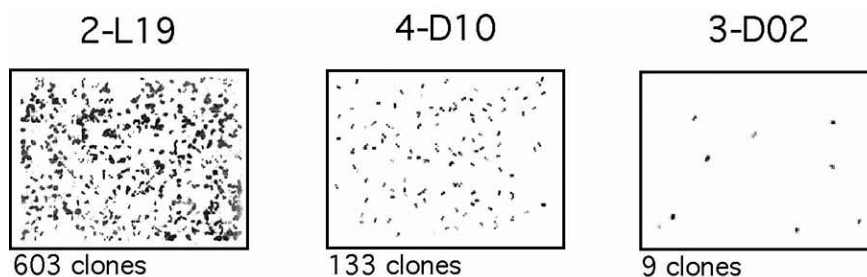


Figure 11. Redundancy of cremSL library. The Southern hybridisation of PCR products of redundant clones with high density filters with spotted cremSL library. The clone 2-L19 is represented in the library in 603 copies, 4-D10 in 133 and 3-D02 in 9 copies.

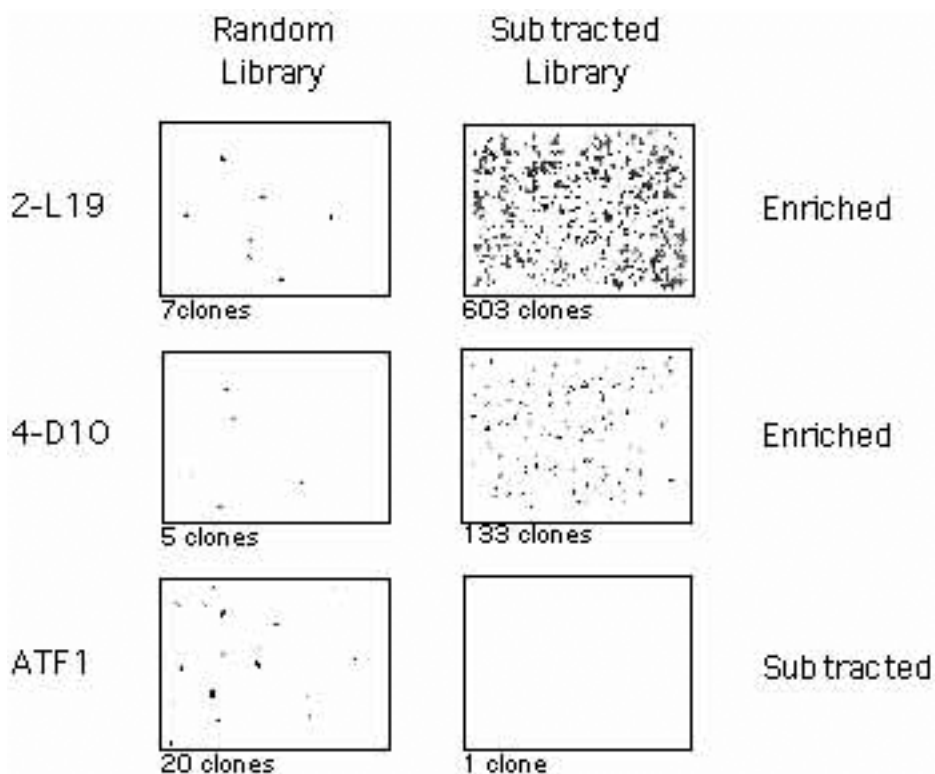


Figure 12. Comparison of subtracted cremSL library with random cDNA wild-type testis library. High density filter Southern hybridisation with ^{32}P labelled clones. Clones nondifferentially expressed in CREM knockout (ATF1 cDNA) are subtracted and differentially expressed clones (2-L19 and 4-D10) are enriched in cremSL library.

3.4. Comparison of clone representation in subtracted cremSL library and random wild-type testis cDNA library

To evaluate the subtraction and enrichment efficiency we compared clone representations in SSH library and common random library (Fig. 12, p. 36). We produced random library high-density filters and hybridised them in parallel with the cremSL library filters with redundant clones and different control clones. These experiments revealed a strong correlation between differentiability of expression and subtraction or enrichment. mRNAs expressed equally in CREM knockout and wild-type mice were subtracted by the SSH, then differentially expressed downregulated in CREM knockout mRNAs were enriched (Fig. 12, p. 36)

3.5. Sequence analysis

3400 clones were sequenced.

Sequence analysis included several steps:

1. Pre-processing of raw sequences - extraction of the pure cDNA RsaI fragments from raw sequences.
2. Sequence clustering - grouping of clones with the same sequence into clusters.
3. Data base search - search for homologies for sequences in DNA sequence data bases.
4. Functional clustering of cloned mRNAs - literature screening, annotation, ontology and grouping of sequences according to their function.

The data about cremSL clones with known sequences are presented in Appendix 1, p. 102.

Data base containing the data about grid positions (plate number - well position) of clones in CremSL library, colony filter hybridisation data, sequences of all cremSL clones are presented in Internet at the address:

<http://www.dkfz.de/tbi/people/beissbarth/crem-project>

3.5.1. Pre-processing of raw sequences

We have sequenced 3400 clones.

The raw data coming out of the automatic sequencer contain a lot of imperfect sequences, which can not be used in further analysis. So, first of all the actual cDNA sequences should be extracted from raw sequences of interest. To solve this problem we have used some programs in the Perl programming language, as well as visual inspection of the trace files. The results of pre-processing of raw sequences are shown on table 2.

The raw sequence contains a vector sequence at the beginning and maybe at the end of the sequence. Also the quality of the traces decreases and the Base Caller

program often reads a nonsense sequence. The vector sequences as well as low quality sequences are removed during the process of vector clipping and the quality clipping. Errors in base calling were corrected manually according to the electrophoregramm.

In ideal case, the raw sequence contains the pBS vector sequence on both ends and the cDNA sequence in the middle surrounded by the two RsaI sites. To get the clean cDNA sequence we cut off the pBS vector sequence in the middle of RsaI sites.

In the case that the cDNA insertion is too long to be sequenced in one run, the raw sequence contains at the beginning the pBS vector sequence, then one RsaI site, then the cDNA sequence and, finally, the low quality sequence at the end. The pBS vector and the low quality sequences were cut off. The result was the incomplete sequence of the RsaI fragment of the cDNA. In these case the sequence remote from the sequencing primer remains unsequenced and unknown.

Due to wrong ligation some clones contain several RsaI fragments from different cDNAs and/or contain primer inside or at the end. These sequences were clipped out if possible and further analysed separately.

Sequence pre-processing procedure	Number of sequences
Clones sequenced	3400
Sequences contained RSA (GTAC) site - split	219
Sequences contained 2 RSA sites - split 2x	11
Sequences contained 3 RSA sites - split 3x	1
Sequences contained primer at the end - cut on one side.	643
Sequences contained primer in the middle - split	22
Sequences consisted only of primer - thrown away	152
Cut Sequences	3351
Resulting Fragments	3221
Sequences used in clustering	3107

Table 2. Summary of the Pre-processing of raw sequences.

3.5.2. Sequence clustering

After the sequence processing, sequences were assembled with the Staden programme package. 3107 sequences were used in clustering. 956 resulting sequence clusters (RSA-Fragments) were obtained. Four sequences were not useful for further analysis because they were shorter than 30 bp. Thus, the final number of clusters with a size more than 30 bp was 952 (Table 3).

Number of sequences in cluster	Number of clusters
1	559
2	138
3	40
4	39
5	35
6	14
7	20
8	18
9	8
10	10
11	15
12	12
12-24	37
24-48	9
57	1
61	1
Total (3107)	956 (952 longer than 30 bp)

Table 3. Summary of sequence clustering.

3.5.3. Data base search.

The resulting RsaI-fragments were used to search against several databases of known sequences to determine known genes. Databases searched were the EMBL Nucleotide Database, the SwissProt Protein Database and the EST Consensus Databases of Mouse and Human from GeneNest (Haas, Beissbarth et al. in press). The found Database Sequences were assembled with the RSA-Fragments using the Staden Package (Staden, Beal et al. 2000).

Amongst sequences we found known mouse genes, homologies to known genes of other species, mouse ESTs, homologies to ESTs from other species, homologies to genomic sequences and a many novel sequences never sequenced before (Table 4, p. 40).

Sequence type	Number of RsaI fragments	Percent of RsaI fragments	Number of contigs
Known mouse genes	259	27%	161
Homologies to known genes (mostly rat and human)	161	17%	119
Identical to mouse ESTs	283	30%	226
Homologies to ESTs from other species	54	6%	48
Novel sequences	199	20%	199
Total	956	100%	753

Table 4. Types of sequences from cremSL library.

3.5.4. Sequence ontology.

There are 420 known and homologous to known genes in the cremSL library. Most of the sequence annotations were retrieved from the Swissprot data base automatically. Swissprot contains very useful information about the function and expression pattern of many genes. Those genes that were not annotated in Swissprot we annotated manually using the PubMed literature data base. Often it was possible to find such an information in abstracts but in some cases it was necessary to extract information directly from papers.

Most cases there were no uniform naming of protein functions. We had to design our own functional nomenclature in order to reasonably classify the genes from cremSL library.

Functional classification of cremSL clones is shown Table 6, p. 41. These data are included in clone description in Appendixes 1 and 2, pp. 102 and 109, and in database located in Internet:

<http://www.dkfz.de/tbi/people/beissbarth/private/crem-project>.

The functional classification consist of functional categories and subcategories. The functional category reflects general function of the protein. The functional subcategory reflects more detailed function or particular pathway protein acts in. For example, GAPDS belongs to category "Metabolic Enzymes" because in general it participate in cell metabolism and to subcategory "Glucose Turnover" because it catalyses one of reaction of glucose turnover pathway.

In some cases we were not able to determine the subcategory because of lack of information in the literature. The function of many proteins is studied only generally and precise *in vivo* function or pathway have not been defined yet. For such genes the field "Functional Subcategory" is empty.

In 47 cases (20%) it was not possible to define any function at all because these proteins were not studied from the functional point of view.

Table 5. (Continued on next page) Functional groups of genes from cremSL library.

	Functional Category	Functional Subcategory
1.	Sperm Structure	a - Calyx Component b - Fibrous Sheath Component c - Actin Polymerisation d - Outer Dense Fiber Component e - DNA Compaction f - Sperm Dynein g - Sperm-Sertoli Cell Adhesion h - Sperm-Egg Fusion
2.	Signal Transduction	a - PKA Pathway b - Phosphatidylinositol Pathway c - Lysophosphatidic Acid Pathway e - Angiotensin Pathway f - Protein Kinase g - PKC h - Protein Phosphatase i - Membrane Receptor k - Orphan Receptor l - GTPase Activator m - Ras GTPase Activator n - Ligands Removal-Accumulation o - Insulin degradation p - Corepressor for Homeodomain Transcription Factors r - Homeotic Protein Kinase
3.	Molecular Chaperon	
4.	Intracellular Transport	a - Vesicle Targeting to Cell Surface b - Vesicle Targeting to Vacuole c - Vesicle Targeting d - Nuclear Pore Transport e - Golgi - ER Transport
5.	Metabolic Enzymes	a - ATP Synthesis b - Glucose Turnover c - Fructose Turnover d - Guanine Nucleotide Synthesis e - Fatty Acids Turnover f - Glycosylphosphatidylinositol Synthesis
6.	Crossmembrane Transport	a - Ion Transport b - Amino Acid Transport
7.	Nuclear skeleton & Motility	a - Lamina Element
8.	Cytoskeleton & Motility	a - Dynein b - Tubulin c - Actin Polymerisation

Table 5. (Continuation).

	Functional Category	Functional Subcategory
9.	Cell Cycle Regulator	a - Entry into S Phase b - Chromosome Condensation c - Sister Chromatid Cohesion
10.	Protein Degradation	a - Ubiquitin Pathway b - Proteasome Inhibitor c - Proteinase Inhibitor d - Protease
11.	Protein Modification	a - Protein Cross-Linking b - Protein Precursor Cleavage c - Signal Peptide Removal
12.	Transcription	a - Transcription Initiation Factor
13.	Transcription Factor	a - Sterol Regulatory Element Repression
14.	RNA Modification	a - RNA Helicase b - Splicing Factor c - RNA Polyadenylation
15.	Translation	a - Translation Elongation Factor b - Ribosomal Protein c - Translation Initiation Factor d - tRNA Synthetase
16.	Mitosis	a - Chromosome Motility
17.	Signal Transmission	a - Neurone Voltage Dependent Ion Channel b - Neurotransmitter Symporter
18.	Telomere length maintenance	
19.	Protein Transport	a - Protein Insertion Into ER
20.	Histones & HMGs	a - Histones b - HMGs
21.	Cell Junction	

Some gene products are studied so exhaustively and have so significant function that in the field "Functional Subcategory" we put more detailed function description or even protein name. For instance, PKC belongs to category "Signal transduction" and to subcategory "PKC". This approach should simplify further analysis of such a complicated process as the spermatogenesis.

Functional classification taken together with expression profiling may provide useful correlations in searching for coexpressed and coregulated groups of genes and, finally, it may be useful for network modelling of process of spermatogenesis.

3.6. High density filter production and hybridisation

There are different approaches for expression profiling: Northern blot, RNase protection, RT PCR, light cycler RT PCR. By all these approaches only one individual mRNA may be studied in one experiment.

The cDNA array approach that was developed in the last five years allows to study the expression of thousands of genes at once. In our expression profiling study we used the high density filter hybridisation with labelled testis cDNA from the CREM *-/-* mutant mice and wild type mice of different ages.

The gene expression profiling includes several steps:

1. High density filter production.
2. High density filter hybridisation with labelled cDNA.
3. High density filter hybridisation evaluation.
4. Expression profiling.
5. Profile clustering.

3.6.1. High density filter production

High density filter have to answer to following requirements:

1. The filter have to contain appropriate DNA amount in each spot;
2. The filter have to contain appropriate controls for normalisation of hybridisation and quality control.

3.6.1.1. Selection of best cluster representatives

The cluster of sequences is the set of several clones with the same or similar sequence. In the cremSL library clusters contain from 1 to 61 clones. Clones belonging to one cluster may be of different quality. So, it is important to choose the best clone which represent the cluster.

Each clone has been sequenced only once. Therefore, the quality of sequences is different because of the different quality of the sequence reactions and gel runs.

Thus, for the high density filter production we chose preferentially the clones with the best sequence quality.

For quality selection we used a system called fuzzy logic. This is the logical system manipulating with the criteria and weights of these criteria. The criteria of clone quality were combined rather intuitively and were improved during the processing. Weights were assigned for each criteria. Some criteria were weak, other were strong. According to the weight criteria influence the overall score. We used the following criteria and weights:

1. Data Base Match - clone has a database homologue, i.e. represents a real sequence (weak)
2. Ns - percent of unrecognised nucleotides in the sequence - if sequencing quality is good it contains few Ns (weak)

3. dist_start, dist_end - as the clones in cluster represent RSA fragments they should all start or end at the same position, if there are many clones an optimal start and end position is computed, clones which start and end at the optimal positions are quite secure (weak).
4. Length - for good hybridisation results clones should have an equal length distribution, if possible, clones with a length between 200-300 bp were preferentially chosen (weak) if the length was lower than 30 bp clones were not used (very strong) (short clones don't give a signal)
5. Contamination - sequences which contain RsaI sites or primers were rated very bad (strong)

The system was set up in a program called FuzzyTech on a PC. For each cluster one optimal clone was chosen.

3.6.1.2. Selection of hybridisation specificity controls

The labelled cDNA is a very complex probe consisting of a huge number of DNA fragments of different sequences and length. It may cause the non-specific cross hybridisation between homologous sequences. To avoid it, high density filters have to contain controls of hybridisation specificity.

Any heterologous DNA from evolutionary remote species which is not highly homologous to mouse DNA may serve as the hybridisation specificity control. One of the controls we used was the pBS plasmid DNA. Another one was the salmon sperm DNA. The high hybridisation signal of these spots containing control DNAs means the high level of cross hybridisation. In this case stringency of hybridisation conditions must be increased.

3.6.1.3. Selection of nondifferentially expressed controls

According to production procedures the subtracted library should contain mostly the differentially expressed genes. To compare filter hybridisation with cDNA from different sources normalisation control is necessary. It means that an additional set of nondifferentially expressed genes has to be spotted on the high density filters in order to be able to normalise raw hybridisation data. The number of nondifferentially expressed genes must be sufficient for statistically significant normalisation. Of course, it is not possible to predict precisely which genes are nondifferentially expressed in a particular tissue or type of cells. Therefore it is necessary to collect potentially nondifferentially expressed genes in excess. We have spotted 54 potentially nondifferentially expressed genes belonging to different functional classes: metabolic enzymes, basic transcription, translation factors, etc.

In the search for nondifferentially expressed genes we used different literature sources but mostly the paper about the use of a cDNA microarray to analyse

gene expression patterns in human cancer (DeRisi, Penland et al. 1996). The set of nondifferentially expressed genes were also provided by Bernd Korn from German resource Center RZPD.

3.6.1.4. Selection of differentially expressed controls

To assess the difference of expression several differentially expressed genes were spotted on the high density filters. There are some genes published in the literature as expressed stage specifically during spermatogenesis. The ACE and TP1 are known to be the direct CREM target genes (Goraya, Kessler et al. 1995; Zhou, Sun et al. 1996). Protamine1 gene is expressed specifically in round spermatids. Expression of these three genes is abolished in CREM knockout (Blendy, Kaestner et al. 1996). These genes may serve as a control of differential expression in gene profiling experiments.

Some other genes with spermatogenic stage or cell specific expression were chosen. In total sixteen differentially expressed genes were spotted on high density filters.

3.6.1.5. DNA preparation of best cremSL library cluster representatives for spotting on high density filters.

The whole cremSL library is kept at -80 C. First step of clone amplification is done by colony PCR. PCR products were kept in the PCR product collection. One part of these PCR products was used for sequencing, another part was used for second step of amplification for high density filter preparation.

The quality of all PCR products was controlled by the agarose gel separation. The concentration was detected by the comparison with the DNA mass ladder. The samples containing several fragments (Fig. 13, line 6 and 25) or of low DNA concentration (Fig. 13, line 5) were discarded.

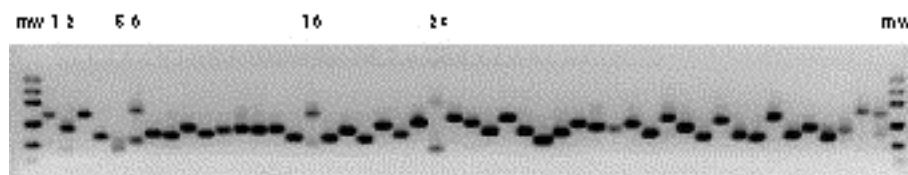


Figure 13. The quality control of the PCR products by separation in an agarose gel.

3.6.1.6. DNA preparation of control clones for spotting on high density filters.

Most of control clones were ordered from German Resource Center RZPD. These clones arrive in agar stabs as bacteria transformed by the plasmid. First of

all we spread it on agar plate by plastic loop. Then particular colonies we placed in the well with LB medium in 96 well microtiter plate. This steps are necessary because clones from RZPD are often cross infected or mixed. By colony PCR clones we amplified and checked by sequencing. Clones with right sequence were amplified by colony PCR. Quality of each PCR product was checked by agarose electrophoresis.

PCR products containing additional bands or of low DNA concentration were discarded. PCR products of satisfactory quality were spotted on high density filters.

3.6.2. High density filter hybridisation

The basic approach for expression profiling was the high density filter hybridisation. Filters with spotted cremSL clones PCR products and control DNAs we hybridised with labelled testis cDNA from CREM knockout and wild type mice of different ages. Labelling was performed by the hot first strand cDNA synthesis using ^{33}P -dATP according to R. Arribas protocol (personal communication). The major advantage of this protocol is that cDNA synthesis reaction carried out in as small a volume as possible. It allows to use small amount of polyA-RNA and increases incorporation efficiency and, indeed, efficiency of hybridisation.

For hybridisation we used special buffer (Clontech, USA) designed especially for high density filter hybridisation with cDNA. Hybridisation in this buffer is 5-10 times more efficient (in actual radioactivity density of hybridised spots) than other tested common buffers for southern or northern hybridisation.

After hybridisation filters were washed in common SSC/SDS washing solutions. It was crucial to wash filters twice in high stringency 0,1xSSC/0,5%SDS solution for significant reduction of cross hybridisation.

Then the filters were exposed on screens and scanned by phosphoimager. After hybridisation and exposure the amount of radioactivity on the membrane was measured using a phosphoimager, resulting in an image file whose grey level correspond to this amount. The grey levels are supposed to be proportional to the amount of radioactivity on the filter over a long range of numbers.

The evaluation of scan data files were performed by AIS ArrayVision array analysis program (Imaging Research, CA, USA). This program in a semiautomatic way recognises spots of array according to specified grid. Then it quantifies intensities of each individual spot. Data come out as a text file of intensity values and array positions for all the spots in the array.

3.6.3. High density filter hybridisation evaluation

3.6.3.1. Background correction

There are several sources of background of nylon filter hybridisation and evaluation. The nylon filter by itself absorbs some radioactivity during hybridisation. It depends on filter quality, radioactivity incorporation efficiency and hybridisation conditions.

Filters of low quality display high level of radioactivity absorption. According to our experience the best nylon filters are Hybond-N+ (Amersham). Hybridisation with Hybond-N+ are always very clean and of low uniform background due to very low level of radioactivity absorption. Experiments presented here were performed on Nunc OmniTray Membrane characterised by higher background level. Background correction of these membranes leads to the loss of spots of low intensity from further analysis.

The background level depends as well on the incorporation efficiency. The higher the incorporation percentage of the radioactivity incorporated in the cDNA the lesser amount of non-specific radioactivity may attach to the filter. The use of fresh radioactive label right after the arrival from the producer always benefits to get better results.

Another source of background is the imaging plate which gradually absorbs background irradiation from the environment. The optimal time should be long enough to allow the accumulation of specific irradiation absorption but short enough to accumulate significant background irradiation.

AIS ArrayVision array analysis program allows to choose various methods of background correction. We evaluated background around each grid's primary element individually and subtracted it from the value of each spot.

3.6.3.2. High density filter hybridisation normalisation

Each hybridisation is a particular experiment. Due to the difference in complexity of mRNA pools, label incorporation efficiency, background level and exposure time there is a variability of intensities measured by the phosphoimager. Indeed, hybridisations under comparison have to be normalised in order to set up the real reference level of zero differences.

There are different methods to normalise hybridisation data. In the case of cremSL the special set of additional nondifferentially expressed genes was spotted on high density filters. Hybridisation values of all spots were normalised according to values of nondifferential controls. Thus, on the scatter plot the values of this controls close to 1/1 ratio line (Fig. 14, p. 49).

3.7. Expression profiling.

3.7.1. The definition of differentially expressed and developmentally regulated genes.

We call the genes for which hybridisation intensity values differ more than three times in adult wild-type in comparison with the adult CREM knockout the differentially expressed genes. The genes which show less than the three times difference we call nondifferentially or equally expressed.

Developmentally regulated are the genes of which hybridisation intensity values differ more than three times between the minimum and maximum values in the time course experiments (hybridisation with mRNA from wild-type prepubertal mice of different ages). If the maximum differs with the minimum less than three times we call this gene constantly or constitutively expressed.

3.7.2. Expression profiles of nondifferentially expressed control clones

The comparison of the expression of the nondifferentially expressed control clones shows that 57 clones were equally expressed in knockout and wild-type mice (red crosses on Fig. 14, p. 49). All these clones did not show big differences of expression level in the time-course experiment. They are expressed constantly during all studied stages of spermatogenesis. In all hybridisations values of hybridisation intensities of these clones did not vary more than 3 times.

3.7.3. Expression profiles of differentially expressed control clones

The differentially expressed control clones (differential controls) spotted on the filters are genes well described in literature. They represent the genes expressed during the post meiotic stages or direct CREM target genes.

The comparison of the high density filter hybridisations with wild-type cDNA versus knockout cDNA show that they are downregulated in the CREM knockout testis. For instance, the ACE cDNA clone known as the direct target of CREM has the ratio of radioactivity intensities of wild-type divided by the knockout intensity of 46 times. For TP1 it is 171,6, for Protamine 1 the ratio is 29. Qualitatively all these data are in agreement with Northern hybridisation data published previously (Blendy, Kaestner et al. 1996).

The time-course experiment (the hybridisations of high density filters with the cDNA from testis of different age mice) revealed that differential controls are specifically regulated during spermatogenesis with no expression in early stages in 9-19 days old mice (spermatogonia, spermatocytes), upregulation at days 21-23 (round spermatids) and reaching maximum at days 25-27 (round and elongated spermatids).

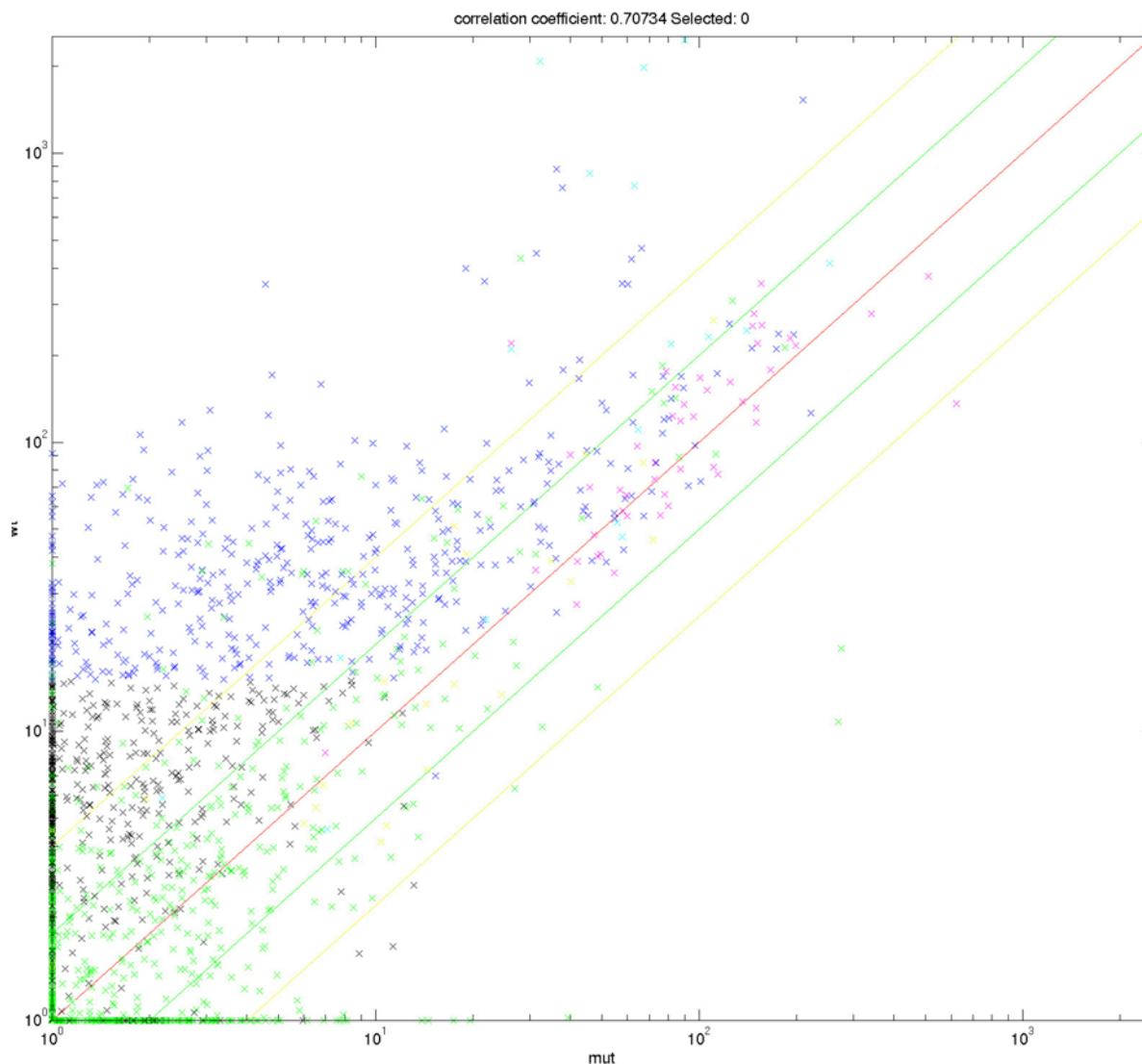


Figure 14. Scatter plot of the measured Intensities of Gene Expression in Mutant vs. Wild type (Numeral values presented in Appendix 2, p. 109)

Housekeeping genes are adjusted to have a median factor of 1.

Designations: red line - 1/1 ratio (equal expression); green lines 1/2 and 2/1 ratios; blue lines - 1/4 and 4/1 ratios; red cross - housekeeping genes; light blue cross - differentially expressed genes; dark blue cross - clones from cremSL library; green cross - empty spot; black cross - clones from cremSL library under the cut-off value of hybridisation signal.

The classical control clone ACE shows no expression from 9 to 21 days, then small upregulation at day 23 and very high maximum expression at days 25 and 27. Another direct CREM τ target gene TP1 has the similar profile of expression but just with some drop of expression at day 27. Thus, it seems possible that the small upregulation at day 23 and the maximum of mRNA level at day 25 is the signature profile of the direct CREM τ target genes.

Taken together these experiments show the clear correlation between the differential expression in wild-type versus CREM knockout and the postmeiotic expression of genes. The wild-type versus knockout nondifferentially expressed genes are constantly expressed during spermatogenesis. It is reasonable to examine this correlation for the mRNAs from the subtracted cremSL library.

The evaluation of nondifferential and differential controls proofs the reliability of the data obtained by the high density filter hybridisation.

3.7.4. Expression profiles of clones from subtracted cremSL library

The cremSL clones may be divided to two groups (clustered expression profiles of all clones are shown on Fig. 15, p. 51).

First group is nondifferentially expressed clones in wild-type and knockout.

Like it is for nondifferential controls their expression is not altered in the CREM knockout and they are expressed before round spermatid stage of spermatogenesis (21 day old prepubertal mice).

The second group of mRNAs is expressed like the differential controls. They are downregulated in CREM knockout and not expressed in young mice and upregulated at postmeiotic stages of spermatogenesis, namely, round spermatids and later (21 and more days old mice).

3.7.5. Types of expression profiles

The expression profile clustering (Fig. 15, p. 51) revealed several types of expression profiles. The various shapes of profiles may be discriminated: from profiles with no expression during early stages and high upregulation at later stages (Krox-like, CREM-target-like profiles, Fig. 16, p. 53) to profiles with maximum of expression at early stages and downregulation at later stages (PGK1-like profiles, Fig. 17(C), p. 55) and different intermediate shapes including constantly expressed mRNAs (b-tubulin-like profiles, Fig. 17(B), p. 55).

The normalised expression profiles presented in figures 16, 17, 19, 20, 21 and 22 are constructed as follows (in collaboration with Tim Beißbarth). In a table of Appendix 2 each row represents a gene and each column a hybridisation timepoint. The values of each column are adjusted by a factor. The factor is chosen on logarithmic values so, that the median difference of each housekeeping gene to its median over all experiments is set to 0 (Beißbarth, Fellenberg et al. 2000). Following this the logarithmic values of each

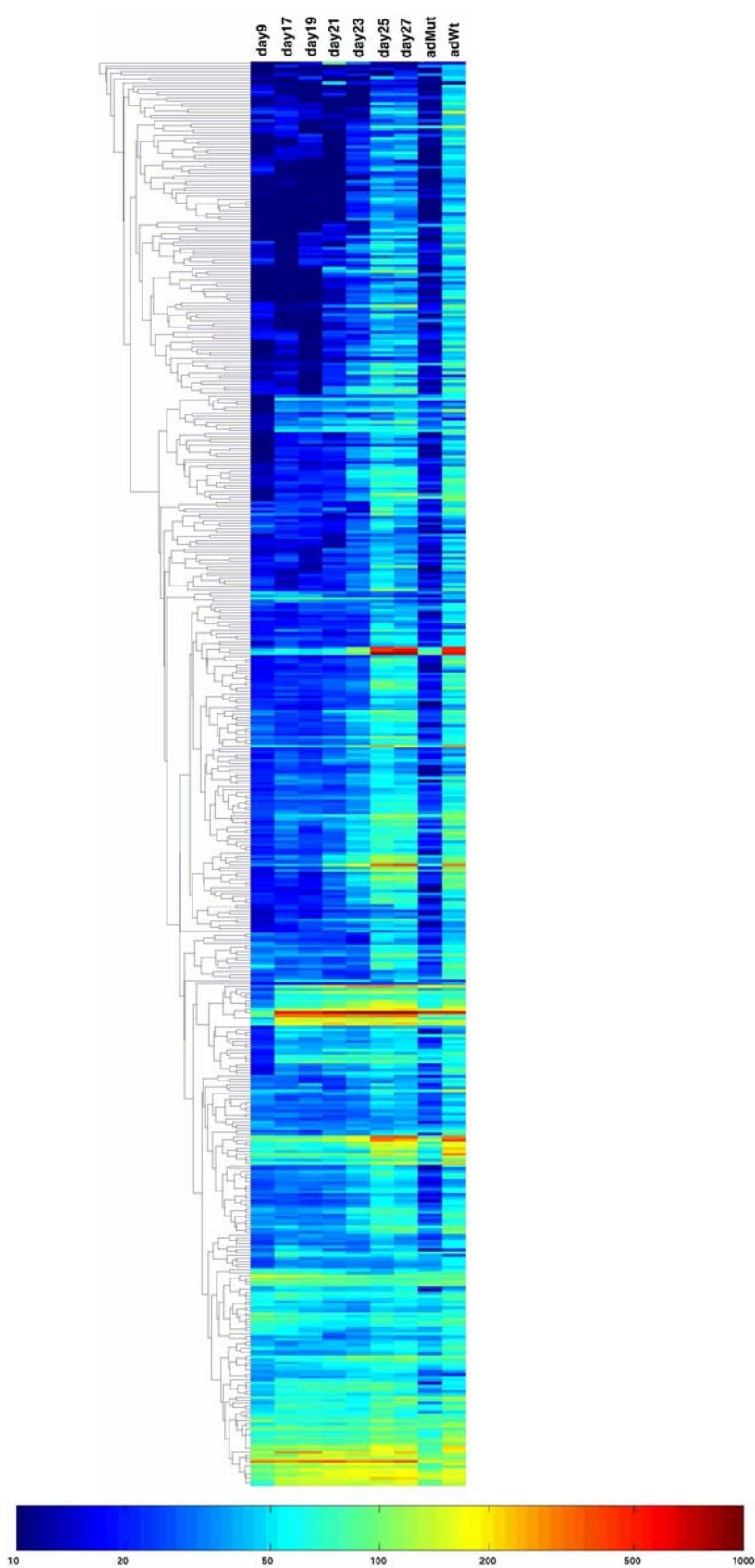


Figure 15. Expression profiles clustered by the modified hierarchical clustering. Each row represents one particular clone. Normalised absolute hybridisation intensities (Appendix 2, p. 109) are coded in colours according to scale presented on lower panel. Designations: p09 - p27 - age of mice; adWt - adult wild type; adMut - adult mutant.

row are adjusted so that the rowsum is equal to 1 by dividing each value by the rowsum. This way the values reflect a distribution of the expression of genes over the timecourse. These values are represented on normalised expression profile graphs of figures 16, 17, 19 20, 21 and 22. The absolute intensity of the gene is irrelevant. For calculation of distances between the profile of two genes the symmetrized relative entropy has been used. The fold of induction between two timepoints 1 and 2 ($F_{1,2}$) may be calculated by the equation $F_{1,2} = e^{(\text{timepoint1} - \text{timepoint2})}$. The values of timepoints are presented in Appendix 2.

3.7.5.1. Profiles of genes downregulated in CREM knockout

3.7.5.1.1. Type 1: Krox20 like expression profile cluster - most lately (stage 5 round spermatids, 25 day old mice) expressed mRNAs

The Krox20 (Chavrier, Janssen-Timmen et al. 1989), Tirp2 and MTDS genes specifically upregulated and display the maximum of expression at day 25 (stage 5 of round spermatids) (Fig. 16(A), p. 53). At day 27 these mRNAs become slightly downregulated. In adult wild type mice these genes are expressed even higher than in 25 day old prepubertal males. In the CREM knockout they are not expressed at all (Fig. 16, panel , p. 53).

3.7.5.1.2. Type 2: CREM-target-like expression profiles with strong upregulation in round spermatids of stage 5 (day 25)

The CREM-target-like profile cluster has this name because these profiles of clones from cremSL library clustered together with two known CREM-target genes ACE and TP1 (Zhou, Sun et al. 1996; Kessler, Rowe et al. 1998). All these profiles have a very similar shape and differ from cluster 1 just by a little upregulation at day 23 (Fig. 16, panel B, p. 53). These mRNAs are not expressed during early spermatogenesis. In round spermatids of stage 3 appearing at day 23 they are slightly upregulated. At day 25 (round spermatids of stage 5) all these mRNAs highly upregulated. The cDNA clones of Protamine 2, β -chimaerin, glucose phosphate isomerase (G6PI), A-kinase anchoring protein 110 (AKAP110), lamina-associated protein 1C (LAP1C), long chain fatty acyl-CoA synthetase and many other clones belonging to ESTs and novel sequences show the same expression profile. It is possible that these genes belong to one coexpression group of genes which expression is activated by the CREM τ transcription factor.

3.7.5.1.3. Type 3: Odf-1-like expression profiles: continuously gradually upregulating from stage 1 (day 21) to stage 5 of round spermatids (day 25)

This cluster is named so because the Odf1 is a gene encoding sperm

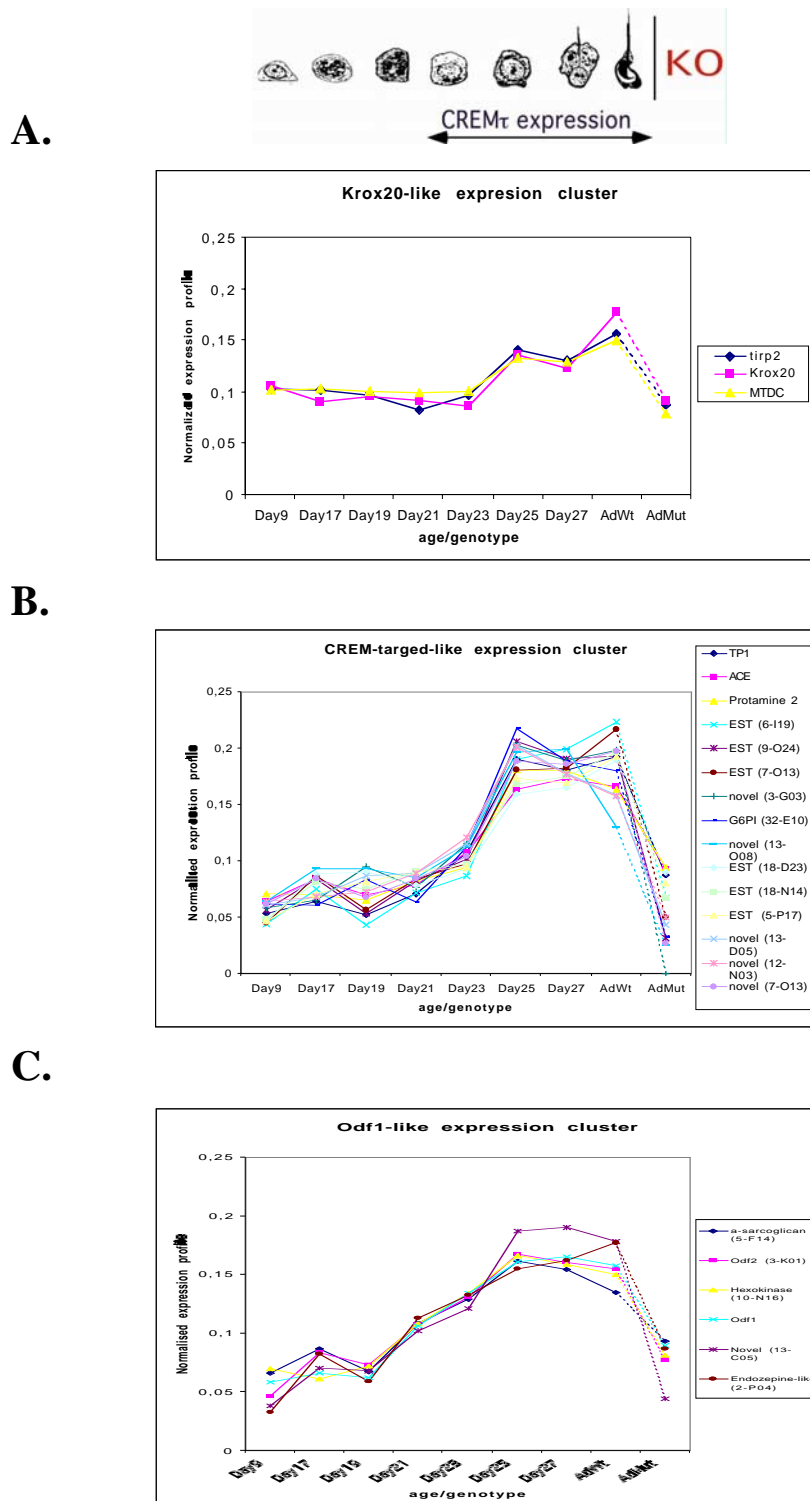


Figure 16. Expression of differentially expressed messages.

A. Krox20-like expression: messages most lately unregulated - at stage 5 of round spermatids (25 days old prepubertal male)

B. CREM-target-like expression: small upregulation at stage 3 of round spermatids and high upregulation at stage 5 (23 and 25 days old prepubertal male).

C. Odf1-like expression: earliest messages amongst differentially expressed - upregulation in stage 1 of round spermatides (21 days old prepubertal male). Designations: KO - CREM KnockOut

outer dense fiber protein (Carrera, Gerton et al. 1994; Chen, Lin et al. 1997). The outer dense fiber protein genes 1 and 2 (Odf1 and Odf2), endozepine-like peptide, α -sarcoglycan, hexokinase mRNA and other mRNAs belonging to this cluster become upregulated at stage 1 of round spermatids (21 days old male) (Fig. 16, panel C, p. 53), then signals of these clones gradually continuously exponentially grow till to the day 25. The level of expression at the day 27 is the same as at day 25 (Fig. 16, panel C, p. 53). All these mRNAs are absent or significantly downregulated in the CREM knockout mice.

3.7.5.2. Profiles of genes nondifferentially expressed in CREM knockout

3.7.5.2.1. Type 4: Tctex1-like expression profiles: mRNAs constantly expressed from pachytene spermatocytes to elongated spermatid stages

Tctex-like cluster contains Tctex1, Tctex2, α -sarcoglycan, α -tubulin and protein phosphatase 1 γ . These mRNAs are not expressed or downregulated at stage 1 of spermatogonia (day 9) (Fig. 17, panel A, p. 55). From stage 9 of pachytene spermatocytes till to the stage 1 of elongated spermatids are constantly expressed.

Tctex-like genes expressed equally in CREM knockout and wild type testes.

3.7.5.2.2. Type 5: β -tubulin-like expression profiles: constitutive expression at different stages of spermatogenesis and nondifferential in CREM knockout.

mRNAs belonging to the β -tubulin group are expressed equally at all spermatogenic stages studied and independently of CREM transcription factor as their expression is not affected in CREM knockout (Fig. 17, panel B, p. 55). This group contains all nondifferentially expressed control genes as well.

3.7.5.1.3. Type 6: Pgk-1-like expression profiles: maximum of expression at stage of spermatogonia (day 9) and gradual diminution to 3d stage of round spermatids

The expression of Pgk-1 in testis is well described in the literature (McCarrey, Berg et al. 1992; Goto, Masamune et al. 1993). The Pgk-1-like cluster contains the tyrosine-threonine dual specificity phosphatase PAC-1, r-ras, HMG-1 and several ESTs and novel sequences. These genes highly expressed at early spermatogenesis (day 9) (Fig. 17, panel C, p. 55) then the expression gradually declines reaching the minimum at stages 1-3 of round spermatids.

All these mRNAs expressed equally in CREM knockout and wild-type testis (Fig. 17, panel C, p. 55).

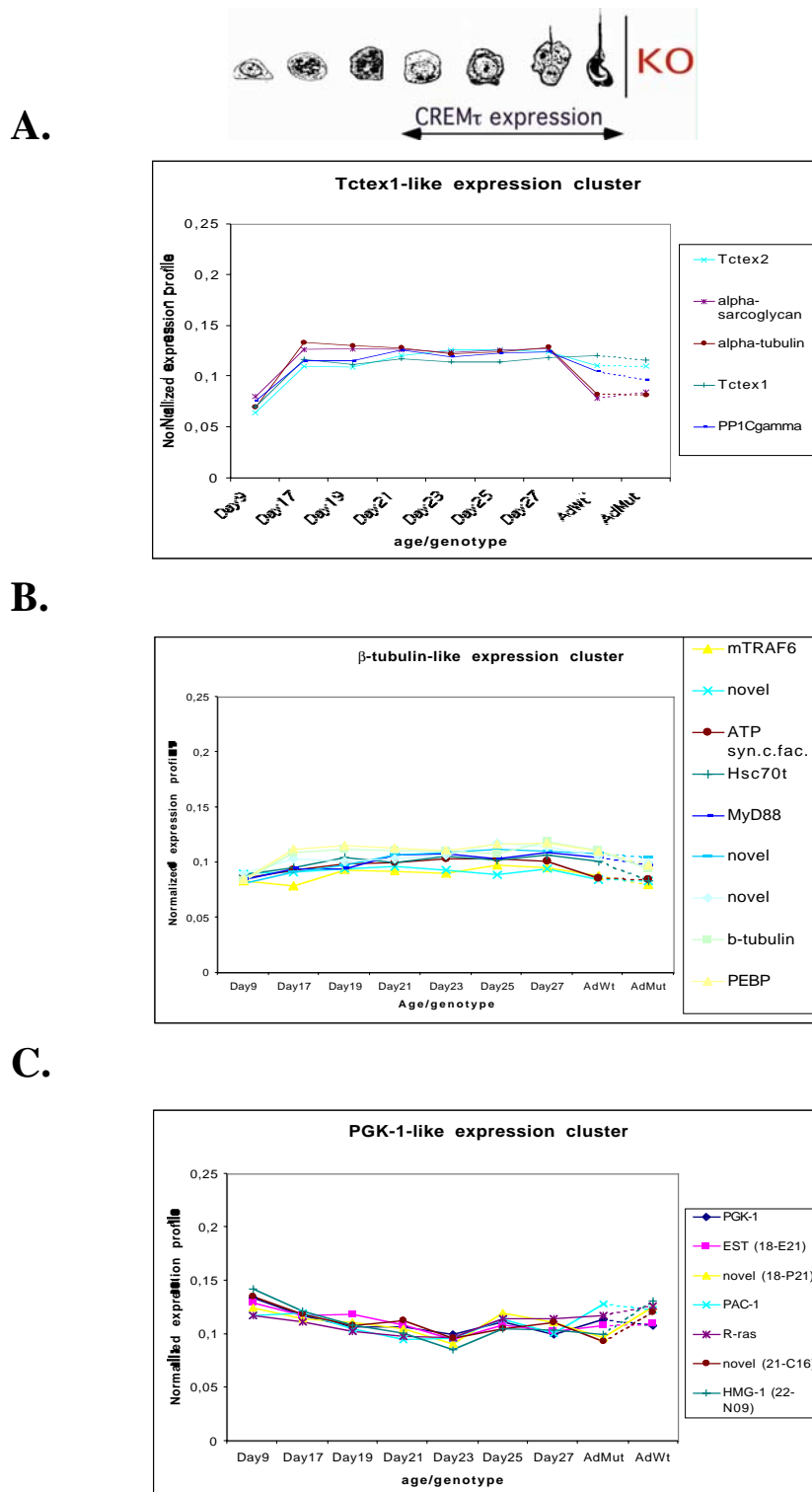


Figure 17. Expression of messages nondifferentially expressed in CREM knockout.

A. Tctex-like expression: No expression at early spermatogenesis then upregulation and constant expression in all later stages.

B. β -tubulin-like expression: little upregulation along spermatogenesis.

C. PGK1-like expression: highest expression level at early stages, then gradual downregulation till to stage 3 of round spermatides (23 day old mice). Designations: **KO** - CREM KnockOt

3.7.6. Expression of genes belonging to different functional groups.

In order to analyse the expression of genes belonging to different functional groups the information about the expression profiles, clone names, gene names and functional characteristics of genes was collected together in one image (Fig. 18, pp. 57-58) and in one Excel file. Using the Excel option "Sort" the genes belonging to one functional group were grouped together and expression profiles were analysed on graphical Excel images (Fig. 19-22). Several groups of coexpressed genes belonging to one functional group were identified.

3.7.6.1 Expression of cremSL clones belonging to genes encoding specific structures of spermatozoon.

There are 13 different genes represented in the cremSL library which encode different specific structures of spermatozoon. 9 of them show the differential and round spermatid stage specific expression, 1 is expressed equally in wild type and CREM knockout and 3 genes can not be analysed due to the low hybridisation signal.

Sperm structural genes may be divided to four groups according to expression profile type:

1. Krox20-like: TP2.
2. CREM-target-like: TP1, protamine 2, DDC8, gsg3, calicin, ADAM4.
3. Odf1-like: Odf1, Odf2, Fsc1.
4. Tctex1-like: Tctex1, Tctex2.

It is important to note that genes having related functions are simultaneously coexpressed. For example, the genes encoding DNA compaction proteins TP1, TP2, and protamine 2 expressed at the same time at stage 3 of round spermatids while the genes encoding core components of sperm tail Odf1, Odf2 and Fsc1 are started to be expressed earlier at stage 1 of round spermatids.

All these mRNAs are not expressed in CREM knockout.

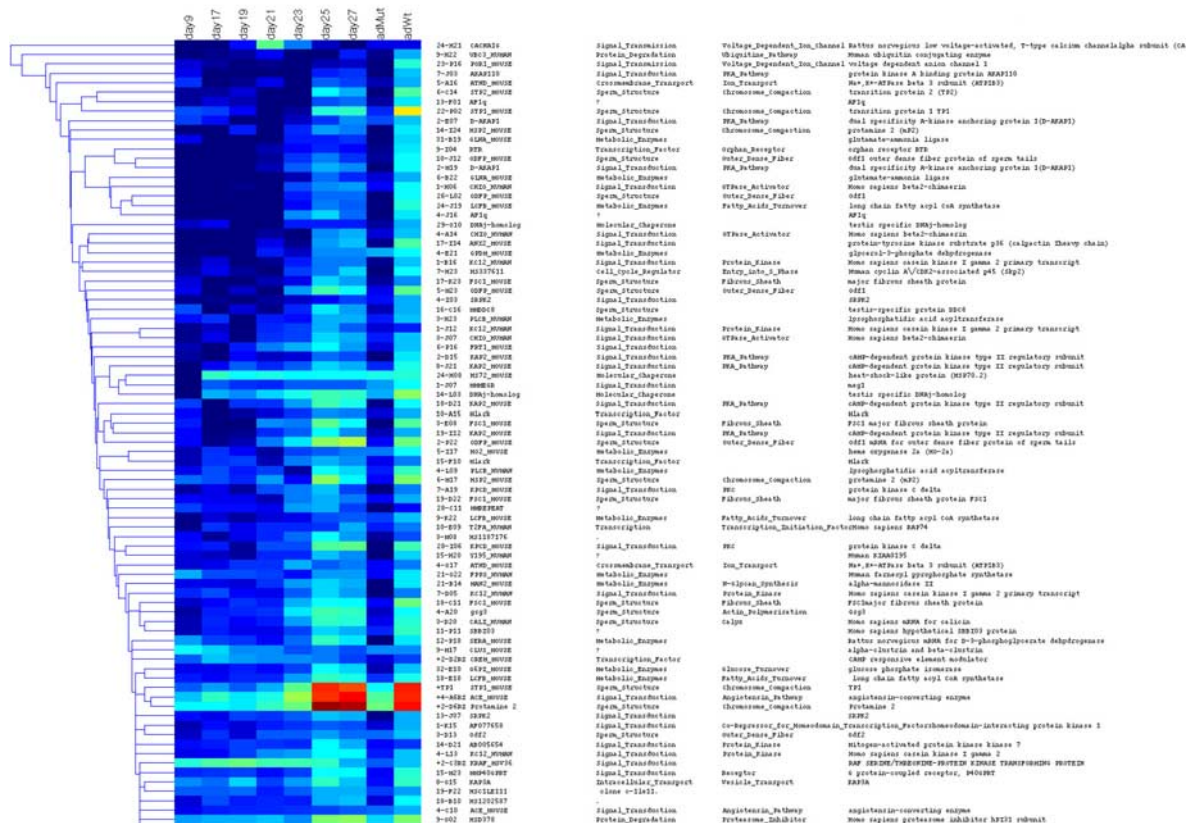


Figure 18 (continued on next page). Clusters of expression profiles of known genes. Text annotations: first column - clone name; second - gene name (from Swissprot database if possible); third - functional category; fifth - functional subcategory; sixth - long gene name.

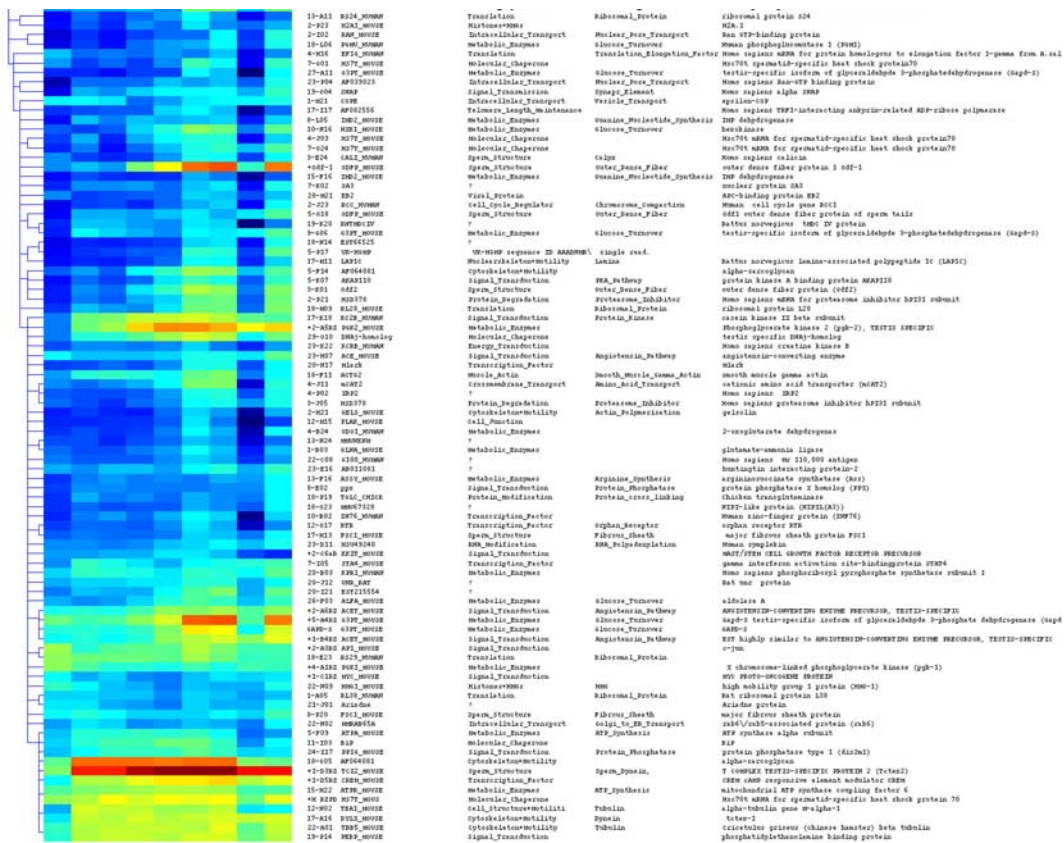
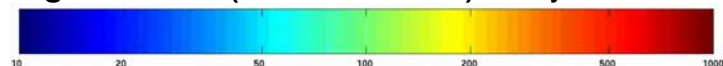


Figure 18 (Continuation). Hybridisation values colour encoding:



3.7.6.2. Expression of cremSL clones belonging to genes encoding transcription factors.

Transcription factor genes show five different expression profile types (Fig. 19, panels A and B, p. 60):

1. RAP74 has a unique type expression. It is expressed most lately - only at stage 1 of elongated spermatids (27 day old mice).
 2. Krox20-like (Fig. 16, panel A, p. 53): Krox20 only.
 3. CREM-target-like (Fig. 16, panel B, p. 53): ZNF76, RTR and Mlark.
All genes belonging to these three groups are not expressed in CREM knockout.
 4. STAT4 is not expressed at early stages of spermatogonia (Fig. 19, panel B, p. 60), upregulated at pachitene spermatocytes of stage 8 (17 day old mice) then downregulated in later stages of spermatocytes and round spermatids, then upregulated at stage of elongated spermatids.
 5. ATF1 is expressed with no significant changes constantly in all mice studied including adult wild-type and CREM knockout (Fig. 19, panel B, p. 60). This data are in agreement with published northern blot data (Nantel, Monaco et al. 1996).
 6. C-jun has two maximums of expression at spermatogonia stage (day 9) and stage 5 of round spermatids (day 25). It is not differentially expressed in CREM knockout probably due to high level of expression at premeiotic spermatogonia stage.
- STAT4 and ATF1 expression are not affected by CREM inactivation in the CREM knockout (Fig. 19, panel B, p. 60) like other mRNAs which are expressed before round spermatid stage.

3.7.6.3. Expression of mRNAs encoding proteins involved in signal transduction.

The mRNAs encoding signal transduction proteins fall into several coregulated groups with different profile types:

1. Krox20-like expression (Fig. 16, panel A, p. 53): LH receptor, FSH receptor, P40GPRT, MAPKK7, progesterone binding protein.
2. CREM-target-like expression (Fig. 16, panel B, p. 53): ACE, CCK, AKAP110, SRPK2, β -chimaerin, LFC.
3. Odf1-like (Fig. 16, panel C, p. 53): PAC-1, casein kinase 1 γ 2, homeodomain interacting protein, PKC δ , PKA regulatory subunit II.
4. Tctex1-like (Fig. 17, panel A, p. 55): PEBP, PTP MEG, Casein kinase 2 β , megl.
5. Constantly expressed: MyD88, PP1 γ , Kit, R-ras, myc, laminin receptor, PPX, raf.

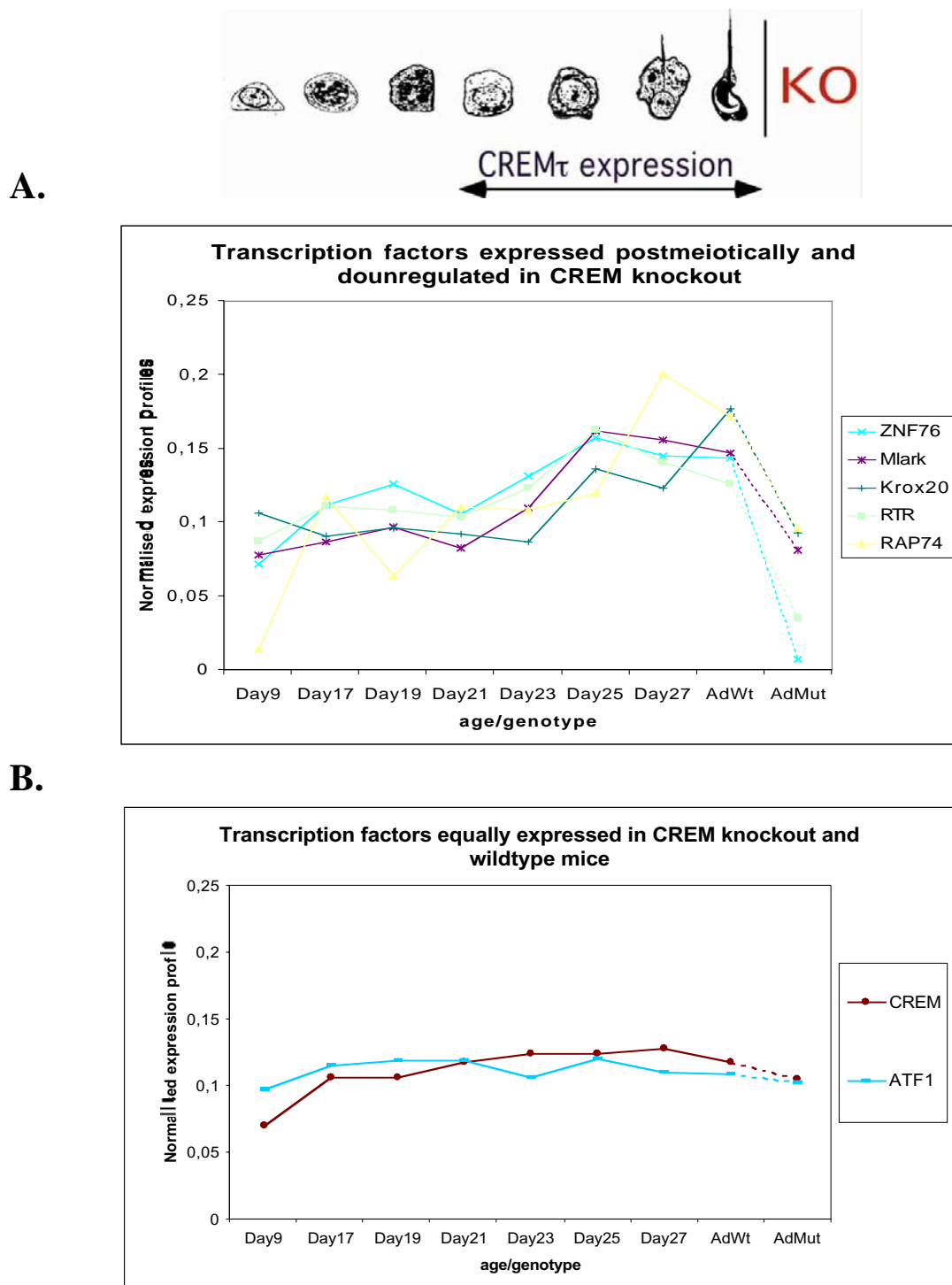


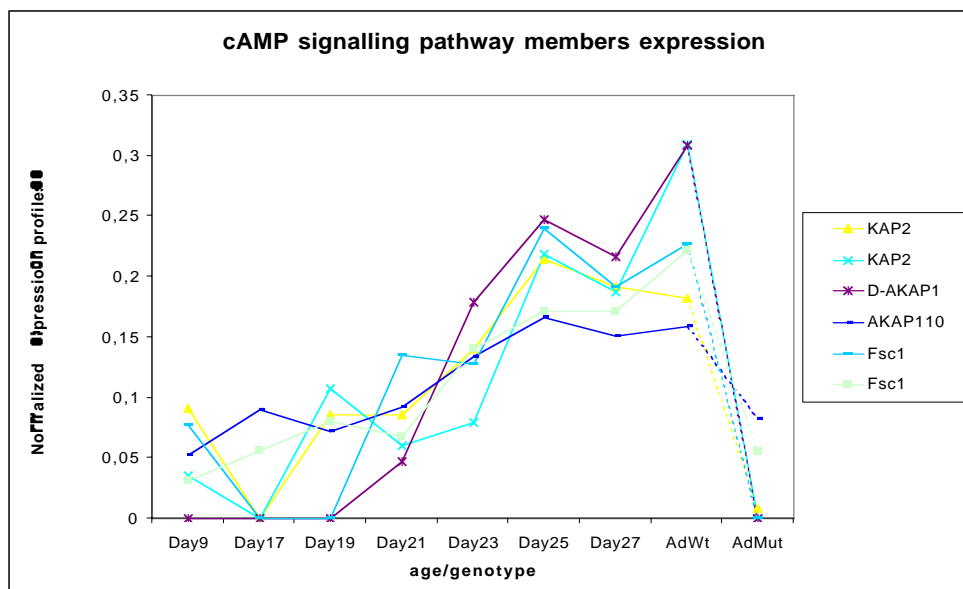
Figure 19. Expression of transcription factors during spermatogenesis

A. Transcription factors downregulated in CREM knockout and expressed postmeiotically.

B. Transcription factors nondifferentially expressed in CREM knockout.

Designations: KO - CREM KnockOut

A.



B.

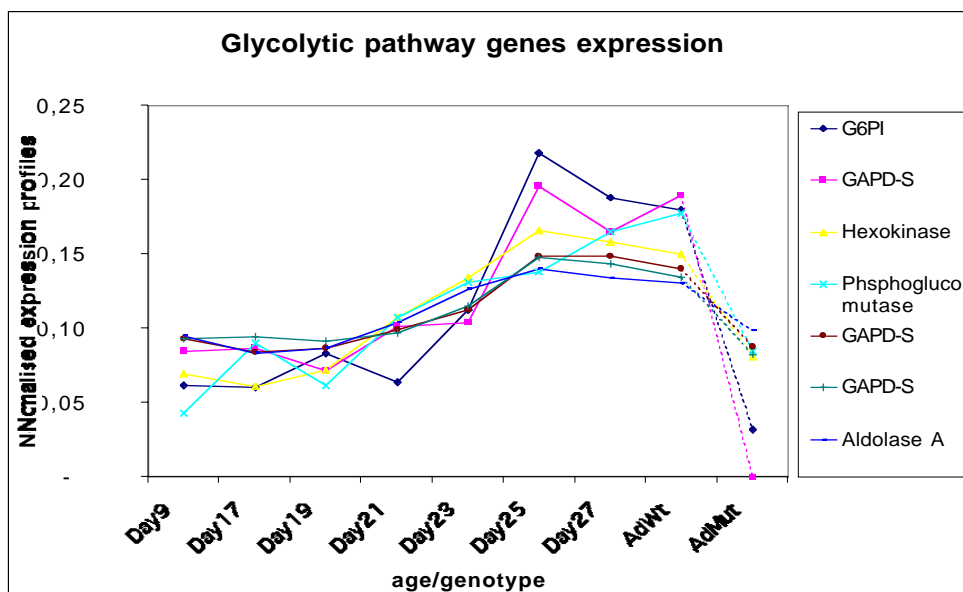


Figure 20. Postmeiotic coexpression of genes belonging to one functional group.

A. Expression profiles of messages encoding proteins involved in cAMP mediated signal transduction pathway.

B. Expression profiles of messages encoding enzymes involved in glycolytic pathway.

Designations: **K O** - CREM KnockOut.

The more detailed analysis may reveal the coexpression of complete signalling networks during spermatogenesis. For example, the postmeiotically expressed proteins AKAP110, Fsc1, D-AKAP1 and protein kinase A regulatory subunit II participate in the cAMP mediated signal transduction (Banky, Huang et al. 1998). All these proteins shown to be localised in the spermatozoon and may participate in processes of sperm capacitation and sperm-egg fusion (Visconti, Johnson et al. 1997).

Thus, there are very complicated signal transduction networks which realise the control on spermatogenesis. Some of these networks function continuously along the whole process of spermatozoon development, another big group of signal transduction proteins is found to be involved in postmeiotic stage specific regulation of sperm development and the process of sperm-egg fusion.

3.7.6.4. Expression of cremSL clones belonging to genes encoding proteins involved in protein turnover and modification

Amongst the proteins involved in translation the ribosomal proteins S24 and L28 are expressed differentially and upregulated at day 23 and later. The S24 have maximum of expression at day 25, at day 27 the level of mRNA returns to normal level. The L28 shows the maximum at days 25 and 27.

The ribosomal proteins L38 and S29 and an mRNA homologous to elongation factor 1 γ are expressed nondifferentially in CREM knockout and expressed constantly during spermatogenesis.

The proteasome inhibitor hPI31 is expressed lower in knockout (2, 2.9 and 5 times less for different RsaI fragments). In prepubertal mice it is lowly expressed till day 23, then highly upregulated at days 25 and 27. Other 9 mRNAs encoding protein degradation proteins were under the level of detection. The mRNAs encoding ribosomal proteins L8 (RPL8), S17 and PO, cysteinyl-tRNA synthetase, Hrs and translation initiation factor eIF3 p40 are under the level of detection.

3.7.6.5. Expression of cremSL clones belonging to genes encoding molecular chaperones

The spermatid specific molecular chaperon heat shock protein 70 (Hsc70t) shows Odf1-like expression.

The molecular chaperon heat shock protein HSP70.2 is expressed in Tctex1-like manner.

The testis specific molecular chaperon DNAj homologue is downregulated in CREM knockout, it is not expressed at day 9, expression is moderate at days 17 and 19 (pachitene spermatocytes), linearly grows next days to the maximum at days 25 and 27 (late round and elongated spermatids).

The molecular chaperon BiP is nondifferentially expressed in CREM knockout and regulated during spermatogenesis. It is moderately expressed in wild-type day 9, linearly unregulated next days, reaches the maximum at day 25 (late round spermatids), then drops down at day 27 (elongated spermatids).

3.7.6.6. Expression of cremSL clones belonging to genes encoding metabolic enzymes.

Metabolic enzymes is the biggest functional group present in the cremSL array. All five glucose turnover enzymes are expressed in CREM-target like manner (Fig. 20, panel A, p. 61). There are testis specific isoform of glyceraldehyde-3-phosphatedehydrogenase (GAPD-S), glucose phosphate isomerase, aldolase A, hexokinase and phosphoglucomutase. All they demonstrate downregulation in the CREM knockout. These enzymes catalyse consequent steps of glycolysis from glucose to 1,3-biphosphoglycerate (see chapter "Discussion" and Fig. 22 on p. 71).

Other genes, the long chain fatty acyl CoA synthetase, D-3-phosphoglycerate dehydrogenase, glutamate-ammonia ligase, 2-oxoglutarate dehydrogenase and lysophosphatidic acid acyltransferase are differentially expressed and regulated during spermatogenesis. They show Krox-20-like expression profiles (Fig. 16, panel A, p. 53).

The nondifferential constitutive expression is shown by the ATP synthetase α chain, ATP synthetase subunit c, the ATP synthetase coupling factor 6, inositolmonophosphate dehydrogenase and subunit I of phosphoribosyl pyrophosphate synthetase.

3.7.6.7. Expression of cremSL clones belonging to genes encoding proteins with other functions

The **histone** H5 and H2A.1 are expressed like CREM-targets (Fig. 16, panel B, p. 53). The histone H5 is downregulated in CREM knockout. The histone H2A.1 is one of very few exceptions which is expressed postmeiotically but nondifferentially expressed in CREM knockout. It is difficult to interpret this fact. Most likely in this case array hybridisation generated artefact and these data should be examined by another expression study method.

The mRNA homologous to the rat **nuclear lamina-associated** polypeptide 1C (LAP1C) demonstrates Krox20-like expression (Fig. 16, panel A, p. 53).

The genes encoding the **nuclear pore transport** proteins Ran GTPase and Ran-GTP binding protein are coexpressed. They are not expressed at spermatogonia stage (day 9), then moderately expressed at pachitene stage (days 17-19) and upregulated at round and later spermatids. Both mRNAs are downregulated in CREM knockout.

The **intracellular vesicle transport** protein KAP3A is downregulated in knockout and expressed like Krox20 (Fig. 16, panel A, p. 53).

The **intracellular vesicle transport** protein BALBVc epsilon-COP shows constant expression in CREM knockout and different spermatogenetic stages.

The **crossmembrane transporters** Na,K-ATPase and cationic amino acid transporter (mCAT2) are downregulated in CREM knockout and show the Krox20 type of expression profiles (Fig. 16, panel A, p. 53).

The **cell junction** ubiquitous protein placoglobin is downregulated in CREM knockout and shows upregulation at day 25.

The **high mobility group 1** protein (HMG1) is nondifferentially expressed in the CREM knockout and shown the Pgk-1-like expression profile (Fig. 17, panel C, p. 55).

The **actin polymerisation regulator** gelsolin is downregulated in the CREM knockout and has the expression profile similar to CREM-target-like profile cluster (Fig. 16, panel B, p. 53).

The **cell cycle regulator** genes homologous to the human cyclin Skp2 and to the chromosome compaction regulator RCC1 are downregulated in the CREM knockout and expressed like CREM-target-like expression group (Fig. 16, pannel B, p. 53).

4. Discussion

4.1 The advantages and problems of expression studies *in vitro* and *in vivo*.

The advantages of *in vivo* study of transcriptional regulation may be deduced from the main problem of *in vitro* and cell culture experiment - reliability of the data obtained. It is obvious now that the functions of homologous proteins often can not be discriminated *in vitro*. Similar proteins interact *in vitro* with the same partners that may never happen *in vivo*. *In vitro* studies usually explore the interaction between two participants (enzyme/substrate in biochemistry, transcription factor/DNA sequence in gene transcription or protein mediator/receptor in signal transduction). *In vivo* interactions are much more complex. One protein may interact with many others simultaneously and exchange counterparts during consequent action. Complex system of membrane structures and anchoring proteins build sophisticated system of compartmentalisation; transport systems give the direction of response. All these determine the specificity of function of particular protein in particular live process. For example, p300 and CBP have the same features *in vitro*. In cell culture experiments they have distinct roles in retinoic-acid-induced differentiation and cell cycle control (Kawasaki, Eckner et al. 1998). Nevertheless, even *in vivo* cell culture experiments may be quite artificial and experiments in organisms may be required in order to understand full complexity of processes.

In vivo study of gene expression have some difficulties and limitations. First of all they are expensive. Second, the microarray experiments demand a lot of RNA and it is difficult to collect appropriate amounts. Third, for expression profiling it is important to study certain time points in accordance to transcription factor activation time. In our CREM τ dependent expression study we were able to do it just approximately. In *in vitro* experiment it is possible to take more short and precise time points and to get expression profiles of early immediate genes which are actually target genes. Fourth, live tissue samples usually are composed of different types of cells and we can not discriminate where exactly a particular mRNA is expressed.

In the case of the CREM τ action in testis the experiments in cell culture hardly may be reliable at all due to the impossibility of cultivation such a complicated multicellular system as seminiferous tubules. The experiments included in this thesis have been done *in vivo* and reflect the real expression in testis.

Applying these thoughts to the data obtained in our study we may conclude the following:

1. We found the genes that are expressed at the time of CREM τ protein expression but we could not determine which of those are early immediate genes.
2. We obtained the data about the genes expressed in testis but our experiments do not give any information about the cell specificity of the gene expression.

3. Expression profiling in mice of different ages reflects the real expression of these genes because we studied wild type mice grown in standard regular conditions without any artificial treatment.
4. We were able to determine gene expression dependent on CREM τ .
5. In order to determine direct CREM τ target genes additional *in vitro* and *in vivo* experiments have to be done.

4.2. Subtractive suppression hybridisation is an efficient method to clone target genes

The first question arising when one starts to establish microarray hybridisation is what set of cDNAs to spot on the microarray. Of course, in ideal case a complete set of all mRNAs should be present on the microarray in order to get the full information. For several reasons such arrays are not available for mouse. First, the mouse genome is not sequenced yet. Second, not all expressed mRNAs are sequenced. Third, it is money and labour consuming to make a complete set for every particular experiment addressed to only one tissue.

In order to avoid all these problems and to obtain the library enriched by the CREM τ targets we used the Subtractive Suppressive Hybridisation. Such an approach appeared to be very efficient. First of all, a quarter of sequences found were novel. The comparison of expression in wild-type and CREM knockout shows that most clones represent differentially expressed mRNAs. Out of nine hundred clones half of them show (about 500) specific expression in time-course expression profiling experiments. For microarrays containing random set of clones this index is much lower. For example, in the study of transcriptional program of human fibroblasts in the response to serum (Iyer, Eisen et al. 1999), out of 8000 random clones only 500 show differential expression.

Thus, subtractive cloning is an efficient approach to obtain a reasonable set of clones for expression profiling experiments.

4.3. Correlation between downregulation in knockout and postmeiotic gene expression.

Two independent sets of experiments have been done. First was the comparison of expression of cloned mRNAs in adult CREM knockout with expression in the adult wild type mice. Second was the time-course expression profiling study addressed to the spermatogenesis stage-specific gene expression. The results of these two sets of experiments appeared to be in agreement with each other.

The differentially expressed mRNAs (downregulated in CREM knockout) always show the regulated (stage-specific) type of expression. All these mRNAs become upregulated in postmeiotic testes (Fig. 16, panels A, B and C, p. 53).

The nondifferentially expressed mRNAs (expressed equally in CREM knockout and wild-type mice) may be divided to two groups: 1) nondifferential-regulated and, 2) nondifferential-nonregulated genes.

Genes belonging to the first group are equally expressed in CREM knockout and wild-type adult testis but expressed at different levels at different stages spermatogenesis. For example, the P_{gk1}-like expressed messages with maximum of expression at early stages (Fig. 17, panel C, p. 55) or the Tctex1-like expression cluster of mRNAs with no expression at early spermatogonia stage (day 9) but upregulated at day 17 and then constantly expressed (Fig. 17, panel A, p. 55).

The genes belonging to the second group are equally expressed in CREM knockout and wild-type adult mice and constantly expressed at all studied prepubertal testis (Fig. 17, panel B, p. 55). It is probable that most of these genes are expressed in housekeeping manner.

Taken together these data mean that if the mRNA is upregulated before stage 1 of round spermatids (day 21) the expression of this mRNA is not altered in CREM knockout. It means that mRNAs dependent on CREM τ (downregulated in CREM knockout) become upregulated concurrently with CREM τ protein expression, namely at stage 1 of round spermatids or later.

mRNAs independent on the CREM τ (nondifferentially expressed in the CREM knockout) become upregulated before the CREM τ protein expression, namely before the stage 1 of round spermatids or expressed constantly.

4.4. Functional systems involved in last stages of spermatogenesis.

The expression profiling experiments revealed the developmental stage specific coexpression of proteins belonging to the particular functional groups.

The biggest number of clones represent signal transduction genes. Out of 44 genes analysed 10 were constantly nondifferentially expressed during spermatogenesis. 34 other mRNAs were expressed postmeiotically and downregulated in CREM knockout. Such a big number of signal transduction proteins reflect the complicated signalling network which regulates the process of spermatogenesis.

Another side of spermatogenesis is the postmeiotic upregulation of both mRNA/protein synthesis and degradation systems. The necessity of it is clear - almost all structures and proteins of round spermatids become substituted by the proteins specific for sperm's structures. It is well known that basal transcriptional machinery proteins and ribosomal proteins are overexpressed postmeiotically. In our subtracted library basal transcription factors are absent but several ribosomal proteins are represented and show postmeiotical overexpression.

Our experiments demonstrate that a number of molecular chaperones are overexpressed postmeiotically as well. Probably they serve for folding of newly

synthesised proteins and for stabilisation of proteins in the spermatozoon during maturation and storage in epididymus.

4.5. Probable role of cAMP-mediated signalling in spermatozoon activity

Many signal transduction proteins are present in the cremSL library. The proteins involved in cAMP mediated signalling are of special interest. Different A kinase anchoring proteins (AKAPs) - AKAP110, D-AKAP1, AKAP82 (fibrous sheath component Fsc1) and protein kinase A regulatory subunit II are found in the cremSL library. AKAPs bind to regulatory subunit and thereby direct the response to increase of cAMP to particular organelle. According to our experiments and in agreement with published data all these genes are upregulated at postmeiotic stages (Fig. 21, p. 69) (Carrera, Gerton et al. 1994; Huang, Durick et al. 1997; Huang, Durick et al. 1997; Huang, Wang et al. 1999; Vijayaraghavan, Liberty et al. 1999). For unknown reasons S-AKAP84 and AKAP220 were not cloned in cremSL library despite the similar expression pattern.

AKAPs possess binding sites for PKA and different specific sites binding to particular organelles (Fig. 21, p. 69). These interactions realise the compartmentalisation of PKA and consequently the direction and specificity of response to cAMP concentration increase (Chen, Lin et al. 1997). The AKAP82 is localised in the fibrous sheath mediating the activation of fibrous sheath slicing and thereby sperm tail movement in response to cAMP (Johnson, Foster et al. 1997; Visconti, Johnson et al. 1997). S-AKAP84 and D-AKAP1 tether the PKA to the mitochondria of spermatozoon and probably mediates the activation of energy production for spermatozoon movement toward the egg (Lin, Moss et al. 1995; Banky, Huang et al. 1998). AKAP110 is located as well in the principal piece of the flagellum as in the acrosomal region of sperm head (Vijayaraghavan, Liberty et al. 1999). It seems that AKAP110 participates in both flagellum movement regulation and acrosomal reaction. Thus, the cAMP signalling system seems to participate in the regulation of sperm action from the very beginning to the end, starting from capacitation (i.e. sperm activation), energy production and movement to the acrosome reaction (Vijayaraghavan, Liberty et al. 1999).

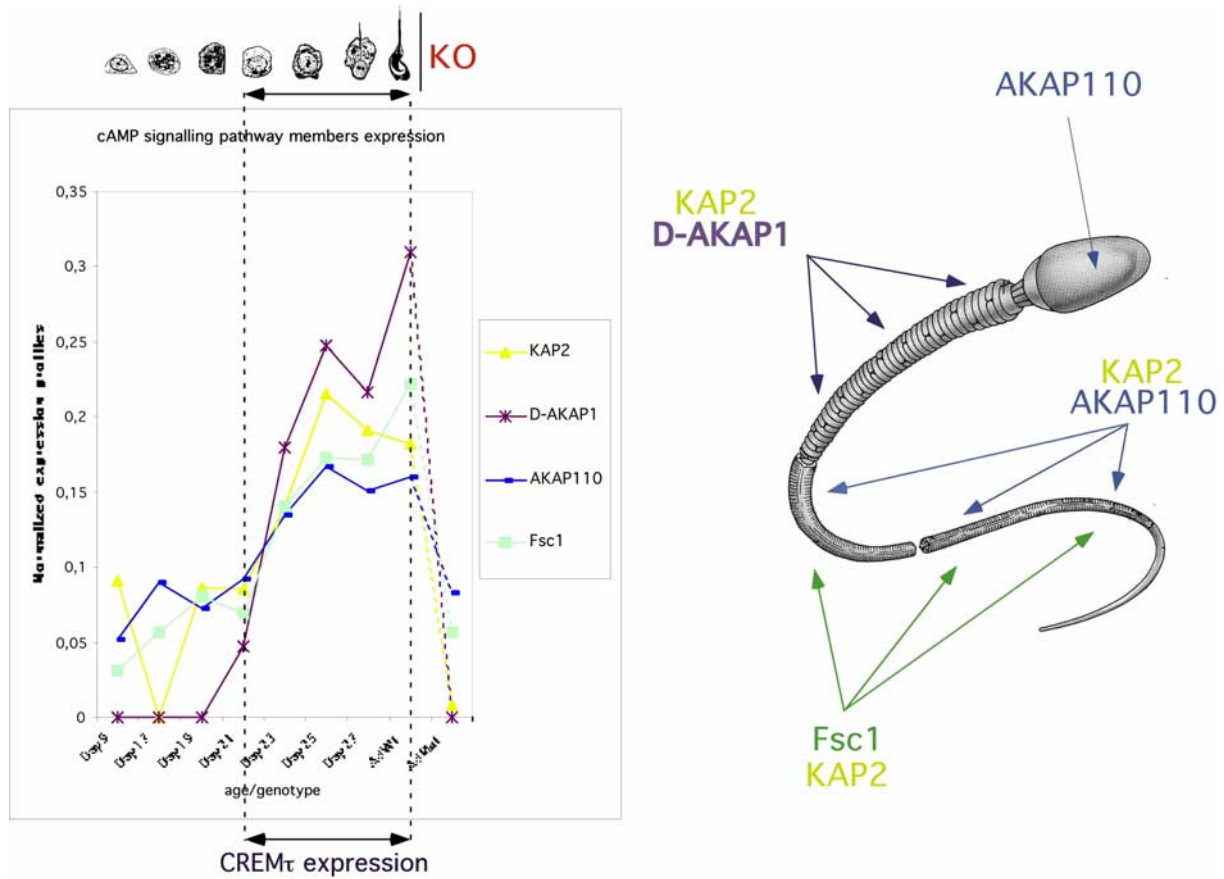


Figure 21. Postmeiotal coexpression of messages encoding proteins involved in cAMP mediated signal transduction.
 Designations: arrows show the localisation of proteins in spermatozoid;
KO - CREM KnockOut.

The complex analysis of this particular cAMP signalling system leads to several important conclusions:

1. Expression of cAMP signalling genes may be directly dependent on CREM τ .
2. Our cloning and expression profiling experiments reflect the real expression of the cAMP system members.
3. It is really possible to extrapolate the expression data to the action of functional network. Of course, one should be very careful in extrapolation and should use all available knowledge about the object of analysis.

4.6. Postmeiotic expression of glycolytic enzymes

It was surprising to find the round spermatid stage specific expression of commonly ubiquitously expressed genes as the members of glycolytic pathway. The cremSL library contains the mRNAs encoding enzymes performing almost all steps of the glycolysis from the very first enzyme hexokinase (catalyses the glucose phosphorylation), glucose phosphate isomerase (isomerisation of glucose-6-phosphate to fructose-6-phosphate), phosphofructokinase (phosphorylation of fructose-6-phosphate to fructose-1,6-biphosphate), aldolase (cleavage of fructose-1,6-biphosphate to dehydroacetone-phosphate and glyceraldehyde-3-phosphate) and, finally, the well studied testis specific isoform of glyceraldehyde-3-phosphate dehydrogenase (oxidation of glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate) (Stryer 1988). Thus, all steps from the conversion of glucose to the energy conservation molecule 1,3-bisphosphoglycerate are represented in the cremSL library. ATP may be easily realised from the 1,3-bisphosphoglycerate in one step reaction catalysed by phosphoglycerate kinase (Stryer 1988) when it is needed (for example for sperm movement to the egg).

The expression of the hexokinase (Kalab, Visconti et al. 1994; Mori, Nakamura et al. 1996; Olds-Clarke, Pilder et al. 1996) and the GAPD-S (Welch, Brown et al. 1995) in spermatids are well studied. The testis expression of all other glycolytic proteins and its mRNA is studied poorly and our information about it is novel.

In conclusion, the glycolytic system is an example that mRNAs encoding a complete set of proteins involved in one process are cloned by differential cloning and have similar expression profiles.

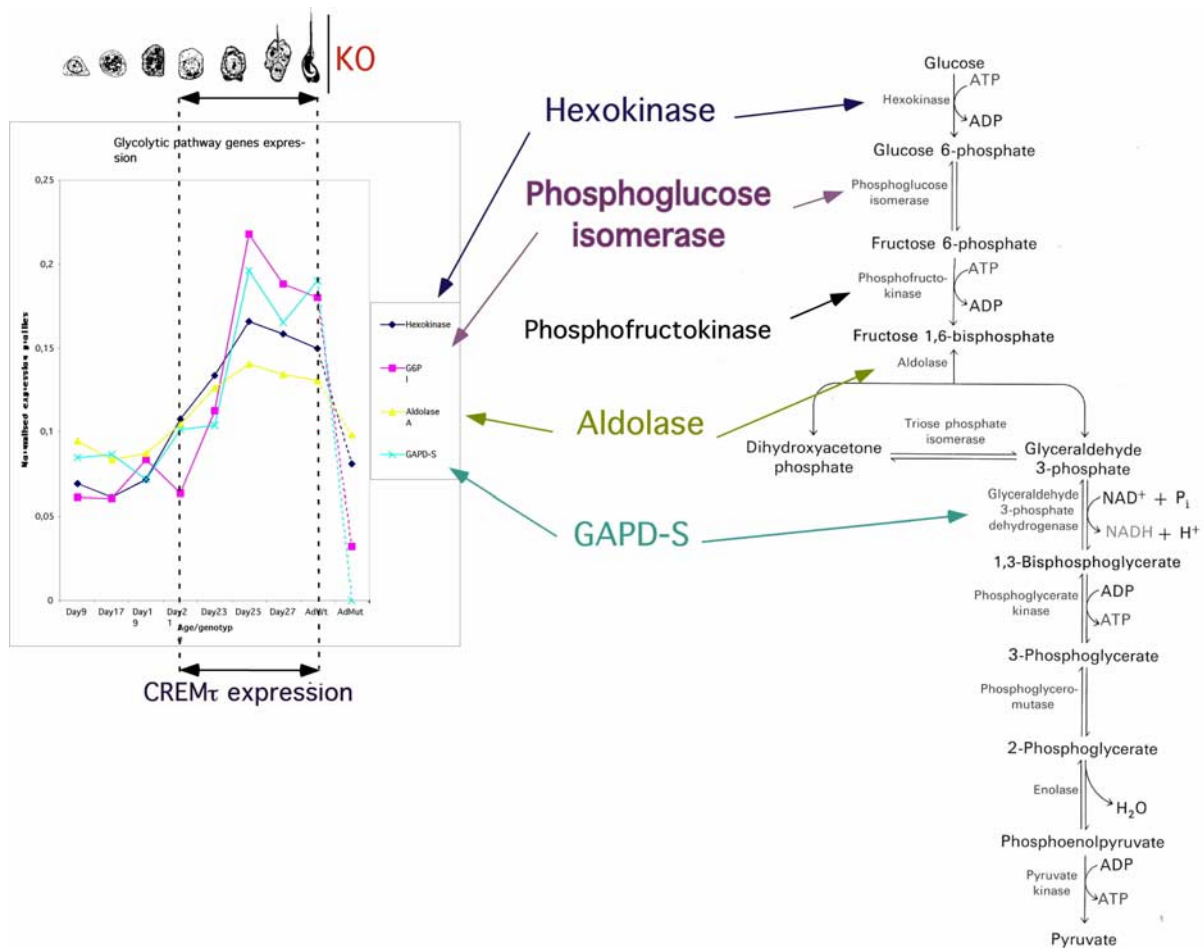


Figure 22. Postmeiotic coexpression of mRNAs encoding glycolytic enzymes.

Designations: **KO** - CREM KnoOt.

4.7. Possible applications of CREM dependent expression study and further development of CREM dependent expression study

Our data open many possibilities for further development of spermatogenic expression study in different areas of research:

1. Analysis of novel sequences from the cremSL library. The clones from the cremSL library are short RsaI fragments with length no more than 600 bp. These short clones may be used for the full-length cDNA libraries screening and the complete cDNA clones may be cloned and sequenced. The full-length cDNA clones in turn may be used for genomic DNA library screening and complete gene sequence and structure may be determined.
2. Gene promoter analysis. Our expression profiling study revealed many groups of contemporary coexpressed genes. Such coexpressed genes probably are coregulated by the same transcription factors. The comparison of promoter regions of these genes may reveal binding sites for the transcription factors. It seems that many postmeiotically expressed genes may be regulated by CREM τ .
3. Search for the direct CREM τ target genes. According to its features the CREM τ is the most probable candidate for the activation of coexpression of many postmeiotically expressed genes. The genes having alternative testis specific mRNA variants or alternative CRE containing promoters are the most probable CREM τ targets.
4. Spermatogenic cell specific expression study. In our expression profiling studies we used the RNA isolated from whole testis from wild type mice of different ages and from adult CREM knockout mice. Therefore, we can say nothing about the cell specific expression but only about expression in the entire testis. In frame of proceeding of spermatogenesis expression study would be reasonable to define the cell specific expression of cloned mRNAs. Different spermatogenic cell types may be separated by the elutriation (Meistrich 1977; Meistrich, Longtin et al. 1981; Bucci, Brock et al. 1986) and fractionation by the velocity sedimentation (Romrell, Bellve et al. 1976). *In situ* hybridisation may be useful for cell specific expression as well.
5. The study of fertilisation potential of CREM deficient spermatid nuclei by the injection of spermatid nuclei into the egg (Sasagawa, Ichianagi et al. 1998). Promising experiment is the ejection of CREM knockout spermatid nucleus into the egg. It will demonstrate whether the CREM dependent expression is important for normal functioning of the zygote. It may be possible that proteins expressed at later stages of spermatogenesis are important just for sperm development but they are not necessary for zygote formation and further development of organism. From the medical point of view it may demonstrate the possibility to perform artificial fertilisation by the samples from patients with deficiency in any gene from group of found by us CREM target genes. Many infertile men display an impaired CREM expression (Lin, Lamb et al. 1998;

Weinbauer, Behr et al. 1998). In case the CREM deficient nuclei injection will be successful, there will be a high probability of successful artificial fertilisation.

4.8. Possible applications of CREM dependent expression study to medicine

The causes of man infertility may be various. One of them is a disturbance of the expression of different genes during spermatogenesis. It was shown that 25% of all infertile man posses the CREM expression impairment. The fastest method to determine the expression variations is the hybridisation of cDNA arrays. The necessary prerequisite for it is the availability of array with appropriate gene set. Our gene set (the cremSL clones) might be a good choice. So far the most informative project about spermatogenetic gene expression was the EST sequencing. It provides the information only about the existence of particular mRNAs in testis with no information about stage and cell specificity. Our CREM target project generated a lot of novel information. At first, we found 230 novel sequences expressed in testis. Second, extensive information about spermatogenetic stage specific expression is collected. Most of this information is novel and is not available from any sources but our database. Taken together our information may serve as a good basis for spermatogenetic cDNA array production.

The simple routine array hybridisation with the labelled RNA isolated from the patient testis sample may provide a complete signature of gene expression that may determine the following treatment or application of artificial fertilisation (Sasagawa, Ichiyanagi et al. 1998). The deviation of expression of certain gene set may provide the information concerning to what kind of cells and at what developmental stage are not normal. Thus, it may be used for simple, fast and precise diagnosis of man infertility.

5. Conclusions

1. The Subtractive Suppression Hybridisation (SSH) is an efficient method to clone differentially expressed mRNAs.
2. The cremSL library constructed by use of SSH contains 259 (27%) RsaI fragments representing 161 known genes, 161 (17%) RsaI fragments representing 119 sequences homologous to known genes of other species (mostly rat and human), 283 (30%) RsaI fragments representing 226 sequences identical to mouse ESTs, 54 (6%) RsaI fragments representing 48 sequences homologous to ESTs of other species and 199 (20%) RsaI fragments representing 199 novel sequences (last update - 7.12.2000). From one hand, these values reflect our poor knowledge about mouse genes, from another hand, high efficiency of SSH method to clone novel sequences.
3. Studied mRNAs show at least six different kinds of expression profile types:
Downregulated in CREM knockout: 1) Krox20-like; 2) CREM-target-like ; 3) Odf1-like (Fig. 16, p. 53);
Nondifferentially expressed in CREM knockout: 4) Tctex1-like; 5) β -tubulin-like; 6) PGK1-like (Fig. 17, p. 56).
4. mRNAs downregulated in CREM knockout are expressed postmeiotically in wild-type mice.
5. Several groups of functionally related genes are coexpressed at postmeiotic stages of spermatogenesis. It may reflect that coregulated genes are regulated by the same transcription factors.
6. Many known and novel mRNAs show the same expression profiles as CREM τ target genes ACE and TP1 and may be CREM target genes as well.

6. MATERIALS AND METHODS

6.1. Materials

6.1.1. Chemicals

- Acetic acid (Merck)
- Acetone (Merck)
- Agar (Roth)
- Agarose (Serva)
- Ammonium Persulfate (Serva)
- Ampicillin (Sigma)
- Bacto Trypton (Difco)
- Bacto Yeast Extract (Difco)
- Boric acid (Baker)
- BSA RNase-free, acetylated (Promega)
- BSA non-acetylated (Boehringer Mannheim)
- Chlorophorm (Merck)
- Dextran sulfate (Pharmacia)
- DMSO (Merck)
- DTT (Gibco)
- EDTA (Sigma)
- EGTA (Sigma)
- Ethanol (Merck)
- Ethidiumbromide (Roth)
- Fetal Calf Serum (Ade laborbedarf)
- Formaldehyde 37% (Merck)
- Formamid (Merck)
- Glycerol (Roth)
- Glycogen, molecular biology grade (Boehringer, Mannheim)
- HCl (Baker)
- HEPES (Sigma)
- Isopropanol (Merck)
- MgSO₄ (Merck)
- Na acetate (Roth)
- NaCl (Sigma, Baker)
- Na citrate (Fluka)
- NaHCO₃ (Merck)
- NaH₂PO₄ (Merck)
- Na₂HPO₄ (Merck)
- NaN₃ (Sigma)
- NaOH (Merck)
- 8-oxychinolin (Serva)

- Paraffin highly liquid (Merck)
- Paraformaldehyde (Merck)
- 10 x PCR buffer (Boehringer, Mannheim)
- Phenol (Merck)
- SDS (Roth)
- TEMED (Serva)
- Trizma base (Merck)
- Triton X-100 (Gerbu)
- Tween-20 (Gerbu)
- Ultrapure sequagel (National diagnostics)

6.1.2. Consumable materials

- Genescreen hybridisation membrane (DuPont/NEN)
- Eppendorf tubes, safe-lock, 0.5, 1.5, 2 ml (Eppendorf)
- Mixed bead resin (BioRad)
- Oligo (dT) Cellulose columns (Gibco, cat 15939-010)
- Pasteur pipettes (neoLab)
- Plastic petri dishes (Greiner)
- Plastic moulds for histology (Polysciences)
- Plastic tips 1 ml, 200 µl, 20 µl, 2 µl (Gilson, Starsted, Matrix, Brand)
- Polygram Cel 300 PEI (Macherey-Nagel GmbH)
- PCR tubes 0.2 ml thin wall (Biozym)
- 15 and 50 ml plastic tubes (Falcon)
- Sephadex G-50 (Pharmacia)
- Sephadex C-25 (Pharmacia)
- Whatman paper

6.1.3. Laboratory equipment

- ABI 377 Sequencer (PE Applied Biosystems)
- Air bath (Biometra)
- Water bath
- Centrifuge with swinging bucket rotor (Haraeus)
- Centrifuge high-speed (Sorvall)
- Centrifuge table-top (Eppendorf)
- Centrifuge table-top, refrigerated, 2K15 (Sigma)
- GS3-Rotor and GS3-tubes (Sorvall)
- Gel chambers (Centipede)
- Gene Pulser Cuvettes (Bio-Rad)
- Magnetic stirrer (Ika Labortechnik)
- Microscopes (Zeiss, Leica)
- Elctrophoresis power supply (Pharmacia, Gibco Brl)
- pH-meter (Beckman)

- Pipettes 1000, 200, 20 μ l (Gilson)
- Programmable thermal cycler PTC 200 (MJ Research)
- Rocking platform (B chler)
- Rolling machine (IDL)
- Shaker (37°C, for bacterial growth) (Inforce AG)
- Spectrophotometer (Beckman)
- Thermomixer (heating block) (Eppendorf)
- UV transilluminator (Bachofer)
- Vacuum Blotter (Bio-Rad)
- Vacuum pump (B chler)
- Water bath (Grant Instruments)
- watchmaker forceps #5 (Dumont)
- Glass and quartz petri dishes (Schott)

6.1.4. Solutions, buffers, media

- 10 x DNase buffer:

200 mM Tris-HCL (pH 7.2)(Sigma)

50 mM MgCl₂(Merck)

10 mM DTT(Gibco)

- DNase Mix:

3 ml 10x DNase buffer

0.25 ml DNase 20U/ml

0.25 ml 25 ml RNA guard 40U/ml

H₂O to 30 ml

- DNase Stop Mix:

100 mM Tris-HCL (pH 8.3)

5 mM EDTA (pH 8.0)

- 0.5 M EDTA, pH 8.0, DEPC-treated, autoclaved

- Ethanol 100% and 70%

- LB - medium:

10 g Bacto Trypton

5 g Bacto Yeast Extract

10 g NaCl

H₂O to 1 l

Adjust pH to 7.5 with 10N NaOH, autoclave. Add 50-100 ___/ml
ampicillin for selection

- LB-amp plates : add 15 mg agar to LB before autoclaving; after cooling to
50°C

add 50-100 μ g/ml ampicillin and pour onto the plates.

- 10x HMF (Hogness modified freezing medium)

36 mM K₂HPO₄

13 mM KH₂

- 5x gel-loading buffer for RNA and DNA

1:1 glycerol/TBE v/v, 0.1% bromphenolblau, autoclave.

- Hybridisation buffer for Northern:(autoclave)

20 mM (NaH₂PO₄ x Na₂HPO₄) pH 7.0

10 mM EDTA

5% SDS

10% dextran sulfate

25 mg/ml sonicated salmon sperm DNA

- NaH₂PO₄ 0.75 M pH3.5

- NaN₃ 1%

- dNTP mix 2mM (dATP, dCTP, dGTP, dTTP 2mM each)

- Phenol-chloroform mix 1/1 v/v (pH 8 to 8.3, containing 8-oxychinolin)

- Phosphate buffer 10X (autoclaved)

100 mM NaH₂PO₄ x Na₂HPO₄) pH 7.0

10 mM EDTA

- Proteinase K mix (75 µl proteinase K solution 2 mg/ml in H₂O, this solution can be stored aliquotted at -80°C; before use add 30 µl EDTA 0.5 M, 45 µl TE buffer)

- SDS 20% in water

- SOC-Medium (autoclave, add glucose to 20 mM, from 2M Glucose stock, sterile-filtrated).

10 mM NaCl

2.5 mM KCl

10 mM MgCl

10 mM MgSO₄

2.0% Bacto tryptone

0.5%Bacto yeast extract

H₂O to 1 l

- 20X SSPE:

175.3 g NaCl

27.6 g NaH₂PO₄XH₂O

7.4 g EDTA

H₂O up to 1 l

pH to 7.4

- TBE buffer 10X

108 g Trisma - base

55 g Boric acid

40 ml (1mM) EDTA (0.5 M, pH8)

H₂O to 1 l

- TE buffer 1X:

- Tris-HCl (pH8) 10 mM
- EDTA (pH8) 1mM

6.1.5. Enzymes

- MMLV reverse transcriptase (Gibco)
- Restriction enzymes (with supplied buffers) (Boehringer Mannheim)
- Proteinase K (Boehringer Mannheim)
- ribonuclease inhibitor (RNA-guard) (MBI)
 - RNAase-free DNAase I (2000 U/ml) (Ambion)
- Taq polymerase (Boehringer Mannheim)
- PfuI polymerase (Boehringer Mannheim)
- T4 DNA ligase (Boehringer Mannheim)
- Polynucleotide kinase (Boehringer Mannheim)

6.1.6. Kits

- Big Dye Terminator Cycle sequencing kit (PE Applied Biosystems)
- Expand High Fidelity PCR system (Boehringer Mannheim)
- Qualex II Gel Extraction kit (Quiagen)
- Qiagen Plasmid Midi kit (Quiagen)
- Ready-To-Go DNA labelling kit (-dCTP) (Pharmacia Biotech)
- cDNA Synthesis Kit (Boehringer Mannheim)
- Atlas Mouse cDNA Expression Array (Clontech)

6.1.7. Nucleotides and DNAs

- (α -³²P)dCTP 10 mCi/ml (Amersham)
- dNTPs (MBI)
- 10x DIG RNA labelling mix (Boehringer cat 1277073)
- NTP solutions (75 mM T3, T7; 50 mM SP6) (Ambion)
- Salmon Testes DNA (Sigma)

6.1.8. Oligonucleotides

Sequence of all oligonucleotides is shown in 5'-3' direction

6.1.8.1. Oligonucleotides for PCR amplification of inserts

Library	Vector	Direct primer	Reverse primer
cremSL	pBSK	AATTAACCCTCACTAA AGGG	GTAATACGACTCACTAT AGGGC

RZPD	pT7T3DPac	GTTTTCCCAGTCACGAC C	AGCGGATAACAATTTCA CACAGGA
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6.1.8.2. Oligonucleotides for cloning of RT-PCR generated fragments

Gene	Direct primer	reverse primer
ACT	TGAGAGCAAGGGCAAAG CAG	TCCTGAGTTATTTTCTTC AAAGCCAAC
ACT	TGAGAGCAAGGGCAAAG CAG	TCAGGTGCAACAAATGC CATC
Odf1	TCAGAGGCCTCCTTTAA AATTAAATGAGCC	GCTTGTGTTCTGTGACCT CACCCACCC
STAT4	AACACACCGACCAACAG CAGGGTCTGC	GAAGACAGGCTTACACA GGTTTGTGGC
gcg3	CAAGACGCTGAGTGACA TGGCCAGTGG	CAGAAGCAAAGCTTCTG AAATGAAGGG
MAPKK	CCAGTGGGAGTTGCAGG GCTGGGG	TAAAAACCAGGGGCATG AAAGGAG
PKC	CTCATAGGAATTGAAGG AGATGCG	GAAGGGTGCCATGATGG AGCCTCC
AKAP84	CCCAGGGTCACAGGAGA TGGAGCC	GCCTCAGGCCCGCTGGT CTTAGCC
TFIID	TTTTCAGTTCTGGAAAAA TGG	AATCCCTTTAAGATGGG GTAG
BAG-1	CTAAGGAATTGCAAGCG GAGG	TCTGTTCCAGAGAGGGC AGGG
Laminin Receptor	GCTGCTCAGCCTGAGGT GGCC	TTTCCTTCCATCTTTTTTC CC
p18ink4	GGGCATCGGAACCATAA GGGG	TAATAAGTGATAGTGAA ACGG
Transferrin	CCTACTACGCTGTGGCTG TGG	CTGTAGTCCATCCTTGGG GGG
Pim1	ACACGGACTTTGATGGG ACCC	CCGGATTTCTTCAAAGG AGGG
Lfc	CCCGTACCTGGCAAGGG CCCC	TCTCGGGACGCTGCATG CACC
Fatty acyl CoA synthase	GACATTCGGCAGTACGT GCGCACCC	CAAGTCGTCCAGGATAG CTTTATTG
Ran	CATCCTCTGATGTTCCAC ACCAGCAG	CCCAAGCCTCACTTTCTC ATAAGTCATC
GCNF	GAGGGCCTCGAGCACCG CCGCATGGAGCGG	GAGGAGCTGCAGCTGCT CCAGGGGCAC

CREM	TGGATTGTGCTGGGAGG TTGTTC	TCTTTGAGGGCCTTGAGT TCCTC
Casein kinase	GTACTAGGCCGGGCGCG AGCTCAGG	GTACATGAACATGTGTC CCAGCG
HSP70	TCGGACAACCAGCCCGG GGTGCTGATC	CCATCGGGATCCCAACG GATGGTGAC
FSC1	TTATGAAAGCTTTGAAA GTACACAGCTG	TCAAAGGAATTCTCAGC TTACAGG
__Chimaerin	ACCCAGGAATTCATGCT TTGCACGTCTCCC	ACTTCCCTCGAGGACTA AAACAGAACATC
FSH	GCTGCTGGAGCAGGCAG AAAGCAG	AGTTCAATGGCGTTCCG GGGGAGG
GAPD-S	AGATCTGAATTCATGTC GAGACGTGACGTG	TGGTGAGCGCCGCGGCC ACCTCGCCAG
G3PDH	ACCACAGTCCATGCCAT CAC	TCCACCACCCTGTTGCTG TA

6.1.9. Vectors

pBSII-KS + (Stratagene); pRN3 (Lemaire *et al.*, 1995)

6.1.10. Bacterial strains

X11-Blue

6.1.11. Animals

Domestic mouse *Mus Musculus*

6.2. Methods

6.2.1. Preparation of electrocompetent bacteria

1. Grow cells overnight in LB.
2. To 400 ml LB add 3 ml of the overnight culture and incubate on the shaker at 37°C until OD 600 reaches 0.5-0.7 (log-phase).
3. Put the cells on ice for 15 min.
4. Spin down the cells in 500 ml GS-3 tubes in the Sorvall centrifuge, 4°C, 4000 rpm, 10 min, GS3-Rotor. The following steps should be performed on ice.
5. Carefully discard LB, resuspend cells in 50 ml cold, sterile distilled H₂O. Fill with H₂O up to 500 ml.

6. Spin down the cells in 500 ml GS-3 tubes in the Sorvall centrifuge, 4°C, 4000 rpm, 15 min, GS3-Rotor.
7. Carefully discard water, resuspend cells in 10 ml cold, sterile distilled H₂O. Fill with H₂O up to 90 ml and place into two precooled 50 ml Falcon-tubes.
8. Spin down the cells in the Haraeus centrifuge 4°C, 4000 rpm, 15 min. Discard water.
9. Carefully resuspend cells in 20 ml cold, sterile 10% Glycerine in distilled H₂O (v/v)
10. Spin down the cells in the Haraeus centrifuge 4°C, 4000 rpm, 15 min. Discard 10% glycerine.
11. Carefully resuspend cells in 2 ml cold, sterile 10% Glycerine in distilled H₂O (v/v) and freeze in 100 ml aliquots in liquid nitrogen.

6.2.2. Transformation of bacteria by plasmid DNA

1. Put in ice SOC-medium, cuvettes, eppendorf tubes, electrocompetent bacteria (for thawing) and probe.
2. Set Gene Pulser at 25 F, 2.5 kV, Pulser Controller at 200 Ohm.
3. Put 40 ml bacteria and 1 ml probe to pre-cooled cuvette, resuspend, cover with the lid and put to the Gene Pulser.
4. Press two buttons on the Gene Pulser until the sound comes (time constant should be 4.5-4.6).
5. Put 1 ml SOC-medium in the cuvette, resuspend, pour to the eppendorf tube.
6. Incubate 0.5 hr in the heating block at 37°C.
7. Plate 1, 10 and 100 ml to LB-Amp Plates, incubate overnight at 37°C.

6.2.3. Plasmid DNA isolation from bacteria

For preparation of the large amounts of highly pure plasmids (e.g. for in-vitro RNA synthesis, sequencing) Qiagen Plasmid Midi kit or Jetstar Plasmid midi kit (Genomed) were used according to manufacturer's instructions.

Mini-preparation was done according to (Sambrook, 1989), by the following protocol:

1. Pick up 1 E.coli colony from agar plate and set up overnight culture in 3 ml LB-ampicillin (37°C, shaking).
2. E. coli cells are pelleted by centrifugation in eppendorf tubes. Remove all traces of medium carefully.
3. Add 300 µl solution 1 to the pellet and resuspend the cells until the suspension is homogeneous.
4. Add 300 µl solution 2 and mix by inverting the tube 3 times until the lysate appears to be homogeneous. Incubate at room temp. for 5 min.
5. Add 300 µl solution 3 and mix immediately by inverting the tube 5 times. Do not vortex! Centrifuge the mixture at table-top centrifuge, maximal speed, for 10

min. Discard pellet.

6. Add 900 ml of 1/1 phenol-chloroform mix (v/v), vortex, centrifuge at table-top centrifuge for 4 min. Take upper phase.

7. Precipitate the DNA with 700 ml of isopropanol. Put at -20°C for 0.5 hr.

8. Centrifuge at table-top centrifuge, maximal speed, for 30 min. Discard supernatant.

9. Wash the plasmid DNA with 70% ethanol and recentrifuge. Discard supernatant.

10. Air dry the pellet for 10 min, and redissolve the DNA in 10 ml H₂O.

6.2.4. DNA separation by agarose gel electrophoresis

1-2.5% Agarose gels were used for analysis of DNA fragments 0.2-5 kb. The agarose gels were prepared using 1X TBE buffer with 1 µg/ml ethidiumbromide; 1X TBE was used as a running buffer. For the estimation of the DNA fragments molecular weight 1 kb molecular weight marker mix (Gibco) was used. The DNA samples were mixed with 5x loading buffer (1:1 glycerol/TBE v/v, 0.1% bromphenolblue) and the electrophoresis was performed at 90V for 15 min. The DNA bands were visualised at the UV-transilluminator; the pictures of the gel were made using the IMAGER computer (Appligene Inc) and the corresponding Appligene software (version 2.03)

6.2.5. Extraction of DNA fragments from agarose gel

For extraction of DNA fragments from agarose gels Qualex II Gel Extraction kit was used according to QUAEX II Handbook.

6.2.6. Radioactive labelling of DNA

Random-prime labelling of DNA was used in this work to generate the probes for Northern blot and high density filter hybridisation.

Random-prime labelling of a DNA fragments was performed with Ready-To-Go DNA labelling kit (-dCTP) (Pharmacia Biotech) in 50 ml reaction according to manufacturer's instructions.

The labelling efficiency was checked by two methods. The first allows to evaluate the approximate size of labelled DNA by the chromatography in Polygram Cel 300 PEI (pre-coated plastic sheets) using 0.75 M NaH₂PO₄ pH 3.5 as a buffer. Unincorporated nucleotides do not move, the longer the labelled DNA is the longer distance is passes during chromatography.

The second allows to evaluate percent of incorporated radionuclides: 1 µl of the sample is dropped on the DE81 paper round filter, total radiation counted by the Cherenkov counter than filter was placed in the syringe and unincorporated nucleotides washed out from the filter by 7 ml of 0,25 M NaHPO₄ pH7, the left

radiation of the filter represents the one incorporated in the DNA. The left counts divided by the total counts is the relative value of radioactivity incorporation.

6.2.7. Molecular cloning

Standard molecular cloning techniques (restriction digest, blunting of the protruding DNA ends with T4 DNA-polymerase, ligation, phosphorylation of DNA ends) were performed according to commonly used manuals (Ausubel, 1987; Sambrook, 1989) or manufacturer's instructions.

6.2.8. PCR-based automatic sequencing

Each clone was sequenced in one run from the T3A primer. By the colony PCR clones were amplified and PCR products were sequenced as described in ABI Prism377 and PERKIN ELMER dye Terminator kit manuals.

Sequencing reactions were performed using Big Dye Terminator Cycle sequencing kit (PE Applied Biosystems, cat. 4303152) according to manufacturer's instructions. For the preparation of the 5% polyacrylamide sequencing gel Ultrapure sequagel (National diagnostics) was prepared according to manufacturer's instructions.

Labelled DNAs were run in ABI 377 Sequencer (PE Applied Biosystems) and analysed at Power Macintosh 7200/120 using the programs 377 DNA Sequencer Data Collection version 1.1 (ABI Prism) and DNA Sequencing Software version 2.1.1 (ABI Prism).

The dye labelled DNA fragments separated according to the size by the electrophoreses in the acrylamide gel. Laser beam excites the fluorescent dyes attached to the fragments and they emit at the specific wavelength for each dye. The sequencer produces a picture of the gel, after lane tracking, for each lane the sequence trace is saved in the trace file. The traces are interpreted through the procedure of base calling through the sequencer software and the raw sequence is coming out.

6.2.9. Sequence processing and database search

Clones extracted from SSH were partially sequenced. The sequences were cleaned from the vector, low quality sequence and primer sequences. Sequences with RsaI sites or primer sequence in the middle were split. The resulting 3400 sequence fragments were assembled using the Staden Package Programs (Staden, Beal et al. 2000). The Assembly resulted in 956 contigs where each contig is likely to represent a unique RSA-Fragment part of the SSH library. The resulting RsaI-fragment were used to search against several databases of known sequences to determine known genes. Data base search was performed by BLAST programs (Altschul, Madden et al. 1997). Databases searched were the EMBL Nucleotide

Database, the SwissProt Protein Database and the EST Consensus Databases of Mouse and Human from GeneNest (Haas, Beissbarth et al. in press). The found Database Sequences were assembled with the RSA-Fragments using the Staden Package (Staden, Beal et al. 2000).

6.2.9. RNA isolation

The testes were disrupted by the rotational homogeniser Ultra Turrax (Janke and Kenkel KG). The total RNA was isolated by the use of RNeasy Midi kit (QIAGEN). The polyA RNA was isolated by the Oligotex midi kit (QIAGEN).

6.2.10. Quality control of RNA

To check the quality of the obtained RNA agarose gel electrophoresis and photometrical determination should be performed routinely. In both cases 0.5 µl of the purified RNA should be enough to detect a clear signal. For agarose gel electrophoresis 0.5 µl of the RNA sample are mixed with about 5 µl RNA loading buffer and heated at 55°C for 2 min. Then the sample can be loaded directly onto a 1.5% agarose gel. For photometrical determination 0.5 µl of the RNA samples are diluted with 100 µl of TE buffer and measured in a quartz cuvette. Typical values for the determined RNA concentration are between 0.5 mg/ml and 2.0 mg/ml. The intensity of the band(s) on the agarose gel should correspond to the determined concentrations. A high concentration in a sample that gives only a weak or no band in the gel hints towards an incomplete removal of the cap analogue. In this case, the RNA should be purified once more as the free cap nucleotide is an inhibitor of protein translation and toxic for the embryos.

6.2.11. Northern blot

6.2.11.1. RNA separation by denaturing electrophoresis

2 µg of poly(A)⁺ RNA were separated on a 1.0% glyoxal gel as following (Sambrook, 1989, with modifications):

1. Prepare RNA-denaturing mix: 100 µl 10X Phosphate buffer, 170 µl Glyoxale 40%, 500 µl Formamid, adjust pH to 6.8-7.0, add DEPC H₂O up to 900 µl.
2. Add 2 µl RNA probe to 18 µl RNA-denaturing mix. Heat at 65°C for 15 min. Put on ice.
3. Treat the gel chamber with 0.1M NaOH for 1 hr; wash with distilled water. Prepare 1% agarose gel on 1X Phosphate buffer.
4. Add to 20 µl of denatured RNA probes 3 µl of RNA loading dye. Load the gel.
5. Run the gel at 200 mA. Use vacuum pump to recycle the buffer.

6.2.11.2. Blotting

Probes from the gel were transferred onto a Genescreen hybridisation membrane (DuPont/NEN) using Vacuum Blotter (Bio-Rad) and 10X SSPE for transfer according to manufacturer's instructions.

6.2.11.3. Hybridisation

The ^{32}P random-priming labelled DNA fragment was used as a probe and hybridisation was carried out as following:

1. Prehybridise the filter 3 hr at 65°C in hybridisation buffer.
2. Add to 50 µl of the labelled probe 50 µl formamid. Heat at 95°C 2 min to denature.
3. Add the denatured probe to the filter; hybridise overnight at 65°C.
4. Wash the filter in 2X SSPE/0.5% SDS at 68°C for 2X15 min each.
5. Wash the filter in 0.1 SSPE/0.5% SDS at 68°C for 15 min.
6. Expose the filter overnight or longer if needed.

6.2.12. SSH: Differential cloning by Subtractive Suppression Hybridisation (SSH)

SSH is efficient and useful methods of differential cloning. It includes several steps: 1) cDNA synthesis & adaptor ligation, 2) two hybridisation, and 3) selective PCR amplification .

cDNA Synthesis & Adaptor Ligation

First, cDNA is synthesised from the two types of tissues or cells being compared (Fig. 23, p. 87). The cDNA in which specific transcripts are to be found is called tester cDNA (in our case it is wild-type testis cDNA, Fig. 10, p. 34), and the reference cDNA is called driver cDNA (in our case it is CREM knockout testis cDNA Fig. 10, p. 34). The tester and driver cDNAs are digested with a four-base-cutting restriction enzyme that yields blunt ends. The tester cDNA is then subdivided into two portions and each is ligated to a different ds cDNA adaptor (Adaptor 1 & Adaptor 2R). The ends of the adaptors lack a phosphate group, so only one strand of each adaptor attaches to the 5' ends of the cDNAs.

6.2.12.1. SSH: Two Hybridisations

In the first hybridisation, an excess of driver cDNA is added to each sample of tester cDNA. The samples are then heat denatured and allowed to anneal. Figure 23 shows the type a, b, c, and d molecules generated in each sample.

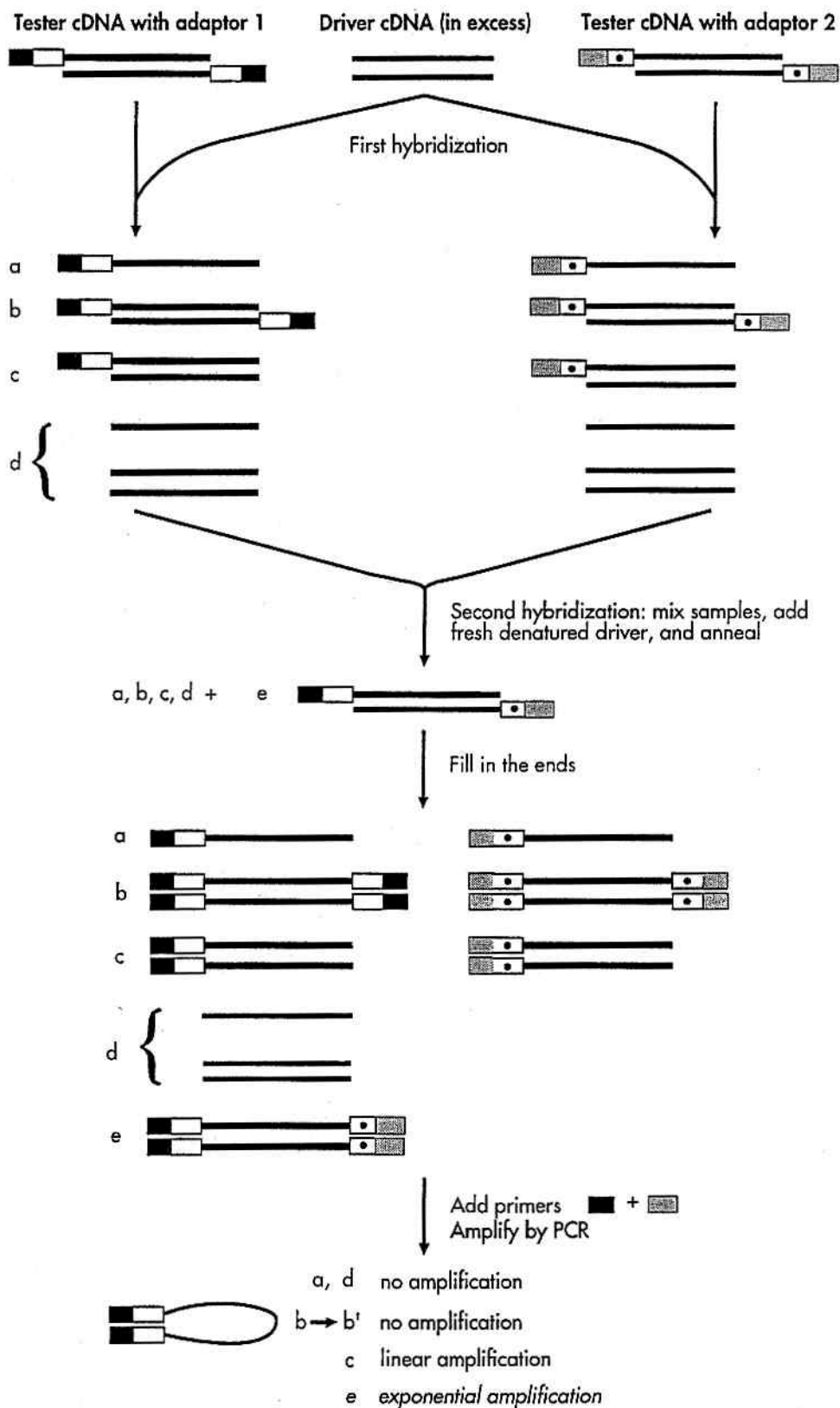


Figure 23. The principles of the Subtractive Suppressive Hybridisation (SSH).

Type a molecules include equal concentrations of high- and low-abundance sequences because reannealing is faster for the more abundant molecules due to the second-order kinetics of hybridisation. At the same time, type a molecules are significantly enriched for differentially expressed sequences, as common non target cDNAs form type c molecules with the driver.

During the second hybridisation, the two primary hybridisation samples are mixed together. Now the type a cDNAs from each tester sample are able to associate and form type b, c, and new type e hybrids. Type e hybrids are ds tester molecules with different ss ends, which correspond to Adaptors 1 and 2R. Fresh denatured driver cDNA is added to further enrich fraction e for differentially expressed sequences.

6.2.12.2. SSH: Selective Amplification.

The entire population of molecules is then subjected to two rounds of PCR to amplify the desired differentially expressed sequences. During the first cycle of primary PCR, the adaptor ends are filled in, creating the complementary primer binding sites needed for amplification. Thus, type a and d molecules are missing primer annealing sites and cannot be amplified. Type b molecules form a pan-like structure that prevents their exponential amplification (8, 9). Type c molecules have one primer annealing site and can only be amplified linearly. Only type e molecules, which have two different primer annealing sites, can be amplified exponentially. These differentially expressed sequences are greatly enriched in the final subtracted cDNA pool. Subtracted cDNA can be used as a hybridisation probe or cloned to create a subtracted library.

6.2.13. Subtraction of CREM knockout testis cDNA from wild-type testis cDNA Subtractive Suppression Hybridisation (SSH)

For the SSH the mRNAs were isolated from wild type and CREM deficient mutant testis and used for the cDNA synthesis. The cDNAs were digested with the short cutting restriction enzyme RsaI which recognises the four nucleotide sequence GTAC and releases blunt ended DNA fragments. Special adaptors were ligated to the obtained cDNA fragments and the PCR-select procedure performed according to the Clontech manual instructions. Wild-type cDNA was used as a tester and CREM knockout cDNA was used as a driver. Driver cDNA was taken in excess of 60 times what should allow to subtract this cDNA efficiently.

6.2.14. CremSL subtracted library construction

The PCR product generated by the SSH procedure was digested by RsaI restriction enzyme, ligated into pBS vector plasmid digested by SmaI, transformed in *E. coli* and grown overnight on the agar in 22x22 cm square Petri

dish. Colonies were arrayed by the robot on 32 microtiter plates of 386 wells filled in with the 90% of LB, 50 µg/ml Ampicilin and 10% of 10x HMFM freezing medium. After overnight growth plates were placed for storage at -80°C.

6.2.15. High density filter production

Colony filter production:

1. The bacteria from 386 well microtiter plates were spotted onto the nylon filters. Each clone was spotted twice in order to control the hybridisation specificity.
2. Colonies were grown on the filter overnight on the LB+agar support in the 22x22 cm Petri dish.
3. Filters were denatured for 5 minutes in the denaturation buffer (1,5 M NaCl, 0,5 M NaOH in H₂O), then incubated in the Tris buffer (1,5 M NaCl, 0,5 M TrisHCl pH7) and the DNA was linked to nylon membrane by baking at 80°C for 1 hour.

Note: for the PCR filters production procedure is the same but PCR products were spotted on a filters and the step 2 was excluded.

6.2.16. High density filter hybridisation with labelled PCR products

PCR products were separated in the agarose gel. Required DNA fragments were isolated from the agarose and labelled with ³²P. High density filter hybridisation with labelled DNA performed according to the Church-Gilbert method (Sambrook, Fritsch et al. 1989).

6.2.17. High density filter hybridisation with labelled oligonucleotides

To identify the empty vectors special oligonucleotide was designed. The cloning site is in the middle of this oligonucleotide. Therefore, if plasmids do not possess the insertion, the oligonucleotide hybridises completely with all 20 nucleotides but only with 10 nucleotides if the plasmids possess the insertion. In appropriate stringency of hybridisation this primer hybridises with empty vectors only.

6.2.18. Radioactive labelling of cDNA

120 ng of the poly(A)⁺ RNA (mRNA) were mixed with 0,5 µg oligo(dT)₁₅ primer in 4 µl in total, incubated at 67°C for 5 minutes, then on ice for 5 minutes and dried in speedvac at room temperature. Reverse transcription performed in mix containing 1,4 µl of 5xMMLV buffer (Promega), 0,4 µl dNTP mix (10 mM dCTP, 10 mM dGTP, 10 mM dTTP, 100 µM dCTP), 4 µl α³³PdATP (10 µCi/µl), 1,2 µl MMLV (200 units/µl, Promega) (final total volume of labelling

mix is 7 μ l). Incubated at 42°C for 2 hours. Incorporation efficiency was 80-90%. Labelled cDNA was separated from unincorporated nucleotides by the chromatography in Chroma Spin-200 columns (Clontech).

6.2.19. High density filter hybridisation with labelled cDNA

Labelled cDNA was denatured according to Clontech Atlas Array manual instruction. High density filters were prehybridised in 8 ml of ExpressHyb hybridisation solution (Clontech) at 65°C for 1-3 hours. Then, hybridisation solution was discarded and 8 ml fresh one added. The labelled cDNA was added in concentration of 2x10⁶ cpm/ml of hybridisation solution. After overnight incubation at 65°C filters were washed in 2xSSC/0,5%SDS twice, then twice in 0,1x SSC/0,1%SDS solutions. Filters were wrapped in polyethylene bags and exposed on phosphoimager screen from overnight to one month.

6.2.20. Image analysis

The phosphoimager provides the image file of particular hybridisation. Images were analysed by the AIS program (Array Vision) installed on a PC. The background was evaluated around each primary element individually and was subtracted from the value of each spot. Values of all spots were saved in text files.

6.2.21. Expression profiling

Hybridisations in text files were compared by use of special image analysis programs developed on the basis of MatLab program package. The data processing involved the normalisation of data according to the values of the nondifferential controls (housekeeping genes, etc.). The normalisation procedure was adjust to be independent on exposure time.

6.2.22. Expression profile clustering

The expression profiles were clustered by the two different methods.

The first was the modified Hierarchical Clustering (Eisen, Spellman et al. 1998). The modification was the way of the distance measure which is different from Eisen approach and called "symmetrized relative entropy".

The second was the a linear ordering giving the shortest overall distance which has not yet been used in this field before. It is called a Travelling Salesman Tour. The TSP (Travelling Salesman Problem) was approximated by Simulated Annealing algorithm (Press, Teukolsky et al. 1992)

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8.ABBREVIATIONS

ACE - angiotensin converting enzyme.

ACT - activator of CREM in testis.

AKAP - A kinase anchoring protein.

ATF - activating transcription factor.

ATP - adenosine 5'-triphosphate.

bZip domain - basic and leucine zipper domain

cAMP - cyclic adenosin monophosphate

CBP - CREB binding protein.

cDNA - complementary DNA.

CRE - cAMP-response element.

CREB - cAMP responsive element binding protein.

CREM - cAMP responsive element modulator.

DBD - DNA binding domain.

DNA - deoxyribonucleic acid.

EDTA - ethylendiaminetetraacetic acid.

FSH - follicle stimulating hormone.

ICER - inducible cAMP early repressor.

kb - kilobase pair.

kD - kilodalton.

KID - kinase inducible domain

l - litre

m - mili (10^{-3})

M - molar

μ - micro (10^{-6})

MAP kinase - mitogen-activated protein kinase.

min - minute

mRNA - messenger RNA.

n - nano (10^{-9}).

OD - optic density.

p - pico (10^{-12}).

PCR - polymerase chain reaction.

PKA - protein kinase A.

PKC - protein kinase C.

RNA - ribonucleic acid.

RNase - ribonuclease.

rpm - rotation per minute

rRNA - ribosomal RNA.

RT-PCR - reverse transcription PCR.

S - Siemens

SDS - sodiumdodecylsulphate.

SSH - subtractive suppressive hybridisation.

TBP - TATA-box binding protein.

V - volt.

wt - wild type.

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10.1. Appendix 1. Annotated list of cremSL clones with known sequences

Complete data about all cremSL clones are presented in Internet website:

<http://www.dkfz.de/tbi/people/beissbarth/private/crem-project>

In this Appendix1 annotations of clones are sorted according to gene function. The clone sequences and all other data about clones may be found in mentioned website.

Designations in Appendixes:

RSA fragment - the clone name of best sequence cluster representatives.

Sequence - accession number of highly homologous sequence from public data bases.

Database Info - the name of gene/clone in public database

Functional Categorie/Subcategorie - the function of protein.

Title - the name of protein/clone retrieved from public databases.

Annotated list of cremSL clones with known sequences

RSAFragment	Sequence	Database Info	Type	Functional_Categorie	Functional_Subcategorie	Title
2	2	2	2	2	2	2
18	18	18	18	18	18	18
37	37	37	37	37	37	37
62	62	62	62	62	62	62
97	97	97	97	97	97	97
281	281	281	281	281	281	281
339	339	339	339	339	339	339
341	341	341	341	341	341	341
489	489	489	489	489	489	489
650	650	650	650	650	650	650
680	680	680	680	680	680	680
741	741	741	741	741	741	741
308	308	308	308	308	308	308
681	681	681	681	681	681	681
76	76	76	76	76	76	76
101	101	101	101	101	101	101
183	183	183	183	183	183	183
239	239	239	239	239	239	239
247	247	247	247	247	247	247
318	318	318	318	318	318	318
419	419	419	419	419	419	419
544	544	544	544	544	544	544
610	610	610	610	610	610	610
622	622	622	622	622	622	622
740	740	740	740	740	740	740
759	759	759	759	759	759	759
67	67	67	67	67	67	67
255	255	255	255	255	255	255
446	446	446	446	446	446	446
442	442	442	442	442	442	442
730	730	730	730	730	730	730
230	230	230	230	230	230	230
273	273	273	273	273	273	273
287	287	287	287	287	287	287
288	288	288	288	288	288	288
297	297	297	297	297	297	297
299	299	299	299	299	299	299
311	311	311	311	311	311	311
418	418	418	418	418	418	418
475	475	475	475	475	475	475
490	490	490	490	490	490	490
547	547	547	547	547	547	547
587	587	587	587	587	587	587
667	667	667	667	667	667	667
9	9	9	9	9	9	9
226	226	226	226	226	226	226
264	264	264	264	264	264	264
313	313	313	313	313	313	313
541	541	541	541	541	541	541
236	236	236	236	236	236	236
275	275	275	275	275	275	275
431	431	431	431	431	431	431
671	671	671	671	671	671	671
689	689	689	689	689	689	689
703	703	703	703	703	703	703
770	770	770	770	770	770	770
6	6	6	6	6	6	6
48	48	48	48	48	48	48
52	52	52	52	52	52	52
199	199	199	199	199	199	199
258	258	258	258	258	258	258
321	321	321	321	321	321	321
464	464	464	464	464	464	464
557	557	557	557	557	557	557
720	720	720	720	720	720	720
439	439	439	439	439	439	439
20	20	20	20	20	20	20
29	29	29	29	29	29	29
32	32	32	32	32	32	32
34	34	34	34	34	34	34
73	73	73	73	73	73	73
89	89	89	89	89	89	89
105	105	105	105	105	105	105
107	107	107	107	107	107	107

Annotated list of cremSL clones with known sequences

225	RSA-23-D11	HSU49240, Mm cluster14097.0.6	MMP40GPRT	Mouse Genes	Signal Transduction	Receptor	Mus musculus mRNA for G protein-coupled receptor, p40GPRT		
254	RSA-15-M23	MMP40GPRT, Mm cluster00685.0.1	ANX2_MOUSE	Mouse Genes	Signal Transduction		Mouse mRNA for protein-tyrosine kinase substrate p36 (calpain1 heavy chain), complete cds.		
259	RSA-13-I02	MMKALLR3, Mm cluster05309.0.1	KC2B_HUMAN	Mouse Genes	Signal Transduction	Protein Kinase	Mouse mRNA for casein kinase II beta subunit (EC 2.7.1.37)		
315	RSA-17-I17	AF082556	PEBP_MOUSE	Mouse Genes	Signal Transduction		Mus musculus phosphatidylethanolamine binding protein mRNA, complete cds.		
323	RSA-28-E07	AF060005, Hs. cluster06200.0.1	INPP_MOUSE	Mouse Genes	Signal Transduction	Phosphatidylinositol Pathway	Mus musculus inositol polyphosphate 1-phosphatase mRNA, complete cds.		
327	RSA-16-F01	MMSOX5, AB006330, Mm cluster01160	KPCD_MOUSE	Mouse Genes	Signal Transduction	PKC	Mouse protein kinase C delta mRNA, complete cds.		
460	RSA-8-P19	Mm. cluster06434.0.3	KPT1_MOUSE	Mouse Genes	Signal Transduction	Protein Kinase	M.musculus of PCTAIRE-1 mRNA encoding protein kinase		
462	RSA-21-J23	MM1209646, Mm. cluster06503.0.2	PPT1_MOUSE	Mouse Genes	Signal Transduction	Protein Phosphatase	Mus musculus protein phosphatase type 1 (dis2m1) mRNA, complete cds.		
509	RSA-1-P02	MMAA10010, Mm. cluster09209.0.3	ACE_MOUSE	Mouse Genes	Signal Transduction	Angiotensin Pathway	Mouse angiotensin-converting enzyme mRNA, 5' end, clone ACE.5.		
527	RSA-2-O22, RSA-9-J15	MM1294447, AA953988, Mm. cluster109	TDXN_MOUSE	Mouse Genes	Signal Transduction	NFkB Pathway	Mus musculus antioxidant enzyme AOE372 mRNA, complete cds.		
532	RSA-27-N08_0	AA874135, Mm. cluster11278.0.26	KAP2_MOUSE	Mouse Genes	Signal Transduction	PKA Pathway	Mouse cAMP-dependent protein kinase type II regulatory subunit mRNA, 3' end.		
538	RSA-8-E01	AA869369, Mm. cluster12477.0.4	PDK1	Mouse Genes	Signal Transduction	Phosphatidylinositol Pathway	Mus musculus phosphoinositide-dependent protein kinase PDK1 mRNA, complete cds.		
558	RSA-14-L11	MMA65988, Mm. cluster13480.0.4	AF077660	Mouse Genes	Signal Transduction	Homeotic Protein Kinase	Mus musculus homeodomain-interacting protein kinase 3 (HIPK3)mRNA, complete cds.		
562	RSA-9-M09	MMAA15901, Mm. cluster13868.0.2	AKAP110	Mouse Genes	Signal Transduction	PKA Pathway	Mus musculus protein kinase A binding protein AKAP110 mRNA, complete cds.		
589	RSA-26-I19	MMA62303, Mm. cluster15244.0.2	AF015811	Mouse Genes	Signal Transduction	Lysosphosphatidic Acid Pathway	Mus musculus putative lysosphosphatidic acid acyltransferase (LPAAT) mRNA, complete cds.		
644	RSA-4-J02_0	MMA28246, Mm. cluster21966.0.1	IkB-beta	Mouse Genes	Signal Transduction	NFkB Pathway	Mus musculus IkB-beta mRNA, complete cds.		
661	RSA-31-B21	MMAA39191, Mm. cluster24250.0.1	ERK3_MOUSE	Mouse Genes	Signal Transduction	Protein Kinase	M.musculus erk3 mRNA		
742	RSA-11-H24_#0	AA794156, Mm. cluster76266.0.1	Mm. cluster01020.0.3	Mouse Genes	Signal Transduction	Protein Kinase	Mus musculus mRNA for testis-specific protein kinase 1, complete cds. (Homolog LIK1_MOUSE (118))		
743	RSA-27-D01	AA798038, Mm. cluster76954.0.1	SRPK2	Mouse Genes	Signal Transduction		Mus musculus mRNA for SRPK2, complete cds.		
751	RSA-24-H17	AI036792, Mm. cluster84804.0.1	FRT1_MOUSE	Mouse Genes	Signal Transduction		Mus musculus proto-oncogene (Frat1) mRNA, complete cds.		
16	RSA-9-K11	AF069072	ISP1_HUMAN	Homologous Other Genes	Signal Transduction	Phosphatidylinositol Pathway	H.sapiens mRNA for InsP3 5-phosphatase.		
68	1-118, RSA-7-H23	HS337611, Hs. cluster10580.0.1	DBX	Homologous Other Genes	RNA Modification	RNA Helicase	Homo sapiens dead box, X isoform (DBX) mRNA, alternative transcript2, complete cds.		
529	RSA-8-A03	MMA61076, Mm. cluster11045.0.2	AF083383	Homologous Other Genes	RNA Modification	Splicing Factor	Homo sapiens 38 kDa splicing factor mRNA, complete cds.		
753	RSA-14-C07, RSA-23-D05	AI037618, Mm. cluster84992.0.1	HSU49240	Homologous Other Genes	RNA Modification	RNA Polyadenylation	Human myplexin mRNA, complete cds.		
604	RSA-4-N14	MMA35868, Mm. cluster16245.0.7	S61A_CANFA	Homologous Other Genes	Protein Transport	Protein Insertion Into ER	Canis familiaris sec61 homologue mRNA, complete cds.		
119	RSA-1-K24	MMD315, Mm. cluster02001	SPC1_HUMAN	Homologous Other Genes	Protein Modification	Signal Peptid Removal	Human mRNA for KIAA0102 gene, complete cds.		
518	RSA-29-D08_1	MMTEST624, Mm. cluster10818.0.6	TGLC_CHICK	Homologous Other Genes	Protein Modification	Protein cross-linking	Chicken transglutaminase mRNA, complete cds.		
638	RSA-14-K08	Mm. cluster21228.0.2	MPPB_HUMAN	Homologous Other Genes	Protein Modification	Protein Precursor Cleavage	Homo sapiens mitochondrial processing peptidase beta-subunit mRNA, complete cds.		
130	RSA-11-L19, RSA-18-E18	MM15977, RNLACS, Mm. cluster03550	Ubp41	Mouse Genes	Protein degradation	Ubiquitin Pathway	Mus musculus ubiquitin-specific protease UBP41 (Ubp41) mRNA, complete cds.		
137	RSA-27-A11_#0, RSA-8-N02	MMA61076, Mm. cluster03005.0.3	CRES_MOUSE	Mouse Genes	Protein degradation	Proteinase Inhibitor	Mus musculus cystatin-related epididymal spermatogenic protein(Cres) mRNA, complete cds.		
482	RSA-10-E02	MM1302841, Mm. cluster08054.0.1	PRST_HUMAN	Homologous Other Genes	Protein degradation	Ubiquitin Pathway	Human mRNA for MSS1, complete cds.		
498	RSA-30-D21	MM1164714, Mm. cluster08845.0.3	HSU96114	Mouse Genes	Protein degradation	Ubiquitin Pathway	Homo sapiens Nedd-4-like ubiquitin-protein ligase WWP2 mRNA, complete cds. (Homolog NED4_MOUSE (119))		
526	RSA-32-D11	MM1294364, Mm. cluster10958.0.1	UBA1_MOUSE	Mouse Genes	Protein degradation	Ubiquitin Pathway	Mus musculus mRNA for ubiquitin activating enzyme E1, complete cds.		
535	RSA-7-C08	Mm. cluster11804.0.6	CATH_MOUSE	Mouse Genes	Protein degradation	Ubiquitin Pathway	Mus musculus cathepsin H prepropeptide (ctsh) mRNA, complete cds.		
537	RSA-14-J01	MM69825, Mm. cluster12297.0.1	UB5B_HUMAN	Mouse Genes	Protein degradation	Ubiquitin Pathway	Mus musculus ubiquitin conjugating enzyme (ubc4) mRNA, complete cds.		
623	RSA-10-L23, RSA-22-F09	MMA23661, Mm. cluster18536.0.8	UBH1	Mouse Genes	Protein degradation	Ubiquitin Pathway	Mus musculus deubiquitinating enzyme (UBH1) mRNA, partial cds.		
707	RSA-14-J17_@0	MMA61539, Mm. cluster51970.0.1	PRCI_HUMAN	Homologous Other Genes	Protein degradation	Ubiquitin Pathway	H.sapiens PROS-27 mRNA		
734	RSA-32-N20	AA647992, Mm. cluster69373.0.1	CATD_MOUSE	Mouse Genes	Protein degradation	Protease	M.musculus mRNA for cathepsin D		
110	RSA-12-O01	HSDOCKP, Mm. cluster36997.0.1	ICAL_RAT	Homologous Other Genes	Protein Degradation	Proteinase Inhibitor	Rat mRNA for calpastatin		
142	RSA-3-A09, RSA-9-H12	MMPKM, Mm. cluster01791.0.186	UBC3_HUMAN	Homologous Other Genes	Protein Degradation	Ubiquitin Pathway	Human ubiquitin conjugating enzyme mRNA, partial cds.		
163	RSA-10-B10	HSD817, AB006710, Mm. cluster92589	HSD378	Homologous Other Genes	Protein Degradation	Proteasome Inhibitor	Homo sapiens mRNA for proteasome inhibitor piP31 subunit, complete cds.		
229	RSA-23-H07, RSA-3-E14, RSA-28-F09	MMACEA, HSAICEA, MMACEC, Mm. cluster03989.0.4	100K_RAT	Homologous Other Genes	Protein Degradation	Ubiquitin Pathway	R.norvegicus mRNA for 100 kDa protein		
659	RSA-6-I19_#0	MMA64424, Mm. cluster23636.0.1	LAP1C	Homologous Other Genes	Nucleoskeleton & Motility	Lamina	Rattus norvegicus lamina-associated polypeptide 1C (LAP1C) mRNA, complete cds.		
187	RSA-2-A04, RSA-8-F02	HSU63743, Mm. cluster15085.0.1, Hs. cluster01000.0.1	ACTG2	Mouse Genes	Muscle Actin	Smooth Muscle Gamma Actin	Mus musculus smooth muscle gamma actin mRNA, complete cds.		
51	RSA-11-P11	AF077599	TCPG_MOUSE	Mouse Genes	Molecular chaperone		Mus musculus matricin mRNA, complete cds.		
599	RSA-3-E07	MMA6542, Mm. cluster16020.0.6	HS77_MOUSE	Mouse Genes	Molecular Chaperon		Mus musculus Hsc70t mRNA for spermatid-specific heat shock protein70, complete cds.		
757	RSA-6-G13	AI119513, Mm. cluster87042.0.1	HSU63743	Homologous Other Genes	Mitosis	Chromosome Motility	Homo sapiens mitotic centromere-associated kinesin mRNA, complete cds. (Homolog KIF2_MOUSE (330))		
26	RSA-10-C04	HS19878, Mm. cluster19574.0.1	LDHM_MOUSE	Mouse Genes	Metabolic Enzymes	Glucose Turnover	Mouse lactate dehydrogenase A-4 (LDH-A) mRNA, complete cds.		
53	RSA-17-M09, RSA-5-K24	MMSURF4A, Mm. cluster02858	DPM2	Homologous Other Genes	Metabolic Enzymes	Metabolic Enzymes	Rattus norvegicus mRNA for DPM2, complete cds.		
54	RSA-20-J12	RNUJNR, Mm. cluster10215.0.9	F26H_MOUSE	Homologous Other Genes	Metabolic Enzymes	Metabolic Enzymes	Glycosylphosphatidylinositol Synthase		
69	RSA-4-B13, RSA-5-A19_#0	MMF9, Mm. cluster01776.0.19	ATPO_HUMAN	Homologous Other Genes	Metabolic Enzymes	Metabolic Enzymes	ATP Synthase		
100	RSA-22-N09	MMHMG1HOM, Mm. cluster02562.0.14	C11A_HUMAN	Homologous Other Genes	Metabolic Enzymes	Metabolic Enzymes	Human cholesterol side-chain cleavage enzyme P450sc mRNA, complete cds.		
108	RSA-5-E18_1	Hs. cluster18049.0.1	GPX4	Mouse Genes	Metabolic Enzymes	Metabolic Enzymes	Mus musculus phospholipid hydroperoxide glutathione peroxidase GPX4 gene, partial cds.		
117	RSA-8-O15_#0	MMD366, Mm. cluster01850.0.2	GLNA_MOUSE	Mouse Genes	Metabolic Enzymes	Metabolic Enzymes	Mus musculus glutamate-ammonia ligase mRNA, complete cds.		
151	RSA-15-F16, RSA-8-L05_#0	MMIMPDP, MMIMPDA, Mm. cluster02896	SERA_MOUSE	Mouse Genes	Metabolic Enzymes	Metabolic Enzymes	R.norvegicus mRNA for D-3-phosphoglycerate dehydrogenase		
216	RSA-32-O17	HSPROS27, Mm. cluster12882.0.24	IMD2_MOUSE	Mouse Genes	Metabolic Enzymes	Metabolic Enzymes	Mouse IMP dehydrogenase mRNA, complete cds.		
220	RSA-14-C01	HSORF02, Mm. cluster08963.0.1	ATPR_MOUSE	Mouse Genes	Metabolic Enzymes	Metabolic Enzymes	Guanine Nucleotide Synthase		
266	RSA-1-H06, RSA-23-B02	(HSB2CHIM, Mm. cluster08865.0.2, Hs. cluster03989.0.4	ATLCB_HUMAN	Homologous Other Genes	Metabolic Enzymes	Metabolic Enzymes	ATP Synthase		
278	RSA-32-N05_0	S61973, Mm. cluster07471.0.15	LCFB_MOUSE	Mouse Genes	Metabolic Enzymes	Metabolic Enzymes	Homo sapiens mitochondrial ATP synthase coupling factor 6 mRNA, nuclear gene encoding mitochondrial protein, complete cds.		
290	RSA-26-G13	RNU8872, Hs. cluster37783.0.3	PGMU_HUMAN	Homologous Other Genes	Metabolic Enzymes	Metabolic Enzymes	Fatty Acids Turnover		
360	RSA-28-M21	MM51204, Mm. cluster03989.0.4	KPR1_HUMAN	Homologous Other Genes	Metabolic Enzymes	Metabolic Enzymes	Glucose Turnover		
388	RSA-30-D08	HS1315818	FPPS_HUMAN	Homologous Other Genes	Metabolic Enzymes	Metabolic Enzymes	Human phosphoglucosyltransferase 1 (PGM1) mRNA, complete cds.		
409	RSA-10-L07	HS169368, Hs. cluster05378.0.65	GLPK_MOUSE	Mouse Genes	Metabolic Enzymes	Metabolic Enzymes	Homo sapiens mRNA for phosphoribosyl pyrophosphate synthetase subunit I, complete cds.		
438	RSA-17-J12	Hs. cluster43833.0.1	ODPT_MOUSE	Mouse Genes	Metabolic Enzymes	Metabolic Enzymes	Human farnesyl pyrophosphate synthetase mRNA, complete cds.		
459	RSA-17-O12_#0	MMA00376, Mm. cluster06330.0.37	HO2_MOUSE	Mouse Genes	Metabolic Enzymes	Metabolic Enzymes	Mus musculus glyceral kinase (Gyk) mRNA, complete cds.		
465	RSA-1-F01	MMA63916, Mm. cluster06693.0.6	G3PT_MOUSE	Mouse Genes	Metabolic Enzymes	Metabolic Enzymes	Mus musculus pyruvate dehydrogenase (pdha-2) mRNA, complete cds.		
476	RSA-8-J07	AI194154, Mm. cluster07673.0.2	MAN2_MOUSE	Mouse Genes	Metabolic Enzymes	Metabolic Enzymes	Glucose Turnover		
494	RSA-5-B13	MMA08314, Mm. cluster08568.0.12	ALFA_MOUSE	Mouse Genes	Metabolic Enzymes	Metabolic Enzymes	Mus musculus heme oxygenase 2a (HO-2a) mRNA, complete cds.		
507	RSA-20-E12	MMAA45651, Mm. cluster09185.0.2	F263_RAT	Homologous Other Genes	Metabolic Enzymes	Metabolic Enzymes	Mus musculus testis-specific isoform of glyceraldehyde 3-phosphatedehydrogenase (Gapd-S) mRNA, complete cds.		
575	RSA-10-D24_#0, RSA-17-L03	MMA34897, Mm. cluster14440.0.3	KPY2_MOUSE	Mouse Genes	Metabolic Enzymes	Metabolic Enzymes	Mouse mRNA for aldolase A		
614	RSA-31-P05	Mm. cluster16864.0.2	ATPA_MOUSE	Mouse Genes	Metabolic Enzymes	Metabolic Enzymes	Rat testis fructose-6-phosphate, 2-kinase/fructose-2, 6-bisphosphatase mRNA, complete cds.		
647	RSA-10-L13, RSA-16-N15	MMAA5308, AA759432, MMAA12520, Mm. cluster42575.0.1	FTDH_RAT	Homologous Other Genes	Metabolic Enzymes	Metabolic Enzymes	Mouse mRNA for pyruvate kinase M.		
688	RSA-7-H15_#0	MM1294366, Mm. cluster42575.0.1	HXK1_MOUSE	Mouse Genes	Metabolic Enzymes	Metabolic Enzymes	Mouse ATP synthase alpha subunit, complete cds.		
701	RSA-16-L17	MMA16790, Mm. cluster49847.0.1	ODO1_HUMAN	Homologous Other Genes	Metabolic Enzymes	Metabolic Enzymes	Homo sapiens 10-formyltetrahydrofolate dehydrogenase mRNA, complete cds.		
723	RSA-19-H19_#0, RSA-21-H02	MMAA52528, Mm. cluster59259.0.1	ASSY_MOUSE	Mouse Genes	Metabolic Enzymes	Metabolic Enzymes	Mouse hexokinase mRNA, complete cds.		
							Human mRNA for 2-oxoglutarate dehydrogenase, complete cds.		
							Mouse argininosuccinate synthetase (Ass) mRNA, complete cds.		

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724	RSA-32-K02_#0	MMAA6193, Mm_cluster60490.0.1	G6PI MOUSE	Mouse Genes	Metabolic Enzymes	Glucose Turnover	Mus musculus glucose phosphate isomerase mRNA, 3' end.				
728	RSA-5-C09	MMAA83563, Mm_cluster64114.0.1	CAOQ RAT	Homologous Other Genes	Metabolic Enzymes		R.norvegicus mRNA for Pristanoyl-CoA Oxidase				
731	RSA-2-H19, RSA-3-G09	MMAA92742, Mm_cluster65814.0.1	GPDM MOUSE	Mouse Genes	Metabolic Enzymes		Mouse mRNA for glycerol-3-phosphate dehydrogenase, complete cds.				
752	RSA-1-H23_#0	AJ036980, Mm_cluster84856.0.1	KDGH_MESAU	Homologous Other Genes	Metabolic Enzymes		Cricetinae gen. sp. diacylglycerol kinase eta mRNA, complete cds.				
769	RSA-18-P23	AU019877, Mm_cluster95030.0.1	DHQV HUMAN	Homologous Other Genes	Metabolic Enzymes		Human quinone oxidoreductase (NQO2) mRNA, complete cds.				
333	RSA-28-A24	MMU58882, Mm_cluster04057.0.3	MMD315	Mouse Genes	Membrane transport		Mouse mRNA for tetraacycline transporter-like protein, complete cds.				
41	RSA-3-K11	Mm_cluster00492.0.11, MMA00416	AF094516	Homologous Other Genes	Intracellular Transport	Vesicle Targeting to Vacuole	Homo sapiens E1-like protein mRNA, complete cds.				
42	RSA-7-N20	AI037658, Mm_cluster85012.0.1	COPE	Mouse Genes	Intracellular Transport	Vesicle transport	Mus musculus strain BALB/c epsilon-COP mRNA, partial cds.				
75	RSA-11-I03_0, RSA-25-A11	MMBPCP_HAP, Hs_cluster18289	MMRAB65A	Mouse Genes	Intracellular Transport	Golgi to ER Transport	Mus musculus rab6/rab5-associated protein (rab6) mRNA, partial cds.				
126	RSA-15-H22	MMU77128, Mm_cluster04244.0.25	SCA1_HUMAN	Homologous Other Genes	Intracellular Transport	Vesicle Targeting to Cell Surface	Homo sapiens secretory carrier membrane protein (SCAMP1) mRNA, complete cds.				
149	RSA-30-H09	AB013359, Mm_cluster06826.0.2	SRPR HUMAN	Homologous Other Genes	Intracellular Transport	Vesicle Targeting	Human mRNA for docking protein (signal recognition particle receptor)				
178	RSA-4-K08	CGU59429, Mm_cluster42540.0.1	Hs_cluster18049	Homologous Other Genes	Intracellular Transport	Vesicle Targeting	Human mRNA for KIAA0263 gene, complete cds				
403	RSA-4-L03	HS335264, Hs_cluster03366.0.5	AF039023	Homologous Other Genes	Intracellular Transport	Nuclear Pore Transport	Homo sapiens Ran-GTP binding protein mRNA, partial cds.				
414	RSA-5-F03	AI090876, Hs_cluster13267.0.1	RAN_MOUSE	Mouse Genes	Intracellular Transport	Nuclear Pore Transport	GTP-binding protein [mice, C3H/HeJ spleens, LDS responder, mRNA, 1166 nt].				
552	RSA-6-A11	MMA60083, Mm_cluster13427.0.10	AP50_HUMAN	Mouse Genes	Intracellular Transport	Vesicle Targeting	Mus musculus clathrin-associated AP-2 complex AP50 subunit mRNA, complete cds.				
607	RSA-22-J01	MMA32965, Mm_cluster16333.0.1	AP47_MOUSE	Mouse Genes	Intracellular Transport	Vesicle Targeting	Mouse clathrin-associated protein (AP47) mRNA, complete cds.				
654	RSA-24-B12	MMA66063, Mm_cluster23176.0.2	AF008935	Homologous Other Genes	Intracellular Transport	Vesicle Targeting	Homo sapiens syntaxin-16A mRNA, complete cds.				
763	RSA-10-J04, RSA-12-B01	AI180544, Mm_cluster92625.0.1	KAP3A	Mouse Genes	Intracellular Transport	Vesicle transport	Mus musculus mRNA for KAP3A, complete cds.				
412	RSA-24-J16	Hs_cluster10869.0.42	HMG1_MOUSE	Mouse Genes	Histones & HMGs	HMG	Mus musculus (clone Clebp-1) high mobility group 1 protein (HMG-1)mRNA, complete cds.				
662	1-L09, RSA-3-N03	MMA64579, Mm_cluster24369.0.1	MMM3A	Mouse Genes	Histones & HMGs	Histone	Mouse histone H3.3 probable processed pseudogene (MH-321), complete cds.				
72	RSA-12-M15	MMPLAKA, Mm_cluster03200.0.2	BH5_MOUSE	Mouse Genes	Histones	H5	M. musculus mRNA for H5 clone				
333	RSA-11-F24_1	MMDBPA, Mm_cluster01662.0.1	H2A1_MOUSE	Mouse Genes	Histones		Mouse histone H2A.1 gene, complete cds.				
684	RSA-1-P22, RSA-23-H06	MM1294179, Mm_cluster42528.0.1	AF044312	Mouse Genes	Erythrocyte Membrane		Mus musculus protein 4.1G mRNA, partial cds.				
434	RSA-2-A15	AA725499, Hs_cluster42349.0.2	KCRB_HUMAN	Homologous Other Genes	Energy transduction		Homo sapiens creatine kinase B mRNA, complete cds.				
159	RSA-21-B14, RSA-25-E01	MMAMANI, Mm_cluster00301.0.1	Wdr1	Mouse Genes	Cytoskeleton & Motility		Mus musculus Wdr1 protein mRNA, complete cds.				
186	RSA-12-P18	RND3PGDEH, Mm_cluster02412.0.22	MM17324	Homologous Other Genes	Cytoskeleton & Motility	Actin Polymerisation	N-r-retinoic acid-regulated gene/profilinII homolog [mice, P19embryonal carcinoma cells, mRNA Partial, 303 nt].				
196	9-H14	RSCLPST, Mm_cluster07410.0.1	Hs_cluster01934	Other EST	Cytoskeleton & Motility	Tubulin	Homolog K00558.1e-129 human alpha-tubulin mRNA, complete cds.				
248	RSA-4-I02_#0	Mm_cluster01020.0.3	DYL1_HUMAN	Homologous Other Genes	Cytoskeleton & Motility	Dynein	Human cytoplasmic dynein light chain 1 (hdc1) mRNA, complete cds.				
249	RSA-6-M12	AF025310, Mm_cluster04905.0.1	DYLX_MOUSE	Mouse Genes	Cytoskeleton & Motility	Dynein	Mouse tctex-1 mRNA, complete cds.				
261	RSA-1-J07	MMMEGR, Mm_cluster00345.0.2	AF064081	Mouse Genes	Cytoskeleton & Motility		Mus musculus alpha-sarcoglycan gene, complete cds.				
332	RSA-8-K11	AB011550, Mm_cluster02217.0.1, Mm_g	GELS_MOUSE	Mouse Genes	Cytoskeleton & Motility	Actin Polymerisation	Mouse gelsolin gene, complete cds.				
390	RSA-3-N10	HSAB2368, Mm_cluster97066.0.1	TB5_MOUSE	Mouse Genes	Cytoskeleton & Motility	Tubulin	Cricetulus griseus (chinese hamster) mRNA for beta tubulin (clone B3T)				
30	RSA-13-O14	HSKIAA09, Hs_cluster19328.0.7	ATND_MOUSE	Mouse Genes	Crossmembrane Transport	Ion Transport	Mus musculus Na ⁺ K ⁺ -ATPase beta 3 subunit (ATP1B3) mRNA, complete cds.				
411	RSA-32-M15	HS1273260, Hs_cluster09289.0.4	RN15176	Homologous Other Genes	Crossmembrane Transport	Ion Transporter	Rattus norvegicus Na,K-ATPase alpha subunit mRNA, complete cds.				
474	RSA-1-M11, RSA-4-H13	AA612279, AF086138, Mm_cluster07446	HSD432	Homologous Other Genes	Crossmembrane Transport	Amino Acid Transport	Human mRNA for KIAA0245 gene, complete cds.				
596	RSA-1-I20	AI050562, Mm_cluster15659.0.6	mCAT2	Mouse Genes	Crossmembrane Transport	Amino Acid Transporter	Mus musculus cationic amino acid transporter (mCAT2) mRNA, 5 UTR.				
124	RSA-23-H17	HSATPSYNT, Mm_cluster17365.0.108	HS72_MOUSE	Mouse Genes	Chaperone		Mouse heat-shock-like protein (HSP70.2) gene, complete cds.				
174	RSA-5-I17	AF029874, RNHO2, Mm_cluster00858.0	BiP	Mouse Genes	Chaperone		Mus musculus mRNA for BiP				
572	RSA-32-G11_1	MM1155093, Mm_cluster14404.0.3	DNAI-homolog	Mouse Genes	Chaperone		Mus musculus testis specific DNAI-homolog mRNA, complete cds.				
253	RSA-8-E02	MMU79747, Mm_cluster04260.0.3	TBA1_MOUSE	Mouse Genes	Cell Structure & Motility	Tubulin	Mouse alpha-tubulin gene M-alpha-1, 3' end.				
144	RSA-1-I19	MMLDHB, MMA4LDHT, Mm_cluster027	PLAK_MOUSE	Mouse Genes	Cell Junction		Mus musculus plakoglobin mRNA, partial cds.				
5	RSA-12-L01	AB014514, Mm_cluster77626.0.1	MMF9	Mouse Genes	Cell Cycle Regulator	Sister Chromatid Cohesion	Mouse NCBP-29 mRNA for PW29, complete cds.				
92	RSA-2-K23, RSA-32-J15_#0	AF064081, Mm_cluster01231.0.4	HS337611	Homologous Other Genes	Cell Cycle Regulator	Entry into S Phase	Human cyclin A/CDK2-associated p45 (Skp2) mRNA, complete cds.				
424	RSA-27-M09	HS1198463, Hs_cluster25577.0.4	RCC_HUMAN	Homologous Other Genes	Cell Cycle Regulator	Chromosome Condensation	Human mRNA for cell cycle gene RCC1				
530	RSA-14-K11, RSA-18-F19	AA690984, AI113283, MM1297089, Mm	GNRP_MOUSE	Mouse Genes	Cell Cycle Regulator		Mouse cell division cycle (CDC25) homologue related mRNA sequence.				
70	RSA-28-A07_0	MMCDC25H, Mm_cluster02410.0.4	PRTC_MOUSE	Mouse Genes	Blood Coagulation		Mus musculus anticoagulant protein C gene, complete cds.				
351	RSA-10-D11_#0	MMHRSH, Mm_cluster01844.0.1	Mm_cluster03360	Mouse Genes	Axon Guidance		M-Sema F=alpha factor in neural network development [mice, neonatal brain, mRNA, 3503 nt].				
1	RSA-5-P17	HSAAADNMR, Hs_cluster30291.0.4	MMUNKNM	Mouse Genes	?		Mouse (clone BALB10N) mRNA, complete cds of unknown function. (Homolog SMY_MOUSE (367))				
40	RSA-13-N24, RSA-6-N12	MMUNKNM, MMUNKND, Mm_cluster02	AB014514	Homologous Other Genes	?		Homo sapiens mRNA for KIAA0614 protein, partial cds.				
56	RSA-19-L09, RSA-4-K10	AF057171, AF057169, Mm_cluster01162	HSICT1GEN	Homologous Other Genes	?		Homo sapiens ICT1 (alias DS-1) mRNA				
58	RSA-4-P02_#0	HS47590, Mm_cluster06974.0.1	HS19878	Homologous Other Genes	?		Human transmembrane protein mRNA, complete cds.				
74	RSA-14-L03_#0, RSA-29	MMU95607, Mm_cluster00662.0.1	SKD3_MOUSE	Mouse Genes	?		Mus musculus SKD3 mRNA, complete cds.				
94	RSA-13-E15	AF020055, MMA65434, Mm_cluster0027	HSAB2349	Homologous Other Genes	?		Human mRNA for KIAA0351 gene, complete cds.				
113	RSA-11-I15, RSA-12-A03,	HSFA5037, RNSCAMP, Mm_cluster6784	Fhl4	Mouse Genes	?		Mus musculus LIM-protein FHL4 (Fhl4) mRNA, complete cds.				
140	RSA-8-N15, RSA-10-N16	MMHEX, Mm_cluster02170	MMREPEAT	Mouse Genes	?		Mouse pEAT11 mRNA with highly repetitive sequence				
160	RSA-6-A22_#0	HSCYPSCC, RNCSCGE, Mm_cluster16	AF1q	Mouse Genes	?		Mouse mRNA for AF1q, complete cds.				
190	RSA-10-A17_#0	MMMATRIC, Mm_cluster02409.0.28	SBBI03	Homologous Other Genes	?		Homo sapiens hypothetical SBBI03 protein mRNA, complete cds.				
200	RSA-23-G10	MMCATD, Mm_cluster00214.0.112	AB018302	Homologous Other Genes	?		Homo sapiens mRNA for KIAA0759 protein, partial cds.				
203	RSA-25-M09	HSMSS1, Mm_cluster04091.0.26	MMU64446	Mouse Genes	?		Mus musculus regulated secretory protein-23 mRNA, partial cds.				
219	RSA-3-P10	AF054182, RNMPPBS, Mm_cluster1965	Y195_HUMAN	Homologous Other Genes	?		Human mRNA for KIAA0195 gene, complete cds.				
237	RSA-6-I24	MM27295, Mm_cluster03697.0.2	Y025_HUMAN	Homologous Other Genes	?		Homo sapiens clone 24560 unknown mRNA, complete cds.				
241	RSA-18-D21, RSA-2-D15,	MMFPK2CMP, Mm_cluster02090.0.1	HSF0480	Homologous Other Genes	?		H. sapiens mRNA for orf (clone ICRFp507F0480).				
256	RSA-17-I14_#0	MMFP36	HSKIAA09	Homologous Other Genes	?		Human mRNA for KIAA0169 gene, partial cds.				
263	RSA-13-J07, RSA-4-J03, R	AB006036, Mm_cluster01106.0.2	SUR4_MOUSE	Mouse Genes	?		Mouse surflet locus surf4 protein mRNA, complete cds.				
284	RSA-31-B06_0	HS095851, Mm_cluster18953.0.1	MMU67328	Mouse Genes	?		Mus musculus NIPL-like protein (NIPL(A3)) mRNA, complete cds.				
317	RSA-10-E09	HSRAP74R, Mm_cluster21544.0.1	UK-HGMP	Homologous Other Genes	?	?	H. sapiens putatively transcribed partial sequence; UK-HGMP sequence ID AAADNMR; single read.				
320	RSA-3-K19	MMTFID, Mm_cluster01574	NY-CO-7, HS1230708	Homologous Other Genes	?		Homo sapiens antigen NY-CO-7 (NY-CO-7) mRNA, complete cds. zw76b08.r1 Soares testis NHT				
336	RSA-10-B02_#0	HSZINC, Mm_cluster20561.0.1	DAL1	Homologous Other Genes	?		Homo sapiens putative lung tumor suppressor (DAL1) mRNA, complete cds.				
365	RSA-4-K24	MDMHTEL2	UNR_RAT	Homologous Other Genes	?		Rat unr mRNA for unr protein with unknown function				
372	RSA-1-N10	HSU58996	VMD2_MOUSE	Mouse Genes	?		Mus musculus bestrophin homolog mRNA, partial cds.				
383	RSA-32-P03_#0	HSZ28471	Ariadne	Mouse Genes	?		Mus musculus mRNA for Ariadne protein, partial				
393	RSA-19-F22	HSC1LE111	EST66525	Homologous Other Genes	?		LNCAP cells I Homo sapiens cDNA 5' end.				
397	RSA-12-J16, RSA-12-M24,	HSD984, Mm_cluster46157.0.1, Hs clus	G100_HUMAN	Homologous Other Genes	?		Human mRNA for Mr 110,000 antigen, complete cds.				
398	RSA-27-N08_1	HSAAS7867, Hs_cluster01755.0.6	EST215554	Homologous Other Genes	?		Normalized rat kidney, Bento Soares Rattus sp. cDNA clone RKIBV87 3' end, mRNA sequence.				
399	RSA-1-A02	AA596097, Hs_cluster02437.0.7	CLUS_MOUSE	Mouse Genes	?		Mus musculus alpha-clustrin and beta-clustrin mRNA, complete cds.				

427	RSA-20-B08	Hs_cluster26376.0.3	AB011081	Mouse Genes	?	Mus musculus mRNA for huntingtin interacting protein-2, complete cds.
468	RSA-14-N06	Mm_cluster06847.0.6	XRP2	Homologous Other Genes	?	Homo sapiens mRNA for XRP2 protein
471	RSA-17-N22_#0	MMA67945, Mm_cluster07118.0.1	Mm_cluster85012	Mouse Genes	?	uh23h02.r1 Barstead mouse testis MPLRB11 Mus musculus cDNA clone 1746291 5', mRNA sequence.
479	RSA-13-H08	MMAA42818, Mm_cluster07739.0.10	HS06631	Homologous Other Genes	?	Human (H326) mRNA, complete cds.
486	RSA-10-E03, RSA-16-D16	AA896206, Mm_cluster08139.0.2	PLRG1	Mouse Genes	?	Mus musculus pleiotropic regulator 1 (PLRG1) mRNA, complete cds.
512	RSA-26-J10	Mm_cluster10335.0.3	MSH3_MOUSE	Mouse Genes	?	Mus Rep-3 protein mRNA, complete cds.
528	RSA-29-M17	MMA08764, Mm_cluster10971.0.23	AI030648	Homologous Other Genes	?	UI-R-C0-jd-b-12-0-UI.s1 UI-R-C0 Rattus norvegicus cDNA clone UI-R-C0-jd-b-12-0-UI 3', mRNA sequence.
533	RSA-7-E20_0	MMA30985, Mm_cluster11379.0.59	LAS1_MOUSE	Mouse Genes	?	Mus musculus SH3 domain-containing protein Lasp-1 mRNA, partial cds.
534	RSA-23-D15	AA990055, Mm_cluster11759.0.1	AB007913	Homologous Other Genes	?	Homo sapiens mRNA for KIAA0444 protein, partial cds.
536	RSA-1-J06_#0	MMA00297, Mm_cluster12024.0.2	MEN1_MOUSE	Mouse Genes	?	Mus musculus menin (Men1) gene, complete cds.
543	RSA-27-J12_#0	MMA30482, Mm_cluster13073.0.2	SA3	Mouse Genes	?	Mus musculus mRNA for nuclear protein SA3
555	RSA-16-M09_0	MMA63934, Mm_cluster13444.0.10	AF070605	Homologous Other Genes	?	Homo sapiens clone 24810 mRNA sequence.
573	RSA-18-I11	Mm_cluster14425.0.21	Hs_cluster27538	Homologous Other Genes	?	Homo sapiens mRNA for KIAA0788 protein, partial cds.
609	RSA-19-N04_1	MMA13852, Mm_cluster16413	Y188_HUMAN	Homologous Other Genes	?	Human mRNA for KIAA0188 gene, partial cds.
612	RSA-3-L01_#0	MM1277708, Mm_cluster16530.0.31	DPY3_MOUSE	Mouse Genes	?	M.musculus mRNA for Ulip protein
621	RSA-9-M24	AA673076, Mm_cluster17645.0.1	Mm_cluster00492.0.1	Mouse Genes	?	M.musculus tex189 mRNA
652	RSA-1-F22, RSA-17-E06	MMAA15695, Mm_cluster22932.0.3	cp151	Homologous Other Genes	?	Rattus norvegicus cp151 mRNA, partial cds.
672	RSA-30-C24_#0	MMA63784, Mm_cluster28224.0.1	RNTMDCIV	Homologous Other Genes	?	R.norvegicus mRNA for tMDC IV protein
47	RSA-26-A10	AF044334, Mm_cluster01054.0.3	Gcap3	Mouse Genes	?	Mus musculus (clone Gcap3) mRNA sequence.
550	RSA-19-P09, RSA-28-K03	MMAA44716, Mm_cluster13386.0.2	EB2	Mouse Genes	?	Mus musculus APC-binding protein EB2 mRNA, partial cds.
691	RSA-24-L17_#0	M1296085, Mm_cluster42937.0.1	ENV1_MOUSE	Mouse Genes	?	Mouse (strain 129 G-IX+) endogenous murine leukemia virus mRNA, clone E1.
754	RSA-3-B03, RSA-4-O05	AA037635, Mm_cluster85000.0.1	MDMHTLE2	Mouse Genes	?	Mouse (C57BL/10J) MHC TL-associated endogenous retrovirus TLv1, 3'LTR and flanks.
4	RSA-23-E16_#0	AB011081, Mm_cluster02181.0.18	Hs_cluster22988	Other EST	?	zw79d12.s1 Soares testis NHT Homo sapiens cDNA clone 782423 3'.
7	RSA-30-D14	AF070605	Mm_cluster09185	Mouse EST	?	mr63d07.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 602125 5' similar to TR:G1067138 G10671381-ACY
8	RSA-11-F13, RSA-13-F01	MMAB733, AI116536, Mm_cluster01019	Mm_cluster11045	Mouse EST	?	ml46d06.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 515051 5'.
10	RSA-21-J01	MMU130977, Mm_cluster01541.0.6	Mm_cluster19317	Mouse EST	?	vi74a05.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 917936 5' similar to WP:T24H10.3 CE03728 DNAJ P1
13	RSA-9-M17	MMSGP2, Mm_cluster01657.0.27	Mm_cluster19317	Mouse EST	?	
14	RSA-10-E12_1	AF092090	Mm_cluster06693	Mouse EST	?	
17	RSA-3-I11, RSA-8-B17	MMRNAULIP, Mm_cluster00565.0.1	Mm_cluster22932	Mouse EST	?	ml42d10.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 514675 5' similar to WP:C47D12.2 CE05430 ;
22	RSA-22-A05_#0	AF053486, Mm_cluster01131.0.1, Mm_c	Mm_cluster39875	Mouse EST	?	vd26h05.s1 Knowles Solter mouse 2 cell Mus musculus cDNA clone 793689 5'.
23	RSA-22-C08	HSGACA, Mm_cluster13713.0.10	Mm_cluster84856	Mouse EST	?	uc8b01.x1 Sugano mouse kidney mkia Mus musculus cDNA clone 14326813' similar to SW:YFE2 YEAST P43560 HYPOTHE
25	RSA-25-F09	HS06631, Hs_cluster27632.0.69	Mm_cluster47348	Mouse EST	?	vi77b04.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 918223 5'.
27	RSA-25-E14	HSAB2349, Hs_cluster09736.0.1	Mm_cluster15659	Mouse EST	?	ub31h10.r1 Soares 2NbMT Mus musculus cDNA clone 1379395 5' similar to SW:GCN5 YEAST Q0330 TRANSCRIPTIONAL A
28	RSA-16-I13	HSF0480, Mm_cluster06712.0.14	Mm_cluster12024	Mouse EST	?	ATPK_MOUSE (175) mq34h01.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 425713 5' similar to PIRA
35	RSA-28-C11_#0	MMREPEAT	Mm_cluster08135	Mouse EST	?	mv86h02.r1 GuayWoodford Beier mouse kidney day 7 Mus musculus cDNA clone 661971 5'.
36	RSA-14-M03_1	MMU64446, Mm_cluster04126.0.5	Mm_cluster24369	Mouse EST	?	ml42b01.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 514633 5' similar to TR:G639691 G639691 XENOPL
43	RSA-27-D11	MMREP3B, Mm_cluster02783.0.2	Mm_cluster68705	Mouse EST	?	M. musculus expressed sequence tag MTEST640
46	RSA-2-B09	Hs_cluster08240.0.20, HS1230709	Mm_cluster09209	Mouse EST	?	ml61f01.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 516505 5'.
50	RSA-7-K02	MMU5678, Mm_cluster01501.0.1	Hs_cluster06678	Other EST	?	H. sapiens partial cDNA sequence; clone c-21b06.
59	RSA-32-K15	AF055001, Mm_cluster09601.0.26	Mm_cluster34325	Other EST	?	
61	RSA-3-H07_0, RSA-5-B05	HSKIAA28, Mm_cluster13659.0.3, Hs_c	Hs_cluster12236	Other EST	?	yyw08e02.s1 Homo sapiens cDNA clone 251642 3'.
65	RSA-25-O02	Mm_cluster03360.0.1	Mm_cluster08054	Mouse EST	?	vc24f10.r1 Ko mouse embryo 11 5dp Mus musculus cDNA clone 7755315'. (Homolog MLZ4_MOUSE (205))
66	10-G13	Mm_cluster00930.0.1	Mm_cluster07528	Mouse EST	?	ml37b08.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 465687 5'.
77	RSA-30-M15	HSD432, Mm_cluster67028.0.1	Mm_cluster15783	Mouse EST	?	mo96f12.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 567599 5'.
78	RSA-28-P07, RSA-4-J11	MM1157631, MM1157671, MMAA5090,	Mm_cluster65316	Mouse EST	?	ml63b05.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 516657 5'.
80	RSA-25-D17, RSA-4-O17	MMU59761, Mm_cluster04076.0.5	AA897680	Other EST	?	o18a03.s1 Soares. NFL. T. GBC. S1 Homo sapiens cDNA clone IMAGE:1504588 3', mRNA sequence.
81	RSA-3-I01, RSA-5-O06, R	RN15176, Mm_cluster43320.0.1	Mm_cluster22387	Mouse EST	?	ml62e10.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 516618 5'.
82	RSA-2-H21	MMGELS, Mm_cluster02157.0.54	Mm_cluster13307	Mouse EST	?	ml75e10.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 420138 5'.
84	RSA-14-C11	MM17324, Mm_cluster03370.0.1	Mm_cluster16824	Mouse EST	?	mq67h08.r1 Soares 2NbMT Mus musculus cDNA clone 583839 5'.
85	RSA-17-M24	HSU32944, Mm_cluster29632.0.1	Mm_cluster08299	Mouse EST	?	
86	RSA-17-A16	MMTCTEX, Mm_cluster02792.0.102	Mm_cluster18536	Mouse EST	?	mh80e10.r1 Soares mouse placenta 4NbMP13.5 14.5 Mus musculus cDNA clone 457290 5' similar to SW:RL22 THEMA P3851
87	RSA-14-K09	Hs_cluster01934.0.4, HS546155	Mm_cluster14600	Mouse EST	?	mv89b12.r1 GuayWoodford Beier mouse kidney day 7 Mus musculus cDNA clone 662207 5' similar to SW:P044 RAT P38718 0
95	RSA-23-K22	HSCKB, Hs_cluster32337	Mm_cluster75907	Mouse EST	?	vs61h05.r1 Stratagene mouse skin (#937313) Mus musculus cDNA clone 1159809 5'.
96	RSA-31-O04	AF044312, Hs_cluster02840.0.1	Mm_cluster58338	Mouse EST	?	mr73f03.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 603101 5' similar to SW:OXYB_HUMAN P22059 OX
98	RSA-2-P23	MMH2A1X, MDH2AH1ST, Mm_cluster00	Hs_cluster05378	Other EST	?	zm60c09.r1 Soares fetal heart NbHH19W Homo sapiens cDNA clone 345040 5'.
99	RSA-30-L21	MMMH3A, Mm_cluster02725	Mm_cluster16933	Mouse EST	?	mm20g01.r1 Stratagene mouse diaphragm (#937303) Mus musculus cDNA clone 522096 5'.
102	RSA-23-P04	AF039023, Mm_cluster20345.0.1	Mm_cluster51209	Mouse EST	?	
103	2-I02, 2-P02, 4-K03	Mm_cluster02508	Mm_cluster14306	Mouse EST	?	mu12d03.r1 Soares 2NbMT Mus musculus cDNA clone 639173 5'.
106	RSA-3-H17	MMAP47A, Mm_cluster03018.0.18	Mm_cluster76266	Mouse EST	?	vr43h10.s1 Knowles Solter mouse 2 cell Mus musculus cDNA clone 1123459 5'.
114	RSA-31-I18	AF094516, Mm_cluster44052.0.1	Mm_cluster27813	Mouse EST	?	vn01h10.r1 Knowles Solter mouse blastocyst B1 Mus musculus cDNA clone 1006531 5' similar to SW:MEPD RAT P24155 THIN
115	RSA-1-M21_#0	U89427, CGEPSCOP, Mm_cluster04332	MMAA5772	Mouse EST	?	ml84g03.r1 Stratagene mouse kidney (#937315) Mus musculus cDNA clone 425744 5'.
121	RSA-13-F16_#0, RSA-18	MMAASSB, Mm_cluster02868.0.141, Hs	HSAB2368	Homologous Other Genes	?	Human mRNA for KIAA0370 gene, partial cds.
123	RSA-5-F09	MMATPSYNX, Mm_cluster02205.0.127	Mm_cluster22431	Mouse EST	?	Homolog GT11_MOUSE (143) mm08b01.r1 Stratagene mouse diaphragm (#937303) Mus musculus cDNA clone 520873 5'.
131	9-K13_#0	RNFKFBP, Mm_cluster0184.0.1	Mm_cluster92625	Mouse EST	?	uc70d01.r1 Soares mouse mammary gland NbMMG Mus musculus cDNA clone 1430977 5', mRNA sequence.
134	RSA-4-F20_#0, RSA-26-F0	MMALDA, Mm_cluster02173.0.15	Mm_cluster15648	Mouse EST	?	mq35a05.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 425744 5'.
138	RSA-32-E10, RSA-4-C13	MMGPI, Mm_cluster02299.0.1	Mm_cluster07344	Mouse EST	?	mw68h02.r1 Soares mouse NML Mus musculus cDNA clone 677619 5'.
145	RSA-18-L06	HSPG11A, RNPHOSPHZ, Hs_cluster00	Mm_cluster12965	Mouse EST	?	mh38d08.r1 Soares mouse placenta 4NbMP13.5 14.5 Mus musculus cDNA clone 444783 5' similar to PIR:SS3818 S53818 XPM
161	RSA-26-O02, RSA-4-E02	RNPRCOX	Mm_cluster10593	Mouse EST	?	mq53d09.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 427505 5' similar to PIR:S47451 S47451 hypoth
162	RSA-4-O09	HSQRE, Mm_cluster12022.0.2	Mm_cluster07739	Mouse EST	?	mq63g06.r1 Soares 2NbMT Mus musculus cDNA clone 583450 5'.
164	RSA-21-O22	HSFAPS, Mm_cluster12956.0.19	Mm_cluster17645	Mouse EST	?	vn45g07.r1 Barstead mouse myotubes MPLRB5 Mus musculus cDNA clone 1024188 5'.
165	RSA-6-P17	AF052732, RN10HCO, Mm_cluster2242	Mm_cluster95030	Mouse EST	?	Mus musculus 8-cell embryo cDNA 3'-end sequence; clone J0523G08.
166	RSA-1-B03, RSA-31-B19,	MM09114, MMGSA, Mm_cluster03003	EST111162	Other EST	?	Rat PC-12 cells, NGF-treated (9 days) Rattus sp. cDNA clone RPNB509.
168	RSA-22-K16	HSGLYKINB, Mm_cluster03953.0.1	Mm_cluster20699	Mouse EST	?	mo97c10.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 567666 5' similar to TR:G747706 G747706 NOVEL
170	RSA-4-E21	MMEC11995, Mm_cluster01855.0.1	Mm_cluster08803	Mouse EST	?	
171	RSA-11-I05, RSA-28-A07	AF044056, Mm_cluster01051.0.1	Mm_cluster29791	Mouse EST	?	vk65c04.s1 Knowles Solter mouse 2 cell Mus musculus cDNA clone 959526 5' similar to qb:M23114 CALCIUM-TRANSPORTIN

Annotated list of cremSL clones with known sequences

179	RSA-20-B03	HSPRSI, RNPRPS1A, Mm cluster16848	Mm cluster14951	Mouse EST	ms07d10.r1 Stratagene mouse skin (#937313) Mus musculus cDNA clone 606259 5'.
181	RSA-4-B24 #0	HS2OGDH, Mm cluster06753.0.1	Mm cluster52803	Mouse EST	m162c02.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 516578 5' similar to TR:G7655 G7655 BETA-H SPEE
185	RSA-7-C18, RSA-3-H23, R	HSAF237, Mm cluster17806.0.19	Mm cluster84992	Mouse EST	uh24a08.r1 Barstead mouse testis MPLRB11 Mus musculus cDNA clone 1746326 5', mRNA sequence.
189	RSA-3-F13, RSA-4-J03, R	D85732, MMHSC70T, Mm cluster01943	Mm cluster70346	Mouse EST	vl17q07.r1 Stratagene mouse Tcell 937311 Mus musculus cDNA clone 972540 5'.
193	RSA-14-D11, RSA-18-F11	MMGAAC, HSACTASK, Mm cluster0211	Hs cluster37608	Other EST	zu18d02.r1 Soares NHMPu S1 Homo sapiens cDNA clone 738339 5' similar to WP:C54D2.5 CE02562 SKELETAL MUSCLE CL
194	RSA-17-M11, RSA-27-L21	RN19614, RN20286, Mm cluster10962.0	Hs cluster05239	Other EST	yx18h08.s1 Homo sapiens cDNA clone 282143 3'.
195	RSA-2-P21, RSA-9-Q02	HSD378, Mm cluster16959.0.4, Mm clu	Mm cluster51270	Mouse EST	m132d06.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 513707 5'.
197	RSA-16-B18	RN100KDP, Mm cluster09925.0.31	Hs cluster46706	Other EST	zw66c09.s1 Soares testis NHT Homo sapiens cDNA clone 781168 3'.
201	RSA-9-J12 #1	AF090691, Mm cluster04407.0.4	Mm cluster16521	Mouse EST	mk19d04.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 4933515'.
202	RSA-26-J24	HSU96114, Mm cluster14492.0.2	Mm cluster13480	Mouse EST	m152c10.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 515634 5'.
205	RSA-27-N18, RSA-28-C21	MMU62483, RNU56407, Mm cluster040	Mm cluster06847	Mouse EST	
207	RSA-21-C04, RSA-28-E12	MMUBA1, Mm cluster01593.0.24, Mm c	Mm cluster92665	Mouse EST	ub91f07.r1 Soares mouse mammary gland NbMMG Mus musculus cDNA clone 1395877 5' similar to SW:UPP_TOXGO Q26998
210	RSA-3-K23	AF027292, Mm cluster00489.0.2	Hs cluster03366	Other EST	yx49f09.s1 Homo sapiens cDNA clone 265097 3'.
215	RSA-12-A23 1	AF079565, Mm cluster01369.0.13	Hs cluster43849	Other EST	zv51c09.r1 Soares testis NHT Homo sapiens cDNA clone 757168 5'.
217	RSA-16-C24 1#0	MMTSH, Mm cluster03444	Mm cluster57614	Mouse EST	mr66e12.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 602446 5'.
218	RSA-18-P19, RSA-7-K15	GGTRANGLU, Mm cluster19910.0.1	Mm cluster20036	Mouse EST	mg33c02.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 425570 5'.
222	RSA-1-P24	CFSEC61A, Mm cluster30476.0.1	Mm cluster21319	Mouse EST	m04b03.r1 Life Tech mouse embryo 15 5dpc 10667012 Mus musculus cDNA clone 556013 5'.
224	RSA-29-D20	AF000982, Hs cluster04912	Mm cluster14724	Mouse EST	mm85e05.r1 Stratagene mouse embryonic carcinomaRA (#937318) Mus musculus cDNA clone 535232 5'.
227	RSA-1-F15	HSINSP35P, Mm cluster24350.0.1	Mm cluster18355	Mouse EST	m156g02.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 516050 5' similar to TR:G243898 G243898 GOR=AN
228	RSA-7-L08	AF015811, Mm cluster00025.0.12	Mm cluster13437	Mouse EST	m166h06.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 468539 5' similar to PIR:A53770 A53770 growthf
231	RSA-29-C15	AF077660, Mm cluster01353.0.1	Mm cluster08568	Mouse EST	mg7h06.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 439067 5'.
233	RSA-30-C23 0	MM19799, Mm cluster03602.0.12	Mm cluster08139	Mouse EST	vx62d10.r1 Stratagene mouse macrophage (#937306) Mus musculus cDNA clone 1279795 5'.
240	RSA-29-D22, RSA-7-J03	AF093406, Mm cluster01439.0.1	Mm cluster11274	Mouse EST	DP1_MOUSE (176) vj29a09.r1 Stratagene mouse diaphragm (#937303) Mus musculus cDNA clone 930424 5' similar to gb:MT4
242	RSA-14-E12, RSA-2-G20	MMPKCDA, MMNPKCD, HSPKSCD, Mm	Mm cluster42622	Mouse EST	v175h06.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 918107 5' similar to SW:GTT1_CHICK P20135 GLUT
243	RSA-14-D21, RSA-9-O15	AB005654, U74464, Mm cluster01097.0	Mm cluster49847	Mouse EST	mh39f08.r1 Soares mouse placenta 4NbMP13.5 14.5 Mus musculus cDNA clone 444903 5'.
244	RSA-30-L12	MMERK3MR, Mm cluster04734.0.1	Mm cluster13444	Mouse EST	m138b10.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 514267 5'.
245	RSA-17-K18	MMCKIIB, Mm cluster00216.0.25	Mm cluster13868	Mouse EST	v40h07.r1 Soares mouse NbMH Mus musculus cDNA clone 846301 5'.
252	RSA-24-I17	MMMP1M, Mm cluster01932.0.34	Mm cluster16333	Mouse EST	m125e10.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 464586 5' similar to SW:YKV8_YEAST P36007 H
260	RSA-9-Q21	MM28495, Mm cluster03717.0.2	Mm cluster22300	Mouse EST	m146g09.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 515104 5'.
262	RSA-19-P14	MMU43206, Mm cluster03905.0.15	Mm cluster14440	Mouse EST	m156g10.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 467586 5'.
267	RSA-26-E06	HSIDE, Mm cluster17370.0.11	HS1315818	Other EST	ng39a01.s1 NCI CGAP_Cc3 Homo sapiens cDNA clone IMAGE:937128similar to TR:E241773 E241773 HYPOTHETICAL 41.3
268	RSA-32-L04	AF044923, Hs cluster16795.0.1	Mm cluster93823	Mouse EST	Mus musculus 2-cell embryo cDNA 3'-end sequence, clone J0738G01.
270	RSA-31-O02	HS264551, Hs cluster14443.0.1	Mm cluster07118	Mouse EST	mm44g09.r1 Stratagene mouse melanoma (#937312) Mus musculus cDNA clone 524416 5'.
272	RSA-4-Q02	AF012872, RS230P4K, Mm cluster1341	Mm cluster06330	Mouse EST	mq24b07.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 424693 5'.
274	RSA-8-P11 0, 8-P11 1	AF048976	Mm cluster00914	Mouse EST	
276	RSA-28-D16, RSA-30-B04	HSORF17, AA895186, Mm cluster17328	Mm cluster75808	Mouse EST	vs66f03.r1 Stratagene mouse skin (#937313) Mus musculus cDNA clone 1151261 5'.
277	RSA-4-O14	HSHPS12, Mm cluster64110.0.1	Mm cluster50354	Mouse EST	m140g08.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 466046 5'.
280	RSA-18-D04, RSA-2-E07	MMU84389, MMU95145, Mm cluster042	Mm cluster28775	Mouse EST	ue15a05.s1 Sugano mouse embryo mewa Mus musculus cDNA clone 14804003', mRNA sequence.
282	RSA-3-J03	Hs cluster19834.0.63, HS091218	Mm cluster36421	Mouse EST	mx03b08.r1 Soares mouse NML Mus musculus cDNA clone 679095 5'.
285	RSA-32-D17	RNU97143, Mm cluster15705.0.1	Mm cluster14425	Mouse EST	
286	RSA-24-H21	AF027984	Mm cluster33762	Mouse EST	
289	RSA-15-N18 1	HSCABCNLS, RNCCB, Mm cluster4812	Mm cluster22271	Mouse EST	mh79b09.r1 Soares mouse placenta 4NbMP13.5 14.5 Mus musculus cDNA clone 457145 5'.
292	RSA-19-M14	MMTPX110	Mm cluster64114	Mouse EST	mo97c08.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 567662 5' similar to SW:ACT_SCHPO P10989 ACT
293	RSA-11-E04, RSA-17-O16	MMMD471, Mm cluster05267.0.1	Mm cluster08372	Mouse EST	mh66b06.r1 Soares mouse placenta 4NbMP13.5 14.5 Mus musculus cDNA clone 455891 5'.
296	RSA-2-K20, RSA-3-D20, R	HSCALICIN, BTALICIN, Hs cluster299	Mm cluster02147	Mouse EST	
298	RSA-6-C14	MMTP2, Mm cluster02107.0.3	Hs cluster15429	Other EST	
300	RSA-25-A09	AF088868, Mm cluster84994.0.1	Mm cluster13459	Mouse EST	z172f11.r1 Soares testis NHT Homo sapiens cDNA clone 727917 5'.
303	RSA-8-P20, RSA-17-K23	MM07423, MM10341, Mm cluster03455	HSC1LE111	Other EST	m160d09.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 516401 5'.
305	RSA-3-D13, RSA-3-K01	AF000968, Mm cluster02741.0.1	Mm cluster42599	Mouse EST	H. sapiens partial cDNA sequence; clone c-1le11.
306	RSA-2-P22, RSA-26-L02	MMODF1, Mm cluster00480.0.1	Mm cluster17638	Mouse EST	mr64d11.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 602229 5'.
309	RSA-30-N24	MM22059, Mm cluster03653.0.1	Mm cluster20069	Mouse EST	mp81c02.r1 Soares 2NbMT Mus musculus cDNA clone 575618 5'.
312	RSA-11-H17, RSA-4-E18	MM22058, Mm cluster05905.0.1	Mm cluster16413	Mouse EST	m173e07.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 4817645'.
314	RSA-24-C01 #0	MMARPP0, Mm cluster00174.0.23	Hs cluster23754	Other EST	mh07a12.r1 Soares mouse placenta 4NbMP13.5 14.5 Mus musculus cDNA clone 441790 5'.
326	RSA-16-O23	U95141, Mm cluster00059.0.2	Mm cluster12477	Mouse EST	zv54f10.s1 Soares testis NHT Homo sapiens cDNA clone 757483 3' similar to TR:G603907 G603907 TRYPsinogen PRECUR
328	RSA-10-I09	MMSOXL22, Mm cluster01528.0.2	Mm cluster26401	Mouse EST	vg07c04.r1 Barstead stromal cell line MPLRB8 Mus musculus cDNA clone 1093542 5' similar to WP:W06D4.4 CE16546 ;.
331	RSA-7-I05	MM09351, Mm cluster03450.0.1	Mm cluster65814	Mouse EST	ua35f08.r1 Soares mouse mammary gland NbMMG Mus musculus cDNA clone 1348743 5', mRNA sequence.
334	RSA-4-H10	MMU69133, Mm cluster06091.0.1	Mm cluster10943	Mouse EST	vi78e07.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 918372 5'.
338	RSA-15-B06, RSA-16-E15	HSNABP, MMCNBPMBR, Hs cluster007	Mm cluster13980	Mouse EST	m134a05.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 513872 5' similar to gb:X63368 cds1 DNAJ PROTEI
342	RSA-18-M09	MMRNAL28, Mm cluster00439.0.156	Mm cluster64097	Mouse EST	
343	RSA-5-P20	MMU67771, Mm cluster04162.0.206	Mm cluster14480	Mouse EST	mo97f01.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 567673 5'.
344	RSA-30-F11	MMIRPS17	Hs cluster09289	Other EST	mo17e05.r1 Life Tech mouse embryo 13 5dpc 10666014 Mus musculus cDNA clone 553856 5'.
345	RSA-13-A11, RSA-26-D17	MMIRPS24, Mm cluster02834	Mm cluster16278	Mouse EST	aa29c04.r1 NCI CGAP_GCB1 Homo sapiens cDNA clone IMAGE:814662 5' similar to TR:G203113 G203113 BETA'-CHAIN CL
346	RSA-18-E23	MMS29RP, Mm cluster02495.0.11	Mm cluster91543	Mouse EST	mr73g10.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 603318 5'.
347	RSA-31-L09 1	HSUBA52A, Mm cluster01863.0.141	Hs cluster25707	Other EST	
349	RSA-1-L04, RSA-4-H16	HSEF1G, Mm cluster13600.0.112	Mm cluster13443	Mouse EST	zu03b05.s1 Soares testis NHT Homo sapiens cDNA clone 730737 3'.
350	RSA-1-L19 1	U54559, Mm cluster11692.0.7	Hs cluster03713	Other EST	m141g02.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 514610 5'.
358	RSA-11-E21	HSCYSTRNA, Mm cluster20483.0.1	Mm cluster16530	Mouse EST	
359	RSA-1-A05	RNRIPRL38, LERPL38A, Mm cluster12	Mm cluster23636	Mouse EST	vb56e03.r1 Ko mouse embryo 11 5dpc Mus musculus cDNA clone 7610205' similar to TR:G505652 G505652 GP36B GLYCOPH
362	RSA-32-E12	MMERE1M	Mm cluster51812	Mouse EST	m148h03.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 515285 5' similar to WP:T26A5.9 CE00788 ;.
369	RSA-8-C12 #0	Mm cluster02314.0.1, HSU79287	Mm cluster93317	Mouse EST	m152g10.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 515682 5' similar to WP:K02B2.3 CE04689 ;.
374	RSA-3-A12	AA772377	Mm cluster20710	Mouse EST	vm40g02.r1 Knowles Solter mouse blastocyst B1 Mus musculus cDNA clone 992690 5'.
375	RSA-1-E13	AA697680	Mm cluster59259	Mouse EST	vi75a01.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 918024 5'.
376	RSA-17-N06	AI003306	Hs cluster41893	Other EST	mq35h06.r1 Soares 2NbMT Mus musculus cDNA clone 582683 5' similarto TR:G285961 G285961 MRNA ;.
377	RSA-23-N01	AI326289	Hs cluster22953	Other EST	zu62a05.s1 Soares testis NHT Homo sapiens cDNA clone 742944 3' similar to TR:G1184318 G1184318 INHIBITOR OF APOPT
378	RSA-3-J06	AI326301	Mm cluster07150	Mouse EST	vi63e12.r1 Soares testis NHT Homo sapiens cDNA clone 742702 5'.
					vi95e09.s1 Knowles Solter mouse 2 cell Mus musculus cDNA clone 944872 5' similar to WP:T10F2.4 CE02043 GUANINE NUCI

380	RSA-13-J09, RSA-4-E05	RS6954	Mm_cluster15460	Mouse EST				va08h04.r1 Soares mouse lymph node NbMLN Mus musculus cDNA clone 722359 5'.						
381	RSA-3-M21	AA850685	Mm_cluster57206	Mouse EST				m158b07.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 516181 5'.						
384	RSA-3-M08	HS1181716	Mm_cluster06503	Mouse EST				vc56h08.s1 Knowles Solter mouse 2 cell Mus musculus cDNA clone 778623 5'.						
387	RSA-18-B10	HS1202587	Mm_cluster20749	Mouse EST				m156h06.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 516059 5'.						
401	RSA-6-F07_#0	Hs_cluster03209.0.124	Mm_cluster16947	Mouse EST										
404	RSA-7-A11	Hs_cluster03713.0.57	Mm_cluster07735	Mouse EST				mq27d10.r1 Barstead MPLRB1 Mus musculus cDNA clone 579955 5'.						
405	RSA-25-G13	HS106265, Hs_cluster05239.0.4	Mm_cluster35623	Mouse EST				mw20q03.r1 Soares mouse 3NME12 5 Mus musculus cDNA clone 671284 5'.						
410	RSA-28-K10	HSC21B061, Hs_cluster06678.0.22	Mm_cluster42941	Mouse EST				vi74e12.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 917998 5'.						
413	RSA-2-K17	HS027252, Hs_cluster12236.0.1	Mm_cluster77934	Mouse EST				uc91c11.r1 Soares mouse uterus NMPu Mus musculus cDNA clone 14330125'.						
415	RSA-2-E04_1#0	HS1222313, Hs_cluster15429.0.6	Mm_cluster42566	Mouse EST				vi73b08.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 917847 5' similar to SW:NCA_HUMAN P40199 NORI						
420	RSA-11-F23	HS1200426, Hs_cluster22953.0.1	Mm_cluster91722	Mouse EST				me93h03.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 403157 5' similar to PIR:S42864 S42864 protein						
422	RSA-2-A03	HS1230902, Hs_cluster22988.0.4	Mm_cluster13618	Mouse EST				mo06e11.r1 Stratagene mouse lung 937302 Mus musculus cDNA clone 552812 5'.						
423	RSA-9-O24	HS1238548, Hs_cluster23754.0.2	Mm_cluster13448	Mouse EST				m156g12.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 516070 5'.						
425	RSA-1-M16	HS1231011, Hs_cluster25696.0.1	Mm_cluster10955	Mouse EST				mm57f05.r1 Stratagene mouse embryonic carcinoma (#937317) Mus musculus cDNA clone 532545 5'.						
426	RSA-7-P06	HS1237282, Hs_cluster25707.0.2	Mm_cluster11759	Mouse EST				ua58c11.r1 Soares 2NbMT Mus musculus cDNA clone 1361684 5' similar to SW:YNOQ3_YEAST P53893 HYPOTHETICAL 124.5						
429	RSA-27-F07	HS1307912, Hs_cluster33753.0.3	Mm_cluster42528	Mouse EST				vi72b06.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 917747 5' similar to TR:G7550 G7550 ACTIN. [1].						
430	RSA-11-O04	AA812713, Hs_cluster35254.0.1	Hs_cluster22673	Other EST				z161d10.r1 Soares testis NHT Homo sapiens cDNA clone 726835 5'.						
433	RSA-28-B04	HS1200382, Hs_cluster41893.0.3	Hs_cluster43833.0.1	Other EST				Homo sapiens chromosome 7q22 sequence						
435	27-L07, RSA-7-P22	HS1301578, Hs_cluster42700.0.1	AI326289	Mouse EST				m141e08.x1 Stratagene mouse testis (#937308) Mus musculus cDNA clone IMAGE:514598 3', mRNA sequence.						
440	RSA-4-P17	HSAA46194, Hs_cluster46706.0.1	Mm_cluster10948	Mouse EST				m158d09.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 516209 5' similar to SW:ACT1_NA P27131 ACT						
443	RSA-11-N16	MMAA5772, Mm_cluster16827.0.1	Mm_cluster06748	Mouse EST				ub26q03.r1 Soares 2NbMT Mus musculus cDNA clone 1378900 5', mRNA sequence.						
444	RSA-30-J04	MMAA83826	OSF-6	Mouse EST				cDNA encoding mouse OSF-6, a transcription regulatory factor.						
445	RSA-20-I22	MMLEUPS	Mm_cluster23176	Mouse EST				m141a05.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 514544 5'.						
451	RSA-6-K04	Mm_cluster00914.0.11	Mm_cluster10424	Mouse EST				vb86e04.r1 Stratagene mouse 3NME12 5 Mus musculus cDNA clone 763902 5' similar to TR:G669045 G669045 PORTION OF HYP						
457	RSA-6-O05	Mm_cluster02147.0.272	Mm_cluster84804	Mouse EST				uh21d03.r1 Barstead mouse testis MPLRB11 Mus musculus cDNA clone 1746053 5', mRNA sequence.						
458	RSA-7-G24	Mm_cluster03547.0.1	Mm_cluster78124	Mouse EST				vw65a05.r1 Stratagene mouse heart (#937316) Mus musculus cDNA clone 1259792 5'.						
467	RSA-22-P07_0, RSA-24-AC	AIO20454, MMAA25190, MMAA82228, M	Mm_cluster12808	Mouse EST				mq75b11.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 438813 5'.						
469	RSA-28-D11_0	MM1175760, Mm_cluster06986.0.2	Mm_cluster26889	Mouse EST				mh88e08.r1 Soares mouse placenta 4NbMP13.5 14.5 Mus musculus cDNA clone 458054 5' similar to SW:MSP1_YEAST P2873						
472	RSA-21-C08	AA545383, Mm_cluster07150.0.2	Mm_cluster86656	Mouse EST										
473	RSA-12-F21_1	MMAA13263, Mm_cluster07344.0.1	Mm_cluster13883	Mouse EST				mq07h10.r1 Soares 2NbMT Mus musculus cDNA clone 578083 5'.						
477	RSA-22-J15	MMAA22462, Mm_cluster07735.0.2	AI003306	Mouse EST				an08h06.s1 Stratagene schizo brain S11 Homo sapiens cDNA clone IMAGE:1685051 3' similar to SW:MIPP_MOUSE P28575 M						
483	RSA-14-L06_1#0	MMAA85411, Mm_cluster08135.0.6	Mm_cluster87042	Mouse EST				uf04q05.y1 Sugano mouse liver mlia Mus musculus cDNA clone 14996725', mRNA sequence.						
496	RSA-13-M17	Mm_cluster08803.0.33	Mm_cluster15244	Mouse EST				m136e04.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 514110 5'.						
497	RSA-6-B21	Mm_cluster08810.0.145	Mm_cluster10335	Mouse EST										
499	RSA-7-H21	Mm_cluster09050.0.1	Mm_cluster12297	Mouse EST										
506	RSA-28-P01_#0	MMA23976, Mm_cluster09177.0.4	Mm_cluster13374	Mouse EST				md15a05.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 368432 5'.						
511	RSA-4-E23	MMAA37794, Mm_cluster09242.0.13	Mm_cluster76954	Mouse EST				M.musculus expressed sequence tag MTEST189						
514	RSA-24-E22_#0	MM1190052, Mm_cluster10424.0.1	Hs_cluster33753	Other EST				vw32a05.r1 Soares mouse mammary gland NbMMG Mus musculus cDNA clone 1245488 5'.						
516	RSA-8-M12	MM1263830, Mm_cluster10460.0.1	Mm_cluster13073	Mouse EST				nf60f09.s1 NCI_CGAP_Co3 Homo sapiens cDNA clone IMAGE:924329						
517	RSA-8-P17	MMA02460, Mm_cluster10593.0.10	Mm_cluster07673	Mouse EST				mi09g09.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 463072 5' similar to WP:B0336_2_CE00696 ARF ;						
519	RSA-23-K19	MM1294185, Mm_cluster10937.0.1	Mm_cluster14171	Mouse EST				ue78e12.r1 Soares mouse uterus NMPu Mus musculus cDNA clone 14972625', mRNA sequence.						
523	RSA-2-I19, RSA-26-K08, R	MMA59999, Mm_cluster10943.0.2	Hs_cluster25677	Other EST				mq98d09.r1 Soares mouse 3NbMS Mus musculus cDNA clone 595985 5'.						
524	RSA-23-P03	MMAA6036, Mm_cluster10948.0.5	Mm_cluster11278	Mouse EST				z189f01.r1 Soares testis NHT Homo sapiens cDNA clone 729529 5'.						
525	RSA-8-L20	MMA68432, Mm_cluster10955.0.3	Mm_cluster23178	Mouse EST				vw87g09.r1 Stratagene mouse skin (#937313) Mus musculus cDNA clone 1261984 5' similar to SW:MPP1_HUMAN Q10713 MI						
531	RSA-8-P01	MM1305294, Mm_cluster11274.0.2	Hs_cluster25696	Other EST				m151h10.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 515587 5' similar to SW:H2A_STRPU P02271 HISTO						
539	RSA-24-K10	MMA08108, Mm_cluster12808.0.4	Mm_cluster06986	Mouse EST				zw72e05.r1 Soares testis NHT Homo sapiens cDNA clone 781760 5'.						
542	RSA-7-H18	MMA16879, Mm_cluster12965.0.7	Hs_cluster03209	Other EST				vb08f11.r1 Soares mouse NML Mus musculus cDNA clone 748365 5' similar to TR:E239919 E239919 CHROMOSOME XIV REA						
545	RSA-26-N22_#0	MMA22519, Mm_cluster13374.0.2	Mm_cluster24381	Mouse EST				Homolog G06868 1e-180 human STS WI-8269						
553	RSA-15-P18	MMA14327, Mm_cluster13437.0.13	Mm_cluster09177	Mouse EST				vi71h04.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 917719 5' similar to SW:ACT1_ABSGL P10982 ACTI						
554	RSA-1-C17, RSA-10-P02, F	MMA63882, Mm_cluster13443.0.1	Hs_cluster01755, Hs	Other EST, Other EST				mh92b09.r1 Soares mouse placenta 4NbMP13.5 14.5 Mus musculus cDNA clone 458393 5' similar to SW:RER1_YEAST P2556						
556	RSA-5-K09	MMAA8909, Mm_cluster13448.0.9	Mm_cluster50866	Mouse EST				z068h02.r1 Stratagene pancreas (#937208) Homo sapiens cDNA clone 592083 5', Homolog G24929 1e-142 human STS EST2						
560	RSA-23-A06_#0	MMAA89229, Mm_cluster13618	Mm_cluster10818	Mouse EST				m157c06.r1 Knowles Solter mouse blastocyst B3 Mus musculus cDNA clone 1110895 5'.						
561	RSA-4-G07	Mm_cluster13849.0.3	Mm_cluster03547	Mouse EST				vo17c02.r1 Barstead mouse myotubes MPLRB5 Mus musculus cDNA clone 1050146 5'.						
563	RSA-5-I08	MMAA16393, Mm_cluster13883.0.3	MM1277293	Mouse EST				m140e07.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 514500 5'.						
566	RSA-2-K02	Mm_cluster13980.0.4	Mm_cluster22634	Mouse EST										
570	RSA-27-L20	MMAA22519, Mm_cluster14171.0.2	MMLEUPS	Mouse EST				vc83d12.r1 Ko mouse embryo 11 5dpc Mus musculus cDNA clone 7896235'.						
571	RSA-11-H06	MMAA964, Mm_cluster14306.0.2	Mm_cluster10971	Mouse EST				m156e02.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 516026 5'.						
576	RSA-2-I24_1	MMAA3135, Mm_cluster14480.0.1	AA772377	Other EST				Mouse leukosialin pseudogene (CD 43)						
577	RSA-4-D08_#0	MMA00577, Mm_cluster14543.0.1	Mm_cluster84692	Mouse EST				mq96h05.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 440889 5' similar to SW:YKQ0_YEAST P36053						
582	RSA-10-N17	MMAA73921, Mm_cluster14600.0.20	Mm_cluster71322	Mouse EST				ai44b10.s1 Soares_parathyroid_tumor_NbHPA Homo sapiens cDNA clone 1359835 3', mRNA sequence.						
584	RSA-31-N24_#0	MMAA62188, Mm_cluster14646.0.7	Mm_cluster11379	Mouse EST				ui20b04.r1 Barstead mouse testis MPLRB11 Mus musculus cDNA clone 1745935 5', mRNA sequence.						
586	RSA-15-K09	MMAA86746, Mm_cluster14724.0.1	Hs_cluster02437	Mouse EST				vr05b04.r1 Knowles Solter mouse blastocyst B3 Mus musculus cDNA clone 1110895 5'.						
591	RSA-21-D05	MM1170614, Mm_cluster15460.0.1	Mm_cluster16020	Mouse EST				m146f10.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 466603 5'.						
592	RSA-12-B19	MMAA0728, Mm_cluster15648.0.54	Mm_cluster09050	Mouse EST				vo17c02.r1 Barstead mouse myotubes MPLRB5 Mus musculus cDNA clone 1050146 5'.						
597	RSA-10-I10, RSA-4-N11_#	MMAA74334, Mm_cluster15783.0.1	Mm_cluster10966	Mouse EST				m140e07.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 514500 5'.						
600	RSA-8-A15_#0, RSA-9-L08	MMA64586, MMAA83568, Mm_cluster1	S83465	Mouse Genes				vi69q07.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 917532 5' similar to SW:KELC_DROME Q04652 RIN						
601	RSA-31-D23	MMAA39033, Mm_cluster16088.0.1	Hs_cluster42700	Other EST				endozepine-like peptide [mice, testis, mRNA, 563 nt].						
606	RSA-2-N03, RSA-8-O24	MMAA40044, MMAA92771, Mm_cluster	Hs_cluster26376.0.3	Other EST				ni05q02.s1 NCI_CGAP_Br2 Homo sapiens cDNA clone IMAGE:967154.						
613	RSA-10-L20	MMAA52950, Mm_cluster16824.0.2	Hs_cluster10869.0.42	Other EST				Homolog G22652 0.0 human STS WI-14109.						
615	RSA-11-F22_#0	MMAA6798, Mm_cluster16933.0.2	AI326301	Mouse EST				Homolog G28167 0.0 human STS SHGC-34133.						
617	RSA-22-I14	Mm_cluster16947.0.4	Mm_cluster10460	Mouse EST				m148f06.x1 Stratagene mouse testis (#937308) Mus musculus cDNA clone IMAGE:515267 3' similar to SW:ACT1_ABSGL P109						
618	RSA-30-J18_0#0	AA756845, Mm_cluster17174.0.1	Mm_cluster00474	Mouse EST				vh09h04.r1 Soares mouse mammary gland NbMMG Mus musculus cDNA clone 874999 5' similar to TR:G200131 G200131 KID						
619	RSA-19-H19_0	MMAA25410, Mm_cluster17638	HSD984	Homologous Other Genes				Human mRNA for KIAA0231 gene, partial cds.						
624	RSA-6-N07	MM1294360, Mm_cluster19317.0.1	Mm_cluster11804	Mouse EST										
625	1-E23_1	Mm_cluster24857.0.1	Mm_cluster08810	Mouse EST				COX1_MOUSE (166)						

Annotated list of cremSL clones with known sequences

626	RSA-15-H15_#0	MMA00336, Mm_cluster20036.0.1	EST193453	Other_EST		Normalized rat ovary_Bento Soares Rattus sp. cDNA clone ROVAJ49 3' end. mRNA sequence.			
628	RSA-19-L04	MMA60070, Mm_cluster20069.0.1	Mm_cluster84802	Mouse_EST		uh21c01.r1 Barstead mouse testis MPLRB11 Mus musculus cDNA clone 1746048 5'. mRNA sequence.			
631	RSA-13-K01	MMAA74405, Mm_cluster20699.0.1	Mm_cluster23479	Mouse_EST		mq11g11.r1 Barstead MPLRB1 Mus musculus cDNA clone 578468 5'.			
633	RSA-21-A02	MM1294504, Mm_cluster20710.0.2	Mm_cluster11079	Mouse_EST		vr83q09.s1 Knowles Solter mouse 2 cell Mus musculus cDNA clone 1135360 5' similar to WP:F53B1.2 CE04642 ;			
634	RSA-21-O19_#0	MMAA18436, Mm_cluster20749.0.1	Mm_cluster51297	Mouse_EST		ml34a12.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 513886 5' similar to SW:TRY2_SALSA P35032 TRY2			
643	RSA-15-J17	MMAA3682, Mm_cluster21319.0.1	Mm_cluster21228	Mouse_EST					
645	RSA-18-K19	MMAA22303, Mm_cluster22271.0.1	Mm_cluster28224	Mouse_EST					
646	RSA-10-D10, RSA-10-H10	MMAA61298, MMAA8974, Mm_cluster22227	HS1202587	Other_EST		mj79a11.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 482300 5'similar to WP:C32D5.9 CE01849 ;			
648	RSA-11-P17, RSA-30-N13	MMA66221, Mm_cluster22431.0.1	Mm_cluster08845	Mouse_EST		zt62g11.r1 Soares testis NHT Homo sapiens cDNA clone 726980 5'similar to SW:ACT_PINCO P24902 ACTIN ;			
649	RSA-29-H22, RSA-4-G23	MMAA20732, Mm_cluster22634.0.1	Mm_cluster29912	Mouse_EST		mw16e10.r1 Soares mouse 3NME12 5 Mus musculus cDNA clone 670890 5'similar to SW:BAT2_HUMAN P48634 LARGE PRO			
655	RSA-4-N15, RSA-8-P04	MMA65460, Mm_cluster23177.0.4, Mm	Mm_cluster78902	Mouse_EST		vx96b05.r1 Stratagene mouse macrophage (#937306) Mus musculus cDNA clone 1293777 5' similar to TR:O35259 O35259 PU			
656	RSA-7-K03, RSA-9-J13_0#	MMA65475, Mm_cluster23178.0.2	MMAA83826	Mouse_EST		mt24f02.r1 Soares mouse 3NbMS Mus musculus cDNA clone 622011 5'.			
657	RSA-3-N16	MMAA16249, Mm_cluster23479.0.1	Mm_cluster17174	Mouse_EST		vu20b07.r1 Barstead mouse myotubes MPLRB5 Mus musculus cDNA clone 1181173 5'.			
660	RSA-17-G06_#0, RSA-31-E	MMAA7258, Mm_cluster23832.0.1	Mm_cluster06506	Mouse_EST		mr76b02.r1 Stratagene mouse heart (#937316) Mus musculus cDNA clone 603339 5'.			
665	RSA-4-L18, RSA-8-E05, RS	MMAA93093, Mm_cluster24381.0.1	Mm_cluster16028	Mouse_EST		ml42f02.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 514683 5'.			
666	RSA-2-G21	AA981455, Mm_cluster26401.0.1	Mm_cluster27109	Mouse_EST		uf01a06.x1 Sugano mouse embryo mewa Mus musculus cDNA clone 14993143', mRNA sequence.			
669	RSA-4-C02_#0	MMA23477, Mm_cluster26988.0.1	Mm_cluster64087	Mouse_EST		mo97f07.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 567685 5'.			
670	RSA-30-N14	AI119718, Mm_cluster27109.0.1	HS1187176	Other_EST		zs90e09.r1 NCI_CGAP_GCB1 Homo sapiens cDNA clone IMAGE:704776 5'similar to WP:B0495.5 CE01763 ;			
673	RSA-18-E21	AA986802, Mm_cluster28775.0.1	Mm_cluster24250	Mouse_EST		mn48g09.r1 Beddington mouse embryonic region Mus musculus cDNA clone 541216 5'.			
675	RSA-19-K03	AA546485, Mm_cluster29791.0.1	Mm_cluster16088	Mouse_EST		mr04f06.r1 Soares mouse 3NbMS Mus musculus cDNA clone 596483 5'.			
676	RSA-30-F02	AA547169, Mm_cluster29912.0.1	Mm_cluster23832	Mouse_EST		ml58h03.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 516245 5'.			
678	RSA-18-I21	Mm_cluster33762.0.1	Mm_cluster47943	Mouse_EST		mc02g08.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 3377905'.			
682	RSA-18-F09	MM1161483, Mm_cluster36421.0.1	Mm_cluster14646	Mouse_EST		mj84b12.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 4827835'.			
683	7-C09_#0	Mm_cluster39875.0.1	Hs_cluster35254	Other_EST		aj31g01.s1 Soares testis NHT Homo sapiens cDNA clone 1391952 3'similar to gb:X62167_cds1 MYELIN P2 PROTEIN (HUMAN			
685	RSA-22-O01_1	MM1294333, Mm_cluster42566.0.1	Mm_cluster16864	Mouse_EST		USF1_MOUSE (252)			
690	RSA-16-I17_#0, RSA-6-J12	MM1294558, Mm_cluster42622.0.1	Hs_cluster22827	Other_EST		zr80f07.s1 Soares testis NHT Homo sapiens cDNA clone 728677 3'similar to TR:G1195552 G1195552 PHOSPHOINOSITIDE-S			
693	RSA-22-M21	MM1296100, Mm_cluster42941.0.1	Mm_cluster14404	Mouse_EST		mx19g01.r1 Soares mouse NML Mus musculus cDNA clone 680688 5'.			
694	RSA-27-J10	MMAA92667, Mm_cluster47348.0.1	Mm_cluster12853	Mouse_EST					
697	RSA-5-D12	MM79610, Mm_cluster47943.0.1	Mm_cluster60490	Mouse_EST		ml59d01.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 516289 5'.			
702	RSA-18-D23	MMA31116, Mm_cluster50354.0.1	Mm_cluster42575	Mouse_EST		vi74b04.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 917935 5'.			
705	11-G20	Mm_cluster51209.0.1	Mm_cluster69373	Mouse_EST		vn08h06.r1 Stratagene mouse Tcell 937311 Mus musculus cDNA clone 1020635 5'.			
709	RSA-8-M19	MMA61775, Mm_cluster51297.0.1	Hs_cluster42349	Other_EST		ai18e05.s1 Soares testis_NHT Homo sapiens cDNA clone 1343168 3'. mRNA sequence.			
710	RSA-20-F20	MMA66018, Mm_cluster51812.0.1	Mm_cluster10958	Mouse_EST		vi74b01.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 917929 5'.			
711	RSA-14-A19	MMAA10447, Mm_cluster52803.0.1	Mm_cluster13386	Mouse_EST		mr68b01.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 602569 5'.			
713	RSA-21-E20, RSA-8-B20	MMAA7224, Mm_cluster57206.0.1, Mm	Mm_cluster13427	Mouse_EST		ml32q10.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 513762 5'.			
717	RSA-15-G03	MMAA40328, Mm_cluster57614.0.1	Mm_cluster13385	Mouse_EST		ml45h03.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 514997 5' similar to TR:G499340 G499340 17BETA-			
721	RSA-4-C23	MMAA44900, Mm_cluster58367.0.1	Mm_cluster26988	Mouse_EST		mh74g07.r1 Soares mouse placenta 4NbMP13.5 14.5 Mus musculus cDNA clone 456732 5' similar to SW:YJJ7_YEAST P40857			
725	RSA-3-F08, RSA-30-O23	MMAA83435, Mm_cluster64087.0.1	Mm_cluster58367	Mouse_EST		mr70h04.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 602839 5'.			
727	RSA-2-L21	MMAA83475, Mm_cluster64097.0.1	Mm_cluster14543	Mouse_EST		mq23h05.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 424665 5' similar to WP:C35D10.10 CE01191 ;			
729	RSA-28-E20, RSA-4-B05,	MMAA8973, Mm_cluster65316.0.1	Mm_cluster10937	Mouse_EST		vi72b12.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 917759 5'.			
732	RSA-1-N03, 10-A19_#0	MTEST640, Mm_cluster68705.0.1	Mm_cluster09242	Mouse_EST		mq90f11.r1 Stratagene mouse heart (#937316) Mus musculus cDNA clone 586029 5'.			
736	RSA-14-E02	AA560417, Mm_cluster70346.0.1	Mm_cluster42937	Mouse_EST		vi74b09.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 917945 5' similar to TR:G6910 G6910 PROTEIN 1. [1			
738	RSA-3-D03	AA683822, Mm_cluster71322.0.1	Mm_cluster13849	Mouse_EST					
739	RSA-3-K03_@1	Mm_cluster74343.0.1	Mm_cluster22741	Mouse_EST		mp79b05.r1 Soares 2NbMT Mus musculus cDNA clone 575409 5'.			
744	RSA-22-N17	AA833486, Mm_cluster77934.0.1	Mm_cluster07445	Mouse_EST		vn97b02.r1 Stratagene mouse skin (#937313) Mus musculus cDNA clone 1039851 5'.			
745	RSA-24-H19	AA839857, Mm_cluster78124.0.1	Mm_cluster21966	Mouse_EST		mi19d04.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 4639755'.			
746	RSA-30-J02	AA867286, Mm_cluster78902.0.1	Mm_cluster06434	Mouse_EST					
749	RSA-12-K09, RSA-21-G24	AI036199, Mm_cluster84692.0.1	Hs_cluster13267	Other_EST		ov44h08.x1 Soares testis_NHT Homo sapiens cDNA clone IMAGE:16402233' similar to SW:PEX5_HUMAN P50542 PEROXISO			
750	RSA-7-B03	AI036784, Mm_cluster84802.0.1	EST96709	Other_EST		Testis I Homo sapiens cDNA 5' end.			
756	RSA-29-L22	Mm_cluster86856.0.1	Mm_cluster74343	Mouse_EST					
758	RSA-2-N10	Mm_cluster91543.0.1	Mm_cluster65776	Mouse_EST		vi76a04.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 918126 5'.			
764	RSA-14-P03	AI180752, Mm_cluster92665.0.1	Mm_cluster16245	Mouse_EST		mi69g08.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 468830 5'.			
765	RSA-20-O07	AA607895, Mm_cluster93317.0.1	Mm_cluster23177	Mouse_EST		ml51d10.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 515539 5' similar to TR:G511639 G511639 C219-RE			
768	RSA-17-N10	AU017407, Mm_cluster93823.0.1	Mm_cluster85000	Mouse_EST		uh24e01.r1 Barstead mouse testis MPLRB11 Mus musculus cDNA clone 1746360 5'. mRNA sequence.			
	RSA-2-P01, RSA-2-P04, R	S83465, Mm_cluster03383.0.1	: HSU58996	Homologous_Other_Genes		Homo sapiens testis calpastatin mRNA, complete cds. R.norvegicus mRNA for Pristanovi-CoA Oxidase			

10.2. Appendix 2. Table of numerical data of clustered expression profiles

Appendix 2 contains the numerical data of expression profiles. The order is the same as on Fig. 15, p. 51. Each value represents the natural logarithm of normalised hybridisation intensity. Each value of this table is coded in colour on colour presentation on Fig. 15, p. 51. Combined numerical/colour presentation may be found in webside:

<http://www.dkfz.de/tbi/people/beissbarth/private/crem-project/>

Designations in Appendix 2:

Day XX- the age of mice from which mRNA for high density filter hybridisation was isolated

AdMut - adult CREM knockout

AdWt - adult wild type

Order - the number of the row in cluster

Position - the position of the clone on high density filter grid.

Clone - clone name, meaning the position of clone in the cremSL library. For instance, 7-E07 clone may be found in plate number 7 in the well located in row E, in column 7.

RSA fragment - the clone name of best sequence cluster representatives.

Sequence - accession number of highly homologous sequence from public data bases.

Database Info - the name of gene/clone in public database

Functional Categorie/Subcategorie - function of protein.

Title - the name of protein/clone retrieved from public databases.

Table of numerical data of clustered expression profiles

Index	Positio	Clone	RSA-Fragmet	Genename	Type	Day9	Day17	Day19	Day21	Day23	Day25	Day27	AdMut	AdWt	#/Non	Categorie	Subcategory	Title
781	1M10	28-M2	RSA-28-A01	EB2	Mouse Gene	11.35	5.59	5.571	7.566	7.41	16.47	15.86	7.353	11.19	d	Viral Protein		Mus musculus APC-binding protein EB2 mRNA, partial cds.
1468	3F19	32-E12	RSA-32-E12	ENV1 MOUSE	Mouse Gene	1.874	1.285	0.601	0.956	2.538	-0.18	4.025	6.024	4.48	d	Viral Protein		Mouse (strain 129 G-IX+) endogenous murine leukemia virus mRNA, clone E1.
282	2I04	18-M09	RSA-18-M09	RL28 MOUSE	Mouse Gene	4.812	17.11	11.93	11.29	21.84	64.81	72.23	6.573	47.65	d	Translation	Ribosomal Protein	M.musculus L28 mRNA for ribosomal protein L28
1143	3E15	30-F11	RSA-30-F11	RS17_CRIGR	Mouse Gene	3.147	0.081	0.729	0.306	0	1.655	-0.1	1.588	5.056	d	Translation	Ribosomal Protein	Mouse rpS17 mRNA for ribosomal protein S17, complete cds.
1407	2J18	20-N17	RSA-20-N17	Mlark	Mouse Gene	5.461	5.966	7.882	5.697	11.02	32.44	27.51	6.031	18.87	d	Transcription Factor		Mus musculus Mlark mRNA, complete cds.
886	1B12	9-I04	RSA-9-I04	RTR	Mouse Gene	0.779	0.046	0.539	1.391	2.421	3.56	2.147	1.289	13.7	d	Transcription Factor	Orphan Receptor	Mus musculus orphan receptor RTR mRNA, complete cds.
102	2E02	10-B02	RSA-10-B02	ZNF76_HUMAN	Homologous	4.148	9.31	12.4	8.343	13.3	20.02	17.22	10.053	13.39	d	Transcription Factor		Human zinc-finger protein (ZNF76) gene, partial cds.
1297	2D17	16-O23	RSA-16-O23	Rnf4	Mouse Gene	3.28	4.291	2.737	3.023	4.117	8.459	7.775	1.981	9.745	d	Transcription Factor		Mus musculus Rnf4 mRNA, partial cds.
1238	3H16	12-O17	RSA-12-O17	RTR	Mouse Gene	5.184	8.09	7.649	7.441	10.39	20.15	12.84	0.103	9.433	d	Transcription Factor	Orphan Receptor	Mus musculus orphan receptor RTR mRNA, complete cds.
816	1D11	8-K11	RSA-8-K11	Tctex-3	Mouse Gene	0.141	0.494	1.476	0.953	0.29	1.658	0.721	2.873	9.119	d	Transcription Factor		Mus musculus Tctex-3 mRNA, complete cds.
1201	1A16	16-F01	RSA-16-F01	SOX5_MOUSE	Mouse Gene	2.512	2.348	3.327	1.333	3.196	4.965	0.971	-0.25	6.939	d	Transcription Factor		M.musculus (testis) Sox-5 mRNA
1781	1E23	16-E15	RSA-16-E15	CNBP_MOUSE	Mouse Gene	1.693	-0.11	0	0.379	0.621	3.041	4.655	-0.53	4.943	d	Transcription Factor	Sterol Regulatory Element Binding Protein	Mus sp. nucleic acid binding protein mRNA, complete cds.
1233	3G16	10-I09	RSA-10-I09	SOX6_MOUSE	Mouse Gene	1.581	0.956	1.469	0.021	0.696	4.686	3.596	0.686	4.017	d	Transcription Factor		Mouse mRNA for SOX-LZ, complete cds.
1626	1F21	10-E09	RSA-10-E09	T2FA_HUMAN	Homologous	1.131	4.394	2.165	3.983	4.097	4.335	12.49	2.183	6.946	d	Transcription	Transcription Initiator	H.sapiens mRNA for RAP74
1643	3I21	17-I17	RSA-17-I17	AF082556	Homologous	4.678	6.237	7.11	7.049	19.88	21.9	15.21	1.5	6.175	d	Telomere Length Maintenance		Homo sapiens TRF1-interacting ankyrin-related ADP-ribose polymerase mRNA, complete cds.
1713	3G22	11-H17	RSA-11-H17	ADAM4	Mouse Gene	0.432	2.354	2.208	1.778	2.773	5.353	4.647	1.23	4.903	d	Sperm-Egg Fusion		Mus musculus ADAM 4 protein precursor (ADAM 4) mRNA, partial cds.
651	1C09	4-E18	RSA-4-E18	ADAM4	Mouse Gene	0.279	0.508	1.226	1.114	2.461	3.57	1.813	0.623	4.149	d	Sperm-Egg Fusion		Mus musculus ADAM 4 protein precursor (ADAM 4) mRNA, partial cds.
617	2L08	22-P02	RSA-22-P02	STP1_MOUSE	Mouse Gene	6.171	4.927	2.347	0.579	1.329	3.543	36.11	5.575	252.5	d	Sperm Structure	Chromosome Comp	Mouse mRNA for transition protein 1 TP1
1457	2D19	3-K01	RSA-3-K01	Odf2	Mouse Gene	3.688	10.31	7.98	22.34	43.96	108.1	91.18	7.473	81.07	d	Sperm Structure	Outer Dense Fiber	Mus musculus outer dense fiber protein (Odf2) mRNA, complete cds.
688	3J09	2-P22	RSA-2-P22	ODFP_MOUSE	Mouse Gene	2.817	0.995	1.387	9.238	23	94.19	110.6	7.237	73.05	d	Sperm Structure	Outer Dense Fiber	M.musculus Odf1 mRNA for outer dense fiber protein of sperm tails
301	1M04	6-M17	RSA-32-I09	HSP2_MOUSE	Mouse Gene	8.42	6.182	3.754	13.27	2.726	94.16	22.62	2.476	72.28	d	Sperm Structure	Chromosome Comp	Mouse protamine 2 (mP2) mRNA.
837	2H11	18-C11	RSA-18-C11	FSC1_MOUSE	Mouse Gene	1.751	2.601	4.46	3.51	14.39	25.71	24.47	2.884	50	d	Sperm Structure	Fibrous Sheath	Mus musculus major fibrous sheath protein mRNA, complete cds.
1846	1B24	3-E08	RSA-3-E08	FSC1_MOUSE	Mouse Gene	3.803	0	0.296	10.46	9.28	65.73	28.5	-0.23	45.61	d	Sperm Structure	Fibrous Sheath	Mus musculus major fibrous sheath protein mRNA, complete cds.
811	1C11	6-C14	RSA-6-C14	STP2_MOUSE	Mouse Gene	1.353	1.357	2.039	0	0.917	24.03	12.95	1.34	44.55	d	Sperm Structure	Chromosome Comp	Mouse, transition protein 2 (TP2) mRNA, complete cds.
142	2M02	8-P20	RSA-8-P20	FSC1_MOUSE	Mouse Gene	28.82	36.87	28.83	18.9	18.76	15.84	22.2	10.7	38.71	d	Sperm Structure	Fibrous Sheath	Mus musculus major fibrous sheath protein mRNA, complete cds.
1413	3K18	3-D20	RSA-10-H16	CALI_HUMAN	Homologous	1.642	3.829	2.541	3.325	13.16	42.96	32.88	0	29.85	d	Sperm Structure	Calyx	H.sapiens mRNA for calicin (partial).
1232	2G16	3-E24	RSA-3-E24	CALI_HUMAN	Homologous	11.56	5.418	3.857	5.745	12.73	29.18	25.42	7.703	22.26	d	Sperm Structure	Calyx	H.sapiens mRNA for calicin (partial).
133	3K02	5-M23	RSA-5-M23	ODFP_MOUSE	Mouse Gene	2.347	0.682	1.707	1.019	11.28	41.12	28.49	5.8	21.52	d	Sperm Structure	Outer Dense Fiber	M.musculus Odf1 mRNA for outer dense fiber protein of sperm tails
891	1C12	14-I24	RSA-14-I24	HSP2_MOUSE	Mouse Gene	0.859	0.237	3.539	0.455	4.01	11.1	8.448	1.812	16.49	d	Sperm Structure	Chromosome Comp	Mouse protamine 2 (mP2) mRNA.
1393	3G18	10-J12	RSA-10-J12	ODFP_MOUSE	Mouse Gene	0.508	0.537	0.521	1.257	4.667	8.668	4.058	1.317	15.59	d	Sperm Structure	Outer Dense Fiber	M.musculus Odf1 mRNA for outer dense fiber protein of sperm tails
1002	2I13	19-D22	RSA-19-D22	FSC1_MOUSE	Mouse Gene	2.716	2.294	2.394	1.38	8.905	12.77	8.142	3.044	10.88	d	Sperm Structure	Fibrous Sheath	Mus musculus major fibrous sheath protein mRNA, complete cds.
173	3C03	26-L02	RSA-26-L02	ODFP_MOUSE	Mouse Gene	0.759	0.85	-0.09	-0.5	2.271	7.534	7.846	-0.02	10.71	d	Sperm Structure	Outer Dense Fiber	M.musculus Odf1 mRNA for outer dense fiber protein of sperm tails
128	3J02	17-K23	RSA-17-K23	FSC1_MOUSE	Mouse Gene	1.946	0.608	0.817	1.462	1.27	16.05	1.321	1.831	8.331	d	Sperm Structure	Fibrous Sheath	Mus musculus major fibrous sheath protein mRNA, complete cds.
491	1C07	16-C16	RSA-16-C16	MMDDC8	Mouse Gene	6.183	0.901	1.979	1.182	3.691	19.02	10.3	1.545	6.115	d	Sperm Structure		M.musculus mRNA for testis-specific protein, DDC8
416	1D06	2-K20	RSA-2-K20	CALI_HUMAN	Homologous	-0.02	1.683	0.574	-0.1	0.089	-1	3.257	1.135	4.937	d	Sperm Structure	Calyx	H.sapiens mRNA for calicin (partial).
1216	1D16	3-E24	RSA-3-E24	CALI_HUMAN	Homologous	2.299	1.794	1.255	0.714	1.876	10.39	3.156	-0.19	4.215	d	Sperm Structure	Calyx	H.sapiens mRNA for calicin (partial).
428	3F06	30-N24	RSA-30-N24	MM2059	Mouse Gene	0.775	0.117	0.785	0	2.032	2.449	1.444	-0.03	4.047	d	Sperm Structure	Sperm-Egg Fusion	Mus musculus ADAM 5 protein precursor (ADAM 5) mRNA, complete cds.
47	2J01	19-O04	RSA-19-O04	SNAP	Homologous	2.252	13.78	10.31	17.67	26.36	26.02	28.24	10.05	33.33	d	Signal Transduction	Synaps Element	Homo sapiens alpha SNAP mRNA, complete cds. (Homolog SNAP_MOUSE (231))
403	3A06	23-P16	RSA-23-P16	POR1_MOUSE	Mouse Gene	1.986	1.302	0.175	1.037	0	0.1539	1.627	-0.16	18.55	d	Signal Transduction	Voltage-Dependent	Mus musculus voltage dependent anion channel 1 mRNA, nuclear gene encoding mitochondrial protein, complete cds.
163	3A03	23-O23	RSA-23-O23	NTTA_MOUSE	Mouse Gene	1.616	0.423	1.571	-0.42	3.304	6.961	4.07	-0.44	10.31	d	Signal Transduction	Neurotransmitter Syn	Mus musculus retinal taurine transporter (mTAUT) mRNA, complete cds.
1066	1D14	15-N18	RSA-15-N18	CCCB_HUMAN	Homologous	1.656	1.275	2.955	1.397	6.329	5.014	10.11	2.661	9.952	d	Signal Transduction	Voltage-Dependent	Human neuronal DHP-sensitive, voltage-dependent, calcium channelbeta-2 subunit mRNA, complete cds.
17	2D01	8-P11	RSA-8-P11	AF048976_AF04897	Homologous	0.829	1.977	3.267	4.686	5.938	17.13	15.36	-0.09	11.78	d	Signal Transduction	Ras_GTPase_Activa	Rattus norvegicus synaptic ras GTPase-activating protein p135SynGAP mRNA, complete cds. Rattus norvegicus synaptic ras G
971	1C13	17-I14	RSA-17-I14	ANX2_MOUSE	Mouse Gene	0.459	0.811	0.089	2.146	1.14	10.46	13.54	1.534	48.83	d	Signal Transduction		Mouse mRNA for protein-tyrosine kinase substrate p36 (catpactin heavy chain), complete cds.
1023	3M13	28-I06	RSA-28-I06	KPCD_MOUSE	Mouse Gene	3.287	3.368	1.27	5.992	5.954	47.73	62	0.185	34.25	d	Signal Transduction	PKC	Mouse protein kinase C delta mRNA, complete cds.
1173	3K02	5-K07	RSA-5-K07	AKAP110_MOUSE	Mouse Gene	3.285	7.517	4.656	8.286	22.15	44.85	31.18	4.888	32.33	d	Signal Transduction	PKA Pathway	Mus musculus protein kinase A binding protein AKAP110 mRNA, complete cds.
881	1A12	8-J21	RSA-8-J21	KAP2_MOUSE	Mouse Gene	0.811	2.365	6.811	3.658	7.111	15.33	13.21	3.885	29.49	d	Signal Transduction	PKA Pathway	Mouse cAMP-dependent protein kinase type II regulatory subunit mRNA, 3' end.
1702	2E22	15-M23	RSA-15-M23	MMMP40GPRT	Mouse Gene	4.149	4.465	5.508	3.051	5.33	25.31	21.64	2.946	26.66	d	Signal Transduction	Receptor	Mus musculus mRNA for G protein-coupled receptor, P40GPRT
801	1A11	2-M19	RSA-2-M19	D-AKAP1	Mouse Gene	0.603	0.657	0.84	1.522	6.425	13.76	9.44	0.164	24.94	d	Signal Transduction	PKA Pathway	Mus musculus dual specificity A-kinase anchoring protein 1(D-AKAP1) mRNA, partial cds.
1051	1C14	1-H06	RSA-1-H06	CHIO_HUMAN	Homologous	0.504	0	0.051	0.852	5.877	9.94	11.75	2.576	23.69	d	Signal Transduction	GTPase_Activator	Homo sapiens beta2-chimerin mRNA, complete cds.
1338	3L17	18-D21	RSA-19-I12	KAP2_MOUSE	Mouse Gene	5.144	0.267	4.44	4.496	11.84	43.97	28.46	1.667	23.01	d	Signal Transduction	PKA Pathway	Mouse cAMP-dependent protein kinase type II regulatory subunit mRNA, 3' end.
1681	1A22	6-P16	RSA-6-P16	FRT1_MOUSE	Mouse Gene	1.546	0	3.515	9.486	15.49	21.88	11.4	2.003	13.15	d	Signal Transduction		Mus musculus proto-oncogene (Frat 1) mRNA, complete cds.
1766	1B23	2-E07	RSA-2-E07	D-AKAP1	Mouse Gene	5.077	3.122	0.211	-0.69	1.688	4.549	9.53	0.763	12.43	d	Signal Transduction	PKA Pathway	Mus musculus dual specificity A-kinase anchoring protein 1(D-AKAP1) mRNA, partial cds.
1861	1E24	4-A14	RSA-4-A14	CHIO_HUMAN	Homologous	0.403	0.224	-0.33	1.278	0.769	9.196	12.89	0.444	12.3	d	Signal Transduction	GTPase_Activator	Homo sapiens beta2-chimerin mRNA, complete cds.
1011	1K13	7-J03	RSA-7-J03	AKAP110_MOUSE	Mouse Gene	2.371	0.07	0.15	0	0.633	3.205	3.955	0	12.17	d	Signal Transduction	PKA Pathway	Mus musculus protein kinase A binding protein AKAP110 mRNA, complete cds.
1161	1K15	1-K15	RSA-1-K15	AF077658	Mouse Gene	1.483	2.911	1.924	3.659	4.312	6.139	5.634	0.242	12.17	d	Signal Transduction	Co-Repressor_for_H	Mus musculus homeodomain-interacting protein kinase 1 mRNA, complete cds.
262	2E04	3-J07	RSA-3-J07	CHIO_HUMAN	Homologous	1.101	2.264	-0.2	4.599	5.675	17.61	12.98	0.446	11.83	d	Signal Transduction	GTPase_Activator	Homo sapiens beta2-chimerin mRNA, complete cds.
86	1B02	2-D15	RSA-2-D15	KAP2_MOUSE	Mouse Gene	1.33	0.537	2.598	1.637	1.945	7.41	5.703	1.049	11.78	d	Signal Transduction	PKA Pathway	Mouse cAMP-dependent protein kinase type II regulatory subunit mRNA, 3' end.
22	2E01	4-L13	RSA-4-L13	KC12_HUMAN	Homologous	2.519	2.545	5.361	10.67	11.62	23.82	24.05	0.391	11.41	d	Signal Transduction	Protein Kinase	Homo sapiens casein kinase gamma 2 primary transcript, complete cds.
1857	2D24	1-B16	RSA-1-B16	KC12_HUMAN														

Table of numerical data of clustered expression profiles

281	1104	6-N12	RSA-6-N12	MMUNKNM	Mouse Gene	1.347	0	0.055	4.229	5.692	5.911	10.04	0	7.692	d	?		Mouse (clone BALB10N) mRNA, complete cds of unknown function. (Homolog SMY_MOUSE (367))
1553	3G20	11-F13	RSA-11-F13	AF1q	Mouse Gene	1.236	0.385	0	2.461	5.71	6.55	5.332	1.074	6.104	d	?		Mouse mRNA for AF1q, complete cds.
1153	3G15	10-H14	RSA-10-H14	SKD3 MOUSE	Mouse Gene	2.424	1.881	0.887	-0.01	3.642	6.5	6.092	1.610	5.116	d	?		Mus musculus SKD3 mRNA, complete cds.
366	1J05	13-O14	RSA-13-O14	HSKIAA09	Homologous	1.655	1.646	1.189	0.429	0.021	0.958	2.873	0.134	4.614	d	?		Human mRNA for KIAA0169 gene, partial cds.
111	1G02	10-H02	RSA-10-H02	Y188 HUMAN	Homologous	1.137	0	0	1.317	0.193	3.884	0.015	-0.29	4.291	d	?		Human mRNA for KIAA0188 gene, partial cds.
1526	1B20	8-B17	RSA-8-B17	DPY3 MOUSE	Mouse Gene	2.364	0.638	-0.12	1.579	8.312	1.886	3.258	-0.64	3.434	d	?		M.musculus mRNA for Ulip protein
502	2E07	2-H02	RSA-2-H02	Mm_cluster23178		2.903	4.349	2.7	7.294	23.71	46.26	42.8	1.722	62.15	d			m151h10.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 515587 5' similar to SW:H2A_STRPU P02271 HISTO
1408	3J18	4-N15	RSA-4-N15	Mm_cluster23177		2.895	3.929	2.056	9.036	23.38	112	78.17	3.657	54.24	d			m151d10.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 515539 5' similar to TR:G511639 G511639 C219-RE
922	2I12	19-B16	RSA-19-B16	Mm_cluster24369		2.063	5.96	5.463	4.233	15.05	46.36	39.06	3.627	49.56	d			m142b01.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 514633 5' similar to TR:G639691 G639691 XENOPU
347	2F05	7-K03	RSA-7-K03	Mm_cluster23178		0.602	2.245	1.01	1.536	4.499	17.62	28.25	1.666	48.27	d			m151h10.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 515587 5' similar to SW:H2A_STRPU P02271 HISTO
1302	2E17	13-K01	RSA-13-K01	Mm_cluster20699		1.166	2.055	3.511	1.54	6.067	20.52	21.06	2.783	47.32	d			mo97c10.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 567666 5' similar to TR:G47706 G47706 NOVEL E
821	1E11	3-N03	RSA-3-N03	Mm_cluster24369		0.329	0.313	1.65	0.72	2.935	7.66	8.516	1.522	43.85	d			mo97c10.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 514633 5' similar to TR:G639691 G639691 XENOPU
1472	2G19	9-O24	RSA-9-O24	Hs_cluster23754		3.114	5.279	2.884	5.186	16.167	68	47.46	4.569	38.88	d			zv54f10.s1 Soares testis NHT Homo sapiens cDNA clone 757483 3'similar to TR:G603907 G603907 TRYPSINOGEN PRECURS
1337	2L17	23-H06	RSA-23-H06	Mm_cluster42528		12.05	5.36	5.122	11.21	27.04	46.66	51.99	10.29	37.57	d			vi72b06.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 917747 5' similar to TR:G7550 G7550 ACTIN. [1]
1903	3M24	30-C24	RSA-30-C24	Mm_cluster28224		3.227	11.86	7.822	2.844	8.331	57.49	34.12	1.083	33.25	d			m179a11.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 482300 5'similar to WP:C32D5.9 CE01849
1782	2E23	8-P04	RSA-8-P04	Mm_cluster23177		2.609	6.384	5.069	6.075	17.48	42.85	31.05	1.895	26.87	d			m151d10.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 515539 5' similar to TR:G511639 G511639 C219-RE
827	2F11	4-C02	RSA-4-C02	#Mm_cluster26988		10.69	9.106	5.687	12.61	11.05	19.85	31.99	8.847	25.29	d			mh74g07.r1 Soares mouse placenta 4Nbmp13.5 14.5 Mus musculus cDNA clone 456732 5' similar to SW:YJ77 YEAST P40857
1382	2E18	3-G03	RSA-3-G03	Mm_cluster10943		2.836	3.231	5.292	4.002	8.356	40.93	29.41	3.012	23.85	d			m134a05.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 513872 5' similar to gb:K63368 cds1 DNAJ PROTEIN
752	2G10	3-G24	RSA-3-G24	#Hs_cluster37608		1.857	4.584	1.76	5.63	11.67	20.75	27.59	1.959	19.78	d			zu18d02.r1 Soares NHHMP S1 Homo sapiens cDNA clone 738339 5'similar to WP:C5402.5 CE02562 SKELETAL MUSCLE CA
757	2H10	18-B10	RSA-18-B10	HS1202587		2.106	5.772	3.441	4.011	3.569	12.99	14.29	2.457	18.69	d			z162g11.r1 Soares testis NHT Homo sapiens cDNA clone 726980 5'similar to SW:ACT_PINCO P24902 ACTIN
1481	1I19	6-G12	RSA-6-G12	Mm_cluster13385		1.463	0.362	-0.12	1.258	4.256	9.948	9.038	5.149	17.38	d			m145h03.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 514997 5' similar to TR:G499340 G499340 17BETA-A
702	2M09	18-N15	RSA-5-C09	Mm_cluster64114		5.71	3.596	2.446	3.833	9.193	16.47	11.43	4.094	16.49	d			mo97c08.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 567662 5' similar to SW:ACT_SCHPO P10989 ACTIN
1171	1K15	2-P11	RSA-2-P11	#Hs_cluster37608		0.601	0.291	-0.11	0.442	2.161	2.895	0.948	2.888	16.23	d			zu18d02.r1 Soares NHHMP S1 Homo sapiens cDNA clone 738339 5'similar to WP:C5402.5 CE02562 SKELETAL MUSCLE CA
742	2E10	6-I19	RSA-6-I19	#Mm_cluster23636		1.818	2.784	1.765	2.581	3.462	14.77	16.88	3.465	16.03	d			m148h03.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 515285 5' similar to TR:V26A5.9 CE00788
542	2M07	9-I5	RSA-9-I5	Mm_cluster10966		5.729	4.687	8.457	8.423	8.238	23.29	15.92	2.998	14.73	d			vi69g07.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 917532 5' similar to SW:KELC_DROME Q04652 RING
1867	2F24	9-P09	RSA-9-P09	Mm_cluster23178		5.285	8.166	4.846	8.799	17.21	37.37	29.3	3.978	13.4	d			m151h10.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 515587 5' similar to SW:H2A_STRPU P02271 HISTO
1403	3I18	16-I17	RSA-16-I17	#Mm_cluster42622		0.905	3.966	3.235	6.412	12.58	36.11	27.46	0.339	13.22	d			vi75h06.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 918107 5' similar to SW:GTT1_CHICK P20135 GLUTA
1091	1K14	5-C05	RSA-7-K03	Mm_cluster23178		0.272	1.045	-0.01	0.76	1.81	6.538	5.429	0	13.06	d			m151h10.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 515587 5' similar to SW:H2A_STRPU P02271 HISTO
1821	1M23	8-M19	RSA-8-M19	Mm_cluster51297		0.242	2.578	5.445	0.569	2.666	3.636	12.77	1.3	12.4	d			m134a12.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 513886 5' similar to SW:TRY2_SALSA P35032 TRYF
861	1M11	5-C09	RSA-5-C09	Mm_cluster64114		3.946	2.535	1.741	0.828	2.144	16.87	12.9	1.217	12.22	d			mo97c08.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 567662 5' similar to SW:ACT_SCHPO P10989 ACTIN
1142	2E15	15-P17	RSA-15-P17	Mm_cluster18355		7.15	7.713	5.308	5.563	2.739	19.74	17.28	9.303	12.11	d			m156g02.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 516050 5' similar to TR:G243898 G243898 GOR-AN
607	2J08	20-F20	RSA-20-F20	Mm_cluster51812		4.137	3.221	1.593	2.74	6.349	20.69	11.94	2.421	11.07	d			m152g10.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 515682 5' similar to WP:K02B2.3 CE04689
528	3J07	2-I19	RSA-2-I19	Mm_cluster10943		1.296	0	0.396	0.075	4.805	10.98	10.58	-0.11	10.99	d			m134a05.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 513872 5' similar to gb:K63368 cds1 DNAJ PROTEIN
213	3K03	6-C10	RSA-6-C10	Mm_cluster58338		0.846	1.101	0	2.965	8.225	11.59	7.412	1.605	10.49	d			mr7303.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 603101 5' similar to SW:OXYB_HUMAN P22059 OXY
426	1F06	6-J12	RSA-6-J12	Mm_cluster42622		0.013	0	0	0.973	3.94	3.961	5.25	1.238	9.977	d			vi75h06.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 918107 5' similar to SW:GTT1_CHICK P20135 GLUTA
1081	1I14	9-K21	RSA-9-K21	Mm_cluster13385		0.275	0.519	0.12	0.198	3.919	17.97	12.78	0	9.903	d			m145h03.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 514997 5' similar to TR:G499340 G499340 17BETA-A
1583	3M20	29-N17	RSA-29-N17	Mm_cluster24543		4.225	5.839	5.748	7.556	16.35	34.58	18.68	2.82	9.616	d			vi71h04.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 917719 5' similar to SW:ACT1_ABSGL P10982 ACTIN
1462	2E19	4-D08	RSA-4-D08	#Mm_cluster14541		2.589	3.649	0.267	4.199	10.75	9.1	9.624	2.164	7.87	d			mq23h05.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 424665 5' similar to TR:C35D10.1 CE01191
511	1K18	11-P01	RSA-11-P01	#Hs_cluster22827		-0.08	0.831	0.292	0.439	2.533	1.78	2.442	0	7.727	d			z18f007.s1 Soares testis NHT Homo sapiens cDNA clone 728677 3'similar to TR:G1195552 G1195552 PHOSPHOINOSITIDE-SF
1491	1G08	3-I01	RSA-3-I01	#Mm_cluster16530		1.173	0.501	0.492	1.918	0.885	2.309	2.434	1.975	7.677	d			vb56h03.r1 Ko mouse embryo 11.5 SdpC Mus musculus cDNA clone 7610205 5' similar to TR:G505652 G505652 G3P6B GLYCOPR
1898	3L24	24-L17	RSA-23-F20	Mm_cluster42937		3.606	7.723	1.71	5.094	7.952	19.45	12.53	1.499	7.323	d			vi74b09.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 917945 5' similar to TR:G6910 G6910 PROTEIN 1. [1]
1572	2K20	22-J01	RSA-22-J01	Mm_cluster16333		7.19	2.929	5.869	6.307	4.326	10.23	6.739	0.362	6.557	d			m125e10.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 464586 5' similar to SW:YKVB YEAST P36007 H
1543	3E20	30-J02	RSA-30-J02	Mm_cluster78902		1.043	1.499	1.219	0.434	1.195	7.237	5.396	0.992	5.357	d			vx96h05.r1 Stratagene mouse macrophage (#937306) Mus musculus cDNA clone 1293777 5' similar to TR:Q35259 O35259 PUP
1151	1G15	8-M12	RSA-8-M12	Mm_cluster10460		2.594	0.357	0	-0.33	1.342	2.034	1.418	0.152	5.295	d			vh09h04.r1 Soares mouse mammary gland NbMMG Mus musculus cDNA clone 874999 5' similar to TR:G200131 G200131 KIDN
1386	1F18	11-E12	RSA-11-E12	Mm_cluster58338		0.239	1.057	-0.09	0.001	0	-0.07	1.201	-1.24	4.952	d			mr7303.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 603101 5' similar to SW:OXYB_HUMAN P22059 OXY
698	3L09	2-F02	RSA-3-G03	Mm_cluster10943		8.033	5.801	4.521	6.662	8.282	10.96	5.77	1.288	4.303	d			m134a05.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 513872 5' similar to gb:K63368 cds1 DNAJ PROTEIN
201	1I03	1-P22	RSA-1-P22	Mm_cluster42528		1.056	0.116	0.929	0.888	2.723	1.416	1.932	0.037	4.216	d			vi72b06.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 917747 5' similar to TR:G7550 G7550 ACTIN. [1]
1807	2J23	21-A24	RSA-21-A24	Mm_cluster59259		2.384	-0.17	0	2.357	3.871	3.221	3.941	1.279	4.203	d			mq55h06.r1 Soares 2NBM1 Mus musculus cDNA clone 582683 5' similar to TR:G285961 G285961 MRNA
1076	1H14	10-L23	RSA-10-L23	Mm_cluster18536		2.161	1.258	0.554	-1.25	1.469	2.724	0.396	0	4.182	d			mh80e10.r1 Soares mouse placenta 4Nbmp13.5 14.5 Mus musculus cDNA clone 457290 5' similar to SW:RL22_THEMA P3851
991	1G13	15-B18	RSA-15-P18	Mm_cluster13437		1.04	0	0.05	-0.28	-0.36	0.666	0.885	1.212	3.982	d			m166h06.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 488539 5' similar to PIR:A53770 A53770 growthfa
778	3L10	3-M08	RSA-3-M08	HS1187176		2.849	1.284	2.199	3.787	8.001	26.52	11.49	0	3.636	d			zs90e09.r1 NCI CGAP GCB1 Homo sapiens cDNA clone IMAGE:704776 5' similar to WP:80495.5 CE01763
93	3C02	26-K08	RSA-26-K08	Mm_cluster10943		0.589	-0.27	-0.02	0.083	0	1.001	0	0.666	3.584	d			m134a05.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 513872 5' similar to gb:K63368 cds1 DNA

Table of numerical data of clustered expression profiles

678	3H09	12-A23	RSA-12-A23	Ubp41	Mouse	Gen	2,416	4,904	6,089	9,079	10,85	27,44	28,23	4,311	7,174	n	Protein Degradatic	Ubiquitine Pathwat	Mus musculus ubiquitin-specific protease UBP41 (Ubp41) mRNA, complete cds.
777	2L10	22-P24	RSA-22-P24	Mm_cluster91722			12,18	8,603	9,514	11,08	14,15	15,99	20,42	19,22	19,23	n	mRNA sequence.		me93h03.r1 Soares mouse embryo NBME13.5 14.5 Mus musculus cDNA clone 403157 5' similar to PIR:S42864 S42864 protein
1097	2L14	23-D15	RSA-23-D15	Mm_cluster11759			8,615	5,761	4,495	6,125	8,885	8,464	8,987	8,663	14,56	n	mRNA sequence.		ua58c11.r1 Soares 2NbMT Mus musculus cDNA clone 1361684 5' similar to SW:YNQ3 YEAST P53893 HYPOTHETICAL 124.5
1402	2I18	19-H19	RSA-19-H19	Mm_cluster17638	Mouse	EST	11,51	16,25	18,6	19,28	16,76	19,49	22,49	23,69	13,96	n	mRNA sequence.		mp81c02.r1 Soares 2NbMT Mus musculus cDNA clone 575618 5', mq55h06.r1 Soares 2NbMT Mus musculus cDNA clone 5826
767	2J10	20-I08	RSA-6-K05	R			13,6	14,32	9,233	13,33	20,14	33,11	48,35	21,6	45,54	n			
783	3M10	27-C21	RSA-27-C20	Mm_cluster22387	Mouse	EST	2,257	6,573	7,158	19,86	26,75	45,3	40,22	10,04	20,19	n			ml62e10.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 516618 5',
697	2L09	22-P07	RSA-22-K10	Mm_cluster06748	Mouse	EST	12,82	4,604	7,416	5,653	5,503	9,508	12,25	10,26	18,94	n			.ub26g03.r1 Soares 2NbMT Mus musculus cDNA clone 1378900 5', mRNAsequence.
1162	2I15	19-F22	RSA-19-F22	HSC1LE111			6,777	7,083	8,013	3,706	6,629	13,58	14,03	7,512	9,804	n			H. sapiens partial cDNA sequence
1111	1O14	+1-D3RZ					87,32	2087	2058	4532	6119	5751	5205	4624	2547	n			O161230 514791 T COMPLEX TESTIS-SPECIFIC PROTEIN 2 (Tctex2) [Mus musculus]expression: from leptotene(week) to late
1511	1O19	+2-A5RZ					9,456	75,43	115,4	357,1	563,6	559,6	488,8	306,6	347,1	n			N091458 602312 Phosphoglycerate kinase 2 (pgk-2), TESTIS SPECIFIC [Mus musculus] IMAGE: 602312expression: from pach
1357	2P17	-					76,84	31,34	39,74	51,63	118,7	314,7	212,6	221,6	245,2	n			
1106	1N14	=Cox1					226,2	475,8	338,7	359,8	259,4	246,2	311,8	448,7	239,6	n			Cox1 cytochrome oxidase 1T3A/T7A PCR product from pBSK containing incertion of110 bp Alu/HaeIII fragment pos. 6624-6717
1352	2O17	=5-A5RZ					199	194,4	229,7	192,1	179,1	240,2	165	142,3	230,9	n			RODIY00225/MMJ1PRO Identities = 332/366 (90%) Murine mRNA for J1 protein, yeast ribosomal protein L3 homologue Length =
1427	2N18	=3-D4RZ					319,7	301,9	274,4	389,7	363,2	280,9	245,8	517,9	222,3	n			RODIM27466/IRCOXIV Identities = 260/287 (90%) Rattus norvegicus liver cytochrome oxidase subunit Vlc (COX-Vlc) mRNA, L
477	2P06	=C RZPD					85,51	217,4	258,6	250,1	301,8	479,1	449,4	133,6	201,5	n			RODIAF079565/AF079565 Identities = 615/651 (94%) Mus musculus ubiquitin-specific protease UBP41 (Ubp41) mRNA, complet
1191	1O15	+1-D5RZ					25,19	122,5	131	224	304,8	270,7	330,2	236,9	200,8	n			P171233 515968 CREM cAMP responsive element modulator (source - testis) IMAGE: 515968not expressed in CREM -/- knock
947	2N12	=3-C2RZ					66,41	72,12	56,74	74,38	107,4	193,6	219,9	69,65	198,9	n			HUM1I221507/HSEF1DELA Identities = 265/297 (89%) H.sapiens EF-1delta gene encoding human elongation factor-1-delta.Len
957	2P12	5-D1RZ					56,84	87,27	126,2	197,9	215	211,3	216,9	185,4	187,4	n			HUM1IAC003042/AC003042 Identities = 233/236 (98%) Homo sapiens chromosome 17, clone HCIT75G16, complete sequence,
876	1P11	-frog	5e23D209	5e23D209			29,14	90,47	62,01	58,04	62,1	88,91	97,1	128,2	187,1	n			(frog Bambi) frog clone constructed by Dasha Onistchouk, gene Bambi, 627bp insert BamHI/XhoI, in vector pCS2+, sense RNA A
1117	2P14	-					77,36	68,94	75,36	99,4	92,14	106,5	145,2	108,2	180,8	n			
1037	2P13	-					95,74	94,52	112,3	192,5	205,3	168,6	181,1	206,7	170,1	n			
797	2P10	=G RZPD					266,7	338,8	1065	1470	819,6	486,6	604,1	1043	169,5	n			ORGIV00665/MIMM01 Identities = 259/261 (99%) Mouse mitochondrial genes coding for three transfer RNAs (specific for Phe, V
391	1O05	+1-B4RZ					26,56	37,87	23,54	42,32	100,2	132,5	148,3	77,3	167,7	n			E20943 404347 mouse EST highly similar to ANGIOTENSIN-CONVERTING ENZYME PRECURSOR, TESTIS-SPECIFIC [Mus r
472	2O06	4-C4RZ					96,27	181,4	171,4	191,2	226,2	354,2	326	161,4	164,4	n			no data
1186	1N15	MyD88					68,36	113,5	105,4	207,1	209,8	160	211,8	241,9	162,9	n			MyD88PCR product from Erich Greiner
236	1P03	=2-D4RZ					16,42	12,04	14,8	12,06	31,69	94,75	43,47	5,488	159,1	n			J052627 1051108 Mouse histone H4 gene IMAGE: 1446370 (cont.) IMAGE: 1051108
1277	2P16	-					116,6	32,58	57,67	107,5	174,6	406,2	342,3	137,9	158,8	n			
1667	2N21	=4-A5RZ					406,8	363,5	298,4	334,5	305	194,2	231,7	146	145,6	n			RODIU52822/IMS2822 Mus musculus ornithine decarboxylase antizyme mRNA, complete cds.
467	2N06	=3-A6RZ					103	97,09	104,5	129,6	134,9	187	236,1	159,7	144	n			RODIY00516/IMMALDA 98% Mouse mRNA for aldolase A Length = 1359
227	2N03	=3-A3RZ					376,4	482,8	444,5	355,5	308	257,9	226,8	191,8	134,4	n			RODIU29402/IMM29402 Mus musculus acidic ribosomal phosphoprotein P1 mRNA, complete cds.Length = 495
317	2P04	=A RZPD					394,1	440,3	373,1	364,1	289,9	191,8	224,6	191,8	125,6	n			RODIU29402/IMM29402 Identities = 466/469 (99%) Mus musculus acidic ribosomal phosphoprotein P1 mRNA, complete cds. Len
1347	2N17	=3-D2RZ					71,42	110,7	109,9	154,5	159,8	128,4	143,6	118,6	118,5	n			HUM1XJ70649/HSQL1042 Identities = 411/467 (88%) Homo sapiens DDX1 gene, complete CDS Length = 2706
1031	1O13	=1-D2RZ					57,89	53,95	55,07	96,01	99,88	84,52	105,1	152	115,6	n			K111144 481666 ESTs, Highly similar to 3-KETOACYL-COA THIOLEASE MITOCHONDRIAL [Rattus norvegicus]from Bernhard K
1192	2O15	5-A3RZ					41,31	41,9	44,67	41,58	44,84	65,32	55,29	67,19	106,6	n			**** No hits found ****
1437	2P18	-					81,59	83,12	95,72	123,6	131,2	73,21	108,8	151,3	105	n			
557	2P07	=D RZPD					61,24	86,53	77,22	44,56	40,91	52,14	65,5	27,24	104	n			RODIM37134/RNCP45Z // RODIM21855/IMMTH16A Identities = 395/437 (90%) Rat cytochrome P-450 mRNA, 3' end. // Identiti
1032	2O13	=4-D5RZ					77,32	70,25	77,35	96,57	86,57	48,72	67,34	136,3	103,2	n			RODI83590/IMMRPL5 Identities = 151/154 (98%) M.musculus mRNA for ribosomal protein L5, 3'end Length = 349
1187	2N15	=3-C6RZ					57,68	80,93	69,97	154,6	144,5	107	148,7	161,5	100,9	n			RODI9X7042/IMMUBCM4GN Identities = 623/640 (97%) Mus musculus mRNA for UBcm4 protein Length = 2621
1267	2N16	=3-D1RZ					82,44	87,47	93,26	132,6	111,1	157,9	99,56	103,5	98,22	n			STSI22985/H5985343 Identities = 877/99 (87%) human STS WL-15071. Length = 348
637	2P08	=E RZPD					219,5	193,2	248,4	195,2	170,6	130,6	145,1	157,1	96,14	n			RODIIM32599/IMMGAPDH Identities = 235/235 (100%) Mouse glyceraldehyde-3-phosphate dehydrogenase mRNA, complete cds
1346	1N17	=G3PDH					62,45	67,7	38,15	57,83	62,8	68,09	79,75	103,3	92,97	n			G3PDHestis cDNA RT-PCR product from Holger's Reichardt primers, PCRRed by Igor Borissevitch
877	2P11	+H RZPD					103,9	142,1	223	189,7	238,1	196,6	238	96,5	92,93	n			RODI885732/D85732 Identities = 596/645 (92%) Mus musculus Hsc70t mRNA for spermatid-specific heat shock protein 70, com
1266	1N16	mTRAF6					90,28	65,79	142,8	133	121,4	152,5	145,9	155,7	87,16	n			mTRAF6PCR product from Erich Greiner
1426	1N18	=G3PDH					61,21	59,63	39,13	62,56	73,91	64,03	67,58	92,62	86,81	n			G3PDHestis cDNA RT-PCR product from Holger's Reichardt primers, PCRRed by Igor Borissevitch
1222	2E16	1-N03	RSA-1-N03	Mm_cluster68705	Mouse	EST	53,9	51,2	49,52	43,49	30,73	73,98	105,3	49,39	85,31	n			M. musculus expressed sequence tag MTEST640
1677	2P21	-					163,5	76,41	100,8	105,8	114,9	76,22	94,86	82,88	82,42	n			
1181	1M15	7-G24	RSA-7-G24	Mm_cluster03547	Mouse	EST	16,45	30,42	27,89	21,55	26,74	30,25	61,4	50,78	79,92	n			POL2_MOUSE (1672)
768	3J10	3-E07	RSA-3-E07	Mm_cluster16020	Mouse	EST	5,709	19	20,51	38,72	40,69	112	110,1	73,58	74,12	n			ml40e07.r1 Stratagene mouse testis (#937308) Mus musculus cDNA clone 514500 5'
872	2O11	4-D3RZ					127,4	132,6	154,9	172,3	135,8	108,6	138,4	83,99	73,35	n			no data
517	2H07	5-D05	RSA-5-D05 #5-D05 #0				1,946	2,505	1,31	6,025	20,5	66,79	30,02	59	72,19	n			
1432	2O18	5-B2RZ					84,7	88,67	75,02	72,08	73,21	65,76	55,67	63,28	70,36	n			no data
1422	2M18	-					73,14	60,65	52,88	88,35	72,29	54,64	69,79	159,9	68,38	n			
151	1O02	=1-A3RZ					22,25	43,07	40,45	38,33	43,83	38,34	35,55	19,51	68,29	n			
1107	2N14	=3-C5RZ					56,94	44,63	44,76	44,07	47,68	44,37	44,72	52,29	67,66	n			J10761 334569 mouse EST highly similar to GLUTAMINYL-TRNA SYNTHETASE [Homo sapiens]from Bernhard Korn
307	2N04	=3-A4RZ					188,4	152,9	124,2	107	67,43	79	80,33	24,72	67,46	n			RODI0X01756/IMMCCYCG Identities = 348/350 (99%) Mouse cytochrome c gene (MC1) Length = 1436
1197	2P15	-					39,12	56,74	58,75	56,58	122,7	46,04	131,8	153,1	67,22	n			RODID28812/IMMAMIATI Mouse mRNA for alpha-1 microglobulin/inter-alpha-trypsin inhibitor light chain.Length = 1234
1751	1O22	=2-B5RZ					383,7	200,7	232,3	299,8	219,6	203,6	195,3	114,2	64,52	n			A182030 821657 ESTs, Highly similar to ATP SYNTHASE GAMMA CHAIN, MITOCHONDRIAL [Rattus norvegicus] IMAGE: 8216
312	2O04	=4-C2RZ					68,18	77,19	73,08	69,25	63,47	50,34	55,43	47,5	63,18	n			RODI0X02621/IMMHISH2B Identities = 240/291 (82%) Mouse gene for histone H2b Length = 688
867	2N11	3-C1RZ					65,52	58,37	62,63	58									

Table of numerical data of clustered expression profiles

237	2P03	=5-C6RZ				43.17	82.24	79.16	83.2	71.76	54.34	70.88	51.18	46.75	n	
556	1P07	=2-B6aR				142.1	97.61	108	82.84	86.08	68.6	66.15	40.38	46.63	n	
711	1O09	=1-C3RZ				181.1	151.8	225.1	152.6	129	94.16	98.39	102.5	46.59	n	
1182	2M15	7-D16	RSA-8-P21	8-P21		17.58	26.58	14.11	15.32	40.69	66.74	55.79	29.55	46.35	n	
716	1P09	=2-C1aR				99.16	115.6	136	148.4	138.4	160.9	179.1	54.6	46.18	n	
1902	2M24	-				75.22	32.78	34.23	39.4	30.12	40.06	35.61	41.99	46.05	n	
387	2N05	=3-A5RZ				51.14	54.27	40.92	45.69	36.27	52.71	36.14	29.98	44.05	n	
547	2N07	=3-B1RZ				50.14	51.66	50.17	36.75	33.19	46.03	40.52	31.46	43.64	n	
1271	1O16	=1-D6RZ				60.96	21.68	38.52	62.6	73.05	54.29	59.12	122.1	43.35	n	
272	2G04	14-L11	RSA-14-L11	Mm_cluster13480	Mouse_EST	1.274	25.1	21.48	34.92	38.69	35.58	43.41	28.45	43.33	n	
1261	1M16	7-J15	RSA-7-J15	7-J15		6.741	6.786	3.724	13.02	11.66	14.25	18.22	37.74	42.25	n	
712	2O09	4-D1RZ				38.63	55.57	44.12	23.52	25.49	31.07	31.34	34.43	42.25	n	
852	2K11	21-A02	RSA-21-A02	Mm_cluster20710	Mouse_EST	20.16	13.79	14.5	17.67	25.49	32.01	43.52	19.49	41.94	n	
1671	1O21	=2-B4RZ				122.7	92.87	88.18	98.02	69.22	64.54	71.43	83.26	41.71	n	
1501	1M19	8-A03	RSA-8-A03	Mm_cluster11045	Mouse_EST	16.26	14.32	19.45	29.2	28.05	25.89	42.09	33.56	41.59	n	
1115	5O14	-				0.678	0	0	0	6.969	5.339	0	54.62	41.55	n	
1342	2M17	-				39.36	23.93	25.93	26.95	24.79	22.33	38.21	69.88	39.77	n	
1351	1O17	+2-A3RZ				116.3	39.69	62.21	67.42	60.53	127.1	82.98	94.81	38.8	n	
67	2N01	=3-A1RZ				20.25	35.71	28.61	33.29	31.16	31.5	23.97	18.06	37.04	n	
1907	2N24	4-B3RZ				85.93	66.33	57.86	54.98	47.51	37.15	37.08	51.33	36.75	n	
546	1N07	PP1c				38.98	246.6	254.6	430	305.8	352.6	372.1	81.01	35.51	n	
152	2O02	4-B5RZ				44.69	45.76	28.26	20.13	20.02	34.95	23.5	13.11	34.7	n	
1587	2N20	+4-A1RZ				112	55.3	39.07	39.87	32.75	45.41	30.6	30.22	34.5	n	
453	3K06	6-K04	RSA-6-K04	Mm_cluster00914	Mouse_EST	3.938	52.25	70.92	72.18	58.9	78.62	54.21	39.33	34.22	n	
842	2I11	18-P23	RSA-18-P23	Mm_cluster95030	Mouse_EST	5.07	31.62	30.01	33.12	38.1	33.36	45.37	33.61	33.43	n	
5	5A01	-				0	18.51	0	13.17	7.332	0	71.3	46.99	32.84	n	
1512	2O19	5-B3RZ				15.94	33.81	31.56	44.69	65.77	56.51	62.97	52.08	32.34	n	
636	1P08	2-B3aRZ				44.88	42.05	35.04	33.51	37.65	53.91	29.31	35.52	32.14	n	
1592	2O20	5-B5RZ				35.61	45.85	42.77	38.18	34.43	42.32	28.18	35.69	31.69	n	
1114	4O14	-				-0.18	1.454	-3.82	3.481	-5.44	-10.1	-2.93	23.13	31.64	n	
1251	1K16	4-J04	RSA-4-J04	4-J04		3.177	2.355	4.211	2.809	2.866	10.76	11.66	11.65	30.27	n	
217	2L03	22-N17	RSA-22-N17	Mm_cluster77934	Mouse_EST	36.81	22.37	21.74	16.11	12.51	27.48	23.86	20.21	30	n	
692	2K09	21-P13	RSA-21-P13	21-P13		7.73	7.148	9.021	3.6	4.57	8.092	12.05	12.36	29.63	n	
717	2P09	-F	RZPD			78.26	56.33	47.97	30.16	27.08	39.62	28.36	29.38	29.39	n	
1036	1P13	=5A5XL				7.151	12.96	9.21	10.78	14.44	9.938	21.16	25.24	29.29	n	
1256	1L16	3-J03	RSA-3-J03	HU-PP-1	Homologous	4.392	6.342	8.808	4.106	3.523	11.38	15.16	10.27	28.78	n	
1421	1M18	7-P06	RSA-7-P06	Hs_cluster25707	Other_EST	15.29	9.067	11.34	15.47	13.54	14.16	24.01	31.46	27.68	n	
1416	1L18	3-D02	RSA-3-D02	3-D02		5.05	2.924	6.34	5.08	1.999	1.124	13.17	15.55	27.05	n	
1516	1P19	=24E11X				53.17	72.01	77.05	78.54	64.58	38.13	64.44	68.07	26.9	n	
232	2O03	=4-C1RZ				29.31	41.61	32.79	22.53	22.85	17.79	20.22	35.49	26.17	n	
1092	2K14	22-A06	RSA-22-A06	22-A06		10.31	5.998	6.475	4.582	6.253	17.56	20.68	10.78	25.07	n	
667	2F09	6-A15	RSA-6-A15	Mm_cluster16028	Mouse_EST	3.375	30.72	27.54	33.18	26.31	28.47	36.44	12.13	24.78	n	
1196	1P15	=13H7XL				21.09	52.35	61.96	118.9	92.57	82.1	62.45	38.98	24.31	n	
1451	1C19	2-K02	RSA-2-K02	Mm_cluster13980	Mouse_EST	1.523	1.575	1.558	-0.03	4.589	18.19	15.37	0.254	24.19	n	
1168	3J15	4-C23	RSA-4-C23	Mm_cluster58367	Mouse_EST	12.54	19.72	19.91	28.37	27.12	33.3	29.52	16.54	24.04	n	
1803	3I23	17-J12	RSA-17-J12	Hs_cluster43833.01	Other_EST	17.69	10.18	11.73	13.74	36.23	44.12	40.87	10.19	23.63	n	
632	2O08	4-C6RZ				10.68	13.66	8.366	10.82	15.87	19.16	16.17	17.56	23.55	n	
1336	1L17	7-I23	RSA-7-I23	7-I23		1.86	6.061	2.874	3.727	5.364	19.19	24.35	8.08	23.28	n	
1919	4P24	-				0.669	2.583	2.186	0.161	6.201	2.698	1.928	15.71	23.09	n	
761	1I10	6-B21	RSA-6-B21	Mm_cluster08810	Mouse_EST	51.22	38.13	41.36	24.21	14	11.81	32.59	52.95	22.8	n	
1152	2G15	11-F23	RSA-11-F23	Hs_cluster22953	Other_EST	13.46	7.777	7.572	4.29	6.888	44.06	36.21	8.144	22.49	n	
912	2G12	7-K04	RSA-7-K04	7-K04		4.811	6.372	3.573	7.458	5.529	26.16	18.71	8.98	22.3	n	
72	2O01	=4-B4RZ				77.36	34.52	38.56	38.04	31.25	25.13	21.04	9.09	21.62	n	
372	2K05	21-J23	RSA-21-J23	Mm_cluster06503	Mouse_EST	7.887	10.37	7.16	4.714	5.744	10.16	10.77	8.319	21.41	n	
1418	3L18	19-G11	RSA-19-G11	Mm_cluster42599	Mouse_EST	1.87	2.468	3.674	4.101	26.24	33.53	20.83	8.281	21.22	n	
937	2L12	23-D08	RSA-23-D08	Mm_cluster84992	Mouse_EST	7.428	7.836	3.278	4.164	10.51	12	11.42	15.45	20.7	n	
212	2K03	21-C16	RSA-21-C16	21-C16		42.48	25.2	18.01	22.25	12.95	14.59	19.1	17.91	20.46	n	
1438	3P18	-				3.646	1.713	4.35	27.27	32.39	6.26	11.52	7.022	20.29	n	
1668	3N21	-				24.47	11.1	25.66	35.48	65.36	68.96	38.21	10.92	20.15	n	
1172	2K15	22-B18	RSA-22-B18	22-B18		6.426	15.54	9.831	13.34	12.83	11.22	19.75	10.57	19.72	n	
631	1O08	=1-C2RZ				50.1	44.62	56.44	48.79	46.06	46.43	32.53	38.43	19.38	n	
308	3N04	-				10.5	9.984	11.96	11.82	13.89	40.92	31.52	12.47	19.21	n	
787	2N10	=3-B5RZ				40.28	26.54	25.79	18.93	17.39	19.65	19.45	28.14	19.09	n	
1276	1P16	=16E2XL				11.64	11.96	11.75	18.2	9.543	21.37	21.34	21.85	18.95	n	
1157	2H15	18-E21	RSA-18-E21	Mm_cluster28775	Mouse_EST	55.72	36.58	37.65	28.02	18.64	25.7	21.68	33.9	18.94	n	
1087	2J14	20-J22	RSA-20-J22	20-J22		7.99	7.632	4.878	6.121	7.291	27.93	20.79	10.57	18.88	n	
1586	1N20	Laminin				44.89	37.6	31.23	24.46	22.65	13.36	22.23	13.46	18.71	n	
1428	3N18	=1-C4RZ				58.75	49.01	55.09	44.83	44.24	46.29	40.88	40.58	18.17	n	
1737	2L22	23-N01	RSA-23-N01	A1326289	Mouse_EST	0.082	1.745	1.363	13.63	45.2	31.63	18.6	14.59	17.59	n	
1508	3N19	-				2.916	7.028	4.847	20.13	62.26	60.73	34.49	20.1	16.3	n	
706	1N09	ATF1				15.17	24.3	26.67	28.09	19.26	26.76	19.13	15.56	16.05	n	
1622	2E21	13-M11	RSA-13-M11	Mm_cluster08803	Mouse_EST	31.46	48.01	38.1	36.27	30.71	31.79	41.03	12.97	15.89	n	
1496	1L19	3-N17	RSA-3-N17	3-N17		0.003	0.46	0.243	5.444	9.771	7.667	13.6	7.732	15.8	n	
1401	1I18	17-G06	RSA-17-G06	Mm_cluster23832	Mouse_EST	3.523	1.007	0.831	0							

