

# New Transcripts Identified for *S. cerevisiae*, *S. pombe* and *Drosophila* Using Novel cDNA Cloning

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## Abstract

Unannotated transcripts, including antisense, noncoding, intergenic, and potential regulatory RNAs, were identified in three different eukaryotic model organisms by cDNA cloning and sequencing. A rapid double-stranded cDNA generation system will be described that uses modifications of standard techniques and a new cloning vector. Short reverse transcription times facilitated cloning short (250-750 nucleotide, nt) cDNAs that were sequenced by two schemes. No size selection of cDNAs other than shorter reverse transcription was used, and a high percentage of cDNAs were apparently full-length. The average cDNA length was approximately 400 nt, a size which is usually excluded by following standard cDNA cloning protocols. Putative capping (transcription initiation) sites and poly(A) addition sites were located for most clones by single-pass sequencing. Sequencing purified cDNA clone plasmids or cDNA library colony PCR products gave similar results. Several cDNAs correlated with ChIP-Chip data showing RNA polymerase II occupancy sites in the *S. cerevisiae* genome (Steinmetz, E.J., *et al.*, *Mol. Cell* **24**, 735-746 (2006)). For several transcription units, cDNA clones contained multiple 5' and/or 3' ends. Many transcripts overlapped with or corresponded to those found using a different cDNA cloning approach (Miura, F., *et al.*, *PNAS* **103**, 17846-17851 (2006)). There was a preponderance of *S. cerevisiae* cDNAs for nuclear-encoded mitochondrial (mt) proteins. We postulate that the mRNAs of nuclear-encoded mt proteins may be more accessible to extraction due to their proximity to the mt network structure near the cell membrane of *S. cerevisiae*. Approximately 50% of the cloned cDNAs from *Drosophila* melanogaster S2 cells encoded ribosomal protein genes, with a strong (11:1) bias toward small ribosomal subunit cDNAs over large subunit clones. The reason for such a discrepancy between small and large subunit cDNAs is under investigation.

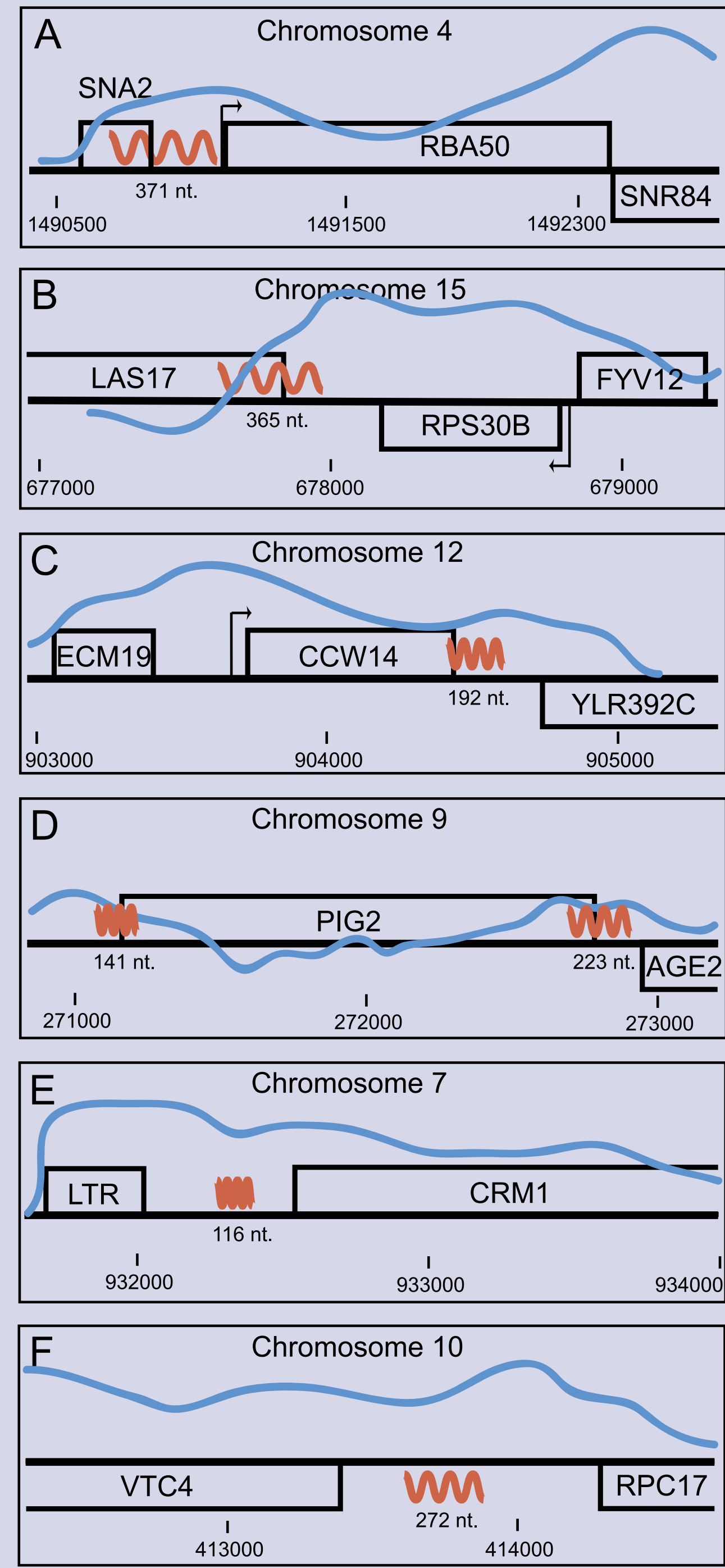
## Introduction

cDNA cloning methods are being developed to meet the needs of bioinformatics and the evolving landscape of the transcriptome. The traditional view of neatly annotated transcripts representing the open reading frames (ORFs) of a genome have given way to a more fluid concept of the transcriptome. Recent articles (1-8) have noted noncoding, antisense, cryptic, unannotated or regulatory transcripts in virtually every system investigated. Some *S. cerevisiae* transcripts appear to be promiscuous intergenic, capped transcripts, called CUTs (1,2). CUTs are rapidly degraded, however, in wild-type yeast. Other yeast studies, such as the large-scale cDNA cloning and sequencing by Miura *et al.* (3), have identified a plethora of new yeast transcripts that are not "CUTs". Analyses of steady-state RNA (4) and RNA polymerase II occupancy (5) using high-density tiled DNA microarrays have also indicated the presence of many intergenic transcripts in *S. cerevisiae*.

We present data for three different eukaryotic model organisms that show a range of new RNAs can be found by cloning cDNAs of "small, but not very small" RNAs, rather than selecting for larger, "gene-sized" cDNAs, as is traditionally done. The repertoire of short-ish RNAs (named shishRNAs), both annotated and unannotated, varies between organisms. If longer (1-3 kb) cDNA is selected prior to cloning, few unannotated or CUT-like transcripts are found. The "oligo capping" method presented is a new technique in the transcriptome analysis arsenal that is fast and straightforward. To show the robustness of the technique, we made no attempt to exclude uncapped or degraded mRNAs from the template pools. Very few degraded, and necessarily 5' phosphorylated, RNAs were cloned by the new method. Several of the newly cloned Saccharomyces cDNAs correspond to transcripts found by analysis of RNA polymerase II occupancy sites by ChIP-Chip assays (5). The transcripts are located in regions of the genome that appear to have significant RNA Pol II occupancy, and several are in conserved regions of the yeast genome.

- Myers, F., *et al.*, Cryptic Pol II transcripts are degraded by a nuclear quality control pathway involving a new poly(A) polymerase. *Cell* **121**:725-737 (2005).
- Davis, C. A and Ares, M. Accumulation of unstable promoter-associated transcripts upon loss of the nuclear exosome subunit Rps6 in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci.* **100**: 3262-3267.
- Miura, F., *et al.*, A large-scale full-length cDNA analysis to explore the budding yeast transcriptome. *PNAS* **103**:17846-17851 (2006).
- David, L., *et al.*, A high-resolution map of transcription in the yeast genome. *PNAS* **103**: 5320-5325 (2006).
- Steinmetz, E.J., *et al.*, Genome-wide distribution of yeast RNA polymerase II and its control by Sen1 Helicase. *Mol. Cell* **24**:735-746 (2006).
- Furuno, M., *et al.*, Clusters of internally primed transcripts reveal novel long noncoding RNAs. *PLoS Genetics* **2**:537-553 (2006).
- Yuan, G. *et al.*, Rhotomys in *Drosophila melanogaster*. *Nucleic Acids Res.* **31**:2495-2501 (2003).
- Watanabe, T., *et al.*, Abundant poly(A)-bearing RNAs that lack open reading frames in *Schizosaccharomyces pombe*. *DNA Res.* **9**:209-215 (2002).

## Examples of *S. cerevisiae* shish RNAs



### Sense transcripts that overlap the 3' (and 5') ends of ORFs:

While these could be degradation fragments of mRNAs or failed primer extension products, the cloning strategy and Pol II ChIP-Chip data suggest otherwise. However, preliminary Northern blot analysis has not yet detected these RNAs.

**A) dSNA2 (3D01):** This 371 nt. cDNA starts in the middle of the SNA2 ORF, which may encode a cation transporter, and almost reaches the RBA50 ORF, which encodes an RNA polymerase II interacting protein of unknown function. Pol II occupancy correlates better with the cDNA than with the SNA2 ORF. Conservation is poor except for the last 100 nt. RT-PCR with oligo(T) gives major 3' ends near 3' end of cDNA and minor 3' ends about 200 nt. upstream (SNA2 mRNA).

**B) dLAS17 (3C04):** This 365 nt. cDNA starts near the end of the LAS17 ORF, which encodes a WASP protein that nucleates branched actin filaments. It underlies a strong Pol II peak that overlaps the convergent RPS30B Pol II peak.

**C) CCW14 (2E04):** This 192 nt. cDNA just over laps the CCW14 ORF, which encodes a cell wall mannoprotein. The DNA sequence is moderately conserved for about 200 bp downstream of the CCW14 ORF.

**D) dPIG2 (3G02) and dPIG2 (2G06):** Strikingly, the PIG2 gene, which encodes a targeting subunit for the Glc7 protein phosphatase, exhibits short cDNAs at both the upstream and downstream ends of the ORF. Each cDNA correlates with a peak of Pol II, and both cDNAs are also found in the Miura *et al.* library.

### Intergenic transcripts:

These transcripts are potential regulatory RNAs for the genes immediately downstream, as has been established previously for SRG1/SEF3 (Martens *et al.*, *Nature* **429**:571-574, 2004). uCRM1 is the strongest candidate so far for such a function.

**B) uCRM1 (2F10):** This 116 nt. cDNA lies upstream of the CRM1 ORF, which encodes a protein required for export of RNAs and proteins from the nucleus to the cytoplasm. RT-PCR with an oligo(dT) primer gave a major cDNA product about 200 bp longer than expected, which would reach the CRM1 ORF. Sequences within and upstream of the 2F10 cDNA are fairly well conserved.

**F) uVTC4 (1C10):** This 272 nt. cDNA lies upstream of the VTC4 ORF, which encodes a vacuolar membrane protein. It did not give a clear RT-PCR product.



Well	Length	Chr.#	Location (bp)	ORF Name (Standard)	ORF Name (Systematic)	ORF location	Sense or Anti-sense to ORF	Intergenic	Watson or Crick Strand	Poly (A) Tail approx	Comments
E04	101	B	904413-904604	CCW14	YLR390W-A	903724-904440	SENSE	Intergenic	Watson	19	Barely skims CCW14, no other ORF on 3' side
F10	115	B	932276-932391	CRM1	YGR118W	932544-935798	SENSE	Intergenic	Watson	22	Near the 5' end of CRM1
G06	222	B	272982-272914	PIG2	YIL045	271160-272776	SENSE		Watson	20	Covers a small part of the 3' end and then beyond
G02	140	X	271066-271206	PIG2	YIL045W	271160-272776	SENSE		Watson	YES	PARTIAL poly(A) 44 nt after ATG; overlaps F Miura transcrs
D01	380	IV	1490684-1491054	RBA50	YDR527W	1491086-1492405	SENSE		Watson	YES	PARTIAL, but protein is not well-annotated
CO4A	375	XV	677807-677971	RPS30B	YOR182C	676794-678192	ANTI		Watson	YES	unannotated RNA near RBA50-lys gene, SAGE tags present. Covers the 3' and of LAS17
C10	290	B	413810-413882	VTC4	YAL012C	413390-411228	SENSE	Intergenic	Crick	40	Upstream of VTC4

## Summaries of cDNA Cloning

Unsize <i>S. cerevisiae</i> cDNAs						
clone	Tag	poly(A)	RNA length	BLASTN best match	Chr. Location	COMMENTS
A1	yes	yes	425	180 sequence		
A2	yes	yes	425	180 sequence		
A3	yes	yes	280	YDR118W a portion of unknown function	IX 143584-143581	Small tag present
A4	yes	yes	700	ORF19850C, subunit of the diacylglycerol cation channel		full length
A5	yes	yes	250	ORF19850C, subunit of the diacylglycerol cation channel	IX 143584-143581	partial 5' end, overlap Miura transcrs
A6	yes	yes	115	ORF19850C, subunit of the diacylglycerol cation channel	IX 143584-143581	partial 5' end, overlap Miura transcrs
A7	yes	yes	790	APP105, 127 kDa, cytoplasmic protein	IX 143584-143581	full length
A8	yes	yes	410	ORF19850C, subunit of the diacylglycerol cation channel		full length
A9	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A10	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A11	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A12	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A13	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A14	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A15	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A16	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A17	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A18	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A19	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A20	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A21	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A22	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A23	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A24	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A25	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A26	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A27	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A28	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A29	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A30	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A31	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A32	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A33	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A34	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A35	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A36	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A37	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A38	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A39	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A40	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A41	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A42	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A43	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A44	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A45	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A46	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A47	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A48	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A49	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A50	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A51	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A52	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A53	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A54	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A55	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A56	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A57	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A58	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A59	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A60	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A61	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A62	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A63	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A64	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A65	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A66	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A67	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A68	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A69	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A70	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A71	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A72	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A73	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A74	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A75	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A76	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A77	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A78	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A79	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A80	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A81	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A82	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A83	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A84	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A85	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A86	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A87	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A88	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A89	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A90	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A91	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A92	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A93	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A94	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A95	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A96	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A97	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A98	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A99	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length
A100	yes	yes	190	ORF19850C, subunit of the diacylglycerol cation channel		full length

"2-4 Kb" Sized <i>S. cerevisiae</i> cDNAs						
clone	Tag	poly(A)	RNA length	BLASTN best match	Chr. Location	COMMENTS
A1	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A2	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A3	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A4	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A5	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A6	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A7	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A8	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A9	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A10	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A11	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A12	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A13	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A14	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A15	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A16	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A17	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A18	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A19	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A20	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A21	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A22	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A23	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A24	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A25	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A26	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A27	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A28	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A29	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A30	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A31	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A32	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A33	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A34	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A35	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A36	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A37	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A38	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A39	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A40	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A41	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A42	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A43	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A44	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A45	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A46	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A47	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A48	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A49	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A50	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A51	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A52	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A53	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A54	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A55	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A56	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A57	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A58	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A59	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A60	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A61	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A62	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A63	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A64	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A65	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A66	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A67	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A68	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A69	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A70	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A71	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A72	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A73	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A74	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A75	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A76	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A77	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A78	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A79	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A80	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A81	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A82	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A83	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A84	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A85	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A86	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A87	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A88	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A89	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A90	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A91	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A92	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A93	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A94	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A95	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A96	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A97	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A98	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A99	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A100	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A101	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A102	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A103	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A104	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A105	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A106	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A107	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A108	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A109	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A110	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A111	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A112	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A113	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A114	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A115	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A116	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A117	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A118	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A119	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A120	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A121	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A122	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A123	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A124	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A125	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A126	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A127	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A128	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A129	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A130	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A131	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A132	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A133	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A134	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A135	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A136	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A137	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A138	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A139	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A140	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A141	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A142	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A143	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A144	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A145	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A146	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A147	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A148	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A149	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A150	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A151	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A152	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A153	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A154	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A155	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A156	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A157	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A158	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A159	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A160	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A161	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A162	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A163	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A164	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A165	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A166	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A167	yes	yes	430	ORF19850C, A	IX 143584-143581	full length
A168	yes	yes	430	ORF19850C, A		