# Suppression of nonsense-mediated mRNA decay permits unbiased gene trapping in mouse embryonic stem cells

# Toshiaki Shigeoka, Masashi Kawaichi and Yasumasa Ishida\*

Division of Gene Function in Animals, Graduate School of Biological Sciences, Nara Institute of Science and Technology (NAIST), 8916-5 Takayama-cho, Ikoma-shi, Nara 630-0192, Japan

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### ABSTRACT

An international collaborative project has been proposed to inactivate all mouse genes in embryonic stem (ES) cells using a combination of random and targeted insertional mutagenesis techniques. Random gene trapping will be the first choice in the initial phase, and gene-targeting experiments will then be carried out to individually knockout the remaining 'difficult-to-trap' genes. One of the most favored techniques of random insertional mutagenesis is promoter trapping, which only disrupts actively transcribed genes. Polyadenylation (poly-A) trapping, on the other hand, can capture a broader spectrum of genes including those not expressed in the target cells, but we noticed that it inevitably selects for the vector integration into the last introns of the trapped genes. Here, we present evidence that this remarkable skewing is caused by the degradation of a selectable-marker mRNA used for poly-A trapping via an mRNA-surveillance mechanism, nonsense-mediated mRNA decay (NMD). We also report the development of a novel poly-Atrap strategy, UPATrap, which suppresses NMD of the selectable-marker mRNA and permits the trapping of transcriptionally silent genes without a bias in the vector-integration site. We believe the UPATrap technology enables a simple and straightforward approach to the unbiased inactivation of all mouse genes in ES cells.

#### INTRODUCTION

With the animal genome sequencing projects approaching their completion, the next big task for our bioscience research communities is to rapidly and efficiently elucidate physiological functions in animals of the vast number of newly discovered genes and gene candidates.

Recently, an international collaborative project has been proposed to inactivate all mouse genes in ES cells within five years using a combination of random and targeted insertional mutagenesis techniques (1). To disrupt as many genes in ES cells as possible within a limited period of time, gene trapping will first be employed because it is simple, rapid and cost-effective (2). Genes incapable of being captured by standard gene-trap techniques will then be subjected to labor-intensive and time-consuming gene-targeting experiments (1). Therefore, it is essential for the success of the project to establish an efficient gene-trap strategy suited to universally target genes in ES cells.

One of the most commonly used gene-trap methods is promoter trapping, which involves a gene-trap vector containing a promoterless selectable-marker cassette (3-6). In promoter trapping, the mRNA of the selectable-marker gene can be transcribed only when the gene-trap vector is placed under the control of an active promoter of a trapped gene. Although promoter trapping is effective at inactivating genes, transcriptionally silent loci in the target cells cannot be identified by this strategy. To capture a broader spectrum of genes including those not expressed in the target cells, poly-A-trap vectors have been developed in which a constitutive promoter drives the expression of a selectable-marker gene lacking a poly-A signal (7-10). In this strategy, the mRNA of the selectablemarker gene can be stabilized upon trapping of a poly-A signal of an endogenous gene regardless of its expression status in the target cells.

Here, we show that despite the broader spectrum of its potential targets, poly-A trapping inevitably selects for the vector integration into the last introns of the trapped genes, resulting in the deletion of only a limited C-terminal portion of the protein encoded by the last exon of the trapped gene. We present evidence that this remarkable skewing is created by the degradation of a selectable-marker mRNA used for poly-A

\*To whom correspondence should be addressed. Tel: +81 743 72 5531; Fax: +81 743 72 5539; Email: ishiday@bs.naist.jp

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trapping via an mRNA-surveillance mechanism, NMD. The NMD pathway is universally conserved among eukaryotes and is responsible for the degradation of mRNAs with potentially harmful nonsense mutations (11–13). We also show that an internal ribosome entry site (IRES) sequence (14) inserted downstream of the authentic termination codon (TC) of the selectable-marker mRNA prevents the molecule from undergoing NMD, and makes it possible to trap transcriptionally silent genes without a bias in the vector-integration site. We believe this novel anti-NMD technology, termed UPATrap, could be used as one of the most powerful and straightforward strategies for the unbiased inactivation of all mouse genes in the genome of ES cells (1).

## MATERIALS AND METHODS

#### Vector construction

To create the UPATrap retrovirus vector, two minor alterations and one major modification were introduced into the conventional RET poly-A-trap vector (10). The loxP signal (a 44 bp EcoRI–BamHI fragment) in the 3' LTR (long terminal repeat) of the RET vector was replaced with a synthetic Flprecombinase target (FRT) sequence (15), and a 726 bp NcoI-NsiI fragment containing an entire coding sequence of hrGFP (green fluorescence protein) (Stratagene), which had been generated by PCR, was ligated with the vector after removal of the original EGFP (enhanced GFP) sequence by NcoI-NsiI double digestion. Then, the IRES sequence flanked by two tandem loxP signals, three initiation codons (ICs) in all reading frames, and a modified version of the mouse hypoxanthine-guanine phosphoribosyl transferase gene (hprt) exon 8 splice donor (SD) sequence generated by PCR were inserted downstream of the NEO (a gene segment that confers resistance to a neomycin analog, G418) coding sequence in the altered RET vector after removal of the corresponding region (213 bp) by MluI-AatII double digestion. Details of these changes in the UPATrap vector are shown in Supplementary Figure 1. The nucleotide sequences of PCR primers used for the construction of the UPATrap vector are available on request.

#### Culture and manipulation of ES cells

ES cell culturing, production of recombinant retroviruses, gene trapping and isolation of cDNA fragments of trapped genes were carried out as previously described (10), except that TC1 (16) and V6.4 (17) ES cell lines were used instead. For excision of the floxed IRES sequence, two ES cell clones (15v-19 and 15v-43) in which different genes (*Pde9a* and *Nf2*) had been trapped by the UPATrap vector were electroporated as previously described (16) with a Cre recombinase-encoding plasmid pCAGGS-NLS/Cre (9). Subclones were then isolated by limiting dilution, and recombination events and clonality of the cells were analyzed by genomic PCR. The primer sequences are available on request.

#### **Bioinformatics**

Before detailed analyses, the nucleotide sequences of the trapped cDNA fragments were filtered using information available in public genome databases [University of California, Santa Cruz (UCSC) mouse genome assembly and Ensembl mouse genome database] to eliminate repetitive and low-quality fragments. To determine the number of exons in the trapped genes and integration sites of the gene-trap vectors, we used the mouse BLAT search provided by UCSC. Homology searches were performed using the non-redundant (NR) and expressed sequence tag (EST) databases of National Center for Biotechnology Information (NCBI) in conjunction with the BLAST algorithm. Identity or similarity extending more than ~100 bp with the probability *E* value of 10<sup>-20</sup> or less was considered to be significant homology. Information regarding the EST clones, especially their origins, was obtained from the Unigene database at NCBI.

#### Gene expression analyses

Total RNA was prepared from ES cells using Sepasol reagent (Nacalai) or from mouse tissues using a guanidine method (18). Reverse transcription was then performed using Moloney murine leukemia virus reverse transcriptase (Invitrogen) and pd (T)<sub>12-18</sub> primer (Amersham) according to the manufacturer's instructions. PCR was carried out using Advantage-GC2 polymerase mix (BD Biosciences) (36 cycles of 94°C for 30 s, 62°C for 30 s and 72°C for 90 s). Real-time PCR was performed following the relative standard curve method (Applied Biosystems 7700 User Bulletin #2) using SYBR Green Master Mix and an Applied Biosystems Prism 7700 sequence detector (Applied Biosystems). Reactions were performed in 3-4 replicates of cDNA samples for each clone. The levels of transcripts to be analyzed were normalized to those of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNAs. For the NMD-inhibition experiments, we treated 60% confluent cells with 100 µg/ml of emetine dihydrochloride hydrate (Sigma) at 37°C for 10 h before preparation of total RNA. The DNA probes used for northern hybridization included a 0.8 kb BamHI-BamHI fragment containing an NEO coding sequence in the original RET vector (9) and a 0.3 kb EcoRI-EcoRI fragment derived from the 3' untranslated (UT) region of the mouse  $\beta$ -actin cDNA (19) labeled with <sup>32</sup>P using a random-priming kit (TAKARA). Hybridization, washing and autoradiographic analysis of the filters were carried out under standard conditions (19). The primer sequences used for PCR are available on request.

#### URLs

The UCSC Genome Browser is available at http://genome. ucsc.edu/. The Ensembl Genome Browser is available at http:// www.ensembl.org/. The BLAST search of NCBI's NR and EST databases is available at http://www.ncbi.nlm.nih.gov/ BLAST/. The Unigene database is available at http://www. ncbi.nlm.nih.gov/UniGene/. The NAISTrap database is available at http://bsw3.naist.jp/kawaichi/naistrap.html.

#### **RESULTS AND DISCUSSION**

# Strong bias in the vector-integration site in poly-A trapping

We have generated a collection of mutated mouse ES cell clones by using a poly-A-trap vector, RET (Figure 1A), established the NAISTrap database, and subsequently constructed DNA arrays of trapped gene fragments (9,10). We found that a



Figure 1. A model showing a biased selection of the vector-integration sites in poly-A trapping. (A) Structure of the RET provirus integrated into the genome of infected cells (10). IC, initiation codon; TC, termination codon; dEn, enhancer deletion; LTR, long terminal repeat; SA, splice acceptor; EGFP, enhanced green fluorescence protein; pA, poly-A-addition signal; CP, constitutive promoter; SD, splice donor. (B) Integration sites of a poly-A-trap retrovirus vector and stability of a selectable-marker mRNA. Trapped exons are depicted as gray boxes. The NEO pre-mRNA driven by a CP must utilize a pA of an endogenous gene to acquire a poly-A tail for its stabilization. When multiple exons are trapped, however, the TC of the NEO cassette is recognized as premature, and the NEO-trapped gene fusion transcripts are degraded by NMD.

high proportion (88%) of the trapped genes had insertional mutations in their last (3'-most) introns (see Table 1 for examples). This phenomenon is not specific to our RET system because another research group using poly-A trapping (20) has also experienced this same bias (see Supplementary Material for details). In general, the chance of producing a null allele becomes small with insertion of the gene-trap vector into the last intron because such an event results in the deletion of only a limited C-terminal portion of the protein encoded by the last exon of the trapped gene. This strong skewing in the vector-integration site was suspected to be caused by an mRNA-surveillance mechanism, NMD (11–13).

In mammalian cells, a TC is recognized as premature if it is located greater than  $\sim 60$  nt 5' to the last exon–exon junction, and an mRNA containing such a premature TC (PTC) is degraded by NMD. In contrast, NMD does not function if a TC is generated within  $\sim 60$  nt upstream from the last exon– exon junction, or anywhere inside the last exon (21,22). We hypothesized that the TC of the NEO cassette of the RET vector would be recognized as a PTC when it is inserted into one of the upstream introns (other than the last one) of a trapped gene, and as a consequence the NEO-trapped gene fusion transcript would undergo NMD (Figure 1B). On the other hand, if the RET vector is integrated into the last intron of a gene, the NEO mRNA would be able to narrowly escape degradation because the distance between the NEO TC and the last exon–exon junction [64 nt in the case of the RET vector (10)] is probably too short to efficiently induce NMD (21,22) (Figure 1B).

# Development of a novel strategy for unbiased poly-A trapping

To test the above hypothesis and also overcome the serious problem of the strongly skewed selection of the vectorintegration sites in poly-A trapping, we developed a novel strategy, termed UPATrap (Figure 2A). In brief, an IRES sequence (14) flanked by two tandem loxP signals and three initiation codons in all three reading frames were inserted between the NEO TC and the SD sequence of the conventional RET vector (10) (see Supplementary Figure 1 for details). By adding these modifications, we attempted to induce internal initiation of translation that would proceed toward the end of the NEO fusion transcript. The inserted IRES sequence is flanked by two loxP signals for the purpose of deleting the IRES-mediated translation of abnormal proteins in mutant mice derived from gene-trapped ES cell clones. In the absence of IRES, only the NEO protein is translated from the NEOtrapped gene fusion transcripts because of the monocistronic rule of eukaryotic translation.

Table 1. Known genes and their exons trapped using the RET poly-A-trap vector

Gene identity		Total number	Number of
Gene symbol	Accession number	of exons	trapped exons
A630021E21Rik	AK080278	4	1
A930013B10Rik	NM 001001497	3	1
A930017E24Rik	NM_177041	9	1
Atox1	NM_009720	4	1
Bach	NM_133348	9	1
BC061259	AK037138	3	1
Bub1b	NM_009773	23	1
Cd47	NM_010581	10	1
Cdc42	NM_009861	6	1
Cfdp1	NM_011801	7	1
Commd5	NM_025536	3	1
Copg	NM_017477	24	1
Crk	NM_133656	3	1
D10Ertd522e	NM_026065	6	1
DITERTAOUSE	NM_020025	20	1
D3JJ11 D420024E16D3k	NM_178608	20	1
E030012C15Dik	AK053136	4	1
E050012C15KiK Ens15-rs	NM 007944	24	1
Epsi5-is Frh	NM_007951	24 4	1
En Falz	BC037661	9	8
Fcgr2h	NM 010187	8	2
Fnth	NM 145927	12	1
Fthfsdc1	NM 172308	28	1
Gabarapl2	NM_026693	5	1
Gpc3	NM_016697	8	1
Gpil	NM_008155	18	16
Gpr1	NM_146250	3	1
Hrasls3	NM_139269	5	1
Ifitm1	NM_026820	3	2
Il17d	NM_145837	3	1
IMAGE:3494995	BC046463	19	3
Immp2l	NM_053122	7	1
Lgals7	NM_008496	4	1
Lman2	NM_025828	8	1
LSM/ Manhal	NM_025349	4	1
Mandal Mms27	AK007399 NM 172757	5 11	1
Mrps27 Mrps31	NM_020560	7	1
Msra	NM_026322	6	1
Ndo?	NM 175329	4	1
Ndufa7	NM_023202	4	1
Nnr1	NM_008727	23	1
Nt5c1b	NM_027588	9	1
Pecam1	BC008519	14	1
Pfdn1	NM_026027	4	1
Plvap	NM_032398	6	1
Popdc2	NM_022318	4	1
Praf2	NM_138602	3	1
Prcc	NM_033573	7	1
Pum1	NM_030722	22	2
Rab27a	NM_023635	6	1
Ralgps1	NM_175211	20	19
Rbks	NM_153196	8	1
Rpl23	NM_022891	5	4
Sdsl	NM_133902	8	1
Sgce	NM_011360	11	1
Sult2b1	NM_017465	7	1
1 add31 Tract1	INM_133932	9	1
1 pSt1 Vars	NM 13/151	13	5
i ui s Vwhaz	NM 011740	15	1
Zechell	NM 026470	4	1
Zfn297h	NM 027947	4	1
1110008L16Rik	AK031144	7	1
1110014C03Rik	NM_026775	4	1

Table 1. Co	ontinued
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Gene identity		Total number	Number of trapped exons <sup>a</sup>
Gene symbol	Accession number	of exons	aupped enons
1110014D18Rik	NM_026746	7	1
1110031B06Rik	NM_144521	5	1
1600010M07Rik	AK005418	4	1
1810013L24Rik	AK007485	4	2
2600006L11Rik	AK011166	3	1
2610033C09Rik	NM_026407	9	1
2810410M20Rik	NM_024428	5	1
2810417J12Rik	NM_029798	3	1
2900010M23Rik	NM_026063	4	1
4930579G22Rik	NM_026916	3	1
4930583C14Rik	NM_029472	5	1
4933427L07Rik	NM_027727	17	1
4933439F18Rik	NM_025757	6	1
9230116N13Rik	AK033832	3	2

<sup>a</sup>Number of exons downstream of vector-integration site. Genes consisting of two exons were excluded from analysis.

The UPATrap retrovirus vector was used to infect mouse ES cells, and G418-resistant clones were then selected. After obtaining cDNA fragments of the trapped genes by 3' rapid amplification of cDNA ends (3' RACE) from the downstream portions of the NEO-trapped gene fusion transcripts, we determined their nucleotide sequences to generate gene-trap sequence tags and assessed intragenic distribution of the vector-integration sites for one hundred known genes. As shown in Figure 2B, 71 clones (71%) had insertional mutations in the upstream regions of the trapped genes, and multiple downstream exons were disrupted by the vector integration (see Table 2 for examples). Only six clones (6%) carried an insertion in the 3'-most intron of the trapped gene. The size of the deduced protein deletions due to gene trapping was also significantly larger for the UPATrap than for the standard poly-A-trapping method (Figure 2C). These data indicate that the strongly biased selection of the vector-integration sites in a standard poly-A-trap strategy was corrected for by the UPATrap system. Rather, ES cell clones which integrated the UPATrap vector near the 5' end of genes were frequently isolated, probably reflecting the integration-site preference of Moloney murine leukemia virus (23). A preliminary analysis (n = 50) also showed that there was no strong skewing in the reading-frame pattern of the trapped gene portion. In 34%, 24% and 42% of the clones, the trapped exons used the reading frames of the ICs #1, #2 and #3, respectively, located downstream of the IRES sequence in the NEO cassette of the UPATrap vector.

Next, we analyzed the nature of genes identified using the UPATrap system. Since 41 out of 80 genes listed in Table 2 do not have corresponding expressed sequence tags (ESTs) in current databases which have been derived from undifferentiated ES cells (data not shown), mRNA expression in ES cells was examined for six of these genes using the reverse transcription-mediated polymerase chain reaction (RT–PCR), and we found that three did not give rise to discrete cDNA bands (Figure 3A). This indicates that transcriptionally inactive genes in ES cells can be trapped using the UPATrap vector as expected for poly-A-trapping strategies in general. Then, the GenBank homology analysis was performed for the whole range of sequence tags isolated in the UPATrap experiments to

A



Integrated UPATrap provirus (3' half) with trapped exons

**Figure 2.** Unbiased gene trapping using the UPATrap vector. (A) Critical elements in the UPATrap vector. The IRES sequence flanked by two tandem *lox*P signals and three ICs (IC  $\times$  3) were inserted between the NEO TC and SD sequence of the RET vector (10) (see Supplementary Figure 1 for details). Components other than the NEO cassette of the RET vector were basically unchanged, and several useful features including the high gene-disruption efficiency of the vector (9,10) have been utilized in the UPATrap system. Trapped exons are depicted as gray boxes. (B) Distribution of the vector-insertion sites within trapped genes. To identify the insertion sites, gene-trap sequence tags were analyzed for 200 ES cell clones in which known genes had been trapped using the RET (100 clones) or the UPATrap (100 clones) vector. Insertion events in the known genes consisting of only two exons were excluded from the analysis. Introns near the 5' and 3' ends were independently counted and excluded from those associated with vector integration into introns near 3' ends. (C) Size of the deduced protein deletions due to gene trapping. The proportions of protein-coding sequences (CDSs) located 3' to the vector-integration sites were analyzed for 200 ES cell clones is were analyzed for 200 ES cell clones in the RET (100 clones) or the UPATrap (100 clones) vector. Insertion events in the known genes consisting of only two exons were excluded from the analysis. Introns near the 5' and 3' ends were defined as being located 5' and 3' to the middle excluded from those associated with vector integration into introns near 3' ends. (C) Size of the deduced protein deletions due to gene trapping. The proportions of protein-coding sequences (CDSs) located 3' to the vector-integration sites were analyzed for 200 ES cell clones in which known genes had been trapped using the RET (100 clones) vector.

elucidate the spectrum of genes disrupted using this system. As shown in Figure 3B, the proportion of matches to known (nonredundant) genes increased by 32% as compared to the previously obtained data using the conventional RET vector, suggesting that the improvement of the vector design reduced the rate of ES cell clones in which non-functional DNA segments had been trapped as artifact. The molecular basis for the contrasting nature of poly-A trapping created by the RET and UPATrap vectors (Figures 2 and 3) can only be explained by the structural difference in their NEO cassettes because a variant of the UPATrap vector containing EGFP instead of hrGFP showed a pattern of poly-A trapping identical to that of the original UPATrap vector (data not shown).

#### Suppression of NMD permits unbiased gene trapping

To examine whether unbiased poly-A trapping was made possible by the IRES-mediated suppression of NMD, we performed Cre-mediated excision of the floxed IRES portion from the genome of two ES cell clones in which multiple

 Table 2. Known genes and their exons trapped using the UPATrap vector

Gene symbol         Accession number         Control         Composition           Adgalt         NM_001004150         3         2           Aldh3b1         NM_026316         10         8           Ankrd25         NM_145611         12         9           App51         NM_0097417         5         4           Blmh         NM_178645         12         6           C33001112Rik         AK049181         12         1           Cracc         NM_016746         12         3           Cend3         AK083384         7         6           Cidea         NM_007702         5         2           Commd7         NM_138500         7         4           Crip1         AK088267         5         3           Daf         NM_029787         9         8           Dan2         NM_007871         19         18           Eifelp1         NM_002788         12         11           Eno1         NM_022448         6         6           Figd1         NM_008001         8         17           Figd1         NM_008035         6         3           Goiph2         NM_010346	Gene identity		Total number of exons	Number of trapped exons
A4galt       NM_001004150       3       2         Aldh3b1       NM_026316       10       8         Ankrd25       NM_145611       12       9         Apg51       NM_007477       5       4         Blz12       NM_007471       3       1         Blmh       NM_178645       12       6         C330011112Rik       AK049181       12       1         C730048E16Rik       NM_144849       8       7         Ccnc       NM_016746       12       3         Ccmain       NM_007702       5       2         Commd7       NM_133850       7       4         Crip1       AK083267       5       3         Cimb11       NM_025680       16       5         Dal       NM_02787       9       8         Dma2       NM_007871       19       18         Eifdelp1       NM_007918       2       11         Emp1       NM_010128       5       4         Eno1       NM_025644       8       6         Fgd1       NM_008001       17       7         Fnb1       NM_010345       16       11         Grb7	Gene symbol	Accession number		aupped enone
Alāhšbi       NN_026316       10       8         Ankrd25       NM_145611       12       9         Apg51       NM_053069       8       3         Arf2       NM_007477       5       4         Bcl2       NM_009741       3       1         Blmh       NM_178645       12       6         C30048E16Rik       NM_144849       8       7         Cenc       NM_007702       5       2         Commd7       NM_014746       12       3         Cidea       NM_007702       5       2         Commd7       NM_133850       7       4         Cripl       AK083384       7       6         Cidea       NM_007702       5       2         Commd1       NM_025680       16       5         Daf3       NM_022994       13       6         Dial       NM_007918       3       2         Eifdelp1       NM_0023119       2       11         Enol       NM_010128       5       4         Enol       NM_023616       13       6         Fabr2       NM_008035       6       3         Fold2       <	A4galt	NM_001004150	3	2
Ankrd25         NM_145611         12         9           Apg51         NM_053069         8         3           Arf2         NM_007477         5         4           Bcl2         NM_007477         5         4           Bcl2         NM_007477         5         4           Bcl2         NM_007477         5         4           C30048E16Rik         NM_178645         12         6           Crac         NM_016746         12         3           Ccnd3         AK083384         7         6           Cidea         NM_007702         5         2           Commd7         NM_13850         7         4           Crip1         AK08267         5         3           Cimub1         NM_022964         18         13           Dap3         NM_022987         9         8           Din1         NM_029787         9         8           Din2         NM_007918         3         2           Emp1         NM_01018         12         11           Enol         NM_010319         12         11           Enol         NM_0025564         8         6 <t< td=""><td>Aldh3b1</td><td>NM_026316</td><td>10</td><td>8</td></t<>	Aldh3b1	NM_026316	10	8
Apg51         NM_053069         8         3           Arf2         NM_007471         5         4           Bcl2         NM_007411         3         1           Blmh         NM_178645         12         6           C330011112Rik         AK049181         12         1           Crac         NM_016746         12         3           Ccnda         AK083844         7         6           Cidea         NM_007702         5         2           Commd7         NM_133850         7         4           Cripl         AK088267         5         3           Cmbl1         NM_025680         16         5           Daff         NM_029787         9         8           Dum2         NM_007871         19         18           Eifdebp1         NM_007918         3         2           Emp1         NM_010128         5         4           Enol         NM_019406         9         3           Foh2         NM_008001         18         17           Fhp1         NM_01345         16         11           Groph2         NM_010346         5         14	Ankrd25	NM_145611	12	9
Arf2       NM_007477       5       4         Bcl2       NM_009741       3       1         Blmh       NM_178645       12       6         C30004BLIGRik       NM_144849       8       7         Ccnc       NM_016746       12       3         Ccnd3       AK083384       7       6         Cidea       NM_007702       5       2         Commd7       NM_133850       7       4         CripJ       AK088267       5       3         CimubII       NM_022660       16       5         Dap3       NM_0229787       9       8         Dap3       NM_029787       9       8         Dmn2       NM_007918       3       2         Emp1       NM_007918       3       2         Emp1       NM_010128       5       4         Eno3       AK002485       12       11         Eno3       AK002485       12       11         Eno3       AK002485       12       11         Eno1       NM_010346       15       14         Grb10       NM_010345       16       11         Grb77       NM_0103	Apg5l	NM_053069	8	3
Bcl2         NM_009741         3         1           C330011112Rik         AK049181         12         1           C730048E16Rik         NM_1178645         12         3           Ccnc         NM_016746         12         3           Ccnd3         AK083384         7         6           Cidea         NM_007702         5         2           Commd7         NM_133850         7         4           Crip1         AK088267         5         3           Commd7         NM_025680         16         5           DBErtd354e         AK035264         18         13           Daja         NM_029787         9         8           Dmn2         NM_007918         3         2           Emp1         NM_007918         3         2           Emp1         NM_010128         5         4           Eno3         AK002485         12         11           Epn2         NM_010148         10         4           Exoscl         NM_027307         10         7           Grip1         NM_01345         16         11           Grb7         NM_016786         7         4	Arf2	NM_007477	5	4
Binn         NM_1/3645         12         6           C330011112Rik         AK049181         12         1           C730048E16Rik         NM_1044849         8         7           Ccnc         NM_016746         12         3           Ccidea         NM_007702         5         2           Commd7         NM_133850         7         4           Crip1         AK088267         5         3           Cmnb11         NM_025980         16         5           DBErtd354e         AK035264         18         13           Dap3         NM_022994         13         6           Dial         NM_007918         3         2           Emp1         NM_001718         5         4           Enol         NM_023119         12         11           Eno3         AK002485         12         11           Eno3         AK002485         12         11           Eno3         AK002485         12         11           Eno3         AK002485         12         11           Grap1         NM_019406         9         3           Folt2         NM_00805         6         3 <td>Bcl2</td> <td>NM_009741</td> <td>3</td> <td>1</td>	Bcl2	NM_009741	3	1
Coston 1112K1K AK049181 12 1 Crade AK049181 12 3 Crade AK083384 7 6 Crade NM_007702 5 2 Commd7 NM_133850 7 4 Crip1 AK088267 5 3 Crimb11 NM_025680 16 5 D8Errd354e AK035264 18 13 Dap3 NM_022994 13 6 Dial NM_029787 9 8 Dnm2 NM_007918 3 2 Emp1 NM_007918 3 2 Emp1 NM_007918 5 4 Eno1 NM_02198 5 4 Eno1 NM_02198 5 4 Eno1 NM_002485 12 11 Epn2 NM_010148 10 4 Exosc1 NM_02304 8 6 Fgdl NM_008003 6 3 Golph2 NM_008035 6 3 Golph2 NM_010345 16 11 Grb7 NM_010345 16 11 Grb7 NM_010346 15 14 Grb10 NM_013541 7 6 Grb10 NM_013541 7 6 Grb10 NM_013541 7 6 Hip2 NM_016884 10 5 Jarid1b NM_152895 27 26 Kif27 NM_016884 10 5 Jarid1b NM_02307 0 Grb10 NM_01345 16 11 Grb7 NM_016864 7 4 Harpc NM_01686 7 4 Harpc NM_01686 15 14 Grb7 NM_016864 10 5 Jarid1b NM_02396 9 7 Lman21 BC046969 8 7 M60rbp1 NM_02396 9 7 Lman21 BC046969 8 7 M60rbp1 NM_025533 9 8 NGC:99447 BC072561 20 7 Mip NM_00800 4 3 M6C:99447 BC072561 20 7 Mip NM_02533 9 8 NM_02533 9 8 Npl NM_025533 9 8 Npl NM_02676 24 13 Np2 NM_0159 44 10 Sirr6 NM_181586 8 6 Sic12ad NM_009195 24 23 Sox5 AK029047 12 11 Src BC039953 14 13 Tacc3 NM_001152 4 6 Sirr6 NM_181582 7 6 Sic12ad NM_0031182 7 6 Sirr6 NM_011524 7 5 Sic244 NM_0031182 7 6 Sirr6 NM_011524 7 5 Sic244 NM_0011524 7 5 Sic244 NM_0011524 7 5 Sic244 NM_0011524 16 Sirr6 NM_181582 7 6 Sic12ad NM_0011582 7 6 Sic12ad N	Blmh C220011110Dil	NM_178645	12	6
$\begin{array}{cccc} Conconstruction NM_124369 & 3 & 7 \\ Cence NM_016746 & 12 & 3 \\ Cend3 & AK083384 & 7 & 6 \\ Cidea & NM_007702 & 5 & 2 \\ Commd7 & NM_133850 & 7 & 4 \\ Crip1 & AK088267 & 5 & 3 \\ Crip1 & AK088267 & 5 & 3 \\ Dap3 & NM_0225980 & 16 & 5 \\ D8Ertd354e & AK035264 & 18 & 13 \\ Dap3 & NM_029787 & 9 & 8 \\ Dnm2 & NM_007918 & 3 & 2 \\ Emp1 & NM_007918 & 3 & 2 \\ Emp1 & NM_001128 & 5 & 4 \\ Enol & NM_023119 & 12 & 11 \\ Eno3 & AK002485 & 6 & 3 \\ Golph2 & NM_0019140 & 9 & 3 \\ Fohr2 & NM_008001 & 18 & 17 \\ Fihp1 & NM_019406 & 9 & 3 \\ Fohr2 & NM_008035 & 6 & 3 \\ Golph2 & NM_01345 & 16 & 11 \\ Grb7 & NM_010345 & 16 & 11 \\ Grb7 & NM_010346 & 15 & 14 \\ Grsp1 & NM_016786 & 7 & 4 \\ Hnrpc & NM_008706 & 4 & 3 \\ mKIAA0978 & AK122413 & 12 & 11 \\ Nbr1 & NM_008676 & 24 & 13 \\ Nfa & NM_00898 & 16 & 11 \\ Nbr1 & NM_00898 & 16 & 11 \\ Nbr1 & NM_00898 & 16 & 11 \\ Nbr2 & NM_00898 & 16 & 11 \\ Nbr1 & NM_008976 & 24 & 13 \\ Nfa & NM_008981 & 9 & 6 \\ Pde9a & NM_008981 & 9 & 15 \\ Pohr2e & BC045521 & 7 & 5 \\ Pomt1 & NM_145145 & 20 & 11 \\ Prkar2a & NM_009981 & 9 & 6 \\ Pde9a & NM_00894 & 19 & 15 \\ Pohr2e & NM_00875 & 24 & 23 \\ Sox5 & AK029047 & 12 & 11 \\ Src & BC039953 & 14 & 13 \\ Tacc3 & NM_001182 & 7 & 6 \\ \end{array}$	C330011J12K1K	AK049181 NM 144840	12	1
Cind AKUS13940 12 5 Cind AKUS13940 7 6 Cidea NM_007702 5 2 Commd7 NM_133850 7 4 Cijpl AKUS8267 5 3 Cimb11 NM_025680 16 5 D8Ertd354e AKUS5264 18 13 Dap3 NM_022994 13 6 Dial NM_02994 13 6 Dial NM_02994 13 2 Eif4ebp1 NM_007871 19 18 Eif4ebp1 NM_007871 19 18 Eif4ebp1 NM_00781 3 2 Emp1 NM_010128 5 4 Enol NM_023119 12 11 Eno3 AKU02485 12 11 Eno3 AKU02485 12 11 Eno3 AKU02485 12 11 Eno3 AKU02485 6 3 Golph2 NM_025644 8 6 Fgd1 NM_008001 18 17 Fnbp1 NM_010346 9 3 Golph2 NM_027307 10 7 Grb10 NM_010345 16 11 Grb7 NM_010345 15 14 Gstp1 NM_01686 7 4 Hip2 NM_01686 7 Jarid1b NM_152895 27 26 Kif27 NM_175214 17 2 Kremen NM_032396 9 7 Lman21 BC046969 8 7 M6prbp1 NM_025836 9 7 Lman21 BC046969 4 3 mKIAA0978 AK122413 12 11 Nbr1 NM_008676 24 13 Nf2 NM_008876 11 Nolc1 AKU34817 13 12 Nolc1 AKU34817 13 12 NM_008676 24 13 Nf2 NM_008876 14 Jarid1b NM_125833 9 8 Npl NM_025733 9 8 Npl NM_025733 9 8 Npl NM_025733 9 8 Npl NM_025833 9 8 Npl NM_028749 12 8 Nip205 AK129093 43 7 Pcyt1a NM_008877 7 4 Socip NM_008874 15 12 Seci4a NM_00981 9 6 Prx NM_01817 7 4 Rarg MM_01817 7 4 Seci4a NM_008924 10 9 Prx NM_018417 13 12 Seci4a NM_008924 10 9 Prx NM_018417 13 12 Seci4a NM_00981 9 6 Prax NM_019412 6 Seci4a NM_008924 10 9 Prx NM_01817 7 4 Seci4 NM_01817 7 4 Seci4 NM_01818 7 Ci 1 Seci4 NM_018459 14 10 Sirr6 NM_181586 8 Ci 22 Seci44 NM_001859 14 10 Sirr6 NM_181586 8 Ci 22 Seci4 NM_011524 16 Sirr6 NM_181586 8 Ci 22 Seci4 NM_001859 14 Sirr6 NM_181586 8 Ci 22 Seci4 NM_001859 14 Sirr6 NM_181586 7 Seci4 Sirr6 Sirr6 Sirr6 15 Seci4 NM_001182 7 Ci 2 Seci4 NM_0018182 7 Seci4 Sirr6 NM_01182 7 Seci4 Sirr6 Sirr6 Sirr6 Sirr	Cr50046ETORIK	NM 016746	0 12	3
Cidea NM_007702 5 2 Commd7 NM_133850 7 4 Crip1 AK088267 5 3 Crimbl1 NM_025680 16 5 D8Ert4354e AK035264 18 13 Dap3 NM_022994 13 6 Dial NM_0229787 9 8 Dnm2 NM_007918 3 2 Eif4ebp1 NM_007918 5 4 Eif4ebp1 NM_007918 5 4 Eno1 NM_023119 12 11 Eno3 AK002485 12 11 Epn2 NM_010148 10 4 Exoscl NM_02485 12 11 Epn2 NM_010148 10 4 Exoscl NM_02544 8 6 Fgd1 NM_008001 18 17 Fnbp1 NM_019406 9 3 Fohr2 NM_008035 6 3 Golph2 NM_01345 16 11 Grb10 NM_01345 16 11 Grb7 NM_010345 15 14 Gstp1 NM_01346 15 14 Gstp1 NM_015895 27 26 Kif27 NM_016786 7 4 Hnrpc NM_016786 8 7 MGC:99447 BC072561 20 7 Mip NM_02533 9 8 NGC:99447 BC072561 20 7 Mip NM_008676 24 13 N/2 NM_00876 24 13 N/2 NM_00876 24 13 N/2 NM_00876 24 13 N/2 NM_00876 24 13 N/2 NM_00877 4 AK124078 AK122413 12 11 Nbr1 NM_028749 12 8 Ng1 NM_025533 9 8 Npl NM_02553 9 8 Npl NM_	Cend3	AK083384	7	6
Commd7       NL_133850       7       4         Cripl       AK088267       5       3         Crinbll       NM_025680       16       5         DBErtd354e       AK035264       18       13         Dag3       NM_022994       13       6         Dial       NM_029787       9       8         Dnm2       NM_007871       19       18         Eifdebpl       NM_0023119       12       11         Enol       NM_010128       5       4         Enol       NM_01148       10       4         Exoscl       NM_025644       8       6         Fgdl       NM_010446       9       3         Folp1       NM_010345       16       11         Grb10       NM_010345       16       11         Grb7       NM_01684       10       5         Jarid1b       NM_175214       17       2         Kremen       NM_032396       9       7         Macf       AF150755       94       93         MGC:99447       BC0245960       4       3         Nk1       NU<088806	Cidea	NM 007702	5	2
Cripl         AK088267         5         3           Cmnbll         NM_022680         16         5           D8Ertd354e         AK035264         18         13           Dap3         NM_022994         13         6           Dial         NM_02787         9         8           Dial         NM_007871         19         18           Eifdebpl         NM_007871         19         18           Eifdebpl         NM_007871         19         18           Enol         NM_010128         5         4           Enol         NM_010148         10         4           Exoscl         NM_025644         8         6           Fgdl         NM_00805         6         3           Golph2         NM_010345         16         11           Grb10         NM_010345         16         14           Grb10         NM_010355         17         26           Kij27         NM_016786         7         4           Hmrpc         NM_016884         10         5           JaridIb         NM_152895         27         26           Kij27         NM_016884         10         5	Commd7	NM 133850	7	4
CrumbllNM_025860165D8Ertd354eAK0352641813Dap3NM_022994136DialNM_022978798Dnm2NM_0078711918EifdebplNM_00791832Emp1NM_01012854Eno1NM_0231191211Epn2NM_010148104ExoscINM_02564486FgdINM_00803563Golph2NM_0103451611Grb10NM_0103451611Grb7NM_0103451611Grb7NM_01678674HmpcNM_01678674HmpcNM_01528952726Kif27NM_175214172KremenNM_02230687MacfAF1507559493MGC:99447BC072561207MipNM_00860043mKLA0078AK1224131211Nbr1NM_0086762413Ng2NM_0108841915PortlaNM_008801915PortlaNM_0088041915PortlaNM_0088041915PortlaNM_0081774RargNM_0184152011Prka2aNM_0080774ScribNM_134089389Prka2aNM_0086015	Crip1	AK088267	5	3
$D8Ertd354e$ AK035264       18       13 $Dap3$ NM_022987       9       8 $Dmm2$ NM_007871       19       18 $Eifdebpl$ NM_007918       3       2 $Empl$ NM_010128       5       4 $Enol$ NM_023119       12       11 $Eno3$ AK002485       6       3 $Golph2$ NM_010366       9       3 $Folr2$ NM_008035       6       3 $Golph2$ NM_010346       15       14 $Gstp1$ NM_013541       7       6 $Hirpc$ NM_016864       0       5 $Jaridlb$ NM_152895       27       26 $Ki27$ NM_175214       17       2 $Kremen$ NM_023396       9       7 $Macrf       AK122413       12       11     $	Ctnnbl1	NM_025680	16	5
$\begin{array}{llllllllllllllllllllllllllllllllllll$	D8Ertd354e	AK035264	18	13
DialNM_02978798Dnm2NM_0078711918EifdebplNM_00791832EmplNM_01012854Eno1NM_0231191211Eno3AK0024851211Epn2NM_010148104ExoxclNM_02564486FgdlNM_0280011817FnbplNM_01940693FolzNM_00800563Golph2NM_027307107Grb10NM_0103451611Grb7NM_0103461514Gstp1NM_0158476Hip2NM_01678674HnrpcNM_016884105JaridlbNM_03239697Lman2lBC04696987MdorfAF1507559493MGC:99447BC072561207MipNM_00880043Nf1NM_0287391611Nbr1NM_008801611Nol<1NM_008801611Nol<1NM_028749128NplNM_0181774RargNM_0181774RargNM_0181774RargNM_0181774RargNM_0181774RargNM_0181774RargNM_0184512ScribNM_134089 </td <td>Dap3</td> <td>NM_022994</td> <td>13</td> <td>6</td>	Dap3	NM_022994	13	6
$\begin{array}{llllllllllllllllllllllllllllllllllll$	Dial	NM_029787	9	8
$Elf4ep1$ NM_00/91832 $Emp1$ NM_01012854 $Eno1$ NM_0231191211 $Eno3$ AK0024851211 $Eno3$ AK0024851211 $Epn2$ NM_010148104 $Exosc1$ NM_02564486 $Fgd1$ NM_01940693 $Folr2$ NM_0080011817 $Fnbp1$ NM_01940693 $Folr2$ NM_00803563 $Golph2$ NM_0103451611 $Grb7$ NM_0103451611 $Grb7$ NM_01354176 $Hip2$ NM_01678674 $Hnrpc$ NM_016884105 $Jaridlb$ NM_1528952726 $Kij27$ NM_01586087 $Macf$ AF1507559493 $MGC:99447$ BC072561207 $Mip$ NM_00860643 $nKIAA0978$ AK1224131211 $Nbr1$ NM_0086762413 $Nf2$ NM_0108981611 $Nolc1$ AK0348171312 $Nolc1$ AK0348171312 $Nolc1$ AK0348171312 $Nolc1$ AK0348171516 $Polr2e$ BC04552175 $Pourla$ NM_008924109 $Prkar2a$ NM_0089389 $Serid$ NM_011244101 <td>Dnm2</td> <td>NM_007871</td> <td>19</td> <td>18</td>	Dnm2	NM_007871	19	18
$Emp1$ NM_01012854 $Enol$ NM_01231191211 $Eno3$ AK0024851211 $Eno3$ AK0024851211 $Epn2$ NM_010148104 $Exoscl$ NM_02564486 $FgdI$ NM_0080011817 $Fnbp1$ NM_01940693 $Folr2$ NM_00803563 $Golph2$ NM_027307107 $Grb10$ NM_0103451611 $Grb7$ NM_0103461514 $Gstp1$ NM_01678674 $Hnrpc$ NM_01678674 $Hnrpc$ NM_016884105 $Jariallb$ NM_1528952726 $Kij27$ NM_175214172 $Kremen$ NM_02583687 $Mopripl$ NM_02583687 $Macf$ AF1507559493 $MGC:99447$ BC072561207 $Mip$ NM_00860043 $mKLA0978$ AK1224131211 $Nbr1$ NM_0086762413 $Nf2$ NM_0108861611 $NoclAK0348171312NosipNM_02553398Np1NM_028749128Nup205AK129093437PetrlaNM_008924109Prkar2aNM_008924109Prkar2aNM_0181774<$	Eif4ebp1	NM_00/918	3	2
$Enol$ $NM_{-0.23119}$ $12$ $11$ $Eno3$ $AK002485$ $12$ $11$ $Epn2$ $NM_{-010148}$ $10$ $4$ $Exoscl$ $NM_{-025644}$ $8$ $6$ $Fgdl$ $NM_{-008001}$ $18$ $17$ $Fnhpl$ $NM_{-019406}$ $9$ $3$ $Folr2$ $NM_{-008035}$ $6$ $3$ $Golph2$ $NM_{-01345}$ $16$ $11$ $Grb7$ $NM_{-010345}$ $16$ $11$ $Grb7$ $NM_{-010345}$ $16$ $11$ $Grb7$ $NM_{-010345}$ $16$ $11$ $Grb7$ $NM_{-013541}$ $7$ $6$ $Hip2$ $NM_{-016884}$ $10$ $5$ $Jarid1b$ $NM_{-15295$ $27$ $26$ $Kij27$ $NM_{-015835$ $8$ $7$ $Macf$ $AF150755$ $94$ $93$ $MGcf$ $AK122413$ $12$ $11$ $Nbr1$ $NM_{-008600$ $4$ $3$ $mKIAA0978$ $AK122413$ $12$ $11$ $Nbr1$ $NM_{-0088749$ $12$ $8$ $Ng205$ $AK122413$ $12$ $11$ $Nbr1$ $NM_{-028749$ $12$ $8$ $Ng205$ $AK12993$ $43$ $7$ $Pcyt1a$ $NM_{-008804$	Emp1	NM_010128	5	4
$Ends$ ANO24651211 $Epn2$ NM_010148104 $Exoscl$ NM_02564486 $Fgdl$ NM_0080011817 $Fnbpl$ NM_01940693 $Folr2$ NM_00803563 $Golph2$ NM_027307107 $Grb10$ NM_0103451611 $Grb7$ NM_0103461514 $Gstp1$ NM_0168674 $Hnpc$ NM_0168674 $Hnpc$ NM_016884105 $Jaridlb$ NM_523952726 $Kij27$ NM_175214172 $Kremen$ NM_02339697 $Macf$ AF1507559493 $MGcr99447$ BC072561207 $Mip$ NM_00860043 $mKIAA0978$ AK1224131211 $Nbrl$ NM_0088762413 $Nf2$ NM_0108981611 $Nolcl$ AK0348171312 $Nosip$ NM_028749128 $Npl$ NM_0088041915 $Polr2e$ BC04552175 $Pom11$ NM_0184774 $Rarg$ NM_011244101 $Rarg$ NM_011244101 $Rarg$ NM_0136591410 $Sirrb$ NM_134089389 $Sec24a$ NM_0136591410 $Sirrb$ NM_134089389 <td>Enol Enol</td> <td>NM_025119</td> <td>12</td> <td>11</td>	Enol Enol	NM_025119	12	11
$Lpnz$ $NM_025644$ $8$ $6$ $Fgdl$ $NM_008001$ $18$ $17$ $Fnbpl$ $NM_0019406$ $9$ $3$ $Folr2$ $NM_008035$ $6$ $3$ $Golph2$ $NM_027307$ $10$ $7$ $Grb10$ $NM_010345$ $16$ $11$ $Grb7$ $NM_010346$ $15$ $14$ $Gstp1$ $NM_013541$ $7$ $6$ $Hip2$ $NM_016786$ $7$ $4$ $Hnrpc$ $NM_016884$ $10$ $5$ $Jaridlb$ $NM_152895$ $27$ $26$ $Kij27$ $NM_015786$ $7$ $4$ $Hnrpc$ $NM_025836$ $8$ $7$ $Mocf$ $AF150755$ $94$ $93$ $MGcr.99447$ $BC072561$ $20$ $7$ $Mip$ $NM_008600$ $4$ $3$ $mKlA00978$ $AK122413$ $12$ $11$ $Nbrl$ $NM_008676$ $24$ $13$ $Ng2$ $NM_010898$ $16$ $11$ $Nolc1$ $AK034817$ $13$ $12$ $Nasip$ $NM_025533$ $9$ $8$ $Npl$ $NM_0028749$ $12$ $8$ $Nup205$ $AK129093$ $43$ $7$ $Pexta<$ $NM_008804$ $19$ $15$ $Polr2e$ $BC045521$ $7$ $5$ $Pomtl$ $NM_01847$ $7$ $4$ $Rarg$ $NM_010817$ $7$ $4$ $Rarg$ $NM_010847$ $10$ $9$ $Prkar2a$ $NM_0108417$ $7$ $4$ $Ra$	Enos Enn?	NM 010148	12	11
Exist: $NM_008001$ 1817Fnbp1 $NM_009001$ 1817Fnbp1 $NM_009035$ 63Folr2 $NM_0027307$ 107Grb10 $NM_010345$ 1611Grb7 $NM_010345$ 1611Grb7 $NM_010346$ 1514Gstp1 $NM_010346$ 1514Gstp1 $NM_016786$ 74Hnrpc $NM_016786$ 74Jarid1b $NM_152895$ 2726Kij27 $NM_016884$ 105Jarid1b $NM_025336$ 87M6prbp1 $NM_025336$ 87Macf $AF150755$ 9493MGC:99447 $BC072561$ 207Mip $NM_008600$ 43mKIAA0978 $AK122413$ 1211Nbr1 $NM_008676$ 2413Nf2 $NM_010898$ 1611Nolc1 $AK034817$ 1312Nosip $NM_025533$ 98Np1 $NM_025533$ 96Polr2e $BC045521$ 75Pomt1 $NM_019412$ 65Pasc $NM_010817$ 74Rarg $NM_011244$ 101Rarg $NM_011244$ 101Rarg $NM_011524$ 1615Scrib $NM_011524$ 1615Scrib $NM_011524$ 1615Scrib $NM_011524$ 16 <t< td=""><td>Epn2 Exosc1</td><td>NM_025644</td><td>8</td><td>6</td></t<>	Epn2 Exosc1	NM_025644	8	6
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Fedl	NM 008001	18	17
$Foir2$ $NM_008035$ 63 $Golph2$ $NM_027307$ 107 $Grb10$ $NM_010345$ 1611 $Grb7$ $NM_010346$ 1514 $Gstp1$ $NM_0113541$ 76 $Hip2$ $NM_016786$ 74 $Hnrpc$ $NM_016884$ 105 $Jarid1b$ $NM_152895$ 2726 $Kij27$ $NM_175214$ 172 $Kremen$ $NM_023296$ 97 $Lman21$ $BC046969$ 87 $Macf$ $AF150755$ 9493 $MGC:99447$ $BC072561$ 207 $Mip$ $NM_008600$ 43 $mKIAA0978$ $AK122413$ 1211 $Nbr1$ $NM_008676$ 2413 $Nf2$ $NM_010898$ 1611 $Nolc1$ $AK034817$ 1312 $Nosip$ $NM_0225533$ 98 $Npl$ $NM_0028749$ 128 $Nup205$ $AK129093$ 437 $Pcytla$ $NM_009981$ 96 $Pde3a$ $NM_008804$ 1915 $Polr2e$ $BC045521$ 75 $Pom11$ $NM_145145$ 2011 $Prkar2a$ $NM_008804$ 1512 $Pcrib$ $NM_134089$ 389 $Sec24a$ $NM_013469$ 3 $Srcib$ $NM_013469$ 43 $Srcib$ $NM_013459$ 1410 $Sirt6$ $NM_013659$ 1410 <td>Fnbp1</td> <td>NM 019406</td> <td>9</td> <td>3</td>	Fnbp1	NM 019406	9	3
Golph2NM_027307107Grb10NM_0103451611Grb7NM_0103461514Gstp1NM_01354176Hip2NM_016884105JaridlbNM_1528952726Kij27NM_015214172KremenNM_02339697Lman21BC04696987M6prbp1NM_02583687M6prbp1NM_02583687MGC:99447BC072561207MipNM_00860043mKIAA0978AK1224131211Nbr1NM_0086762413Nf2NM_0108981611NosipNM_02573398NplNM_0088041915Polr2eBC04552175Pomt1NM_1451452011Prkar2aNM_0088041915Polr2eBC04552175Pomt1NM_1451452011Prkar2aNM_01941265Psmd7NM_01081774RargNM_011244101Raff111NM_036041512ScribNM_1134089389Sec24aNM_0136591410Sirt6NM_18158686Slc12a4NM_0091952423Sox5AK0290471211SrcBC03995314<	Folr2	NM_008035	6	3
Grb10NM_0103451611Grb7NM_0103461514Gstp1NM_01354176Hip2NM_01678674HmrpcNM_016884105Jarid1bNM_1528952726Kij27NM_175214172KremenNM_023239697Lman21BC04696987Móprbp1NM_02583687MacfAF1507559493MGC:99447BC072561207MipNM_00860043mKIAA0978AK1224131211Nbr1NM_0086762413Nf2NM_0108981611Nolc1AK0348171312NaipNM_022573398Nup205AK12093437Pcyt1aNM_0088041915Polr2eBC04552175Pomt1NM_1451452011Prka2aNM_008924109PrxNM_01081774RargNM_011244101RargNM_0134089389Sec24aNM_134089389Sec24aNM_0136591410Sirt6NM_118158686Slc12a4NM_0091952423Sox5AK0290471211SrcBC0399531413Tacc3NM_01152416 <t< td=""><td>Golph2</td><td>NM_027307</td><td>10</td><td>7</td></t<>	Golph2	NM_027307	10	7
$Grb7$ NM_0103461514 $Gstp1$ NM_01354176 $Hip2$ NM_01678674 $Hnrpc$ NM_016884105 $JaridIb$ NM_1528952726 $Kif27$ NM_175214172 $Kremen$ NM_03239697 $Lman2l$ BC04696987 $Macf$ AF1507559493 $MGC:99447$ BC072561207 $Mip$ NM_00860043 $mKIAA0978$ AK1224131211 $Nbr1$ NM_0086762413 $Nf2$ NM_0108981611 $Nolcl$ AK0348171312 $Nosip$ NM_02553398 $Npl$ NM_0088041915 $Polr2e$ BC04552175 $Pomtl$ NM_008924109 $Prx$ NM_0181774 $Rarg$ NM_0124101 $Rrf111$ NM_0336041512 $Scrib$ NM_134089389 $Sec24a$ NM_0136591410 $Sir6$ NM_0136591413 $Sac3$ NM_0115241615 $Sca3$ NM_0115241615 $Sca4$ NM_03118276	Grb10	NM_010345	16	11
Gstp1       NM_013541       7       6         Hip2       NM_016786       7       4         Hmrpc       NM_016884       10       5         JaridIb       NM_152895       27       26         Kif27       NM_175214       17       2         Kremen       NM_032396       9       7         Lman21       BC046969       8       7         M6prbp1       NM_025836       8       7         Macf       AF150755       94       93         MGC:99447       BC072561       20       7         Mip       NM_008600       4       3         mKIAA0978       AK122413       12       11         Nbr1       NM_008676       24       13         Nf2       NM_010898       16       11         Nolc1       AK034817       13       12         Nosip       NM_025533       9       8         Npl       NM_028749       12       8         Nup205       AK129093       43       7         Pcyt1a       NM_008804       19       15         Polr2e       BC045521       7       5         Pomt1	Grb7	NM_010346	15	14
Hip2       NM_016786       7       4         Hnrpc       NM_016884       10       5         Jarid1b       NM_152895       27       26         Kij27       NM_175214       17       2         Kremen       NM_032396       9       7         Lman21       BC046969       8       7         Mdorbp1       NM_025836       8       7         Macf       AF150755       94       93         MGC:99447       BC072561       20       7         Mip       NM_008600       4       3         mKIAA0978       AK122413       12       11         Nbr1       NM_008676       24       13         Nf2       NM_010898       16       11         Nolc1       AK034817       13       12         Nosip       NM_028749       12       8         Nup205       AK129093       43       7         Pcyt1a       NM_008804       19       15         Polr2e       BC045521       7       5         Pomt1       NM_145145       20       11         Prkar2a       NM_008924       10       9         Psmd7 <td>Gstp1</td> <td>NM_013541</td> <td>7</td> <td>6</td>	Gstp1	NM_013541	7	6
$Hnrpc$ NM_016884105 $Jarid1b$ NM_1528952726 $Kif27$ NM_175214172 $Kremen$ NM_03239697 $Lman21$ BC04696987 $M6prbp1$ NM_02583687 $Macf$ AF1507559493 $MGC:99447$ BC072561207 $Mip$ NM_00860043 $mKIAA0978$ AK12241312 $Nbr1$ NM_0086762413 $Nf2$ NM_0108981611 $Nolc1$ AK0348171312 $Nosip$ NM_02553398 $Npl$ NM_0088041915 $Poyla$ NM_00998196 $Pde9a$ NM_0088041915 $Polr2e$ BC04552175 $Pomt1$ NM_1451452011 $Prkar2a$ NM_01941265 $Psmd7$ NM_01081774 $Rarg$ NM_011244101 $Rnf111$ NM_036041512 $Scrib$ NM_134089389 $Sec24a$ NM_1752552322 $Sem4b$ NM_0136591410 $Sirt6$ NM_18158686 $Slc12a4$ NM_0091952423 $Sox5$ AK0290471211 $Src$ BC0399531413 $Tacc3$ NM_0115241615 $Tacc3$ NM_0118276<	Hip2	NM_016786	7	4
Jariato $NM_1122895$ $27$ $26$ Kif27 $NM_175214$ $17$ $2$ Kremen $NM_032396$ $9$ $7$ Lman2l $BC046969$ $8$ $7$ M6prbp1 $NM_025836$ $8$ $7$ Macf $AF150755$ $94$ $93$ MGC:99447 $BC072561$ $20$ $7$ Mip $NM_008600$ $4$ $3$ mK1AA0978 $AK122413$ $12$ $11$ Nbr1 $NM_008676$ $24$ $13$ Nf2 $NM_010898$ $16$ $11$ Nolc1 $AK034817$ $13$ $12$ Nosip $NM_025533$ $9$ $8$ Npl $NM_0028749$ $12$ $8$ Nup205 $AK129093$ $43$ $7$ Pcyt1a $NM_009981$ $9$ $6$ Pde9a $NM_008804$ $19$ $15$ Polr2e $BC045521$ $7$ $5$ Pomt1 $NM_145145$ $20$ $11$ Prkar2a $NM_008924$ $10$ $9$ Prx $NM_01817$ $7$ $4$ Rarg $NM_011244$ $10$ $1$ Rnf111 $NM_03604$ $15$ $12$ Scrib $NM_134089$ $38$ $9$ Sec24a $NM_013659$ $14$ $10$ Sirt6 $NM_181586$ $8$ $6$ Slc12a4 $NM_009195$ $24$ $23$ Sox5 $AK029047$ $12$ $11$ Src $BC039953$ $14$ $13$ Tacc3 $NM_011524$ $16$ $15$ </td <td>Hnrpc</td> <td>NM_016884</td> <td>10</td> <td>5</td>	Hnrpc	NM_016884	10	5
$Klg27$ $Klm_113214$ $17$ $22$ $Kremen$ $NM_032396$ $9$ $7$ $Lman2l$ $BC046969$ $8$ $7$ $M6prbp1$ $NM_025836$ $8$ $7$ $Macf$ $AF150755$ $94$ $93$ $MGC:99447$ $BC072561$ $20$ $7$ $Mip$ $NM_008600$ $4$ $3$ $mK1AA0978$ $AK122413$ $12$ $11$ $Nbrl$ $NM_008676$ $24$ $13$ $Nf2$ $NM_010898$ $16$ $11$ $Nolcl$ $AK034817$ $13$ $12$ $Nosip$ $NM_025533$ $9$ $8$ $Npl$ $NM_028749$ $12$ $8$ $Nup205$ $AK129093$ $43$ $7$ $Pcytla$ $NM_009981$ $9$ $6$ $Pde9a$ $NM_008804$ $19$ $15$ $Polr2e$ $BC045521$ $7$ $5$ $Pomtl$ $NM_145145$ $20$ $11$ $Prkar2a$ $NM_008924$ $10$ $9$ $Prx$ $NM_019412$ $6$ $5$ $Psmd7$ $NM_010817$ $7$ $4$ $Rarg$ $NM_011244$ $10$ $1$ $Rnf111$ $NM_03604$ $15$ $12$ $Scrib$ $NM_134089$ $38$ $9$ $Sec24a$ $NM_013659$ $14$ $10$ $Sirt6$ $NM_013659$ $14$ $10$ $Sirt6$ $NM_00195$ $24$ $23$ $Sax5$ $AK029047$ $12$ $11$ $Src$ $BC039953$ $14$ $13$ <th< td=""><td>Jaria10 K;f77</td><td>NM 175214</td><td>27</td><td>20</td></th<>	Jaria10 K;f77	NM 175214	27	20
InternalInt_OD200 $3$ $7$ Lman2lBC0469698 $7$ MacfAF1507559493MGC:99447BC07256120 $7$ MipNM_00860043mKIAA0978AK1224131211Nbr1NM_0086762413Nf2NM_0108981611Nolc1AK0348171312NosipNM_02553398NplNM_028749128Nup205AK12909343 $7$ PcytlaNM_0098196Pde9aNM_0088041915Pol2eBC045521 $7$ 5Pomt1NM_1451452011Prkar2aNM_008924109PrxNM_01941265Psmd7NM_010817 $7$ 4RargNM_011244101Rnf111NM_036041512ScribNM_134089389Sec24aNM_1752552322Sema4bNM_0136591410Sirt6NM_18158686Slc12a4NM_0091952423Sox5AK0290471211SrcBC0399531413Tacc3NM_0115241615Tcfap4NM_03118276	Kıj27 Kremen	NM 032396	9	27
LinkerDiscussion $3$ $7$ MoprippiNM_02583687MacfAF1507559493MGC:99447BC072561207MipNM_00860043mKIAA0978AK1224131211Nbr1NM_0086762413Nf2NM_0108981611Nolc1AK0348171312NosipNM_02553398NplNM_028749128Nup205AK129093437PcytlaNM_0088041915Polr2eBC04552175PomtlNM_1451452011Prkar2aNM_008924109PrxNM_01941265Psmd7NM_01081774RargNM_011244101Rnf111NM_036041512ScribNM_1814089389Sec24aNM_1752552322Sema4bNM_0136591410Sirt6NM_18158686Slc12a4NM_0091952423Sox5AK0290471211SrcBC0399531413Tacc3NM_0115241615Tcfap4NM_03118276	Lman21	BC046969	8	7
MacfAF1507559493MGC:99447BC072561207MipNM_00860043mKIAA0978AK1224131211Nbr1NM_0086762413Nf2NM_0108981611Nolc1AK0348171312NosipNM_02553398NplNM_028749128Nup205AK129093437PcytlaNM_0088041915Polr2eBC04552175Pomt1NM_1451452011Prkar2aNM_008924109PrxNM_01081774RargNM_011244101Rnf111NM_036041512ScribNM_134089389Sec24aNM_18158686Slc12a4NM_0091952423Sox5AK0290471211SrcBC0399531413Tacc3NM_0115241615Tcfap4NM_03118276	M6prbp1	NM 025836	8	7
$MGC:99447$ $BC072561$ $20$ $7$ $Mip$ $NM_008600$ $4$ $3$ $mKIAA0978$ $AK122413$ $12$ $11$ $Nbr1$ $NM_008676$ $24$ $13$ $Nf2$ $NM_010898$ $16$ $11$ $Nolc1$ $AK034817$ $13$ $12$ $Nosip$ $NM_025533$ $9$ $8$ $Npl$ $NM_025533$ $9$ $6$ $Nup205$ $AK129093$ $43$ $7$ $Pcytla$ $NM_009981$ $9$ $6$ $Pde9a$ $NM_008804$ $19$ $15$ $Polr2e$ $BC045521$ $7$ $5$ $Pomt1$ $NM_019817$ $7$ $4$ $Rarg$ $NM_019412$ $6$ $5$ $Psmd7$ $NM_010817$ $7$ $4$ $Rarg$ $NM_011244$ $10$ $1$ $Rnf111$ $NM_033604$ $15$ $12$ $Scrib$ $NM_134089$ $38$ $9$ $Sec24a$ $NM_013659$ $14$ $10$ $Sirt6$ $NM_013659$ $14$ $10$ $Sirt6$ $NM_001915$ $24$ $23$ $Sax5$ $AK029047$ $12$ $11$ $Src$ $BC039953$ $14$ $13$ $Tacc3$ $NM_011524$ $16$ $15$ $Tcfap4$ $NM_031182$ $7$ $6$	Macf	AF150755	94	93
MipNM_00860043mKIAA0978AK1224131211Nbr1NM_0086762413Nf2NM_0108981611Nolc1AK0348171312NosipNM_02553398Np1NM_028749128Nup205AK129093437Pcyt1aNM_00998196Pde9aNM_0088041915Polr2eBC04552175Pomt1NM_01941265Prkar2aNM_01941265Psmd7NM_01081774RargNM_011244101Rnf111NM_036041512ScribNM_134089389Sec24aNM_1752552322Sema4bNM_0031591410Sirt6NM_18158686Slc12a4NM_0091952423Sax5AK0290471211SrcBC0399531413Tacc3NM_0115241615Tcfap4NM_03118276	MGC:99447	BC072561	20	7
mKIAA0978AK1224131211Nbr1NM_0086762413Nf2NM_0108981611Nolc1AK0348171312NosipNM_02553398NplNM_028749128Nup205AK129093437Pcyt1aNM_0088041915Pohr2eBC04552175Pomt1NM_1451452011Prkar2aNM_008924109PrxNM_01941265Psmd7NM_01081774RargNM_011244101Rnf111NM_134089389Sec24aNM_135552322Sema4bNM_0031591410Sirt6NM_18158686Slc12a4NM_0091952423Sox5AK0290471211SrcBC0399531413Tacc3NM_0115241615Tcfap4NM_03118276	Mip	NM_008600	4	3
Nbr1NM_0086762413Nf2NM_0108981611Nolc1AK0348171312NosipNM_02553398NplNM_028749128Nup205AK129093437Pcyt1aNM_00998196Pde9aNM_0088041915Pohr2eBC04552175Pomt1NM_1451452011Prkar2aNM_008924109PrxNM_01941265Psmd7NM_01081774RargNM_011244101Rnf111NM_0336041512ScribNM_134089389Sec24aNM_0136591410Sirt6NM_18158686Slc12a4NM_0091952423Sox5AK0290471211SrcBC0399531413Tacc3NM_0115241615Tcfap4NM_03118276	mKIAA0978	AK122413	12	11
Nf2       NM_010898       16       11         Nolc1       AK034817       13       12         Nosip       NM_025533       9       8         Npl       NM_025749       12       8         Nup205       AK129093       43       7         Pcytla       NM_008804       19       15         Poh2e       BC045521       7       5         Pomtl       NM_008924       10       9         Prx       NM_010817       7       4         Rarg       NM_011244       10       1         Rnf111       NM_03604       15       12         Scrib       NM_134089       38       9         Sec24a       NM_175255       23       22         Sema4b       NM_0013659       14       10         Sirt6       NM_181586       8       6         Slc12a4       NM_009195       24       23         Sox5       AK029047       12       11         Src       BC039953       14       13         Tacc3       NM_011524       16       15         Tacc3       NM_011524       16       15	Nbr1	NM_008676	24	13
Note1AK03481/1312NosipNM_02553398NplNM_025749128Nup205AK129093437Pcyt1aNM_00998196Pde9aNM_0088041915Polr2eBC04552175Pont1NM_008924109PrxNM_01081774RargNM_01081774RargNM_011244101Rnf111NM_036041512ScribNM_134089389Sec24aNM_15552322Sema4bNM_0091952423Sox5AK0290471211SrcBC0399531413Tacc3NM_0115241615Tcfap4NM_03118276	Nf2	NM_010898	16	11
NostpNM_0235398 $Npl$ NM_028749128 $Nup205$ AK129093437 $Pcyt1a$ NM_00998196 $Pde9a$ NM_0088041915 $Polr2e$ BC04552175 $Pont1$ NM_1451452011 $Prkar2a$ NM_008924109 $Prx$ NM_01941265 $Psmd7$ NM_01081774 $Rarg$ NM_011244101 $Rnf111$ NM_036041512 $Scrib$ NM_134089389 $Sec24a$ NM_1752552322 $Sem4b$ NM_0136591410 $Sirt6$ NM_18158686 $Slc12a4$ NM_0091952423 $Sox5$ AK0290471211 $Src$ BC0399531413 $Tacc3$ NM_0115241615 $Tcfap4$ NM_03118276	NolCI	AK034817	13	12
$N\mu$ $NM_0050747$ $12$ $16$ $Nup205$ $AK129093$ $43$ $7$ $Pcyt1a$ $NM_009981$ $9$ $6$ $Pde9a$ $NM_008804$ $19$ $15$ $Polr2e$ $BC045521$ $7$ $5$ $Pont1$ $NM_145145$ $20$ $11$ $Prkar2a$ $NM_008924$ $10$ $9$ $Prx$ $NM_019412$ $6$ $5$ $Psmd7$ $NM_010817$ $7$ $4$ $Rarg$ $NM_011244$ $10$ $1$ $Rnf111$ $NM_03604$ $15$ $12$ $Scrib$ $NM_134089$ $38$ $9$ $Sec24a$ $NM_175255$ $23$ $22$ $Sem4b$ $NM_013659$ $14$ $10$ $Sirt6$ $NM_181586$ $8$ $6$ $Slc12a4$ $NM_009195$ $24$ $23$ $Sox5$ $AK029047$ $12$ $11$ $Src$ $BC039953$ $14$ $13$ $Tacc3$ $NM_011524$ $16$ $15$ $Tcfap4$ $NM_031182$ $7$ $6$	Nosip Nol	NM_028740	9	0 8
$Map205$ $MM_{200}$ $MM_{200}$ $H12505$ $H5$ $H7$ $Pcyt1a$ $NM_{009981}$ 96 $Pde9a$ $NM_{008804}$ 1915 $Polr2e$ $BC045521$ 75 $Pomt1$ $NM_{145145}$ 2011 $Prkar2a$ $NM_{008924}$ 109 $Prx$ $NM_{019412}$ 65 $Psmd7$ $NM_{010817}$ 74 $Rarg$ $NM_{011244}$ 101 $Rnf111$ $NM_{033604}$ 1512 $Scrib$ $NM_{134089}$ 389 $Sec24a$ $NM_{175255}$ 2322 $Sem4b$ $NM_{013659}$ 1410 $Sirt6$ $NM_{181586}$ 86 $Slc12a4$ $NM_{009195}$ 2423 $Sox5$ $AK029047$ 1211 $Src$ $BC039953$ 1413 $Tacc3$ $NM_{011524}$ 1615 $Tcfap4$ $NM_{031182}$ 76	Nun205	AK129093	43	7
Pde9a       NM_008804       19       15         Polr2e       BC045521       7       5         Pomt1       NM_145145       20       11         Prkar2a       NM_008924       10       9         Prx       NM_019412       6       5         Psmd7       NM_010817       7       4         Rarg       NM_011244       10       1         Rnf111       NM_033604       15       12         Scrib       NM_134089       38       9         Sec24a       NM_013659       14       10         Sirt6       NM_181586       8       6         Slc12a4       NM_009195       24       23         Sox5       AK029047       12       11         Src       BC039953       14       13         Tacc3       NM_011524       16       15         Tcfap4       NM_031182       7       6	Pcvtla	NM 009981	9	6
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	Pde9a	NM_008804	19	15
Pomtl         NM_145145         20         11           Prkar2a         NM_008924         10         9           Prx         NM_019412         6         5           Psmd7         NM_010817         7         4           Rarg         NM_011244         10         1           Rnf111         NM_03604         15         12           Scrib         NM_134089         38         9           Sec24a         NM_175255         23         22           Sema4b         NM_013659         14         10           Sirt6         NM_181586         8         6           Slc12a4         NM_009195         24         23           Sox5         AK029047         12         11           Src         BC039953         14         13           Tacc3         NM_011524         16         15           Tcfap4         NM_031182         7         6	Polr2e	BC045521	7	5
Prkar2a         NM_008924         10         9           Prx         NM_019412         6         5           Psmd7         NM_010817         7         4           Rarg         NM_011244         10         1           Rnf111         NM_03604         15         12           Scrib         NM_134089         38         9           Sec24a         NM_013659         14         10           Sirt6         NM_181586         8         6           Slc12a4         NM_009195         24         23           Sox5         AK029047         12         11           Src         BC039953         14         13           Tacc3         NM_011524         16         15           Tcfap4         NM_031182         7         6	Pomt1	NM_145145	20	11
$Prx$ NM_01941265 $Psmd7$ NM_01081774 $Rarg$ NM_011244101 $Rnf11$ NM_036041512 $Scrib$ NM_134089389 $Sec24a$ NM_0136591410 $Sirt6$ NM_18158686 $Slc12a4$ NM_0091952423 $Sox5$ AK0290471211 $Src$ BC0399531413 $Tacc3$ NM_0115241615 $Tcfap4$ NM_03118276	Prkar2a	NM_008924	10	9
Psmd7         NM_010817         7         4           Rarg         NM_011244         10         1           Rnf111         NM_033604         15         12           Scrib         NM_134089         38         9           Sec24a         NM_175255         23         22           Sema4b         NM_013659         14         10           Sirt6         NM_181586         8         6           Slc12a4         NM_009195         24         23           Sox5         AK029047         12         11           Src         BC039953         14         13           Tacc3         NM_011524         16         15           Tcfap4         NM_031182         7         6	Prx	NM_019412	6	5
Rarg         NM_011244         10         1           Rnf111         NM_033604         15         12           Scrib         NM_134089         38         9           Sec24a         NM_175255         23         22           Sema4b         NM_013659         14         10           Sirt6         NM_181586         8         6           Slc12a4         NM_009195         24         23           Sox5         AK029047         12         11           Src         BC039953         14         13           Tacc3         NM_011524         16         15           Tcfap4         NM_031182         7         6	Psmd7	NM_010817	7	4
Rnf111         NM_035604         15         12           Scrib         NM_134089         38         9           Sec24a         NM_175255         23         22           Sema4b         NM_013659         14         10           Sirt6         NM_181586         8         6           Slc12a4         NM_009195         24         23           Sox5         AK029047         12         11           Src         BC039953         14         13           Tacc3         NM_011524         16         15           Tcfap4         NM_031182         7         6	Rarg	NM_011244	10	1
Scrib         NM_134089         58         9           Sec24a         NM_175255         23         22           Sema4b         NM_013659         14         10           Sirt6         NM_181586         8         6           Slc12a4         NM_009195         24         23           Sox5         AK029047         12         11           Src         BC039953         14         13           Tacc3         NM_011524         16         15           Tcfap4         NM_031182         7         6	Rnf111	NM_033604	15	12
Stel24         NM_013253         25         22           Sema4b         NM_013659         14         10           Sirt6         NM_181586         8         6           Slc12a4         NM_009195         24         23           Sox5         AK029047         12         11           Src         BC039953         14         13           Tacc3         NM_011524         16         15           Tcfap4         NM_031182         7         6	Sec24a	NM 175255	30 23	9 22
Scirito         NM_181586         8         6           Sirito         NM_181586         8         6           Slc12a4         NM_009195         24         23           Sox5         AK029047         12         11           Src         BC039953         14         13           Tacc3         NM_011524         16         15           Tcfap4         NM_031182         7         6	Sec24u Sema4h	NM 013650	23 14	10
Slc12a4         NM_009195         24         23           Sox5         AK029047         12         11           Src         BC039953         14         13           Tacc3         NM_011524         16         15           Tcfap4         NM_031182         7         6	Sirt6	NM 181586	8	6
Sox5         AK029047         12         11           Src         BC039953         14         13           Tacc3         NM_011524         16         15           Tcfap4         NM_031182         7         6	Slc12a4	NM 009195	24	23
Src         BC039953         14         13           Tacc3         NM_011524         16         15           Tcfap4         NM_031182         7         6	Sox5	AK029047	12	11
Tacc3         NM_011524         16         15           Tcfap4         NM_031182         7         6	Src	BC039953	14	13
<i>Tcfap4</i> NM_031182 7 6	Tacc3	NM_011524	16	15
	Tcfap4	NM_031182	7	6

Table 2.	Continued
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Gene identity		Total number	Number of trapped exons <sup>a</sup>
Gene symbol	Accession number	of exons	unpped exons
Tef	AY540631	4	3
Terf2	NM_009353	9	7
Tgfb1i4	NM_009366	3	2
Ube2d2	NM_019912	7	6
Y1G0138J11	AK201545	4	2
Zfp346	NM_012017	7	2
1100001D10Rik	BC061692	15	4
1110003H10Rik	BC064456	4	3
1700073K01Rik	NM_026626	7	4
2200001I15Rik	NM_183278	3	2
2310061L18Rik	BC072561	20	7
4632409L19Rik	AK040612	19	18
4930447K03	AK015408	5	1

<sup>a</sup>Number of exons downstream of vector-integration site. Genes consisting of two exons were excluded from analysis.



**Figure 3.** Nature of genes trapped using the UPATrap vector. (A) Disruption of genes that are not expressed in ES cells. The absence and presence of gene expression in undifferentiated ES cells and mouse tissues, respectively, were confirmed by RT–PCR. The ubiquitously expressed *Fnbp3* mRNA (24) was used as an internal control. (B) Identity of genes disrupted using the UPATrap vector. After eliminating repetitive and low-quality sequences, 100 trapped genes were randomly chosen for each strategy (RET or UPATrap) and classified as previously described (10).

downstream exons of different genes had been trapped using the UPATrap vector (Figure 4A). Our prediction was that the IRES removal would result in the destabilization of the NEOtrapped gene fusion transcripts because such mRNAs must be regarded by the cell as being typical NMD-prone molecules containing PTCs (Figure 4A). As shown in Figure 4B, the levels of the NEO fusion transcripts were markedly lower



**Figure 4.** Suppression of NMD permits unbiased poly-A trapping. (A) Cre/*lox*P-mediated removal of the IRES sequence inserted between the NEO TC and SD sequence. (**B**) IRES insertion is required to suppress NMD of the NEO fusion transcripts. Two ES cell clones in which different genes (*Pde9a* and *Nf2*) had been trapped using the UPATrap vector were transiently transfected with a Cre recombinase-encoding plasmid. After isolating subclones by limiting dilution, recombination events were screened for using genomic PCR, and northern hybridization analysis of the NEO mRNAs was carried out using  $\beta$ -actin mRNA as an internal control. The estimated sizes of the transcripts are 3.3, 3.1 and 1.4 kb for the NEO-*Pde9a* fusion, the NEO-*Nf2* fusion and the feeder-derived NEO, respectively. Real-time PCR was also used to evaluate the relative quantities of the NEO fusion transcripts in emetine-treated and untreated cells. The GAPDH-normalized levels of the NEO fusion transcripts are represented relative to those of the emetine-untreated IRES-positive subclones. For real-time PCR, the upstream and downstream primers were designed in the *hprt*-SD region of the trap vector and in the most proximal exon in the affected region of the trapped gene, respectively, in order to detect only the NEO-trapped gene fusion transcripts, neglecting the feeder-derived NEO mRNAs or endogenous mRNAs for the trapped genes. In addition, the resistance/sensitivity of the ES cell subclones to G418 (200 µg/ml) was also examined.

in the IRES-negative subclones as compared with those of the IRES-positive ones, and all of the IRES-negative subclones had lost their G418 resistance while the IRES-positive ones had not. The decrease of the NEO fusion transcripts in the

IRES-negative subclones was considered to be due to the mRNA degradation by NMD because the levels of the fusion transcripts were significantly recovered after the treatment of the cells with a translation inhibitor, emetine, an efficient

blocker of the NMD pathway (25) (Figure 4B). In contrast, the same treatment did not affect the amount of the fusion transcripts in the IRES-positive subclones (Figure 4B). These observations indicate that the IRES sequence inserted downstream of the NEO TC was required to prevent the fusion transcripts from undergoing NMD, and this manipulation of the mRNA-surveillance pathway in turn made it possible to trap multiple downstream exons of genes in ES cells using a novel poly-A-trap strategy.

Although we discovered that the IRES insertion between a PTC and a downstream SD sequence suppresses NMD, the molecular mechanism responsible for this phenomenon needs to be determined. We assume that the IRES-mediated internal translation would proceed toward the end of the NEO fusion transcript, displacing the exon-exon junction complexes (EJCs) from downstream exon-exon junctions (26) (Supplementary Figure 2). The EJCs remaining attached to PTCcontaining mRNAs are believed to be an essential triggering factor for NMD (11-13,27-30). Alternatively, the IRES sequence itself predicted to generate a highly complex secondary structure at the RNA level (31) might simply interfere with the interaction between components of the translation termination complex formed at a PTC and the downstream EJCs, thereby canceling the essential initial steps for NMD (27-30) (Supplementary Figure 2).

## CONCLUSION

Suppression of NMD by the UPATrap strategy permits the trapping of genes (i) regardless of their transcriptional status in the target cells and (ii) without a bias in the vector-integration site. We believe this novel anti-NMD strategy enables a simple and straightforward approach to the unbiased inactivation of all mouse genes in the genome of ES cells (1). Conventional poly-A trapping, on the other hand, can be applied for more specialized purposes including the production of hypomorphic or C-terminal-tagged alleles of the disrupted genes. Combinatorial usage of the two poly-A-trap strategies (i.e. UPATrap and conventional) will significantly increase the diversity of mutations created by random intragenic vector integrations.

#### SUPPLEMENTARY MATERIAL

Supplementary Material is available at NAR Online.

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