Targeted Gene Disruption by Ectopic Induction of DNA Elimination in *Tetrahymena*

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ABSTRACT *Tetrahymena* is a useful eukaryotic model for biochemistry and molecular cell biology studies. We previously demonstrated that targeted ectopic DNA elimination, also called co-Deletion (coDel), can be induced by the introduction of an internal eliminated sequence (IES)-target DNA chimeric construct. In this study, we demonstrate that coDel occurs at most of the loci tested and can be used for the production of somatic gene KO strains. We also showed that coDel at two loci can be simultaneously induced by a single transformation; thus, coDel can be used to disrupt multiple gene loci in a single cell. Therefore, coDel is a useful tool for functional genetics in *Tetrahymena* and further extends the usefulness of this model organism.

KEYWORDS *Tetrahymena*; DNA elimination; RNAi; gene knockout

THE ciliated protozoan *Tetrahymena thermophila* can grow at an exceptionally high rate (its doubling time is approximately 2 hr) and can reach a high density (a few million cells per milliliter) under simple and inexpensive culture conditions (reviewed in Orias *et al.* 2000). In combination with robust genetic manipulation methods (reviewed in Chalker 2012), *Tetrahymena* is a useful model eukaryote for biochemistry and molecular cell biology studies (reviewed in Collins and Gorovsky 2005).

Three strategies for loss-of-function genetic studies have been established for this organism. The first strategy is a germline knockout (KO), in which one of the two gene copies in the diploid micronucleus (MIC) is replaced with a drug-resistance gene by homologous recombination, and two heterozygous strains are then sexually crossed to obtain a homozygous germline KO strain (Cassidy-Hanley *et al.* 1997). The second strategy is somatic KO, in which one of the ~45 copies of a gene in the polyploid macronucleus (MAC) is replaced with a drug-resistance gene by homologous recombination, and "phenotypic assortment" is used to obtain cells with the drug-resistance gene at all loci (Merriam and Bruns 1988). Phenotypic assortment is possible because MAC chromosomes are randomly segregated into daughter cells in vegetative cell division and a stepwise increase of the drug in culture selects for cells that have more drug-resistance genes. The final strategy is gene knockdown (KD) by RNA interference (RNAi), in which a construct expressing long hairpin RNA that is complementary to the gene of interest is introduced into a nonessential locus in the MAC and small RNAs produced from the hairpin RNA post-transcriptionally silence the expression of the gene (Howard-Till and Yao 2006).

Although the first two gene KO strategies have been reliably used to study individual gene functions, they are not suitable for high-throughput genetic screening because the integration of a KO construct into the MIC germline occurs at very low efficiency and the phenotypic assortment process used in somatic KOs require the careful control of drug concentrations in each strain and culture step. Moreover, it takes ~1 month to obtain KO strains using these methods. For germline KO, two sexual crosses are necessary to produce a homozygous KO, and the *Tetrahymena* have a sexually immature stage that lasts ~2 weeks after the first sexual cross. For somatic KO, phenotypic assortment requires at least 2–3 weeks for completion. In contrast to the gene KO strategies, RNAi KD can be done in a shorter period of time and is thus suitable for high-throughput genetic screening. However, RNAi KD often only incompletely shuts down expression of a target gene (Howard-Till and Yao 2006; Cheng *et al.* 2010; Chung and Yao 2012; Lukaszewicz *et al.* 2013) and may thus result in the misinterpretation of the gene function.

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During the development of the new MAC of *Tetrahymena*, >8000 DNA segments called internal eliminated sequences (IESs) were reproducibly eliminated. IESs are recognized by 26- to 32-nt small RNAs called scnRNAs (Schoeberl *et al.* 2012). IESs can be classified into two types: type A IESs that are recognized by scnRNAs produced by themselves and type B IESs that are recognized by scnRNAs produced by type A IESs (Noto *et al.* 2015). In a recent study, we demonstrated that ectopic DNA elimination can be induced in a targeted manner through the introduction of a chimeric construct of a type A IES and a fragment of a target locus into the new MAC (Noto *et al.* 2015). We call this phenomenon co-Deletion (coDel). Several pieces of experimental evidence indicate that coDel is induced by the molecular mechanism shown schematically in Figure 1A. First, scnRNAs produced from the endogenous type A IES in the MIC (Figure 1A, i) recognize the endogenous copy of the IES in the new MAC (Figure 1A, ii) and the type A IES in the introduced chimeric construct (Figure 1A, iii); scnRNA production is then induced from the target sequence adjacent to the type A IES (Figure 1A, iv), which recognizes the endogenous target locus in *trans* (Figure 1A, v) and thus induces ectopic DNA elimination.

Because coDel can be induced by the simple introduction of an IES-target DNA chimeric construct, we reasoned that coDel would be suitable as a gene KO technology in *Tetrahymena*. In this study, we tested the versatility and specificity of coDel as a tool to produce somatic gene KO strains.

Materials and Methods

Strains and culture conditions

Wild-type B2086 and CU428 strains of *T. thermophila* were obtained from the *Tetrahymena* Stock Center at Cornell University. Cells were grown in SPP medium (Gorovsky *et al.* 1975) containing 2% proteose peptone at 30°. For conjugation, growing cells (~5–7 × 10^5/ml) of two different mating types were washed, prestarved (~12–24 hr), and mixed in 10 mM Tris (pH 7.5) at 30°.

coDel

pMcoDel vector (Noto *et al.* 2015; see also Figure 1B) was digested with *Not*I, and the target sequences were inserted by Gibson Assembly (NEB). The genomic location of the targets and the primer sets used for their amplification are listed in Table S1. The primer sequences are listed in Supporting Information, Table S2. To insert the *XRN2* and *XRN4* target sequences into the pMcoDel vector, two target sequences were first connected by overlapping PCR before insertion via Gibson Assembly. The vectors were introduced into conjugating wild-type cells using a biolistic gun, and the cells were cultured in 10 mM Tris (pH. 7.5) overnight and for an additional ~3 hr in 1× SPP. Next, the cells possessing pMcoDel-derived rDNAs were selected using 100 μg/ml paromomycin in 1× SPP. Total genomic DNA was then extracted from paromomycin-resistant cells using a NucleoSpin Tissue Kit (Macherey-Nagel), and DNA eliminations at the endogenous target loci were determined by PCR using the primer sets listed in Table S1.

Production of gene KO strains and DNA elimination assays

Progeny lines showing higher coDel efficiency were selected and cultured for 12 passages. In each passage, 5 μl of the previous culture was incubated in 1 ml 1× SPP at 30° for 1–2 days. Next, 6–8 clones from each cell line were isolated, and their target locus was analyzed by genomic PCR. Clones showing a complete lack of the target gene were established as KO strains. Combinations of KO strains that mate were determined by directly checking their mating ability. DNA elimination of the *Tlr1* element was analyzed by DNA FISH, as described previously (Noto *et al.* 2010).

Complementation assay

pHA-*pur4* was produced by replacing the neo gene of pHA-*neo4* (Kataoka *et al.* 2010) with the puromycin-resistance gene (*pac*) of the *pur4* cassette (Iwamoto *et al.* 2014). The *pur4* cassette expresses a puromycin-resistance gene in the presence of cadmium ions. To make a *DED1* rescue construct (Figure 6C), the 3′ flanking region of the *DED1* gene was amplified by PCR using DED1_Res_3FW and DED1_Res_3RV and cloned into the *Xho*I site of the pHA-pur4 vector using Gibson Assembly (NEB). The resulting plasmid was then digested with *Bam*HI, and the 5′ flanking region of *DED1* gene, which was amplified by PCR using DED1_Res_5FW and DED1_ResHA_5RV, was inserted using Gibson Assembly (NEB). The sequences of the PCR primers are listed in Table S2. The resulting plasmid DNA was digested with *Bam*HI and *Xho*I and introduced into the MACs of starved *DED1* KO strains by biolistic gun. Then, cells were cultured in 1× SPP for ~4 hr, and cells that had the rescue constructs were selected in 1× SPP containing 200 μg/ml puromycin (InvivoGen) and 1 μg/ml $CdCl_2$. Phenotypic assortment was then performed until cells grew in 1–1.5 mg/ml puromycin.

Data availability

Strains and plasmids used in this study are available upon request.

Results

coDel occurs at many target loci

In the previous study, we showed that coDel was induced at all three of the targeted loci (Noto *et al.* 2015). To ask if coDel occurs generally at different loci, we target 14 genes for coDel that are expressed predominantly at early conjugation stages (Figure S1). This was done because the complete deletion of an essential gene causes a loss of progeny and underestimation of coDel efficiency at that gene locus, whereas gene expression at early conjugation stages occurs in the maternal MAC and deletions from the new (zygotic) MAC most likely would not affect progeny viability.

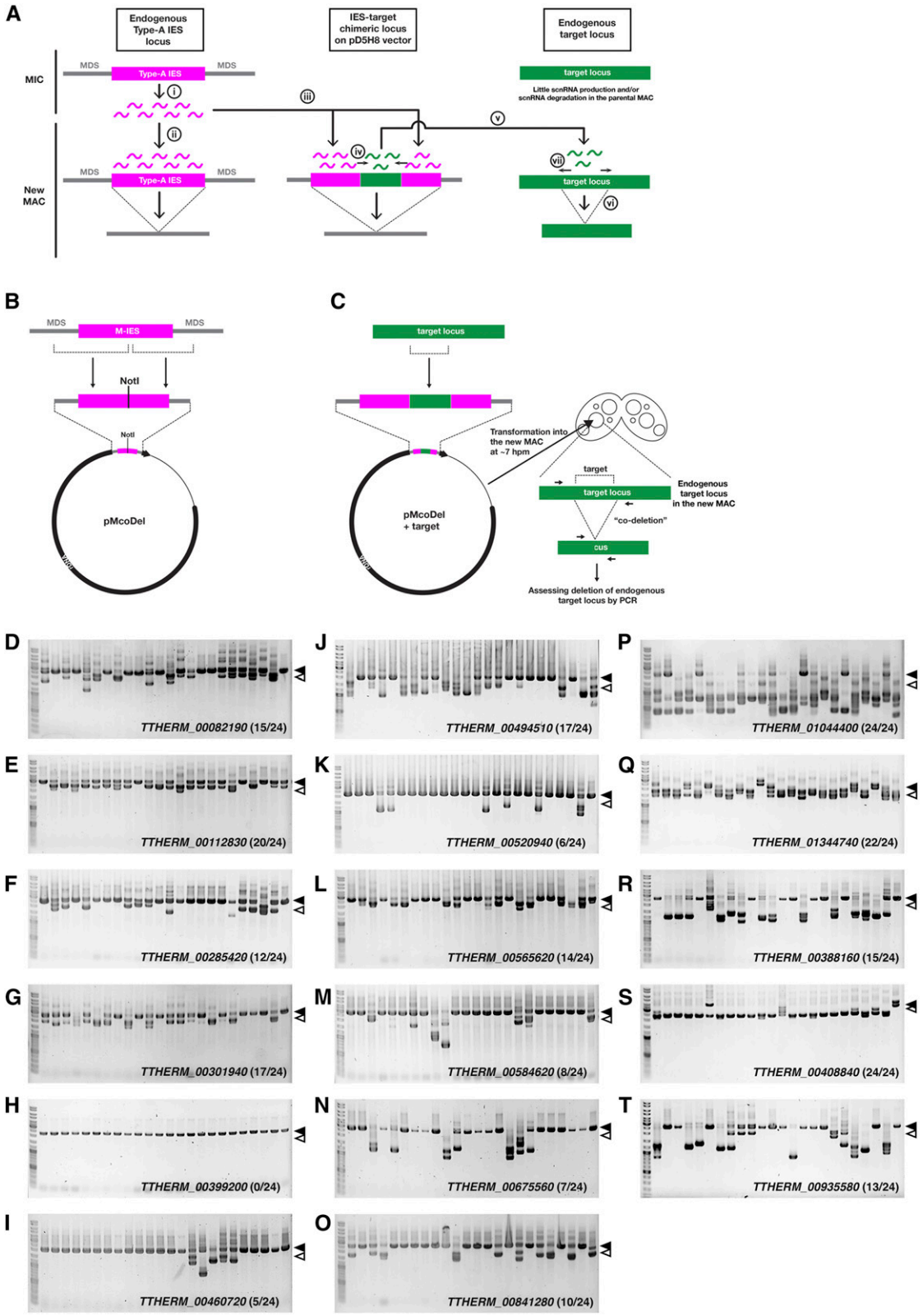

Figure 1 Versatility of coDel. (A) The proposed actions of early and late scnRNAs in coDel. Early scnRNAs produced from an endogenous type A IES in the MIC (i) interact not only with the endogenous type A IES (ii) but also with the type A IES on the pMcoDel vector introduced into the new MAC (iii). The latter interaction triggers late scnRNA production from the adjacent target sequence in *cis* (iv), which then interacts with the endogenous target locus in *trans* (v) and induce ectopic DNA elimination (vi). Late scnRNAs likely further induces production of late scnRNAs in *cis* at the target locus (vii). (B) Construction of the pMcoDel vector. (C) A schematic of the coDel experiment. A PCR-amplified fragment (~580–710 bp) of each target gene was

We used the pMcoDel vector (Figure 1B) (Noto *et al.* 2015), in which the M-IES (a type A IES) (Austerberry *et al.* 1984) and its flanking MAC-destined sequences were inserted into pD5H8 (gift from Dr. Meng-Cao Yao, Academia Sinica, Taiwan). The pD5H8 vector contains *Tetrahymena* MIC rDNA with a paromomycin-resistant allele of the 17S rRNA (Spangler and Blackburn 1985). Upon introduction of pD5H8 into the new MAC, the rDNA is excised from the vector and forms an rDNA chromosome. At the 3′ nontranscribed region of the rDNA, we can insert a sequence of interest (in this study, we used M-IES and its flanking sequences) without disturbing the functions of the rDNA (Sweeney and Yao 1989). The unique *Not*I site was made in the middle of M-IES where we can insert the target DNA sequence (Figure 1B). Our experimental scheme is shown in Figure 1C. A PCR-amplified fragment (∼580–710 bp) of each target gene was inserted into the *Not*I site of the pMcoDel vector and introduced into the conjugating cells at ∼7 hpm. Progeny cells that have pD5H8-derived rDNA were then selected on the basis of their resistance to paromomycin and ectopic elimination of the endogenous target gene in the MAC analyzed by genomic PCR.

Of the 14 early conjugation-specific genes that were subjected to coDel using the strategy described above, ectopic DNA elimination occurred in the new MAC at 13 gene loci (Figure 1, D–T). These results indicate that coDel can be induced at many different genomic loci. DNA elimination induced by coDel removed regions wider than the targets at several of the tested loci (the open arrowheads in Figure 1, D–W, indicate the expected sizes of the PCR products lacking the exact target sequences). As previously suggested (Noto *et al.* 2015), this likely indicates that Late-scnRNAs further induce Late-scnRNA production at the target loci in *cis*, as shown in Figure 1A, vi.

To test whether coDel occurs independently of the expression pattern of the target gene, we targeted loci encoding two genes that were expressed predominantly in exponentially growing cells (Figure 1, R and S) and one gene that was expressed predominantly in starved cells (Figure 1T) for coDel. We found that coDel also occurs at these vegetative gene loci. Altogether, we conclude that coDel occurs at a variety of genomic loci, irrespective of their gene expression pattern.

coDel is efficiently induced by introduction of an IES-target chimeric construct into the early developing MAC

We then investigated whether the timing of the introduction of an IES-target chimeric construct affects the efficiency of coDel. For this purpose, we used the pMcoDel vector containing a 592-bp DNA fragment complementary to the *TTHERM_00301940* locus, which was also used for the experiment shown in Figure 1G. This construct was introduced into conjugating cells at 7, 8.5, and 10 hpm, and DNA elimination at the *TTHERM_00301940* locus in the new MAC of the progeny was analyzed by genomic PCR. It is important to note that the formation of the new MAC starts at ∼7 hpm but that most DNA eliminations occur at ∼12–16 hpm. We found that the earlier we introduced the construct, the higher the efficiency of coDel was (Figure 2). Therefore, introducing a coDel-inducing construct into the new MAC in its early development likely induces coDel more efficiently.

Longer target sequences increase the efficiency for coDel

We then investigated whether an extension of the target sequence improves the efficiency of coDel. We prepared pMcoDel vectors with 672-, 672-, 1021-, 1479-, or 2022-bp DNA fragments complementary to the *TTHERM_00285420* locus (Figure 3A) and introduced them into conjugating cells at 7 hpm to induce coDel. We found that coDel occurred at a higher frequency with the 1021-bp target sequence than with the two different 672-bp target sequences (Figure 3, B–D), whereas the 1021-, 1479-, and 2022-bp target sequences induced coDel at a similar efficiency (Figure 3, D–F). Therefore, we conclude that longer target sequences increase the efficiency for coDel and an ∼1-kb target sequence is necessary and sufficient to induce coDel at the highest efficiency at the *TTHERM_00285420* locus in our experimental condition.

Off-target coDel occurs at extremely similar loci

To test how much homology between a target sequence in the pMcoDel vector and a locus in the new MAC is necessary to induce coDel, we introduced a pMcoDel vector containing a 1022-bp DNA fragment that was complementary to part of the *TWI2* coding sequence to determine whether other loci (Couvillion *et al.* 2009) were also targeted for coDel. *TWI6* and *TWI7* have 90.6 and 65.5% identity at the nucleotide level, respectively, to the target sequence for *TWI2*. We found that the introduction of the *TWI2*-targeting construct induced DNA elimination not only at the *TWI2* locus (Figure 4A) but also at the *TWI6* locus (Figure 4B). In contrast, we did not detect DNA elimination at the *TWI7* locus (Figure 4C). These results suggest that off-target coDel can be induced at a locus that shares high similarity with a target sequence.

inserted into the *Not*I site of pMcoDel vector and introduced into the conjugating cells at ∼7 hpm. Progeny cells with the pMcoDel-derived rDNA were then selected and ectopic elimination of the endogenous target gene loci in the MAC analyzed by genomic PCR. (D–T) Results of coDel experiments. Sequences complementary to the indicated target loci (D–Q) conjugation-specific genes; (R and S) growing cell-specific genes; (T) starved cell-specific genes) were cloned into pMcoDel and introduced into the new MACs of conjugating wild-type cells. Deletions at the endogenous target loci in progeny cells were analyzed by PCR (C). The number of progeny lines showing any deletions at the endogenous target loci was determined. The solid and open arrowheads, respectively, indicate the expected positions of PCR products from the target loci without deletions and with deletions corresponding to the exact target sequences.

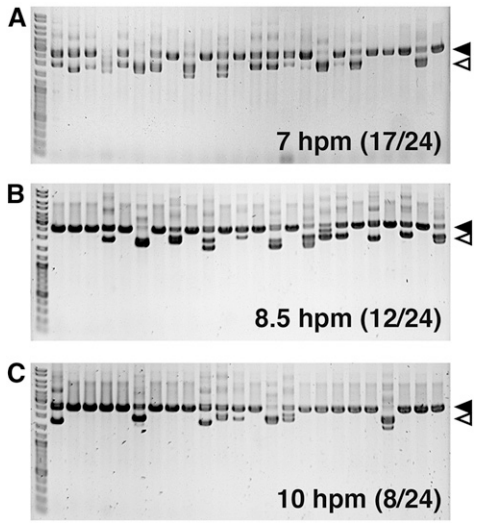

Figure 2 Effect of the introduction of a coDel-inducing vector at different developmental stages. The pMcoDel–*TTHERM_00301940* vector used for the experiment shown in Figure 1G was introduced into the conjugating cells at 7, 8.5, or 10 hpm and DNA eliminations at the endogenous *TTHERM_00301940* loci in the MAC were analyzed by genomic PCR as in Figure 1. The number of progeny lines showing any deletions at the endogenous target loci was determined. The solid and open arrowheads, respectively, indicate the expected positions of PCR products from the target loci without deletions and with deletions corresponding to the exact target sequences.

Two-target loci can be simultaneously deleted by coDel

Because >2-kb target sequence can be inserted into the pMcoDel vector without reducing the coDel efficiency (Figure 3E) and an ~1-kb target sequence is sufficient to efficiently induce coDel (Figure 3C), we reasoned that coDel may be simultaneously induced at two independent loci by introducing a pMcoDel vector with two target sequences. To test this idea, we introduced a pMcoDel vector containing 998 bp complementary to the *XRN2* gene and 1083-bp DNA complementary to the *XRN4* gene into conjugating cells at 7 hpm (Figure 5A). *XRN2* and *XRN4* share only 51.9% identity at the nucleotide level in their coding regions; thus, we expect no off-target coDel between these loci. We found that in 7 of the 24 cell lines, DNA elimination was induced at both *XRN2* and *XRN4* loci via the introduction of the coDel vector with two target sequences (Figure 5B, marked with asterisks). Therefore, coDel can be simultaneously induced at two independent loci using the pMcoDel vector. In most of the clones we tested, we observed either the simultaneous coDel of the both genes or no deletion of them. This tendency might be caused by introduction of the vector at variable stages of development, because coDel efficiency depends on timing of the introduction of a coDel vector (Figure 2).

Gene KO strains can be produced by coDel

Next, we aimed to establish somatic KO strains for a gene by coDel and to analyze the function of the gene using the established KO strains. The establishment process of *TTHERM_00301940* KO cell lines is shown in Figure 6A. In most of the cell lines subjected to coDel, some wild-type copies of the target loci remained (Figure 6A, top). This is likely because DNA elimination occurs when each new MAC has four to eight copies of the genome following one to two rounds of endoreplication (Allis *et al.* 1987) and because coDel does not always induce DNA elimination in all copies of

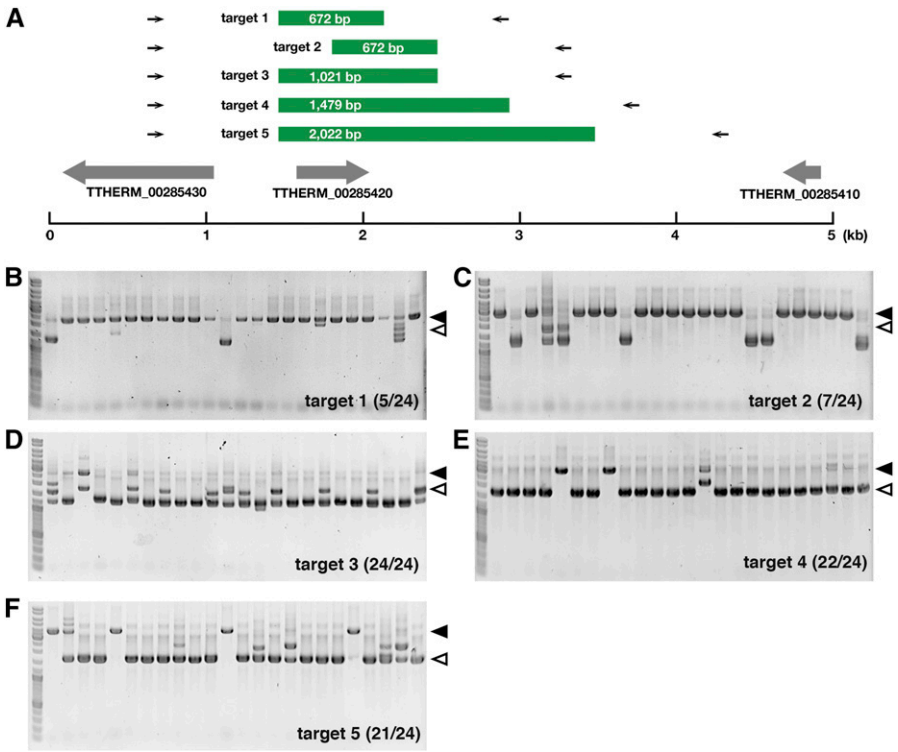

Figure 3 Effect of the introduction of coDel-inducing vectors of different target length. (A) Schematic of the *TTHERM_00285420* MAC locus. Green boxes indicates target sequences complementary to the *TTHERM_00285420* gene. Primers used for genomic PCR for each target are shown as arrows adjacent to the target. (B–E) Results of the coDel experiments. The indicated targets were cloned into pMcoDel and introduced into the new MACs of the conjugating cells at 7 hpm. Deletions at the endogenous *TTHERM_00285420* locus in the MAC were analyzed by genomic PCR with primers indicated in (A). The number of progeny lines showing any deletions at the endogenous target loci was determined. The solid arrowheads indicate PCR products from the target loci without deletions. The open arrowheads show the expected positions of PCR products from the target loci with deletions corresponding to the exact target sequences.

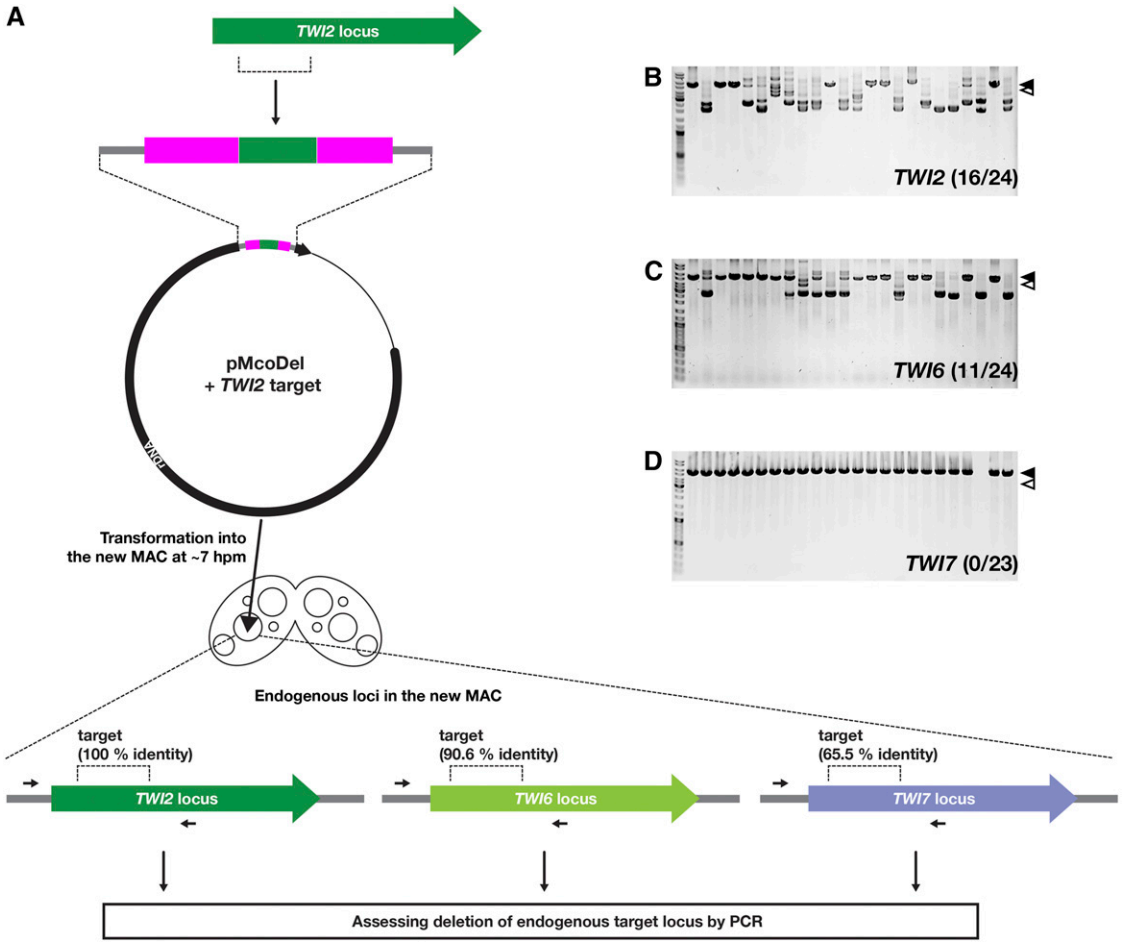

Figure 4 Analysis of off-target coDel. (A) A schematic of the coDel experiment targeting the *TWI2* locus. A part (1022 bp) of the *TWI2* locus was cloned into pMcoDel and introduced into the new MACs of conjugating cells at 7 hpm. Deletions at the endogenous *TWI2* locus in the MAC were analyzed by genomic PCR. Deletions at the *TWI6* and *TWI7* loci, which share 90.6 and 65.5% identities at the nucleotide level with the *TWI2* locus, respectively, were also analyzed by genomic PCR. (B–D) Results of genomic PCR detecting deletions at the endogenous *TWI2* (B), *TWI6* (C), *and TWI7* (D) loci in the MAC. PCR products from the same progeny lines were separated in the same lane positions. The number of progeny lines showing any deletions at the endogenous target loci was determined. The solid arrowheads indicate PCR products from the target loci without deletions. The open arrowheads show the expected positions of PCR products from the target loci with deletions corresponding to the exact target sequences.

the target locus. Therefore, to establish the *TTHERM_00301940* KO strains, we first chose cell lines that had the highest ratios of deleted target loci to corresponding nondeleted (wild-type) loci (cell lines 4, 6, 9, and 17 in Figure 6A, top), cultured them through several passages to allow for the random assortment of MAC chromosomes, and then isolated single cells to find and establish clonal cell lines in which all of the target loci in the MACs were deleted (Figure 6A bottom). Several KO strains were then chosen and test crossed to each other to identify clones with different mating types. The clones 6-1, 9-2, 9-6, 17-1 of *TTHERM_00301940* KO cells were selected for further study.

TTHERM_00301940 is exclusively expressed during conjugation and many conjugation-specific genes are required for DNA elimination (Mochizuki *et al.* 2002; Taverna *et al.* 2002; Liu *et al.* 2007; Cheng *et al.* 2010; Shieh and Chalker 2013; Woehrer *et al.* 2015); we sought to determine whether *TTHERM_00301940* is also involved in DNA elimination. *TTHERM_00301940* KO strains (6-1 × 9-6 [= cross 1 in Table 1] or 9-2 × 17-1 [= cross 2 in Table 1]) were mated, and the DNA elimination in their exconjugants (progeny) was analyzed by DNA FISH using probes complementary to the Tlr1 element. The Tlr1 element is a transposon-related IES that is moderately repeated in the MIC genome. In the exconjugants from the wild-type cells, DNA complementary to the Tlr1 element was detected in the MIC but not in the new MAC (Figure 6B, top, and Table 1). In contrast, DNA complementary to the Tlr1 element was detected in both the MIC and the new MAC in the exconjugants from the *TTHERM_00301940* KO cells (Figure 6B, middle, and Table 1), indicating that *TTHERM_00301940* plays an important role in DNA elimination. According to the phenotypes of *TTHERM_00301940* KO cells, we decided to call this gene *DNA Elimination Defective 1* (*DED1*).

Establishment of a complementation assay system

As shown above, coDel occurs not only at the target locus but also potentially at loci that have high homology to the target sequence

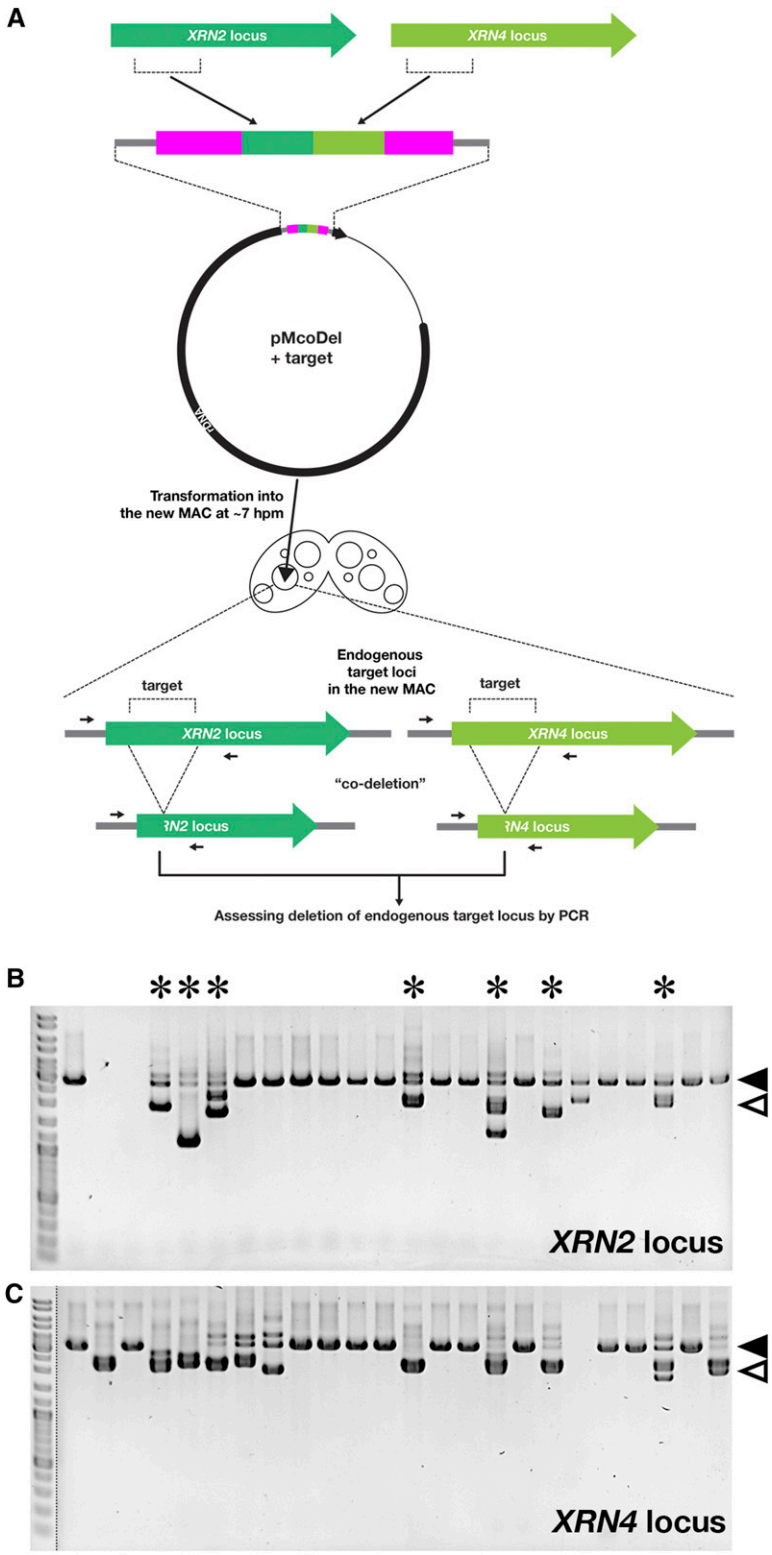

Figure 5 Double coDel. (A) A schematic of the coDel experiment simultaneously targeting *XRN2* and *XRN4* loci. A part of the *XRN2* locus and a part of the *XRN4* locus were cloned into pMcoDel and introduced into the new MACs of the conjugating cells at 7 hpm. Deletions at the endogenous *XRN2 and XRN4* loci in the MAC were analyzed by genomic PCR. (B and C) Results of genomic PCR detecting deletions at the endogenous *XRN2* (B) and *XRN4* (C) loci in the MAC. PCR products from the same progeny lines were separated in the same lane positions. Asterisks indicate progeny lines showing deletions at both *XRN2* and *XRN4* loci. The solid arrowheads indicate PCR products from the target loci without deletions. The open arrowheads show the expected positions of PCR products from the target loci with deletions corresponding to the exact target sequences.

(Figure 4). In addition, because the regulation of DNA elimination has not been fully elucidated, unintended DNA elimination might be induced during coDel. Therefore, it is important to confirm whether phenotypes of gene KO strains produced by coDel are caused only by the absence of the target genes and not by unintended disruption of nontarget genes.

We designed a genetic complementation system and tested it by complementing the *DED1* KO strains. We produced the *DED1* rescue construct (Figure 6C) by combining the 5′ flanking region and the coding region of *DED1* (Figure 6C, i), the HA-*pur4* cassette (Figure 6C, ii), and the 3′ flanking region of *DED1* (Figure 6C, iii). The *DED1* KO locus was replaced with

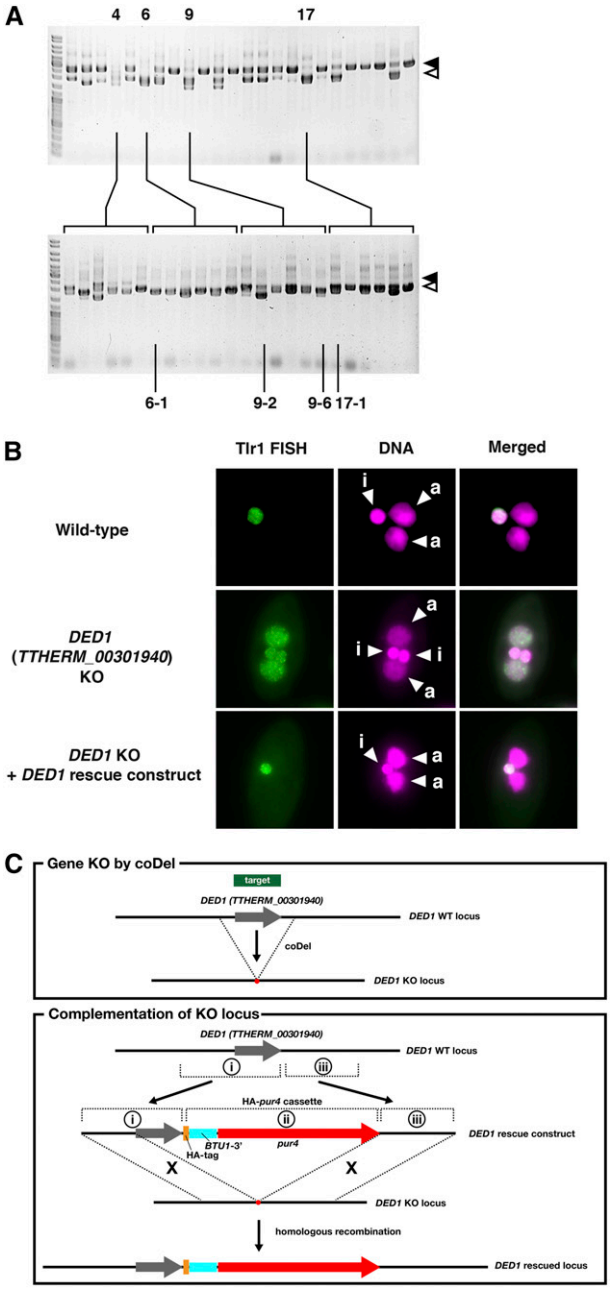

Figure 6 Establishment and analyses of *DED*1 KO strains produced by coDel. (A) Establishment of *TTHERM_00301940* (*DED*1) KO strains by coDel. Progeny lines showing nearly complete deletion (nos. 4, 6, 9, and 17) in the coDel experiment shown in Figure 1G were cultured for 12 passages to allow random assortment of *DED1* MAC locus and sexual maturation. Six clonal cell lines were then established from each progeny line and their *DED1* locus analyzed by genomic PCR. Clones 6-1, 9-2, 9-6, and 17-1 were used as *DED1* KO cells. (B) Analyses of DNA elimination. Exconjugants (progeny) of wild-type cells (top), *DED1* KO cells (middle) or *DED1* KO cells rescued with a *DED1* rescue construct (bottom) at ~36 hpm were used to detect Tlr1-IES elements by fluorescent *in situ* hybridization (Tlr-FISH, green). The DNA was stained with DAPI (DNA, magenta). The MICs (i) and the new MACs (a) are marked. (C) Complementation assay for *DED1* KO locus. Top: A schematic drawing of *DED1* KO by coDel. Bottom: A schematic drawing of the strategy for genetic rescue of *DED1* KO. The *DED1* rescue construct was produced by combining the 5′ flanking region and the coding region of the *DED1* gene (i), HA-*pur4* cassette (ii), and the 3′ flanking region of *DED1* (iii) and the *DED1* KO locus was replaced with the *DED1* rescue construct by homologous recombination.

the *DED1* rescue construct by homologous recombination (Figure 6C). The HA-*pur4* cassette expresses a puromycin-resistance gene (Iwamoto *et al.* 2014); thus, cells containing the rescue construct were selected on the basis of their resistance to puromycin. Phenotypic assortment was performed to increase copy number of *DED1* rescue loci in the macronucleus to produce the *DED1* rescue strains. The *DED1* rescue strains were then mated with the *DED1* KO cells, and the DNA elimination in their exconjugants was analyzed by DNA FISH using probes complementary to the Tlr1 element. We found that DNA complementary to Tlr1 was absent in the new MACs in these exconjugants (Figure 6B, bottom, and Table 1). Therefore, we conclude that the DNA elimination-defective phenotype of the *DED1* KO cells was due to loss of the *DED1* gene and that the rescue system above can be used for genetic complementation analyses of KO strains made by coDel.

CoDel can be used for functional genetic screening

Next, we aimed to perform functional genetic screening by coDel. For this purpose, we asked whether some of the early conjugation-specific genes subjected to coDel (Figure 1, E–U) are important for programmed DNA elimination, such as *DED1*. In addition, we studied two early conjugation-specific genes [*TTHERM_00079530* (also called *COI5*; Woehrer *et al.* 2015) and *TTHERM_00313180*] that we subjected to coDel in our previous study (Noto *et al.* 2015). For *TTHERM_00285420*, *TTHERM_00460720*, and *TTHERM_00675560*, the original coDel attempts with ~600- to 700-bp targets (Figure 1, F, I, and N) did not provide sufficient DNA elimination to produce KO strains. Therefore, we induced coDel for these genes using ~900- to 1000-bp targets.

The establishment process of KO cells for these genes is shown in Figure S2. We established KO strains for the 14 genes (including *DED1*) in which we detected DNA elimination at the target locus by coDel. Then, two KO strains for each gene were mated, and the DNA elimination in their exconjugants was analyzed by DNA FISH for the *Tlr1* element. A summary of the DNA elimination analyses is shown in Table 1. Because the KO cells for *TTHERM_00079530*, *TTHERM_00112830*, *TTHERM_01044400*, *TTHERM_00494510*, and *TTHERM_00675560* did not produce exconjugants for unknown reasons, we could not check whether these genes played any role in DNA elimination. Two of 11 conjugation-specific genes were also determined as genes required for completing conjugation in our previous conventional homologous recombination-based gene KO studies (Woehrer *et al.* 2015). Because conjugation involves multiple complex processes, it is not surprising that many conjugation-specific genes are required for producing exconjugants.

The KO strains for the rest of the genes formed exconjugants and we found that in addition to *DED1* KO cells, a strong DNA elimination defect was detected in *TTHERM_00285420* KO cells. Therefore, we named *TTHERM_00285420 DNA Elimination Defective 2* (*DED2*). The KO strains for *TTHERM_00082190*, *TTHERM_01344740*, *TTHERM_00520940*, and *TTHERM_00460720* also showed weaker defects in DNA elimination, and these minor defects may be consequences of some disturbance in the conjugation

Table 1 Results of DNA elimination assays

Genotypes	DNA elimination assay results	No. counted	Exconjugants		
			++	+	−
Wild type		100	0	0	100
TTHERM_00079530 (COI5) KO	Cross 1	0 (no exconjugant)	—	—	—
	Cross 2	0 (no exconjugant)	—	—	—
TTHERM_00082190 KO	Cross 1	100	7	7	86
	Cross 2	100	9	5	86
TTHERM_00112830 KO	Cross 1	0 (no exconjugant)	—	—	—
	Cross 2	0 (no exconjugant)	—	—	—
TTHERM_00285420 (DED2) KO	Cross 1	100	73	25	2
	Cross 2	100	64	31	5
TTHERM_00301940 (DED1) KO	Cross 1	100	100	0	0
	Cross 2	100	100	0	0
TTHERM_00313180 KO	Cross 1	100	0	0	100
	Cross 2	100	0	0	100
TTHERM_00460720 KO	Cross 1	100	0	1	99
	Cross 2	100	0	2	98
TTHERM_00494510 KO	Cross 1	0 (no exconjugant)	—	—	—
	Cross 2	0 (no exconjugant)	—	—	—
TTHERM_00520940 KO	Cross 1	100	5	1	94
	Cross 2	100	9	0	91
TTHERM_00565620 KO	Cross 1	100	0	0	100
	Cross 2	100	0	0	100
TTHERM_00584620 KO	Cross 1	100	0	0	100
	Cross 2	100	0	0	100
TTHERM_00675560 KO	Cross 1	0 (no exconjugant)	—	—	—
	Cross 2	0 (no exconjugant)	—	—	—
TTHERM_00841280 KO	Cross 1	60	0	0	60
	Cross 2	100	0	0	100
TTHERM_01044400 KO	Cross 1	0 (no exconjugant)	—	—	—
	Cross 2	0 (no exconjugant)	—	—	—
TTHERM_01344740 KO	Cross 1	100	5	2	93
	Cross 2	100	2	3	95
TTHERM_00301940 (DED1) KO	Cross 1	100	0	0	100
+ *DED1* rescue construct	Cross 2	100	0	0	100

progression. Altogether, we conclude that a medium-throughput functional genetic screen can be performed using coDel.

Discussion

CoDel as a tool for the production of gene KO strains

We detected coDel at 16 of the 17 target loci (Figure 1). These 16 loci include genes expressed in exponentially growing cells, starved cells, and conjugating cells. In addition, we previously showed that coDel can occur at noncoding genomic loci (Noto *et al.* 2015). Therefore, coDel can be induced at many different loci, irrespective of their transcriptional state and coding capacity. However, the current protocol for coDel is not suitable for production of gene KO strains at some loci, as we could not detect coDel at the *TTHERM_00399200* locus. Currently it is not clear what makes the *TTHERM_00399200* locus insensitive to coDel. We need a larger-scale study to determine whether there are necessary conditions for target loci to be eliminated by coDel and to make coDel a more versatile gene KO technology. Nonetheless, because gene KO strains can be produced in a week by coDel, instead of in a month by the conventional homologous recombination-based strategies (Cassidy-Hanley *et al.* 1997; Chalker 2012), coDel is a useful tool to facilitate *Tetrahymena* genetics.

High-throughput genetic screening

To make a somatic gene KO strain, all copies of a target gene in the polyploid MAC must be disrupted. This can be achieved either by coDel of all copies of a target gene or by coDel of some copies of a target gene followed by random assortment of MAC chromosomes and then choosing cells in which all copies of a target gene are eliminated. In Figure 6 and Table 1, all KO strains were produced by the latter strategy because some wild-type copies remained in many strains in which coDels were detected. Because the assortment and the following selection of KO cells is a cumbersome process, improving the efficiency of coDel is important for establishing this technology as a tool for high-throughput genetic screening. It may be useful to compare coDel efficiency using different IESs in a coDel-inducing vector to find a better IES for coDel experimentation. In addition, because we found that extension of the target sequence in the pMcoDel vector improves not only the number of progeny cells showing coDel but also the completeness of coDel within each progeny

line (Figure 3), it would be important to use 1 kb or longer sequences as a target for coDel for a high-throughput genetic screening attempt. In addition, because we found that off-target coDel occurs when a locus shares ~90% identity with a target sequence (Figure 4), using unnecessarily long target sequences should be avoided. In addition, target sequences need to be chosen carefully if a target locus shares high-sequence identity with other loci. Because off-target coDel could occur in any experimental design, it is always necessary to confirm a cause–effect relationship between coDel-induced disruption of a target gene and a detected phenotype by (i) observing multiple independent coDel cell lines and (ii) asking if reintroduction of the target gene into a coDel line can restore the phenotype.

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Targeted Gene Disruption by Ectopic Induction of DNA Elimination in *Tetrahymena*

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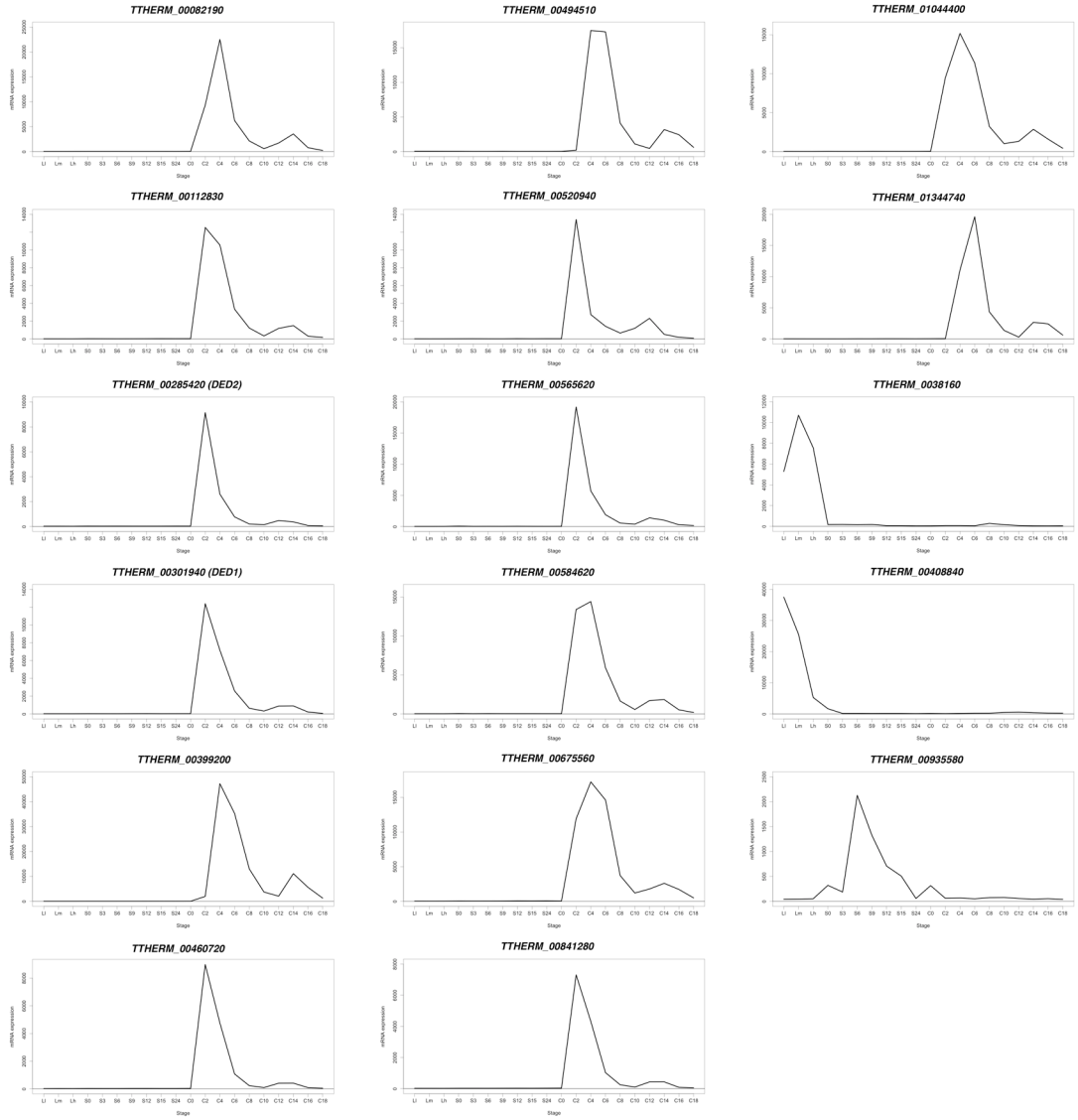

Figure S1. Gene expression profiles of target genes

The expression levels of mRNAs analyzed by microarray hybridization at different stages of the life cycle (Miao et al. 2009) are plotted. Growing cells at three different concentrations: ~1 X 105 cells/mL, ~3.5 X 105 cells/mL and ~1 X 106 cells/mL (Ll, Lm, and Lh, respectively); cells starved for 0, 3, 6, 9, 12, 15 and 24 hrs (S0, S3, S6, S9, S12, S15 and S24, respectively); and conjugating cells at 2, 4, 6, 8, 10 and 12 hrs post-mixing (C2, C4, C6, C8, C10 and C12, respectively) are indicated. The data were obtained from the Tetrahymena Functional Genomics Database (http://tfgd.ihb.ac.cn/).

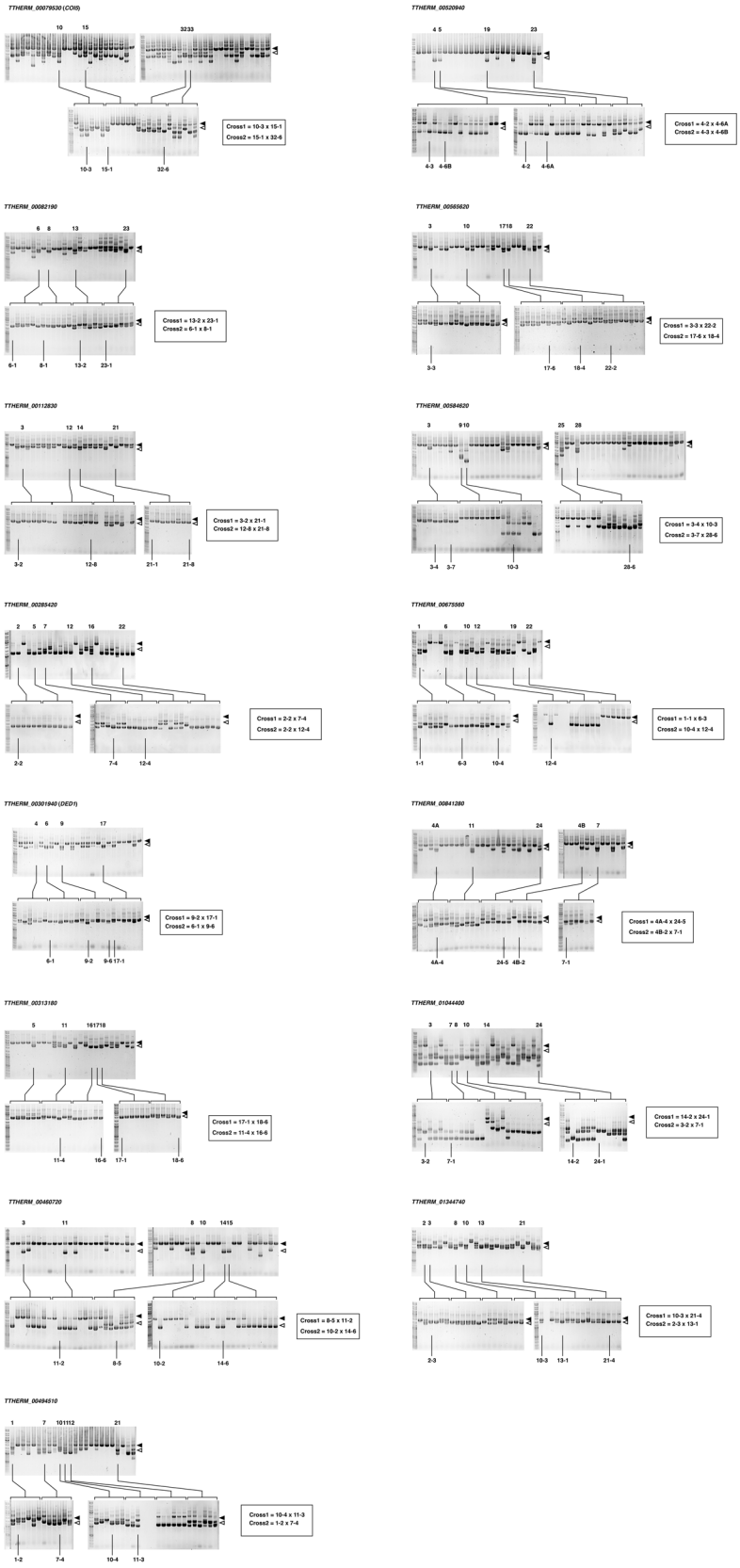

Figure S2. Establishment of KO strains by coDel

Establishment of KO strains by coDel. Progeny lines with nearly complete deletion at the indicated gene loci by coDel (top) were cultured for 12 passages to allow random assortment of the loci and sexual maturation. Then, 6-8 clonal cell lines were established from each progeny and analyzed by genomic PCR (bottom). Indicated cells lines were chosen and used for a DNA elimination assay.

Table S1. The genomic locations of the coDel targets and the names of the primers used for target amplification and for genomic PCR checking coDel

Target gene	Corresponding	MAC genomic position of target			Primer set used for target amplification		Primer set used for deletion check	
	Figs/Tables	MAC SC#	start	end	Primer 1	Primer 2	Primer 1	Primer 2
TTHERM_00079530 (COI5)	Fig. 1; Table 1	3828	1004795	1005509	cDelSa1_FW	cDelSa1_RV	TTHERM_00079530_DelCheck_FW	TTHERM_00079530_DelCheck_RV
TTHERM_00082190	Fig. 1; Table 1	3836	1201352	1201965	cDelSa3_FW	cDelSa3_RV	TTHERM_00082190_DelCheck_FW	TTHERM_00082190_DelCheck_RV
TTHERM_00112830	Fig. 1; Table 1	3812	682512	683218	cDelSa4_FW	cDelSa4_RV	TTHERM_00112830_DelCheck_FW	TTHERM_00112830_DelCheck_RV
TTHERM_00285420 (DED2)	Fig. 1, 3; Table 1	3688	58874	59545	cDelSa17_FW	cDelSa17_RV	TTHERM_00285420_DelCheck_FW	TTHERM_00285420_DelCheck_RV
TTHERM_00285420 (DED2)	Fig. 3	3688	58525	59196	cDelSa17_FW5	cDelSa17_RV2	TTHERM_00285420_DelCheck_FW	Sa17_DelCheck_RV2
TTHERM_00285420 (DED2)	Fig. 3	3688	58525	59545	cDelSa17_FW	cDelSa17_RV2	TTHERM_00285420_DelCheck_FW	Sa17_DelCheck_RV2
TTHERM_00285420 (DED2)	Fig. 3	3688	58067	59545	cDelSa17_FW	cDelSa17_RV3	TTHERM_00285420_DelCheck_FW	Sa17_DelCheck_RV3
TTHERM_00285420 (DED2)	Fig. 3	3688	57524	59545	cDelSa17_FW	cDelSa17_RV4	TTHERM_00285420_DelCheck_FW	Sa17_DelCheck_RV4
TTHERM_00301940 (DED1)	Fig. 1, 2, 6; Table 1	3691	710271	710862	cDelSa2_FW	cDelSa2_RV	TTHERM_00301940_DelCheck_FW	TTHERM_00301940_DelCheck_RV
TTHERM_00399200	Fig. 1; Table 1	3713	73945	74523	cDelSa7_FW	cDelSa7_RV	TTHERM_00399200_DelCheck_FW	TTHERM_00399200_DelCheck_RV1
TTHERM_00313180	Fig. 1; Table 1	3810	469974	470526	cDelSa19_FW	cDelSa19_RV	Sa19_DelCheck_FW	Sa19_DelCheck_RV
TTHERM_00460720	Fig. 1	3823	98721	99339	cDelSa15_FW	cDelSa15_RV	TTHERM_00460720_DelCheck_FW1	TTHERM_00460720_DelCheck_RV
TTHERM_00460720	Table 1	3823	98677	99568	cDelSa15_FW2	cDelSa15_RV2	TTHERM_00460720_DelCheck_FW2	TTHERM_00460720_DelCheck_RV
TTHERM_00494510	Fig. 1; Table 1	3711	398243	398863	cDelSa12_FW	cDelSa12_RV	TTHERM_00494510_DelCheck_FW	TTHERM_00494510_DelCheck_RV
TTHERM_00520940	Fig. 1; Table 1	3717	18388	18969	cDelSa13_FW	cDelSa13_RV	TTHERM_00520940_DelCheck_FW	TTHERM_00520940_DelCheck_RV
TTHERM_00565620	Fig. 1; Table 1	3721	288798	289473	cDelSa6_FW	cDelSa6_RV	TTHERM_00565620_DelCheck_FW	TTHERM_00565620_DelCheck_RV
TTHERM_00584620	Fig. 1; Table 1	3675	48731	49313	cDelSa5_FW	cDelSa5_RV	Sa5_DelCheck_FW	Sa5_DelCheck_RV
TTHERM_00675560	Fig. 1	101	212542	213240	cDelSa16_FW	cDelSa16_RV	TTHERM_00675560_DelCheck_FW	TTHERM_00675560_DelCheck_RV1
TTHERM_00675560	Table 1	101	212542	213580	cDelSa16_FW	cDelSa16_RV2	TTHERM_00675560_DelCheck_FW	Sa16_DelCheck_RV2
TTHERM_00841280	Fig. 1; Table 1	3741	38458	39065	cDelSa18_FW	cDelSa18_RV	Sa18_DelCheck_FW	Sa18_DelCheck_RV
TTHERM_01044400	Fig. 1; Table 1	3760	14886	15592	cDelSa11_FW	cDelSa11_RV	TTHERM_01044400_DelCheck_FW	TTHERM_01044400_DelCheck_RV1
TTHERM_01344740	Fig. 1; Table 1	336	36510	37116	cDelSa10_FW	cDelSa10_RV	TTHERM_01344740_DelCheck_FW	TTHERM_01344740_DelCheck_RV
TTHERM_00388160	Fig. 1; Table 1	41	199509	200155	Log1_cDelFW	Log1_cDelRV	Log1_DelCheckFW	Log1_DelCheckRV
TTHERM_00408840	Fig. 1; Table 1	3714	89022	89690	Log2_cDelFW	Log2_cDelRV	Log2_DelCheckFW	Log2_DelCheckRV
TTHERM_00935580	Fig. 1; Table 1	169	58015	58654	Stv1_cDelFW	Stv1_cDelRV	Stv1_DelCheckFW	Stv1_DelCheckRV
TWI2	Fig. 4	scf_8254654	64114	65135	TWI2_cDelFW	TWI2_cDelRV	TWI2_DelCheckFW2	TWI2_DelCheckRV2
TWI2	Fig. 4	scf_8254654	64114	65135	TWI2_cDelFW	TWI2_cDelRV	TWI6_DelCheckFW	TWI6_DelCheckRV
TWI2	Fig. 4	scf_8254654	64114	65135	TWI2_cDelFW	TWI2_cDelRV	TWI7_DelCheckFW2	TWI7_DelCheckRV2
XRN2	Fig. 5	3702	598484	599481	XRN2_cDelFW	XRN2_cDelRV_XRN4c	XRN2_DelCheck_FW	XRN2_DelCheck_RV
XRN4	Fig. 5	3813	329272	330354	XRN4_cDelFW_XRN2c	XRN4_cDelRV	XRN4_DelCheck_FW	XRN4_DelCheck_RV

Table S2. The sequences of the primers used in this study

target	primer name	sequence (5' to 3')
TTHERM_00079530 (COI5)	cDelSa1_FW	CTTTATTGTTATCATCTTATGACCGCTGTTCAGCTCTAATTACTCG
	cDelSa1_RV	CTCATCAAGTTGTAATGCTAAAATGCTAATCATTACCATTCTTCTACAGGC
	TTHERM_00079530_DelCheck_FW	ATGAACGATAGTAAACAGCTACTCAC
	TTHERM_00079530_DelCheck_RV	AGCTTGATGAGCAATTTCACGACAG
TTHERM_00082190	cDelSa3_FW	CTTTATTGTTATCATCTTATGACCGCTTGTATGGTCTGTTCCATCCCTC
	cDelSa3_RV	CTCATCAAGTTGTAATGCTAAAATGCTTCTGTTTTTAGGATATCTAGTGG
	TTHERM_00082190_DelCheck_FW	ATTTTCATTTTCGCTCGCTAAGCAGC
	TTHERM_00082190_DelCheck_RV	AGCATCATATAGAAGCATCGATAGAG
TTHERM_00112830	cDelSa4_FW	CTTTATTGTTATCATCTTATGACCGCTTAATGGAGTAATTAATTTCACATG
	cDelSa4_RV	CTCATCAAGTTGTAATGCTAAAATGCAGATTCCACGCCTTCAGTTGTTGC
	TTHERM_00112830_DelCheck_FW	TGTTAGTGTAAGTTTAGATACCAAG
	TTHERM_00112830_DelCheck_RV	ATCAGTGGAGTTAGATAATTCAGG
TTHERM_00285420 (DED2)	cDelSa17_FW	CTTTATTGTTATCATCTTATGACCGCTGAATACACTGAAATTCATTCAGAG
	cDelSa17_FW5	CTTTATTGTTATCATCTTATGACCGCCATAGAGAAAGATACCATTCTTAACCG
	cDelSa17_RV	CTCATCAAGTTGTAATGCTAAAATGCAGGACTGTTAGAAACTTAGTTC
	cDelSa17_RV2	CTCATCAAGTTGTAATGCTAAAATGCAATCCAAATGATGTCATATGTGAG
	cDelSa17_RV3	CTCATCAAGTTGTAATGCTAAAATGCTTGAGGATAATTCTTCTAAGCTAGCAG
	cDelSa17_RV4	CTCATCAAGTTGTAATGCTAAAATGCACTAGTTTACACTTTAATGCTCTGC
	TTHERM_00285420_DelCheck_FW	TCTCCGCAAAGAAGGAACTAATCCTC
	TTHERM_00285420_DelCheck_RV	TTGAGGATAATTCTTCTAAGCTAGCAG
	Sa17_DelCheck_RV2	TCAATCAATAAAAGAGTGAATACTTGTTC
	Sa17_DelCheck_RV3	TTATCTCAACTTGTTTTTTAATCCCTC
	Sa17_DelCheck_RV4	ATCTGTTTTTAATACATGCTAGCTGTC
TTHERM_00301940 (DED1)	cDelSa2_FW	CTTTATTGTTATCATCTTATGACCGCTGATTGGAGGATTGAATCAGAAGC
	cDelSa2_RV	CTCATCAAGTTGTAATGCTAAAATGCATATTTGATTTTTTAGCACATGGC
	TTHERM_00301940_DelCheck_FW	TCTTACGAATCCCATAGATCCCACTG
	TTHERM_00301940_DelCheck_RV	TGCTACGAAAGAAGTTAATTAGCTCC
TTHERM_00399200	cDelSa7_FW	CTTTATTGTTATCATCTTATGACCGCATTCAAAGACACCTATGATGATGC
	cDelSa7_RV	CTCATCAAGTTGTAATGCTAAAATGCTGGCTATTGTTGTATTCCCAAGTG
	cDelSa7_FW2	CTTTATTGTTATCATCTTATGACCGCCACATTTACTTAAGAAATACTCAAAGG
	cDelSa7_RV2	CTCATCAAGTTGTAATGCTAAAATGCTAAATAGTAAGTTATTGTTCCCACTG
	TTHERM_00399200_DelCheck_FW	ATATGCCAGTAACTACGTATTAAGAGG
	TTHERM_00399200_DelCheck_RV1	TAACCACTCGGCCAAAGTGTCC
	TTHERM_00399200_DelCheck_RV2	AAGTCAATCAATACCTTAATAAGTACAG
TTHERM_00313180	cDelSa19_FW	CTTTATTGTTATCATCTTATGACCGCTATAAACTTTCTAAAATGAGCTCC
	cDelSa19_RV	CTCATCAAGTTGTAATGCTAAAATGCGATTTTATACCAGAATTGAATCC
	Sa19_DelCheck_FW	TTAGTTAAAGCCCTCATTAAGATCAGC
	Sa19_DelCheck_RV	TGTATAAATTTTATTAATATTTAATGAGCTACC
TTHERM_00460720	cDelSa15_FW	CTTTATTGTTATCATCTTATGACCGCTGGAGAATAATTATATCGAAGAGCTG
	cDelSa15_RV	CTCATCAAGTTGTAATGCTAAAATGCTTGATATGTAATTCTATTTCATTTTCC
	cDelSa15_FW2	CTTTATTGTTATCATCTTATGACCGCAGACAATTTAGATTGGTATAGCAG
	cDelSa15_RV2	CTCATCAAGTTGTAATGCTAAAATGCTTGCAAATTGAATTTCATTCCATC
	TTHERM_00460720_DelCheck_FW2	TGGATGGATCCAAAAGCTATTCTCC
	TTHERM_00460720_DelCheck_FW1	TTAGTCACCTACAAAGCTTAAAGCAC
	TTHERM_00460720_DelCheck_RV	AGTTGCCATTTTCTTCATTTGGCATCC
TTHERM_00494510	cDelSa12_FW	CTTTATTGTTATCATCTTATGACCGCACAAATCATCAATGAAAACACCC
	cDelSa12_RV	CTCATCAAGTTGTAATGCTAAAATGCAGATTAAAACTTCTTTGTTTAAACTCC
	TTHERM_00494510_DelCheck_FW	TAAGAGCCAGAATGTCTTCCAGC
	TTHERM_00494510_DelCheck_RV	TAACTGTTTCCTGAGTTTTCGAGCTC
TTHERM_00520940	cDelSa13_FW	CTTTATTGTTATCATCTTATGACCGCAGGGGTAGAAGCTAAAATGACCGC
	cDelSa13_RV	CTCATCAAGTTGTAATGCTAAAATGCATCTTCTACATGAAGGATATCTCGC
	TTHERM_00520940_DelCheck_FW	TATGTTTACTATGATTTCTTCTTGCC
	TTHERM_00520940_DelCheck_RV	ATCGCATCATCAAATGCAAATGCACC
TTHERM_00565620	cDelSa6_FW	CTTTATTGTTATCATCTTATGACCGCAAACAAGTCTATATGGTAGC
	cDelSa6_RV	CTCATCAAGTTGTAATGCTAAAATGCAGGAGAATGATTCTGAGCTGTGC
	TTHERM_00565620_DelCheck_FW	TGACTCACTTAACAAGTACTGTTCG
	TTHERM_00565620_DelCheck_RV	TTTATTCATCCTAGCTGCTTAGGC
TTHERM_00584620	cDelSa5_FW	CTTTATTGTTATCATCTTATGACCGCTTATGTTGATAAAGCTGACCATCC
	cDelSa5_RV	CTCATCAAGTTGTAATGCTAAAATGCAGATTTTGTTCTTCTAATTATTCGATGCC
	Sa5_DelCheck_FW	ACCTCTGAAAATCAATGCCTTATAGCC
	Sa5_DelCheck_RV	AGCTTATTTATTGAATGCAGCTGGTTGG
TTHERM_00675560	cDelSa16_FW	CTTTATTGTTATCATCTTATGACCGCTTAATTCTGCTGCCACTTCAG
	cDelSa16_RV	CTCATCAAGTTGTAATGCTAAAATGCTTAAAGGACTCACTAAGATTTACAGG
	cDelSa16_RV2	CTCATCAAGTTGTAATGCTAAAATGCATAAACATCCATGTCTTCAAACGAG
	TTHERM_00675560_DelCheck_FW	TCATTTTCATCATTTTTCAATAGGTGTC
	TTHERM_00675560_DelCheck_RV1	TCTGTAAAATGTTTTAAGCTCCTCCAAGC
	Sa16_DelCheck_RV2	TGCTTAAGGATTCATCGCTGTTCTGC

TTHERM_00841280	cDelSa18_FW	CTTTATTGTTATCATCTTATGACCGCGAATTGAAGTAGAAAGACGTGCGTG
	cDelSa18_RV	CTCATCAAGTTGTAATGCTAAAATGCAGGATCAAAATTTGAGAACTGCTG
	Sa18_DelCheck_FW	TGATTCAGCACCAATTTTATCTCCAGGC
	Sa18_DelCheck_RV	ACCATTAGAGTGGGGATCTTCAATAGC
TTHERM_01044400	cDelSa11_FW	CTTTATTGTTATCATCTTATGACCGCAAATGGAGAAATCGTTAGCTTACAC
	cDelSa11_RV	CTCATCAAGTTGTAATGCTAAAATGCTGTATTTTGATTGTCTGCTTTCTGTGATG
	TTHERM_01044400_DelCheck_FW	AGATAAGATATTTCTCCTATTTCTCAGC
	TTHERM_01044400_DelCheck_RV1	ATGAATTCAAAAGGTTGGCATCATAG
TTHERM_01344740	cDelSa10_FW	CTTTATTGTTATCATCTTATGACCGCATAGTAGAAATTAGAGATGGCCAG
	cDelSa10_RV	CTCATCAAGTTGTAATGCTAAAATGCTGATGTGTTAGACTGTAAGTGTGACTAG
	TTHERM_01344740_DelCheck_FW	AGGTATTTCACTCTAGTTAATTAGGAG
	TTHERM_01344740_DelCheck_RV	TCTATAAAAAAACAGTATTGAGGAACTAC
TTHERM_00388160	Log1_cDelFW	CTTTATTGTTATCATCTTATGACCGCAGGTATTGTTGCTTCTTTTCTATGCC
	Log1_cDelRV	CTCATCAAGTTGTAATGCTAAAATGCTACTCACTAAGTACTTGACTGC
	Log1_DelCheckFW	TCTAAGACTGCTGAAACAAAGAGTGAC
	Log1_DelCheckRV	ACATAATTGGATGTAAAACTACATTCCTC
TTHERM_00408840	Log2_cDelFW	CTTTATTGTTATCATCTTATGACCGCTTATCCTGATTGTACGATTATGACTC
	Log2_cDelRV	CTCATCAAGTTGTAATGCTAAAATGCTACTTCTAATTTACATCCTGAGAGGAC
	Log2_DelCheckFW	ACCCTCAACAAATAACTTTAAGCAACC
	Log2_DelCheckRV	TATACAAATTTATTAAAATTCTGGAAGTC
TTHERM_00935580	Stv1_cDelFW	CTTTATTGTTATCATCTTATGACCGCAGATTTAGTGGTAGGCAATTCAGTTGC
	Stv1_cDelRV	CTCATCAAGTTGTAATGCTAAAATGCAACAGAATTACCAGGAGCAGCAGC
	Stv1_DelCheckFW	TTAGAAAGGCTGAATGACTTCCCATG
	Stv1_DelCheckRV	ATCATAGCTAATTAAAATTTCGCACATC
TWI2, TWI6 and TWI7	TWI2_cDelFW	CTTTATTGTTATCATCTTATGACCGCTGATGAACCTCAAGGATTTGTG
	TWI2_cDelRV	CTCATCAAGTTGTAATGCTAAAATGCTTGTTTTGCTGCTTAAAAATCTG
	TWI2_DelCheckFW2	ATTTTAAGTTTTTAAGATCGCTGTTTGC
	TWI2_DelCheckRV2	AGTTATCAAGTAGTTAGTTCTGGCTGG
	TWI6_DelCheckFW	TGCTTTGTTAGCAGTATCTATTAATTGC
	TWI6_DelCheckRV	TTGTTAAAATAATAAAATTTCGATGAAGC
	TWI7_DelCheckFW2	TCAAGCTTCACTTATTGCCATTAATGCTC
	TWI7_DelCheckRV2	TCAAATCTATCTCTTTAGTCTCGCATGC
XRN2 and XRN4	XRN2_cDelFW	CTTTATTGTTATCATCTTATGACCGCTGTTCCAGCATTCTTTAGGTGGC
	XRN2_cDelRV_XRN4c	GCTGTGTATTGATGGAATTCTCTAGGGCTCATGAGTACTTAATCC
	XRN4_cDelFW_XRN2c	GGATTAAGTACTCATGAGCCCTAGAGAATTCCATCAATACACAGC
	XRN4_cDelRV	CTCATCAAGTTGTAATGCTAAAATGCTCCATTCAAGAAAGGATACATACGC
	XRN2_DelCheck_FW	TCCTATTTATTATTTAAATAAATCCTTAGTC
	XRN2_DelCheck_RV	ACTTTTTTCTCATCCTTTAATCTCCACTC
	XRN4_DelCheck_FW	TAATAAATAATGGCCCTTCTACACAG
	XRN4_DelCheck_RV	TGTTTAATAAGAAAAACACTCGCTTGC
complementation of DED1	Sa2_Res_5FW	CTCTAGAGCATGCGCTAGCGGATCCTGTTGATGAAATGTGCCATTCAGC
	Sa2_Res_5RV	GACCGATTCAGTTCGCTCAATCATTGCATATTTGATTTTTTAGCACATGGC
	Sa2_ResHA_5RV	CAGGAACATCATAAGGATAggaaccTTGCATATTTGATTTTTTAGCACATGGC
	Sa2_Res_3FW	GCTTATCGATACCGTCGACCTGATAATTTAGAAACTTTCAACAAGAG
	Sa2_Res_3RV	GGGTACCGGGCCCCCCCTCGAGAAGAGACTCGTTTCAATTCATCC