Premature termination codons signal targeted gene repair by nonsense-mediated gene editing in *E. coli*

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Abstract

Frameshift mutations yield truncated proteins, leading to loss-of-function, genetic disorders or even death. Reverse mutations, which restore the wild-type phenotype of a mutant, have been assumed to be far rarer than forward mutations. However, in this study, screening tests showed that the revertants of a frameshift mutation are detected more frequently than expected in *E. coli*. Sanger sequencing of the revertants revealed that the reversion of the frameshift mutation is not caused by random mutagenesis but by active targeted gene repair. Molecular studies suggest that premature termination codons (PTCs) in the nonsense mRNAs signal the repair of the frameshift mutation. Genome survey indicate that the genome sequence of a revertant is stable, confirming that the DNA replication proofreading/mismatch repair system of the revertant is not defective. Transcriptome profiling identified dozens of upregulated genes/pathways that possibly involve in frameshift repair, including DNA replication, RNA processing, RNA editing, mismatch repair and homologous recombination. Introducing synthetic DNA or RNA oligonucleotides into the frameshift can promote the gene repair. Based on these data and an in-depth review of previous studies, we hypothesized a molecular model for frameshift repair referred to as *nonsense-mediated gene editing* (NMGE): nonsense mRNAs are recognized by mRNA surveillance by PTC signaling, edited by RNA editing and then used to direct the repair of their defective coding gene through mismatch repair and homologous recombination. Moreover, NMGE may also serve as a driving force for molecular evolution and a new source of genetic diversity, leads to a widespread existence of frameshift homologs.

1. Introduction

DNA replication and DNA repair happen daily in cell division and multiplication. DNA damage, point mutations, insertions and deletions (indels) can be induced by chemical and physical mutagens, such as radiation, pollutants, or toxins. In addition, because of the imperfect nature of DNA replication, mutations and indels also occur spontaneously as replication errors or slipped-strand mispairing. In a protein-coding gene, if the size of an indel is not a multiple-of-three, it causes a frameshift mutation, which results in a substantial change of its encoded amino acid sequence downstream; in addition, it often produces a number of nonsense codons and yields truncated and dysfunctional proteins [1], which may lead to a genetic disorder or even death [2-5].

The molecular mechanisms/pathways involved in the repair of DNA damages and point mutations have been intensively studied [6], including mismatch repair, base- or nucleotide-excision repair, homologous recombination and non-homologous end joining. The maintenance of reading frames of protein-coding genes is fundamentally important for cells. A reverse mutation phenomenon was discovered as early as in the 1960s [7]. Point mutations have been explained by random mutagenesis, and it has been assumed that reverse mutations occur at a lower rate than forward mutations. But, it remains unclear whether a molecular mechanism exists for the repair of frameshift mutations (hereafter frameshift repair). However, here we report that the reversion of a frameshift mutation occurs far more frequently than expected in *E. coli*, and that they are not randomly produced but involve active targeted gene repair.

2. Materials and Methods

2.1 Frameshift mutagenesis and back mutation screening

A plasmid containing a frameshifted β-lactamase gene (*bla*), pBR322-(bla-), was constructed by site-directed mutagenesis (Fig 1): in plasmid pBR322, a G:C base pair, located in the upstream (+136) of the wild-type *bla* gene (*bla*+), was deleted by using overlapping extension polymerase chain reaction (OE-PCR). Competent cells of *Escherichia coli* strain DH5α were transformed with pBR322-(bla-), propagated in tetracycline broth (TCB), and then diluted cultures were plated on a tetracycline plate

(TCP) to count the total number of bacteria (N_0) and on ampicillin plates (ACPs) to screen for and count the number of revertants (N_1). The recovery rates were calculated as N_1/N_0. The screened revertants were picked and propagated in ampicillin broth (ACB) at 37°C with 200 rpm shaking and with 1 mL of overnight seed culture in 10 mL ACB. The growth rates of the revertants were evaluated by the doubling time during their exponential growth phase. Their plasmids DNA were extracted and their *bla* genes were Sanger sequenced.

2.2 Construction and expression of a PTC-free frameshifted bla

A *PTC-free frameshifted bla*, denoted as *bla#*, was derived from *bla-* by replacing each nonsense codon with a sense codon according to the *readthrough rules* (Table 1), and by adding a stop codon (TAA) in the 3'-end. The *bla#* gene was chemically synthesized by *Sangon Biotech, Co. Ltd* (*Shanghai*), inserted into an expression vector, pET28a, and transformed into competent cells of *E. coli* strain BL21. Plasmid pET28a contains a kanamycin-resistance gene, therefore the transformed bacteria were plated on a kanamycin plate (KCP), the transformants were propagated in kanamycin broth (KCB), plated on ACPs to screen for revertants, and then their *bla* genes were Sanger sequenced. The expression of the frameshifted BLA was induced by 0.1 mM IPTG. Their total protein samples were extracted, frameshifted BLA was purified by nickel column chromatography and analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The activity of BLA was measured by iodometry.

2.3 Introduction of DNA or RNA oligonucleotides into the frameshift mutant

Competent cells of the frameshift mutant, which contains the defective plasmid pBR322-(bla-), were transformed with a 39-nt synthetic DNA or RNA oligonucleotide, which has a wild-type or mutated sequence restoring the base pair which was deleted in OE-PCR. The transformed cells were cultured in TCB at 37°C for 6 hours with 200 rpm shaking, and then diluted cultures were plated on TCPs to count the total number of bacteria (N_0), and on ACPs to screen for and count the number of revertants (N_1). The recovery rates were calculated by N_1/N_0. The revertants were subcultured in ACB to test their growth rates, and their *bla* were Sanger sequenced. The growth rates were

evaluated by the doubling time during the exponential growth phase by culturing at 37°C with 200 rpm shaking and with 1 mL of overnight seed culture in 10 mL ACB.

2.4 Genome resequencing and structure/variation analysis of the E. coli strains

Genomic DNA samples were extracted from wild-type (*bla*+) and revertant (*bla**) strains. Library preparation and genome sequencing were conducted by a commercial service provided by *Novogene Co. Ltd*. The library was sequenced on an Illumina HiSeq 250PE platform and paired-end reads were obtained. Raw reads in fastq format were processed by removing the adapter sequence and low-quality reads. The clean reads with high quality were mapped onto the reference genome *E. coli* K12 MG1655 (NC_000913.3) to identify single nucleotide polymorphisms (SNPs), indels and structure variations (SVs) of each sample. Samtools v0.1.18 was used to sort the reads and reorder the bam alignments [8]. SNP typing was performed using the genome analysis tool kit (GATK) [9]. Circos was used to display the coverage of the reads and the distribution of SNPs and indels on the ring diagram [10]. BreakDancer was used to detect structure variations [11, 12].

2.5 Transcriptome analysis of the E. coli strains

Total RNA samples were extracted from the wild type (*bla*+), the frameshift (*bla*-) and the revertants (*bla**) *E. coli* strains. Library preparation and RNA sequencing were conducted by a commercial service provided by *Novogene Co. Ltd*. After library preparation and cluster generation, the library was sequenced on an Illumina HiSeq platform. The paired-end raw reads were processed by removing the adapter sequence and low-quality reads. The high-quality clean reads were mapped onto the annotated reference genome, *E. coli* K12 MG1655 (NC_000913.3), and the expression levels of each gene were calculated for each sample and compared to each other to identify differentially expressed genes (DEGs), enriched Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways.

(1) *Quantification of gene expression level*

Bowtie (v2-2.2.3) was used for index building and aligning the clean reads to the reference genome [13]. HTSeq (v0.6.1) was used to count the numbers of clean reads mapped to each gene [14]. And then expected number of Fragments PerKilobase of

transcript sequence per Millions base pairs sequenced (FPKM) of each gene was calculated based on their lengths and read counts.

(2) *Differential expression analysis*

Prior to differential expression gene analysis, for each strain the read counts were adjusted by the edgeR program [15]. Differential expression of the read counts of two conditions was performed using the DEGSeq R package (1.20.0) [16]. The P values were adjusted by the Benjamini & Hochberg method. The threshold for significantly differential expression were set as: corrected P-value (qvalue) < 0.005 and log2 (Fold change) ≥ 1.

(3) *GO and KEGG enrichment analysis of differentially expressed genes*

GO enrichment analysis was implemented by GOseq R package [17], in which gene length bias was corrected. GO terms with corrected P-value less than 0.05 were considered significantly enriched by DEGs. KOBAS 2.0 [18] was used to test the statistical enrichment of DEGs in KEGG pathways (http://www.genome.jp/kegg/).

3. Results and Analysis

3.1 The growth of the frameshift mutant and the revertants

The plasmid pBR322 contains two wild-type resistance genes, a β-lactamase gene (*bla*+) and a tetracycline resistance gene (*tcr*+). Using OE-PCR, one single G:C base pair in the upstream of *bla*+ was deleted (Fig 1). In the resulting frameshift *bla* (*bla*-), seventeen nonsense codons appeared. Because the active sites of BLA, including the substrate binding site, all locate in the region downstream the deletion, this frameshift mutation is expected to be a loss-of-function. The plasmid containing a wild-type or a frameshifted *bla* gene was transformed into *E. coli* (strain DH5α and BL21), then the transformants were propagated in tetracycline broth (TCB), dilution series were plated on tetracycline plate (TCPs) to estimate the total number of viable bacteria and plated on ampicillin plates (ACPs) to test their ampicillin resistance. The wild-type (*bla*+) bacterial grew well on both ACP and TCP (Fig 2A, left); and it was expected that the deficiency-type (*bla*-) bacterial would not grow on ACP. However, repeatedly a few ampicillin-resistant colonies (*bla**) were observed (Fig 2A, middle). The possibility of

carryover- or cross-contamination was ruled out by blank controls (Fig 2A, right). So, these ampicillin-resistant colonies were considered as revertants whose *bla*- genes were repaired by reverse mutations.

In addition, although most of the revertants can grow in ACB, the growth rates of the revertants are often very much slower when compared to the wild-type strain: by culturing at 37°C with 200 rpm shaking, the wild type took 12 to 24 hrs. to reach the late log phase, while the revertants took up to 36 to 48 hrs., and many of the revertants failed to grow when they were subcultured in ACB, even without adding ampicillin.

Hitherto, it seems that there is nothing unusual, because the reverse mutations are fairly common phenomenon, arising from simple random mutations (Fig 3): in DNA replication, natural or induced mutations occur randomly, a forward mutation causes a defective mutant or a loss-of-function, and a reverse mutation restores the wild-type phenotype. Under this view, these revertants survived only because they were 'lucky': their defective *bla* gene restored coincidently by a reverse mutation, while most of the other bacteria died without a reverse mutation.

3.2 frameshift repair is not a random mutation but a targeted gene repair

The classical random mutagenesis model for reverse mutation sounds faultless in explaining the reversions of point mutations. However, in a 'thought experiment', we noticed that the model is insufficient to explain the frameshift repair observed herein: (1) in the model, a bacterium survives if its frameshifted *bla* gene is restored through random mutagenesis. However, a reverse mutation must occur in the process of DNA replication or repair in a living cell. If the mutant itself could not live in ampicillin, the reverse mutations could not occur after they were cultured in ampicillin but must have occurred before they were cultured in ampicillin; (2) a frameshift mutation can only be repaired by an appropriate insertion or deletion restoring the reading frame of the frameshifted gene. However, even if relatively small, a bacterial genome consists of millions of base pairs over thousands of genes. If the cells did not 'know' which gene is frameshifted, it will be almost impossible to repair it through random insertion or deletion; (3) In *E. coli*, the rate of point mutation is very low. As determined by whole-genome sequencing [19], the baseline mutation rate of the wild-type *E. coli* is

~10^{-3} per genome (or ~10^{-9} per nucleotide) per generation. The frameshift mutation rate measured are even about ten-times lower than that of point mutations [19]. It is generally assumed that reverse mutations occur at a lower rate than forward mutations. For a certain frameshift mutation, the reverse mutation rate should be extremely low if it depends on random mutagenesis, because insertions/deletions are far rarer than substitutions. Therefore, the revertants of a frameshift should be very difficult to be detected. However, through repeated screening tests, we found that the revertants of *bla-* can always be detected easily, and it seems that the reversions of the frameshift mutation occur much more frequently than expected. The measured average recovery rate of *bla-* was 2.61×10^{-8} per cell division in DH5α and reached up to 1.19×10^{-7} per cell division in BL21, both significantly higher than the baseline.

Therefore, it is possible that an efficient mechanism specific for frameshift repair has been developed in the history of life. However, how a frameshifted gene is being identified and repaired at the molecular level is unknown.

3.3 Sanger sequencing of the bla genes of the revertants

To investigate how the frameshift gene is repaired, the revertants were picked and subcultured in ACB. The initial revertants usually grew very slowly, and many of the subcultures failed to grow, but the surviving subcultures grow faster and faster. Their plasmid DNA was extracted, and their *bla* were Sanger sequenced. Unexpectedly, in these initial revertants and subcultures, the *bla* genes are often neither a wild type nor a revertant, but still frameshifted (Fig 2B, top). It is puzzling how these frameshifted *bla* could be functional. To clarify this, the subcultures were re-subcultured and their *bla* were re-sequenced for over 300 replications.

In the later subcultures, the sequencing results of the *bla* genes are also often still frameshifted, but their Sanger diagrams often contain two sets of superimposed peaks (Fig 2B, middle), indicating that they consist of two types of *bla* sequences: one is the original frameshifted *bla*, and the other is the repaired one. By visual inspection of the Sanger diagrams, the repaired sequences of the revertants (*bla**) were read from the secondary peaks. Some sequencing diagrams even contain three or more sets of peaks, but the sequences are nonreadable. In the final fast-growing subcultures, most of their

bla copies were repaired, and their Sanger diagrams contain few superimposed peaks (Fig 2B, bottom).

As shown in Fig 2C, by aligning *bla** sequences against *bla*+ and *bla*- sequences, it was found that they contain a variety of indels and substitutions, especially G:C←→A:T transitions (Fig 2C, circles). Previously, we developed a novel tool for multiple sequence alignment, *codon and aa unified alignment* (CAUSA) [20], which can align and visualize coding sequences and their encoded protein sequences in the same user interface. As shown in Fig 2D, in CAUSA, it is clearly demonstrated that these *bla** coding genes often contain some stop codons, and the translated protein sequences are different from, but highly similar to, that of the wild-type BLA if the stop signals were ignored. In other words, the frameshifted genes are restored far from completely or immediately, but partially and progressively, resulting in a variety of frameshift variants, called *frameshift homologs*.

3.4 The genome of the revertant is not highly variable but very stable

As is well known, an extraordinary high rate of spontaneous mutagenesis occurs over its whole genome in a mutator, such as *E. coli* strain mutD5, due to its defective proofreading and mismatch repair during DNA replication [21]. The *E. coli* strain we used in this study, either DH5α or BL21, is not a mutator, but the frameshift/revertants obtained may change into mutators if their proofreading and mismatch repair system is damaged during culturing, so that high-rate of mutagenesis can occur in their genomes, not only *bla* gene but also other genes.

To clarify whether the other genes had also changed, the tetracycline-resistant (*tcr*) gene in the same plasmid of the frameshift/revertants was also sequenced by the Sanger method, but almost no overlapping peaks were observed in their sequencing diagrams, suggesting that the high-level variations observed in *bla** are not a result of random mutation, but more probably an active gene repair targeting specifically to the frameshifted *bla* gene, but not to other normal genes.

To clarify further whether the frameshift/revertants had changed into mutators, the genome sequences of a frameshift (*bla*-) and a revertant (*bla**) were sequenced by

high-throughput NGS sequencing. The obtained clean reads were mapped onto and compared with the reference genome sequence of the *E. coli* strain K12 (Fig 4A). As shown in Fig 4B and Table 2, the SNP/indel levels of the revertant and frameshift tested are both very low, suggesting that their proofreading/mismatch repair system is not defective. Therefore, they are not mutators, but adopt a stringent proofreading and mismatch repair strategy during DNA replication. In addition, if the variations of *bla* are the result of whole-genome random mutagenesis, the number of variations in *bla* should be proportional to the number of genomic variations, and therefore the genome sequences of the revertants should contain more variations when compared to that of the frameshift. But in fact, as shown in Fig 4B and Table 2, the SNP/indel levels of the revertant are not higher but even lower than those of the frameshift. The number of variations of *bla* are independent on that of genome variations, suggesting that the high-level of variations observed in their *bla* are not caused by whole-genome random mutagenesis, but more probably through an active mechanism specifically targeting the frameshifted *bla* gene.

In addition, genome structure analysis shows that there are quite some structural variations (SVs) in each of the two genomes tested. However, as shown in Fig 4C and Table 3, most of the SV sizes are 100~200 bp, approximate to the length of the reads. The lengths of the SVs should follow a normal or any other skewed distribution, these equal-length SVs are therefore considered as mostly falsely-mapped reads rather than true SVs. As is known, NGS sequencing technologies have several major limitations, especially short read lengths and amplification bias [22], posing serious problems in sequence assembly, genome mapping and structures analysis. However, thanks to the extremely high sequencing depth, the SNP/indel data of NGS is considered as valid and reliable.

3.5 PTCs signal the repair of the frameshift mutation

We speculated that it was the PTCs presented in the nonsense mRNAs that signal the repair of frameshift mutations. To validate this, a *PTC-free bla-* (denoted as *bla#*) was derived from *bla-* by replacing each nonsense codon with an appropriate sense codon in accordance with the *in vivo readthrough rules* (Table 1). The *bla#* gene was

synthesized chemically, cloned in the plasmid vector pET-28a and expressed in *E. coli* strain BL21. The *bla#* transformants were plated on ACPs, but no revertant was found. The *bla#* gene of the transformants were Sanger sequenced, while few variations were observed, suggesting that the *bla#* gene is not repaired as efficiently as the *bla-* gene. Analysis of total and purified protein by SDS-PAGE revealed a band corresponding to the predicted molecular mass (34-kDa), representing the product expressed from the PTC-free frameshifted *bla* (Fig 5A). No lactamase activity was detected by iodimetry test of the product (the frameshifted BLA) (Fig 5B), suggesting that it is indeed a loss-of-function.

In summary, the *bla#* gene was kept unchanged and a dysfunctional product was expressed in its wrong reading frame in *E. coli*. The only difference between *bla#* and *bla-* is that *bla-* consists of a certain number of PTCs but *bla#* does not. Therefore, it must be the PTCs that signaled the frameshift repair in the frameshifts. We postulated that the transcripts of the frameshifted gene (the nonsense mRNAs) must be involved, because presumably these PTCs can only be recognized in a nonsense mRNA in the process of translation but not likely possible in a DNA double helix.

3.6 Identifying genes/proteins involved in frameshift repair

We speculated that frameshift repair requires the upregulation of relevant genes, proteins and pathways. By transcriptome sequencing and profiling, as shown in Fig 6A, the gene expression patterns of these strains are significantly different from each other. When compared to the wild-type (*W*), respectively 292 or 247 genes displayed upregulated transcription (Fig 6A, Supplementary dataset 1-2) in the frameshift (*FS*) or revertant (*RE*). In the pathway enrichment analysis, respectively 65 or 58 pathways were identified as upregulated (Supplementary dataset 3-4). However, the upregulated genes/pathways are mostly nutrition-related, which do not likely involve in frameshift repair but more probably due to the different growth conditions of these different cells. Therefore, we selected the upregulated genes/pathways that most probably involve in frameshift repair manually. The criterion of selection is whether the gene/pathway is involved in the processing of the storage of genetic information (DNA or RNA). As shown in Fig 6B-6C and Table 4-5, the selected upregulated genes/pathways include

DNA replication (Fig 6D), *RNA degradation/processing* (Fig 6E), *mismatch repair* and *homologous recombination* (Fig 6F).

In addition, the selenocysteinyl-tRNA-specific translation factor (*selB*) gene was upregulated in one of the two revertants tested (Supplementary dataset 5). SelB is a specialized translation factor that takes the place of elongation factor EF-Tu, in the case of selenocysteines being inserted into a peptide chain at the site of a UGA codon [23, 24]. The upregulation of *selB* suggested that the nonsense codons remained in the mRNAs might be readthrough in the revertants.

By carefully inspecting the list of DEGs (Supplementary dataset 6), we noticed that the expression levels of the cytosine or isoguanine deaminase gene (*codA*) and the adenosine deaminase gene (*add*) were upregulated in the frameshift, and those of the cytidine/deoxycytidine deaminase gene (*cdd*) and the tRNA-specific adenosine deaminase gene (*tadA*) were also upregulated in the revertant:

(1) In *E. coli*, cytosine or isoguanine deaminase (CodA) catalyzes the hydrolytic deamination of cytosine to uracil, and also the deamination of isoguanine, a mutagenic oxidation product of adenine in DNA, and of isocytosine [25, 26]. In eukaryotes, cytosine deaminase is involved in RNA editing [27].

(2) In *E. coli*, cytidine/deoxycytidine deaminase (Cdd) scavenges exogenous and endogenous cytidine and 2'-deoxycytidine for uridine monophosphate (UMP) synthesis [28-33]. In eukaryotes, cytidine/deoxycytidine deaminase is also involved in RNA editing [34, 35].

(3) In *E. coli*, adenosine deaminase (Add) is an inducible enzyme that converts adenine, adenosine, and deoxyadenosine to guanine nucleotides [36-38]. In eukaryotes, adenosine deaminase (ADAR2) is an important enzyme which is responsible for RNA editing [39-51].

(4) The tRNA-specific adenosine deaminase (TadA) catalyzes the deamination of adenosine to inosine at the wobble position 34 of tRNA-Arg2. TadA was first identified in yeast [52] and the first prokaryotic RNA editing enzyme to be identified in *E. coli* [53] and found widely exist in bacterial [53-56]. TadA

is related to the eukaryotic RNA editing enzymes, ADAR1 and ADAR2 [52], which widely exist in human [57] mouse [58] and fruit fly [59].

As above mentioned, Sanger sequencing showed that the base substitutions in the repaired *bla* are mostly G:C←→A:T transitions, suggesting that they are derived from deamination of DNA bases. As is known, DNA deamination generates deoxyinosine from deoxyadenosine, which links DNA mismatch repair and RNA editing [60]. Here we show that DNA deamination and mismatch repair pathways are upregulated in the frameshift, suggesting that RNA-editing and mismatch-repair are probably both involved in frameshift repair. These genes are also upregulated in the revertant, suggesting that gene repair is still ongoing in the revertants. As mentioned above, the genome sequence of the revertant is not highly variable but stable, suggesting that these upregulated genes are tightly controlled, their function is restricted specifically to the target gene, and thus do not cause many off-targeted variations in their genomes. However, further experimental investigation is needed to validate the regulation and the function of many of these genes in the repair of frameshift mutations.

3.7 Both DNA and RNA can direct the repair of frameshift mutation

Synthetic DNA oligonucleotides have been widely used for targeted gene repair [61-63]. Here, competent cells of the frameshift mutants (*E. coli* strain DH5α or BL21) were transformed by heat-shocking with a 39-nt synthetic sense (ss-), antisense (as-) or double-stranded (ds-) DNA, having a wild-type (WT) or mutated (MT) sequence to recover the base which was deleted in the OE-PCR. The recovery rate (f_r) was very low and unstable in DH5α, but much higher and more stable in BL21. We performed statistical analysis on the data of BL21. As shown in Fig 7A-7B, in the blank or heat-shock-only control groups, the average f_r is 1.19×10^{-7} and 1.30×10^{-7}, respectively, suggesting that heat-shock does not bring significant difference to the recovery rate by itself. Comparing with the blank control group, on average f_r increased to 1.93-fold in the ss-DNA group, 6.72-fold in the as-DNA group and 2.94-fold in the ds-DNA group, respectively, of which differences are all significant (F-test $P<0.01$). These data are

consistent with other's previous studies that both sense- and antisense-DNA can induce targeted gene repair [64].

It has also been reported that synthetic RNA oligos, DNA/RNA complexes and endogenous transcripts can direct the repair of DSBs of DNA [65-70]. We postulated that synthetic RNAs can also promote frameshift repair if the NMGE model is correct. To validate this, the frameshift mutants (strain DH5α or BL21) were transformed with a 39-nt WT or MT RNA to recover the base deleted. The MT RNA caused an increase of f_r to 3.58-fold, however, it is surprising that the WT RNA caused a decrease of f_r to 0.51-fold (Fig 7B). The differences of the recovery rates among different groups are all significant by F-test, unfortunately, however, are not significant by t-test, because their standard deviations are very large (Fig 7B).

For each group, the *bla* genes were sequenced to test whether they were repaired or not. The Sanger diagram showed that a designed base was inserted into the repaired *bla* gene in both the ssDNA (Fig 7C) and the ssRNA group (Fig 7D). In the control group, the type and location of the inserted base are both unpredictable; in the ssDNA or ssRNA group, however, the type of the insertional base is often consistent with the designed type, and the location of the inserted base is exactly at the site of deletion, suggesting that, like DNA, RNA can also direct targeted gene repair by itself.

Homologous recombination must play an important role in frameshift repair, as the average recovery rate measured in BL21 (recA+) is significantly higher than that of DH5α (recA-). However, homologous recombination may not be the only pathway responsible for frameshift repair. In all DNA, RNA and control groups, G:C→A:T substitutions were often observed in the repaired *bla* (Fig 7C). In the ssRNA group, G:C→A:T substitutions were also often observed. In addition, MT-ssRNA-136-A, a ssRNA designed with an G→A substitution, not only resulted in an increased average recovery rate in BL21 (Fig 7B), but also efficiently introduced the substitution into the repaired *bla* (Fig 7D), suggesting that mismatch repair and/or RNA editing might be involved in the repair.

4. Discussion

4.1 A new model for the repair of frameshift mutations

The mechanisms for the repair of DNA damages and point mutations have been well-understood. However, the molecular mechanism for the recognition and repair of frameshift mutations is unknown at present. To gain further insight, we conducted an in-depth literature survey of previous studies that covered a wide range of prokaryotic and eukaryotic organisms. We put many of these fragmentary evidences together by presuming that the molecular mechanism of frameshift repair exists and is conserved among prokaryotic and eukaryotic species. Based on our studies and many others, we hypothesize a molecular model for frameshift repair, referred to as *nonsense-mediated gene editing* (NMGE), consisting of six main steps as follows (Fig 8):

Step 1. ***Nonsense mRNA recognition***: in principle, a frameshift coding gene cannot be recognized at DNA level, and therefore must be first transcribed before it can be recognized. When a frameshift gene is transcribed, a number of nonsense mRNAs are produced. The frameshifted transcripts are recognized as nonsense mRNAs by RNA surveillance [71-77]. The stop codons emerge inside a frameshifted gene or mRNAs are called premature termination codons (PTCs) [78-80] , also known as hidden stop codons (HSCs) [1]. When an nonsense mRNA is translated, the ribosomes are blocked when they encounter the PTCs [81]. Nonsense mRNAs are identified through mRNA surveillance, and usually causes mRNA decay [82-85].

Step 2. ***Nonsense mRNA processing***: In eukaryotes, nonsense-mediated mRNA decay (NMD) is the best-studied nonsense mRNA processing pathway [86-88]. NMD targeting mRNAs harboring PTCs for degradation enhances their decay rate and reduces the abundance of the mRNA transcribed from that gene. In bacteria, it is not yet clear how nonsense mRNAs are processed, but it is known that the quality of their mRNAs is also tightly controlled [89]. Therefore, we speculate that NMD may also exist in bacteria, although it has not been characterized. Nevertheless, in both prokaryotes and eukaryotes, nonsense

mRNAs must be processed by one of the pathways, either degradation (step 2), translation (step 3), or editing (step 4).

Step 3. ***Nonsense mRNA translation*:** Nonsense mRNAs are not always fully degraded but sometimes translated. Frameshifted or nonsense-mutated coding genes can be expressed through some special mechanisms, including programmed translational frameshifting [90-97] and translational readthrough [98-101]. Especially, if the target gene is essential, a cell survives only if it produces functional proteins by translating a nonsense transcript/mRNA and dies if no protein is produced or the products are truncated or nonfunctional.

Step 4. ***RNA editing***: nonsense mRNAs must be involved in the recognition and repair of the frameshift mutation, but a nonsense mRNA is defective by itself, and thus, cannot template the repair of its coding gene directly, therefore, it is more likely that the nonsense mRNAs are modified by RNA editing prior to templating the repair. Nonsense mRNAs can be recognized by PTC signaling, but it might be impossible for cells to locate the frameshift-causing indel exactly, either in the genomic DNA or transcripts, and thus, not always possible to restore the original coding sequence. In addition, a cell cannot distinguish whether a PTC is caused by an indel or by a point mutation. Therefore, the cells can only insert, delete and substitute nucleotides in the nonsense mRNAs within or nearby a PTC, resulting in a variety of variants. After editing, the repaired mRNAs are translated again, and the remaining PTCs are translated each into an appropriate amino acid by translational readthrough.

Step 5. ***RNA-directed DNA repair***: to repair the frameshifted coding gene, mRNAs are transported back to chromosomal DNA to localize its defective coding sequence. the defective coding gene is repaired by using an edited mRNA as the template. A bacterium recovers if functional mRNAs and proteins are produced by a normal transcription and translation process.

As abovementioned, substantial evidence suggests a direct link between RNA editing and DNA repair, which can also support this NMGE model. However, further

systematic experimental investigations are needed to elucidate this process in detail and validate it in other species, especially the eukaryotes.

4.2 *The NMGE model better explains the variations of the bla genes*

As shown by a question mark in Fig 8, there exist two possible strategies: (1) *direct NMGE*: using an original nonsense mRNA to identify the defective coding sequence, and then editing it by DNA-level mutagenesis; however, whether or not the edited CDS is repaired, it must be transcribed and translated again to be functional; (2) *indirect NMGE*: the nonsense mRNAs are first edited, and then transported back to DNA to identify the CDS and to template the gene repair, *i.e.*, a nonsense mRNA is functionalized through RNA editing prior to directing the repair of its own CDS. Compared to the direct strategy, indirect NMGE may be more efficient, because DNA repair only happens in a living cell gaining a functional mRNA by RNA editing.

As described above, distinct types of bases were substituted, inserted or deleted in the *bla* genes of the revertants. These editing events are mostly located near to or in the downstream of the frameshift-causing indel, but not in the distant upstream (Fig 2B-2C). This phenomenon can only be explained by the PTC-signaled RNA editing in the nonsense mRNAs: the PTCs presented downstream the indel may not only signal the recognition of nonsense mRNA but serve as flags for RNA editing. Because the PTCs appear in nonsense mRNAs only downstream of the indel, PTC-signaled RNA editing are restricted near to or in the downstream of the indel, potentially also restrict DNA editing to the corresponding region in the coding gene. Therefore, the frameshift mutation might be repaired by editing the nonsense mRNAs rather than the CDS directly. It is also possible that sometimes editing may cause even more problems in a nonsense mRNA, while these non-repaired mRNAs can be recycled or degraded. This process is repeated in cells until the PTCs in their *bla* genes are completely eliminated. When the cells are cultured in ampicillin media, the fate of a cell depends on whether it can produce a sufficient amount of functional proteins. By transcribing, each copy of a target gene can produce many copies of nonsense mRNAs, each of which might be edited in a different way, without changing the coding DNA sequences. Therefore,

the probability of producing a functional mRNA by editing nonsense mRNAs can be much higher than that of random mutagenesis in their coding sequences.

4.3 *The NMGE model better explains the growth of the revertants*

As abovementioned, frameshift repair is not genially a sudden + complete event but a progressive process. Interestingly, the sizes of wild-type colonies are uniform (Fig 2A), showing that they are uniform in growth rates; while the sizes of revertant colonies varied greatly (Fig 7A), indicating varied growth rates associate with the diversified activities of their BLA. Compared with random mutagenesis, NMGE not only better explains the high-level variations of the *bla* genes, but also the diversified growth rates of the revertants:

(1). In the initial revertants, whose *bla* genes were still frameshifted, survival can be explained in three possible ways: (a) the frameshifted *bla* is translated by *ribosomal frameshifting*, producing a wild-type BLA; (b) the frameshifted *bla* is translated by *translational readthrough*, producing a functional, partially frameshifted BLA; (c) the initial slowly-growing revertants may also contain a few copies of repaired *bla*, with the proportion of the repaired *bla* being too low to be detected by Sanger sequencing. As pBR322 is a multi-copy plasmid, it might be sufficient to support the survival of the host in ampicillin even when only a small proportion of its copies are repaired. The host bacterial grow slowly, and many of them failed to grow, probably due to these abnormal processes are very time- and resource-consuming, as cell energy and resources would be depleted for gene repair if the defective *bla* is not repaired soon.

(2). By NMGE, the *bla* genes were repaired progressively, not only before but also after culturing in the ampicillin media. As the substrate of BLA, ampicillin is neither the cause nor the terminator of the gene repair, but a selection pressure to screen and display the revertants, which is the result of gene repair.

(3). NMGE is triggered by the PTCs presented in the *bla* mRNAs, and the aim of NMGE is to eliminate these PTCs. In the initial revertants, there are so many PTCs in the *bla* mRNAs that the probability of producing a functional product

by mRNA editing is very low, so their growth rates and survival rates are very low, as most revertants died before their *bla* were sufficiently repaired.

(4). Later, in revertants whose *bla* are being repaired, with less and less remaining PTCs, energy and resources are used less and less for gene repair, more and more for growth, their growth rates and survival rates recover gradually.

(5). Finally, gene repair complete if all nonsense codons are eliminated. The *bla* coding genes are recovered, not necessarily the same as the original wild-type, but consisting of a variety of variants, whose growth rates may become normal, slower, or sometimes even faster.

Previously, we have reported that the genetic code, the protein-coding genes and genomes of all species are nearly optimal for frameshift tolerance [102]. Widespread premature termination codons were recently observed in human [78] and fruit fly [103] populations, suggest the ongoing evolutionary plasticity at the genetic level in modern human and other species. In this study, we further demonstrate that PTCs signal the frameshift repair. Frameshift mutations in protein-coding genes are repaired through NMGE, resulting in a variety of frameshift homologs, many of which are functional and preserved in nature. Therefore, the widespread existence of frameshift homologs among species may be considered as natural evidence for this model. In short, NMGE is not only a molecular mechanism for frameshift repair, but also be a driving force for molecular evolution and a source of genetic diversity.

4.4 *Previous studies are supportive to this NMGE model*

In the above, we proposed that NMGE depends on RNA-directed DNA repair but is triggered by PTCs, and the nonsense mRNAs are edited prior to directing the repair of the coding DNA. When a frameshift mutation occurs, the defective gene is repaired not by direct gene editing but by NMGE. Although it may seem surprising, but in fact it is more reasonable than direct gene editing. The above proposed NMGE model well explains our observations and is supported by numerous previous studies. In fact, the model is an integration of several key links, which have already been quite clear:

(1). mRNA decay is linked to other nonsense mRNA processing pathways

Nonsense-mediated mRNA decay (NMD) is the best-studied nonsense mRNA processing pathway [86-88]. In eukaryotes, NMD uses the presence of an exon junction complex (EJC) downstream of PTCs as a second signal to distinguish a PTC from a true stop codon [104]. In addition to NMD, the nonsense mRNAs may also be subject to other nonsense-mediated pathways, such as *translational repression* [105], *transcriptional silencing* [106], and *alternative splicing* [107], all of which are connected to NMD [105-107]. In eukaryotes, the NMD regulating factors, including three interacting proteins, UPF1, UPF2 (also known as NMD2) and UPF3, are encoded by highly conserved genes originally identified in yeast [108].

Frameshifted or nonsense-mutated coding genes can be expressed through some special mechanisms, including ribosomal frameshifting [90-97] and translational readthrough [98-101]. Ribosomal frameshifting occurs in all known organisms, from *E. coli* to mammals [109-112], whereby the ribosome is guided toward a triplet codon that is shifted one base position to the upstream (+1) or downstream (−1). In yeast, telomere maintenance is globally controlled by the ribosomal frameshifting process and NMD [97]. Translational readthrough is a process wherein a nonsense mRNA is translated by translating PTCs each into a specific amino acid [113-117]. Mutations in *upf* genes caused nonsense suppression and subsequent readthrough in yeast and 5-20 fold decreases in the rates of NMD [118], suggested a direct link between NMD, nonsense suppression and readthrough. The three Upf proteins all interact with Sup35, and Upf1 interact further with a second release factor, Sup45 (eRF1), and binding of a SMG-1-Upf1-eRF1-eRF3 (SURF) complex to EJC triggers Upf1 phosphorylation and NMD [119]. In addition, the nonsense suppression caused by *upf* mutations is additive to those by *sup35* and *sup45* mutations [120], suggesting that nonsense suppression is a nonsense-mRNA processing pathway alternative to NMD. In summary, although nonsense mRNAs usually lead to translation errors and NMD, when NMD is inhibited, they can also be translated by translational frameshifting, nonsense suppression or translational readthrough.

(2). RNA can direct DNA repair

We here demonstrated that many G:C→A:T substitutions were observed in the repaired *bla* in the revertants; in addition, four deaminase genes (*codA, cdd, add* and *tadA*) and the mismatch repair genes/pathways were upregulated in the frameshift and/or the revertant. We therefore speculate that mismatch repair and RNA editing are both involved in frameshift repair. The details of the mechanism of nonsense mRNA editing and how it connects to DNA repair has not been made clear.

All types of RNAs are subject to translation, degradation or processing, and a variety of molecular/cellular mechanisms are involved in RNA processing [121]. In the last few decades, studies have established a link between DNA repair and RNA processing. For example, several proteins respond to DNA damage and base-excision repair enzymes, such as SMUG1, APE1 and PARP1, have been shown to participate in RNA surveillance and processing [122].

As is known, RNA is transcribed in the nucleus and then transported to the cytoplasm in eukaryotes [123]. Recently, there is growing evidence that RNAs can also be transported back to the nucleus to repair DNA double strand breaks (DSBs), a process known as *RNA-directed DNA Repair* [65-70]. In yeast [69, 70] and human embryonic kidney (HEK-293) cells [124], not only a synthetic DNA/RNA hybrid, but also a RNA-only oligonucleotide can serve as a template for DNA synthesis for the repair of a DSB in a homologous DNA. In *E. coli*, short RNA patches can also direct DNA modification [124, 125]. However, this is the first report that RNA also can direct precise and targeted repair of a frameshift mutation by itself. Although this process is not yet clear in detail at the molecular level, mismatch repair and homologous recombination are likely to be involved, as they are both upregulated in the frameshift and the revertant tested (Fig 6F).

(3). RNA editing is linked to DNA repair:

RNA editing has been studied extensively and intensively [126-130], suggesting that RNA editing exists in many species, including *E. coli* [34], kinetoplastids [131], trypanosomes [132], plants [133, 134], mammals [135] and human [136-143]. Although the are several different mechanisms of RNA editing, such as A-to-I editing

[144-147], C-to-U editing [148-152], and the insertion/deletion of uridine [153-155], the basic schematism are conserved among species. In particular, the nonsense *apoB* mRNA editing complex suppresses its own decay by NMD [156], suggesting that nonsense mRNAs can be subjected to RNA editing, which is linked to NMD.

Both DNA mismatch repair and RNA editing involve deamination, the removal of an amine group from a DNA/RNA molecule. Enzymes that catalyze this reaction are called DNA/RNA deaminases. Deamination of DNA deoxyadenosine bases generates deoxyinosine, create missense mutations predisposing humans to cancer, and interfere with other molecular genetic processes. Cytidine deamination was discovered first in wheat as a mechanism of mRNA editing [157, 158]. The characterization of the tRNA-specific adenosine deaminases (ADATs) has led to the suggestion that these enzymes, as well as the cytidine/adenosine deaminases (CDARs/ADARs) acting on pre-mRNAs, belong to a superfamily of RNA dependent deaminases [159]. The first example of C→U editing is the apolipoprotein B (apoB) mRNA, in which a single, site-specific cytidine deamination introduces a stop codon (UAA) into the reading frame of the edited transcript [160]. It is well known that deamination based RNA editing plays many important roles, and aberrant or dysregulated RNA editing might contribute to genomic instability in cancer [161]. In eukaryotes, several studies have provided evidence that deamination is related to both DNA repair and RNA editing. For example, endonuclease V, which is highly conserved from *E. coli* to human [60], is involved in excision repair that removes deoxyinosine from DNA.

TadA, the first prokaryotic deaminase for RNA editing identified in *E. coli*, displays sequence similarity to the yeast tRNA deaminase subunit Tad2p [53]. In addition, cytidine deaminase (CodA) has been used as a molecular model for RNA editing and a mechanism for RNA substrate recognition [34]. These studies suggest that RNA editing is conserved between prokaryotes and eukaryotes, and a direct link between the regulation of mismatch repair and RNA editing, and therefore support our speculation that mismatch repair and RNA editing may both involve in frameshift

repair. However, the details of the mechanism of nonsense mRNA editing and how it connects to DNA repair has not been made clear.

4.5 The possibility of RNA-only gene/genome editing

CRISPR/Cas9 for RNA-guided genome editing [162-164] has been widely used for the introduction or correction of specific mutations, which can be used to repair disease-causing frameshift/point mutations [165, 166]. However, present RNA-guided genome editing requires the transferring of foreign genes or proteins in the host cells. Deoxyoligonucleotide-based targeted gene repair [61, 62] could be more favorable and convenient due to its non-transgenic properties, but suffers from inefficiency because it relies on the induction of the endogenous DNA repair system while in the meantime being suppressed by mismatch repair [63].

In addition, synthetic DNA oligonucleotides have been widely used for targeted gene repair [61-63]. And it has also been reported that synthetic RNA or DNA/RNA complexes can direct the repair of double-strand breaks of DNA [65-70], but it has never been reported that endogenous mRNA can be edited and used to direct targeted gene repair. By transforming short synthetic RNAs, here we demonstrated that RNA can direct targeted gene repair by itself alone. It seems that RNA-directed gene repair is very inefficient and unstable, probably owing to the degradation of the naked RNAs by intra- or extra-cellular RNases. If the short RNA strands were protected by certain artificial packaging materials, it would probably result in increased efficiencies for RNA gene editing. In eukaryotes, liposome, polyethylenimine, and nano materials has been widely used for carrier agents to improve the transportation, distribution and retention of therapeutic nucleic acids within host cells, but methods for transferring of DNA/RNA into bacterial are restricted to heat shock or electroporation. Nevertheless, the components of NMGE, including RNA editing, mismatch repair and homologous recombination, are essential pathways highly conserved among various species from bacteria to human. Therefore, if NMGE is confirmed to be conserved, it is potentially possible that genes and genomes can be edited by using RNA-only molecules without introducing any exogenous gene or protein.

Author Contributions

Xiaolong Wang conceived the study, designed the experiments, analyzed the data and wrote the paper. Xuxiang Wang, Chunyan Li, Haibo Peng and Yalei Wang performed the experiments. Gang Chen and Jianye Zhang provided some equipment and materials, Michael Lynch supported this study, gave conceptual suggestions, and revised the manuscript.

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Figure legends

Fig 1. The introduction of a frameshift mutation in the upstream of the *bla* gene in the plasmid pBR322. Sanger sequencing diagram of: (A) the wild type (*bla*+); (B) the frameshift mutant (*bla*-). (C) Alignment of the nucleotide sequence of *bla*+ and *bla*-.

Fig 2. Growth of the wild-type/frameshift *E. coli* and sanger sequencing of the *bla* genes:

(A) Growth of the wild-type and frameshift *E. coli* on ampicillin- or tetracycline plates: *bla*+: wild type; *bla*-: frameshift; *bla**: revertants; *blank*: blank control

(B) The Sanger diagram of the *bla* genes of the revertants: *Top*: in the initial revertants, most of the *bla* genes are still frameshifted; *Middle*: in the later subcultures, sequencing diagrams contains two sets of overlapping peaks, the main peaks are the frameshifted *bla*, the second peaks are the repaired *bla*; *Bottom*: in the final fast-growing subcultures, sequencing diagrams contains very few overlapping peaks.

(C) The ClustalW alignment of the wild-type, frameshifted and repaired *bla* gene sequences: ***bla*+**: wild-type; *bla*-: frameshift; *bla*A-E*: different (independent) revertants; *box*: the base G deleted in the OE-PCR; *underlined*: the bases (in blue) inserted or deleted in the revertants; *circles*: base substitutions (G:C←→A:T transitions).

(D) The codon and amino acid unified alignment of *bla* and their translated protein sequences: *underlined*: the base G deleted in codon 46; *red box*: stop codons; *red star*: stop signals.

Fig 3. The traditional model for frameshift repair: *random mutagenesis*: *Step 1*: a frameshift mutation occurs in a coding gene, many hidden stop codons (*red bars*) emerge in the CDS, and in the nonsense mRNAs, cause *translational termination* and resulted in truncated products; *Step 2*: the coding gene is repaired by a *reverse mutation* through random mutagenesis, the reading frame is restored immediately, stop codons are hidden (*green bars*), and translation proceeds, producing functional mRNAs and proteins, which are identical and same to the original genes and proteins.

Fig 4. NGS resequencing and variation analysis the genomes of the frameshift/revertant:

(A) The genome-wide variation map of the frameshift /revertant: to visualize the levels of read coverages and the distribution of mutations, Circos software was used to show the clean reads of each sample, the depth of coverage (number of clean reads covering a given base position of the reference sequence), and the SNP/indel, using the reference genome sequence as a ruler. The outermost circle: the coordinate position of the reference genome, and then, outside-in, the distribution of indel/SNPs (red dots), the depth of coverage (grey), the GC content (green) and the GC skew value (pink) in the reference genome, respectively. There are two samples, the frameshift

(*bla*-) and the revertant (*bla**), so there are two sets of indel/SNP, and reads coverage depth mapped onto the reference genome sequences.

(B) The distribution of the numbers of SNPs/indels of the frameshift/revertant on the reference genome: The abscissa axis shows the chromosome coordination of the reference genome sequence, and the ordinate axis represents the SNP/indel density on the genome sequence in per 10 kb. The left scale: SNP (red peaks); the right scale: indel (blue peaks). See Table 2 for data.

(C) The types and lengths of the reported "structure variations" of the frameshift/revertant: The horizontal axis represents the range of SV lengths, and the vertical axis represents the number of SVs. See Table 3 for data.

Fig 5. Expression of the PTC-free frameshifted *bla* in *E. coli* BL21:

(A) SDS-PAGE of the lysates of pET28a-*bla#* recombinant *E. coli* cells; Lane 1: protein marker; Lane 2 and 3: whole-cell lysates of uninduced and induced cells; lane 4 and 5: the supernatant of uninduced and induced cells; lane 6 and 7: the precipitate of uninduced and induced cells;

(B) Detection of β-lactamase activity by iodimetry: (1) the revertants of *E. coli* BL21/pET28a-bla- caused the fading of the iodine/starch solution, suggesting that their *bla*- was repaired, produced active β-lactamases, and transformed ampicillin into penicillium thiazole acid, which bind with starch and competitively inhibit the binding of iodine to starch; (2) *E. coli* BL21/pET28a-bla# did not cause fading, suggesting that it was not repaired but expressed a frameshifted BLA (BLA#) , which is an inactive product; (3) the negative control (*E. coli* BL21), no β-lactamase, no fading.

Fig 6. The transcriptome analysis of different *E. coli* strains: *W-1*: wildtype; *FS-2*: frameshift; *R-3*: a revertant; *RE-4*: a subculture of the revertant; (A) The number of differentially expressed genes (DEGs) and the cluster analysis of DEGs; (B) the most enriched GO terms by comparing the frameshift (FS_2) with the wild type (W_1); (C) the most enriched GO terms by comparing the revertant (R_3) with the frameshift (FS_2); (D) DNA replication pathway: (E) genes upregulated in the RNA degradation/processing pathway; (F) genes upregulated in the mismatch repair and homologous recombination pathways. *Underlined* in B, C: GO terms involve in the processing of DNA or RNA, which probably also involve in frameshift repair; in D, E, F: *green box:* genes involve in the pathways; *rex box*: genes upregulated in the frameshift or revertant.

Fig 7. Targeted repair of the frameshift mutation induced by DNA or RNA oligonucleotides.

(A) Both sense- and antisense-strand of DNA and RNA oligonucleotides can promote the repair of the frameshift mutation: *Control*: no oligo; *ssDNA*: sense-strand DNA; *asDNA*: antisense DNA; *dsDNA*: double-stranded DNA; *ssRNA*: sense-strand RNA; *asRNA*: antisense RNA; *dsRNA*: double-stranded RNA; each group were transformed with 10.0 nM of the corresponding oligo. (B) The statistical chart of the average recovery rates, which are the numbers of revertants found in each of the ampicillin plates divided by the numbers of colonies in the parallel tetracycline plates; (C) Sanger sequencing diagram of the DNA-induced revertants: *top*: induced by a wild-type ssDNA; *bottom*: induced by a mutant ssDNA with a C-to-A substitution; (D) Sanger sequencing diagram of the RNA-induced revertants: *top*: induced by a wild-type ssRNA; *bottom*: induced by a mutant ssRNA with a G-to-A substitution.

Fig 8. The new model for frameshift repair: nonsense-mediated gene editing (NMGE): *Step 1*: when the frameshifted gene is transcribed and translated, the nonsense mRNAs containing many PTCs (*yellow bars*) are identified by mRNA surveillance through PTCs signaling; *Step 2*: some of the nonsense mRNAs causing *translational termination* are degraded by NMD; *Step 3*: some of the nonsense mRNAs are translated by translational frameshifting or readthrough; *Step 4*: some of

the nonsense mRNAs are repaired by *RNA editing*, PTCs are replaced by sense codons (*green bars*) through base insertion, deletion and substitution; *Step 5*: some of the original or repaired nonsense mRNAs are transported to identify and localize the defective coding gene; *Step 6*: the coding gene is repaired by *RNA-directed DNA repair*, the reading frame is restored partially, and the translation proceed, the remaining stop codons were readthrough, producing functional mRNAs and proteins with many variations, and so that the host recovered gradually.

Table 1. The natural suppressor tRNA for nonsense mutations (the *readthrough rules*).

Site	tRNA (AA)	Wild type		Correction	
		Code	**Anti-code**	**Code**	**Anti-code**
supD	Ser (S)	→ UCG	CGA←	→ UAG	CUA←
supE	Gln (Q)	→ CAG	CUG←	→ UAG	CUA←
supF	Tyr (Y)	→ UAC	GUA←	→ UAG	CUA←
supG	Lys (K)	→ AAA	UUU←	→ UAA	UUA←
supU	Trp (W)	→ UGG	CCA←	→ UGA	UCA←

Table 2. The summary of SNP/indel variations in the *bla**/- genome

Sample	Indels			SNPs				
	INS	DEL	Total	ts	tv	ts/tv	Total	Density
bla-	2	4	6	43	27	1.59	70	0.02/Kb
*bla**	2	3	5	42	27	1.56	69	0.01/Kb

Note: INS: insertion; DEL: deletion; ts: transitions; tv: transversions;

Table 3. The types and lengths of the "structure variations"

Sample	Total	Type			Length				
		INS	DEL	INV	100-200	200-700	700-800	700-1000	>=1000
Frameshift	77	65	10	2	70 (90.91%)	0	2 (2.6%)	0	5 (6.49%)
Revertant	60	50	8	2	53 (88.33%)	0	2 (3.33%)	0	5 (8.33%)

Note: INS: insertion; DEL: deletion; INV: inversion.

Table 4. Possible NMGE-relevant genes and pathways that were upregulated in the frameshift

Pathway Term	Database	ID	Input number	Background number	P-Value	Corrected P-Value
ABC transporters	KEGG	eco02010	32	171	0.0001	0.0090
RNA degradation	KEGG	eco03018	1	15	0.7419	0.9762
DNA replication	KEGG	eco03030	1	17	0.7822	0.9762
Mismatch repair	KEGG	eco03430	1	22	0.8575	0.9796
Homologous recombination	KEGG	eco03440	1	27	0.9069	0.9796

Table 5. Possible NMGE-relevant genes and pathways that were upregulated in the revertant

Pathway Term	Database	ID	Input number	Background number	P-Value	Corrected P-Value
ABC transporters	KEGG	eco02010	32	171	3.53E-05	0.0018
Homologous recombination	KEGG	eco03440	2	27	0.6539	0.9519
RNA degradation	KEGG	eco03018	3	15	0.1017	0.5470
DNA replication	KEGG	eco03030	1	17	0.7564	0.9519
Mismatch repair	KEGG	eco03430	1	22	0.8357	0.9519

References

1. Seligmann, H. and D.D. Pollock, *The ambush hypothesis: hidden stop codons prevent off-frame gene reading.* DNA Cell Biol, 2004. **23**(10): p. 701-5.
2. Owens, M., et al., *SOS1 frameshift mutations cause pure mucosal neuroma syndrome, a clinical phenotype distinct from multiple endocrine neoplasia type 2B.* Clin Endocrinol (Oxf), 2016. **84**(5): p. 715-9.
3. Yan, S.E., et al., *In-depth analysis of hyaline fibromatosis syndrome frameshift mutations at the same site reveal the necessity of personalized therapy.* Hum Mutat, 2013. **34**(7): p. 1005-17.
4. Amiel, J., et al., *Polyalanine expansion and frameshift mutations of the paired-like homeobox gene PHOX2B in congenital central hypoventilation syndrome.* Nat Genet, 2003. **33**(4): p. 459-61.
5. Kang, S., et al., *GLI3 frameshift mutations cause autosomal dominant Pallister-Hall syndrome.* Nat Genet, 1997. **15**(3): p. 266-8.
6. Jensen, N.M., et al., *An update on targeted gene repair in mammalian cells: methods and mechanisms.* J Biomed Sci, 2011. **18**: p. 10.
7. Streisinger, G., et al., *Frameshift mutations and the genetic code. This paper is dedicated to Professor Theodosius Dobzhansky on the occasion of his 66th birthday.* Cold Spring Harb Symp Quant Biol, 1966. **31**: p. 77-84.
8. Li, H., et al., *The Sequence Alignment/Map format and SAMtools.* Bioinformatics, 2009. **25**(16): p. 2078-9.
9. DePristo, M.A., et al., *A framework for variation discovery and genotyping using next-generation DNA sequencing data.* Nat Genet, 2011. **43**(5): p. 491-8.
10. Krzywinski, M., et al., *Circos: an information aesthetic for comparative genomics.* Genome Res, 2009. **19**(9): p. 1639-45.
11. Fan, X., et al., *BreakDancer: Identification of Genomic Structural Variation from Paired-End Read Mapping.* Curr Protoc Bioinformatics, 2014. **45**: p. 15 6 1-11.
12. Chen, K., et al., *BreakDancer: an algorithm for high-resolution mapping of genomic structural variation.* Nat Methods, 2009. **6**(9): p. 677-81.
13. Langmead, B., *Aligning short sequencing reads with Bowtie.* Curr Protoc Bioinformatics, 2010. **Chapter 11**: p. Unit 11 7.
14. Anders, S., P.T. Pyl, and W. Huber, *HTSeq--a Python framework to work with high-throughput sequencing data.* Bioinformatics, 2015. **31**(2): p. 166-9.
15. Robinson, M.D., D.J. McCarthy, and G.K. Smyth, *edgeR: a Bioconductor package for differential expression analysis of digital gene expression data.* Bioinformatics, 2010. **26**(1): p. 139-40.
16. Wang, L., et al., *DEGseq: an R package for identifying differentially expressed genes from RNA-seq data.* Bioinformatics, 2010. **26**(1): p. 136-8.
17. Young, M.D., et al., *Gene ontology analysis for RNA-seq: accounting for selection bias.* Genome Biol, 2010. **11**(2): p. R14.
18. Xie, C., et al., *KOBAS 2.0: a web server for annotation and identification of enriched pathways and diseases.* Nucleic Acids Res, 2011. **39**(Web Server issue): p. W316-22.

19. Lee, H., et al., *Rate and molecular spectrum of spontaneous mutations in the bacterium Escherichia coli as determined by whole-genome sequencing.* Proc Natl Acad Sci U S A, 2012. **109**(41): p. E2774-83.

20. Wang, X. and C. Yang, *CAUSA 2.0: accurate and consistent evolutionary analysis of proteins using codon and amino acid unified sequence alignments.* PeerJ PrePrints **3**.

21. Schaaper, R.M., *Mechanisms of mutagenesis in the Escherichia coli mutator mutD5: role of DNA mismatch repair.* Proc Natl Acad Sci U S A, 1988. **85**(21): p. 8126-30.

22. Roberts, R.J., M.O. Carneiro, and M.C. Schatz, *The advantages of SMRT sequencing.* Genome Biol, 2013. **14**(7): p. 405.

23. Itoh, Y., S. Sekine, and S. Yokoyama, *Crystal structure of the full-length bacterial selenocysteine-specific elongation factor SelB.* Nucleic Acids Res, 2015. **43**(18): p. 9028-38.

24. Baron, C. and A. Bock, *The length of the aminoacyl-acceptor stem of the selenocysteine-specific tRNA(Sec) of Escherichia coli is the determinant for binding to elongation factors SELB or Tu.* J Biol Chem, 1991. **266**(30): p. 20375-9.

25. Ireton, G.C., et al., *The structure of Escherichia coli cytosine deaminase.* J Mol Biol, 2002. **315**(4): p. 687-97.

26. Danielsen, S., et al., *Characterization of the Escherichia coli codBA operon encoding cytosine permease and cytosine deaminase.* Mol Microbiol, 1992. **6**(10): p. 1335-44.

27. MacGinnitie, A.J., S. Anant, and N.O. Davidson, *Mutagenesis of apobec-1, the catalytic subunit of the mammalian apolipoprotein B mRNA editing enzyme, reveals distinct domains that mediate cytosine nucleoside deaminase, RNA binding, and RNA editing activity.* J Biol Chem, 1995. **270**(24): p. 14768-75.

28. Vita, A., et al., *Cytidine deaminase from Escherichia coli B. Purification and enzymatic and molecular properties.* Biochemistry, 1985. **24**(21): p. 6020-4.

29. Ashley, G.W. and P.A. Bartlett, *Purification and properties of cytidine deaminase from escherichia coli.* J Biol Chem, 1984. **259**(21): p. 13615-20.

30. von Dippe, P.J., et al., *Deoxycytidine transport in the presence of a cytidine deaminase inhibitor and the transport of uracil in Escherichia coli B.* J Biol Chem, 1975. **250**(10): p. 3666-71.

31. Hosono, H. and S. Kuno, *The purification and properties of cytidine deaminase from Escherichia coli.* J Biochem, 1973. **74**(4): p. 797-803.

32. Cohen, R.M. and R. Wolfenden, *Cytidine deaminase from Escherichia coli. Purification, properties and inhibition by the potential transition state analog 3,4,5,6-tetrahydrouridine.* J Biol Chem, 1971. **246**(24): p. 7561-5.

33. Ashley, G.W. and P.A. Bartlett, *Inhibition of Escherichia coli cytidine deaminase by a phosphapyrimidine nucleoside.* J Biol Chem, 1984. **259**(21): p. 13621-7.

34. Navaratnam, N., et al., *Escherichia coli cytidine deaminase provides a molecular model for ApoB RNA editing and a mechanism for RNA substrate recognition.* J Mol Biol, 1998. **275**(4): p. 695-714.

35. Navaratnam, N., et al., *Evolutionary origins of apoB mRNA editing: catalysis by a cytidine deaminase that has acquired a novel RNA-binding motif at its active site.* Cell, 1995. **81**(2): p. 187-95.

36. Nygaard, P., *Adenosine deaminase from Escherichia coli.* Methods Enzymol, 1978. **51**: p. 508-12.

37. Koch, A.L. and G. Vallee, *The properties of adenosine deaminase and adenosine nucleoside phosphorylase in extracts of Escherichia coli.* J Biol Chem, 1959. **234**(5): p. 1213-8.

38. Remy, C.N. and S.H. Love, *Induction of adenosine deaminase in Escherichia coli.* J Bacteriol, 1968. **96**(1): p. 76-85.

39. Kim, U., et al., *Molecular cloning of cDNA for double-stranded RNA adenosine deaminase, a candidate enzyme for nuclear RNA editing.* Proc Natl Acad Sci U S A, 1994. **91**(24): p. 11457-61.

40. Kim, U. and K. Nishikura, *Double-stranded RNA adenosine deaminase as a potential mammalian RNA editing factor.* Semin Cell Biol, 1993. **4**(4): p. 285-93.

41. Oakes, E., et al., *Adenosine Deaminase That Acts on RNA 3 (ADAR3) Binding to Glutamate Receptor Subunit B Pre-mRNA Inhibits RNA Editing in Glioblastoma.* J Biol Chem, 2017. **292**(10): p. 4326-4335.

42. Ward, S.V., et al., *RNA editing enzyme adenosine deaminase is a restriction factor for controlling measles virus replication that also is required for embryogenesis.* Proc Natl Acad Sci U S A, 2011. **108**(1): p. 331-6.

43. Herbert, A., et al., *A Z-DNA binding domain present in the human editing enzyme, double-stranded RNA adenosine deaminase.* Proc Natl Acad Sci U S A, 1997. **94**(16): p. 8421-6.

44. Yang, J.H., et al., *Purification and characterization of a human RNA adenosine deaminase for glutamate receptor B pre-mRNA editing.* Proc Natl Acad Sci U S A, 1997. **94**(9): p. 4354-9.

45. Maas, S., et al., *Structural requirements for RNA editing in glutamate receptor pre-mRNAs by recombinant double-stranded RNA adenosine deaminase.* J Biol Chem, 1996. **271**(21): p. 12221-6.

46. Polson, A.G., B.L. Bass, and J.L. Casey, *RNA editing of hepatitis delta virus antigenome by dsRNA-adenosine deaminase.* Nature, 1996. **380**(6573): p. 454-6.

47. Dabiri, G.A., et al., *Editing of the GLuR-B ion channel RNA in vitro by recombinant double-stranded RNA adenosine deaminase.* EMBO J, 1996. **15**(1): p. 34-45.

48. Hurst, S.R., et al., *Deamination of mammalian glutamate receptor RNA by Xenopus dsRNA adenosine deaminase: similarities to in vivo RNA editing.* RNA, 1995. **1**(10): p. 1051-60.

49. Wang, Y., et al., *Genomic organization and chromosomal location of the human dsRNA adenosine deaminase gene: the enzyme for glutamate-activated ion channel RNA editing.* J Mol Biol, 1995. **254**(2): p. 184-95.

50. Chen, Z., A. Hirano, and T.C. Wong, *Isolation and characterization of intranuclear ribonucleoprotein complexes associated with double-stranded RNA adenosine deaminase from brain cells: implications for RNA-editing and hypermutation of viral RNA in the CNS.* J Neurovirol, 1995. **1**(3-4): p. 295-306.

51. Lai, F., R. Drakas, and K. Nishikura, *Mutagenic analysis of double-stranded RNA adenosine deaminase, a candidate enzyme for RNA editing of glutamate-gated ion channel transcripts.* J Biol Chem, 1995. **270**(29): p. 17098-105.

52. Gerber, A., et al., *Tad1p, a yeast tRNA-specific adenosine deaminase, is related to the mammalian pre-mRNA editing enzymes ADAR1 and ADAR2.* EMBO J, 1998. **17**(16): p. 4780-9.

53. Wolf, J., A.P. Gerber, and W. Keller, *tadA, an essential tRNA-specific adenosine deaminase from Escherichia coli.* EMBO J, 2002. **21**(14): p. 3841-51.

54. Losey, H.C., A.J. Ruthenburg, and G.L. Verdine, *Crystal structure of Staphylococcus aureus tRNA adenosine deaminase TadA in complex with RNA.* Nat Struct Mol Biol, 2006. **13**(2): p. 153-9.

55. Kuratani, M., et al., *Crystal structure of tRNA adenosine deaminase (TadA) from Aquifex aeolicus.* J Biol Chem, 2005. **280**(16): p. 16002-8.

56. Lee, W.H., et al., *Crystal structure of the tRNA-specific adenosine deaminase from Streptococcus pyogenes.* Proteins, 2007. **68**(4): p. 1016-9.

57. Maas, S., A.P. Gerber, and A. Rich, *Identification and characterization of a human tRNA-specific adenosine deaminase related to the ADAR family of pre-mRNA editing enzymes.* Proc Natl Acad Sci U S A, 1999. **96**(16): p. 8895-900.

58. Maas, S., Y.G. Kim, and A. Rich, *Sequence, genomic organization and functional expression of the murine tRNA-specific adenosine deaminase ADAT1.* Gene, 2000. **243**(1-2): p. 59-66.

59. Keegan, L.P., et al., *The properties of a tRNA-specific adenosine deaminase from Drosophila melanogaster support an evolutionary link between pre-mRNA editing and tRNA modification.* Mol Cell Biol, 2000. **20**(3): p. 825-33.

60. Kuraoka, I., *Diversity of Endonuclease V: From DNA Repair to RNA Editing.* Biomolecules, 2015. **5**(4): p. 2194-206.

61. de Semir, D. and J.M. Aran, *Targeted gene repair: the ups and downs of a promising gene therapy approach.* Curr Gene Ther, 2006. **6**(4): p. 481-504.

62. Parekh-Olmedo, H., et al., *Gene therapy progress and prospects: targeted gene repair.* Gene Ther, 2005. **12**(8): p. 639-46.

63. Dekker, M., C. Brouwers, and H. te Riele, *Targeted gene modification in mismatch-repair-deficient embryonic stem cells by single-stranded DNA oligonucleotides.* Nucleic Acids Res, 2003. **31**(6): p. e27.

64. Liu, L., et al., *Strand bias in targeted gene repair is influenced by transcriptional activity.* Mol Cell Biol, 2002. **22**(11): p. 3852-63.

65. Trott, D.A. and A.C. Porter, *Hypothesis: transcript-templated repair of DNA double-strand breaks.* Bioessays, 2006. **28**(1): p. 78-83.

66. Keskin, H., C. Meers, and F. Storici, *Transcript RNA supports precise repair of its own DNA gene.* RNA Biol, 2016. **13**(2): p. 157-65.

67. Keskin, H., et al., *Transcript-RNA-templated DNA recombination and repair.* Nature, 2014. **515**(7527): p. 436-9.

68. Wei, L., et al., *DNA damage during the G0/G1 phase triggers RNA-templated, Cockayne syndrome B-dependent homologous recombination.* Proc Natl Acad Sci U S A, 2015. **112**(27): p. E3495-504.

69. Storici, F., *RNA-mediated DNA modifications and RNA-templated DNA repair.* Curr Opin Mol Ther, 2008. **10**(3): p. 224-30.

70. Storici, F., et al., *RNA-templated DNA repair.* Nature, 2007. **447**(7142): p. 338-41.

71. Ge, Z., et al., *Polypyrimidine tract binding protein 1 protects mRNAs from recognition by the nonsense-mediated mRNA decay pathway.* Elife, 2016. **5**.

72. Schweingruber, C., et al., *Nonsense-mediated mRNA decay - mechanisms of substrate mRNA recognition and degradation in mammalian cells.* Biochim Biophys Acta, 2013. **1829**(6-7): p. 612-23.

73. Niu, D.K. and J.L. Cao, *Nucleosome deposition and DNA methylation may participate in the recognition of premature termination codon in nonsense-mediated mRNA decay.* FEBS Lett, 2010. **584**(16): p. 3509-12.

74. Muhlemann, O., et al., *Recognition and elimination of nonsense mRNA.* Biochim Biophys Acta, 2008. **1779**(9): p. 538-49.

75. Muhlemann, O., *Recognition of nonsense mRNA: towards a unified model.* Biochem Soc Trans, 2008. **36**(Pt 3): p. 497-501.

76. Muhlrad, D. and R. Parker, *Recognition of yeast mRNAs as "nonsense containing" leads to both inhibition of mRNA translation and mRNA degradation: implications for the control of mRNA decapping.* Mol Biol Cell, 1999. **10**(11): p. 3971-8.

77. Zhang, J. and L.E. Maquat, *Evidence that the decay of nucleus-associated nonsense mRNA for human triosephosphate isomerase involves nonsense codon recognition after splicing.* RNA, 1996. **2**(3): p. 235-43.

78. Fujikura, K., *Premature termination codons in modern human genomes.* Sci Rep, 2016. **6**: p. 22468.

79. Wong, T.Y., et al., *Role of premature stop codons in bacterial evolution.* J Bacteriol, 2008. **190**(20): p. 6718-25.

80. Cirulli, E.T., et al., *A whole-genome analysis of premature termination codons.* Genomics, 2011. **98**(5): p. 337-42.

81. Hwang, J. and Y.K. Kim, *When a ribosome encounters a premature termination codon.* BMB Rep, 2013. **46**(1): p. 9-16.

82. Son, H.G., et al., *RNA surveillance via nonsense-mediated mRNA decay is crucial for longevity in daf-2/insulin/IGF-1 mutant C. elegans.* Nat Commun, 2017. **8**: p. 14749.

83. Fang, Y., et al., *Nonsense-mediated mRNA decay of collagen -emerging complexity in RNA surveillance mechanisms.* J Cell Sci, 2013. **126**(Pt 12): p. 2551-60.

84. Behm-Ansmant, I. and E. Izaurralde, *Quality control of gene expression: a stepwise assembly pathway for the surveillance complex that triggers nonsense-mediated mRNA decay.* Genes Dev, 2006. **20**(4): p. 391-8.

85. Wilusz, C.J., W. Wang, and S.W. Peltz, *Curbing the nonsense: the activation and regulation of mRNA surveillance.* Genes Dev, 2001. **15**(21): p. 2781-5.

86. Le Hir, H., et al., *The exon-exon junction complex provides a binding platform for factors involved in mRNA export and nonsense-mediated mRNA decay.* EMBO J, 2001. **20**(17): p. 4987-97.

87. Gonzalez, C.I., W. Wang, and S.W. Peltz, *Nonsense-mediated mRNA decay in Saccharomyces cerevisiae: a quality control mechanism that degrades transcripts harboring premature termination codons.* Cold Spring Harb Symp Quant Biol, 2001. **66**: p. 321-8.

88. Zhang, J. and L.E. Maquat, *Evidence that translation reinitiation abrogates nonsense-mediated mRNA decay in mammalian cells.* EMBO J, 1997. **16**(4): p. 826-33.

89. Richards, J., et al., *Quality control of bacterial mRNA decoding and decay.* Biochim Biophys Acta, 2008. **1779**(9): p. 574-82.

90. Xu, J., R.W. Hendrix, and R.L. Duda, *Conserved translational frameshift in dsDNA bacteriophage tail assembly genes.* Molecular Cell, 2004. **16**(1): p. 11-21.

91. Baykal, U., A.L. Moyne, and S. Tuzun, *A frameshift in the coding region of a novel tomato class I basic chitinase gene makes it a pseudogene with a functional wound-responsive promoter.* Gene, 2006. **376**(1): p. 37-46.

92. Chen, J., et al., *Dynamic pathways of -1 translational frameshifting.* Nature, 2014. **512**(7514): p. 328-32.

93. Gaudin, C., et al., *Structure of the RNA signal essential for translational frameshifting in HIV-1.* J Mol Biol, 2005. **349**(5): p. 1024-35.

94. Bekaert, M., et al., *Identification of programmed translational -1 frameshifting sites in the genome of Saccharomyces cerevisiae.* Genome Res, 2005. **15**(10): p. 1411-20.

95. Licznar, P., et al., *Programmed translational -1 frameshifting on hexanucleotide motifs and the wobble properties of tRNAs.* EMBO J, 2003. **22**(18): p. 4770-8.

96. Klobutcher, L.A. and P.J. Farabaugh, *Shifty ciliates: frequent programmed translational frameshifting in euplotids.* Cell, 2002. **111**(6): p. 763-6.

97. Advani, V.M., A.T. Belew, and J.D. Dinman, *Yeast telomere maintenance is globally controlled by programmed ribosomal frameshifting and the nonsense-mediated mRNA decay pathway.* Translation (Austin), 2013. **1**(1): p. e24418.

98. Claisse, M.L., M. Boguta, and W. Zagorski, *Translational readthrough of a termination codon in the yeast mitochondrial mRNA VAR1 as a result of mutation in the release factor mRF1.* Acta Biochim Pol, 2005. **52**(1): p. 129-37.

99. Halvey, P.J., D.C. Liebler, and R.J. Slebos, *A Reporter System for Translational Readthrough of Stop Codons in Human Cells.* FEBS Open Bio, 2012. **2**: p. 56-59.

100. Eswarappa, S.M., et al., *Programmed translational readthrough generates antiangiogenic VEGF-Ax.* Cell, 2014. **157**(7): p. 1605-18.

101. Staff, P.G., *Correction: Functional Translational Readthrough: A Systems Biology Perspective.* PLoS Genet, 2016. **12**(11): p. e1006434.

102. Wang, X., et al., *Why are frameshift homologs widespread within and across species?* bioRxiv, 2016.

103. Yang, H., et al., *Expression profile and gene age jointly shaped the genome-wide distribution of premature termination codons in a Drosophila melanogaster population.* Mol Biol Evol, 2015. **32**(1): p. 216-28.

104. Scofield, D.G., X. Hong, and M. Lynch, *Position of the final intron in full-length transcripts: determined by NMD?* Mol Biol Evol, 2007. **24**(4): p. 896-9.

105. Isken, O., et al., *Upf1 phosphorylation triggers translational repression during nonsense-mediated mRNA decay.* Cell, 2008. **133**(2): p. 314-27.

106. Ward, A.J., et al., *Nonsense-mediated decay as a terminating mechanism for antisense oligonucleotides.* Nucleic Acids Res, 2014. **42**(9): p. 5871-9.

107. Lewis, B.P., R.E. Green, and S.E. Brenner, *Evidence for the widespread coupling of alternative splicing and nonsense-mediated mRNA decay in humans.* Proc Natl Acad Sci U S A, 2003. **100**(1): p. 189-92.

108. He, F., A.H. Brown, and A. Jacobson, *Upf1p, Nmd2p, and Upf3p are interacting components of the yeast nonsense-mediated mRNA decay pathway.* Mol Cell Biol, 1997. **17**(3): p. 1580-94.

109. Namy, O., et al., *A mechanical explanation of RNA pseudoknot function in programmed ribosomal frameshifting.* Nature, 2006. **441**(7090): p. 244-7.

110. Dinman, J.D., *Control of gene expression by translational recoding.* Adv Protein Chem Struct Biol, 2012. **86**: p. 129-49.

111. Dinman, J.D., *Mechanisms and implications of programmed translational frameshifting.* Wiley Interdiscip Rev RNA, 2012. **3**(5): p. 661-73.

112. Ketteler, R., *On programmed ribosomal frameshifting: the alternative proteomes.* Front Genet, 2012. **3**: p. 242.

113. Kopelowitz, J., et al., *Influence of codon context on UGA suppression and readthrough.* J Mol Biol, 1992. **225**(2): p. 261-9.

114. Weiss, R.B., *Ribosomal frameshifting, jumping and readthrough.* Curr Opin Cell Biol, 1991. **3**(6): p. 1051-5.

115. Engelberg-Kulka, H., L. Dekel, and M. Israeli-Reches, *Regulation of the escherichia coli tryptophan operon by readthrough of UGA termination codons.* Biochem Biophys Res Commun, 1981. **98**(4): p. 1008-15.

116. Seidman, J.S., B.D. Janssen, and C.S. Hayes, *Alternative fates of paused ribosomes during translation termination.* J Biol Chem, 2011. **286**(36): p. 31105-12.

117. Su, D., Y. Li, and V.N. Gladyshev, *Selenocysteine insertion directed by the 3'-UTR SECIS element in Escherichia coli.* Nucleic Acids Res, 2005. **33**(8): p. 2486-92.

118. Leeds, P., et al., *Gene products that promote mRNA turnover in Saccharomyces cerevisiae.* Mol Cell Biol, 1992. **12**(5): p. 2165-77.

119. Kashima, I., et al., *Binding of a novel SMG-1-Upf1-eRF1-eRF3 complex (SURF) to the exon junction complex triggers Upf1 phosphorylation and nonsense-mediated mRNA decay.* Genes Dev, 2006. **20**(3): p. 355-67.

120. Keeling, K.M., et al., *Leaky termination at premature stop codons antagonizes nonsense-mediated mRNA decay in S. cerevisiae.* RNA, 2004. **10**(4): p. 691-703.

121. Isken, O. and L.E. Maquat, *Quality control of eukaryotic mRNA: safeguarding cells from abnormal mRNA function.* Genes Dev, 2007. **21**(15): p. 1833-56.

122. Vohhodina, J., D.P. Harkin, and K.I. Savage, *Dual roles of DNA repair enzymes in RNA biology/post-transcriptional control.* Wiley Interdiscip Rev RNA, 2016.

123. Nakielny, S. and G. Dreyfuss, *Transport of proteins and RNAs in and out of the nucleus.* Cell, 1999. **99**(7): p. 677-90.

124. Shen, Y., et al., *RNA-driven genetic changes in bacteria and in human cells.* Mutat Res, 2011. **717**(1-2): p. 91-8.

125. Thaler, D.S., G. Tombline, and K. Zahn, *Short-patch reverse transcription in Escherichia coli.* Genetics, 1995. **140**(3): p. 909-15.

126. Kugita, M., et al., *RNA editing in hornwort chloroplasts makes more than half the genes functional.* Nucleic Acids Res, 2003. **31**(9): p. 2417-23.

127. Bock, R., *Sense from nonsense: how the genetic information of chloroplasts is altered by RNA editing.* Biochimie, 2000. **82**(6-7): p. 549-57.

128. Horvath, A., E.A. Berry, and D.A. Maslov, *Translation of the edited mRNA for cytochrome b in trypanosome mitochondria.* Science, 2000. **287**(5458): p. 1639-40.

129. Yoshinaga, K., et al., *Extensive RNA editing and possible double-stranded structures determining editing sites in the atpB transcripts of hornwort chloroplasts.* Nucleic Acids Res, 1997. **25**(23): p. 4830-4.

130. Sloof, P., et al., *RNA editing in mitochondria of cultured trypanosomatids: translatable mRNAs for NADH-dehydrogenase subunits are missing.* J Bioenerg Biomembr, 1994. **26**(2): p. 193-203.

131. Simpson, L. and J. Shaw, *RNA editing and the mitochondrial cryptogenes of kinetoplastid protozoa.* Cell, 1989. **57**(3): p. 355-66.

132. Alfonzo, J.D., O. Thiemann, and L. Simpson, *The mechanism of U insertion/deletion RNA editing in kinetoplastid mitochondria.* Nucleic Acids Res, 1997. **25**(19): p. 3751-9.

133. Takenaka, M., et al., *RNA editing in plants and its evolution.* Annu Rev Genet, 2013. **47**: p. 335-52.

134. Graves, P.V., et al., *Direct protein sequencing of wheat mitochondrial ATP synthase subunit 9 confirms RNA editing in plants.* J Mol Biol, 1990. **214**(1): p. 1-6.

135. Slavov, D., M. Clark, and K. Gardiner, *Comparative analysis of the RED1 and RED2 A-to-I RNA editing genes from mammals, pufferfish and zebrafish.* Gene, 2000. **250**(1-2): p. 41-51.

136. Zougman, A., et al., *Evidence for insertional RNA editing in humans.* Curr Biol, 2008. **18**(22): p. 1760-5.

137. Athanasiadis, A., A. Rich, and S. Maas, *Widespread A-to-I RNA editing of Alu-containing mRNAs in the human transcriptome.* PLoS Biol, 2004. **2**(12): p. e391.

138. Wang, Y., et al., *Systematic characterization of A-to-I RNA editing hotspots in microRNAs across human cancers.* Genome Res, 2017.

139. Picardi, E., D.S. Horner, and G. Pesole, *Single cell transcriptomics reveals specific RNA editing signatures in the human brain.* RNA, 2017.

140. Xu, G. and J. Zhang, *Human coding RNA editing is generally nonadaptive.* Proc Natl Acad Sci U S A, 2014. **111**(10): p. 3769-74.

141. Bazak, L., et al., *A-to-I RNA editing occurs at over a hundred million genomic sites, located in a majority of human genes.* Genome Res, 2014. **24**(3): p. 365-76.

142. Shtrichman, R., et al., *Altered A-to-I RNA editing in human embryogenesis.* PLoS One, 2012. **7**(7): p. e41576.

143. Bahn, J.H., et al., *Accurate identification of A-to-I RNA editing in human by transcriptome sequencing.* Genome Res, 2012. **22**(1): p. 142-50.

144. Amariglio, N. and G. Rechavi, *A-to-I RNA editing: a new regulatory mechanism of global gene expression.* Blood Cells Mol Dis, 2007. **39**(2): p. 151-5.

145. Silberberg, G., et al., *Deregulation of the A-to-I RNA editing mechanism in psychiatric disorders.* Hum Mol Genet, 2012. **21**(2): p. 311-21.

146. Terajima, H., et al., *ADARB1 catalyzes circadian A-to-I editing and regulates RNA rhythm.* Nat Genet, 2017. **49**(1): p. 146-151.

147. Valente, L. and K. Nishikura, *ADAR gene family and A-to-I RNA editing: diverse roles in posttranscriptional gene regulation.* Prog Nucleic Acid Res Mol Biol, 2005. **79**: p. 299-338.

148. Blanc, V. and N.O. Davidson, *C-to-U RNA editing: mechanisms leading to genetic diversity.* J Biol Chem, 2003. **278**(3): p. 1395-8.

149. Bourara, K., S. Litvak, and A. Araya, *Generation of G-to-A and C-to-U changes in HIV-1 transcripts by RNA editing.* Science, 2000. **289**(5484): p. 1564-6.

150. Giege, P. and A. Brennicke, *RNA editing in Arabidopsis mitochondria effects 441 C to U changes in ORFs.* Proc Natl Acad Sci U S A, 1999. **96**(26): p. 15324-9.

151. Sharma, S., et al., *Transient overexpression of exogenous APOBEC3A causes C-to-U RNA editing of thousands of genes.* RNA Biol, 2016: p. 1-8.

152. Fossat, N., et al., *C to U RNA editing mediated by APOBEC1 requires RNA-binding protein RBM47.* EMBO Rep, 2014. **15**(8): p. 903-10.

153. Simpson, L., S. Sbicego, and R. Aphasizhev, *Uridine insertion/deletion RNA editing in trypanosome mitochondria: a complex business.* Rna, 2003. **9**(3): p. 265-76.

154. Kable, M.L., et al., *RNA editing: a mechanism for gRNA-specified uridylate insertion into precursor mRNA.* Science, 1996. **273**(5279): p. 1189-95.

155. Ringpis, G.E., J. Stagno, and R. Aphasizhev, *Mechanism of U-insertion RNA editing in trypanosome mitochondria: characterization of RET2 functional domains by mutational analysis.* J Mol Biol, 2010. **399**(5): p. 696-706.

156. Chester, A., et al., *The apolipoprotein B mRNA editing complex performs a multifunctional cycle and suppresses nonsense-mediated decay.* EMBO J, 2003. **22**(15): p. 3971-82.

157. Yu, W. and W. Schuster, *Evidence for a site-specific cytidine deamination reaction involved in C to U RNA editing of plant mitochondria.* J Biol Chem, 1995. **270**(31): p. 18227-33.

158. Blanc, V., S. Litvak, and A. Araya, *RNA editing in wheat mitochondria proceeds by a deamination mechanism.* FEBS Lett, 1995. **373**(1): p. 56-60.

159. Gerber, A.P. and W. Keller, *RNA editing by base deamination: more enzymes, more targets, new mysteries.* Trends Biochem Sci, 2001. **26**(6): p. 376-84.

160. Young, S.G., *Recent progress in understanding apolipoprotein B.* Circulation, 1990. **82**(5): p. 1574-94.

161. Anant, S. and N.O. Davidson, *Hydrolytic nucleoside and nucleotide deamination, and genetic instability: a possible link between RNA-editing enzymes and cancer?* Trends Mol Med, 2003. **9**(4): p. 147-52.

162. Shalem, O., et al., *Genome-scale CRISPR-Cas9 knockout screening in human cells.* Science, 2014. **343**(6166): p. 84-7.

163. Ran, F.A., et al., *Genome engineering using the CRISPR-Cas9 system.* Nat Protoc, 2013. **8**(11): p. 2281-308.

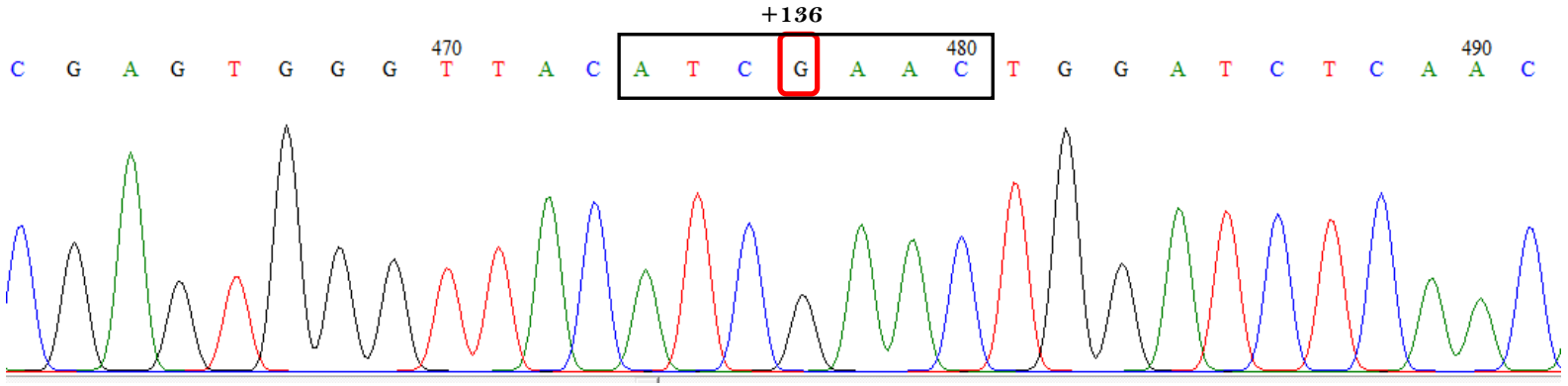
164. Ran, F.A., et al., *Double nicking by RNA-guided CRISPR Cas9 for enhanced genome editing specificity.* Cell, 2013. **154**(6): p. 1380-9.

165. Paquet, D., et al., *Efficient introduction of specific homozygous and heterozygous mutations using CRISPR/Cas9.* Nature, 2016. **533**(7601): p. 125-9.

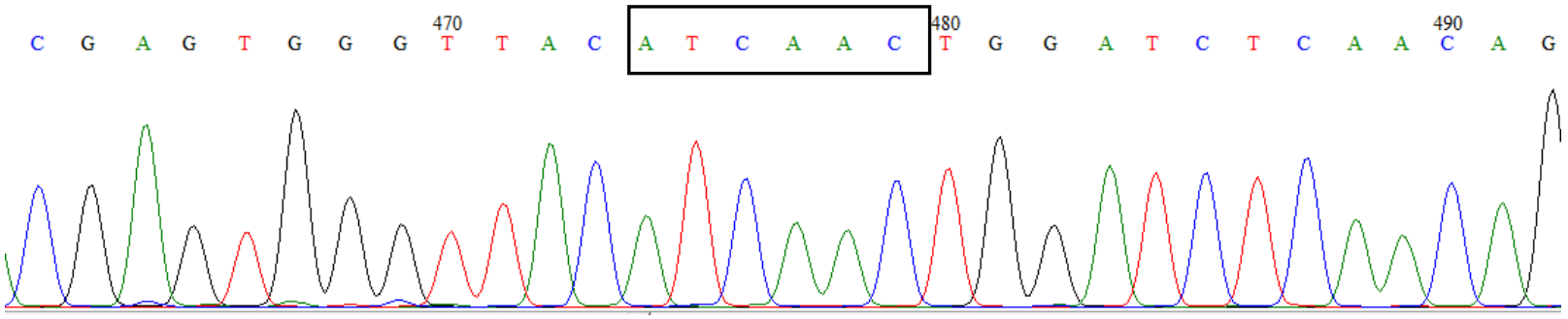
166. Zimmer, C.T., et al., *A CRISPR/Cas9 mediated point mutation in the alpha 6 subunit of the nicotinic acetylcholine receptor confers resistance to spinosad in Drosophila melanogaster.* Insect Biochem Mol Biol, 2016. **73**: p. 62-9.

Fig 1. The frameshift mutagenesis of the *bla* gene in the plasmid pBR322

1A



1B



1C

				136			
Wild-type (*bla* +):	**…A**	**T**	**C**	**G**	**A**	**A**	**C…**
	|	|	|		|	|	|
Frameshift (*bla* -):	**…A**	**T**	**C**	**-**	**A**	**A**	**C…**

Fig 2A

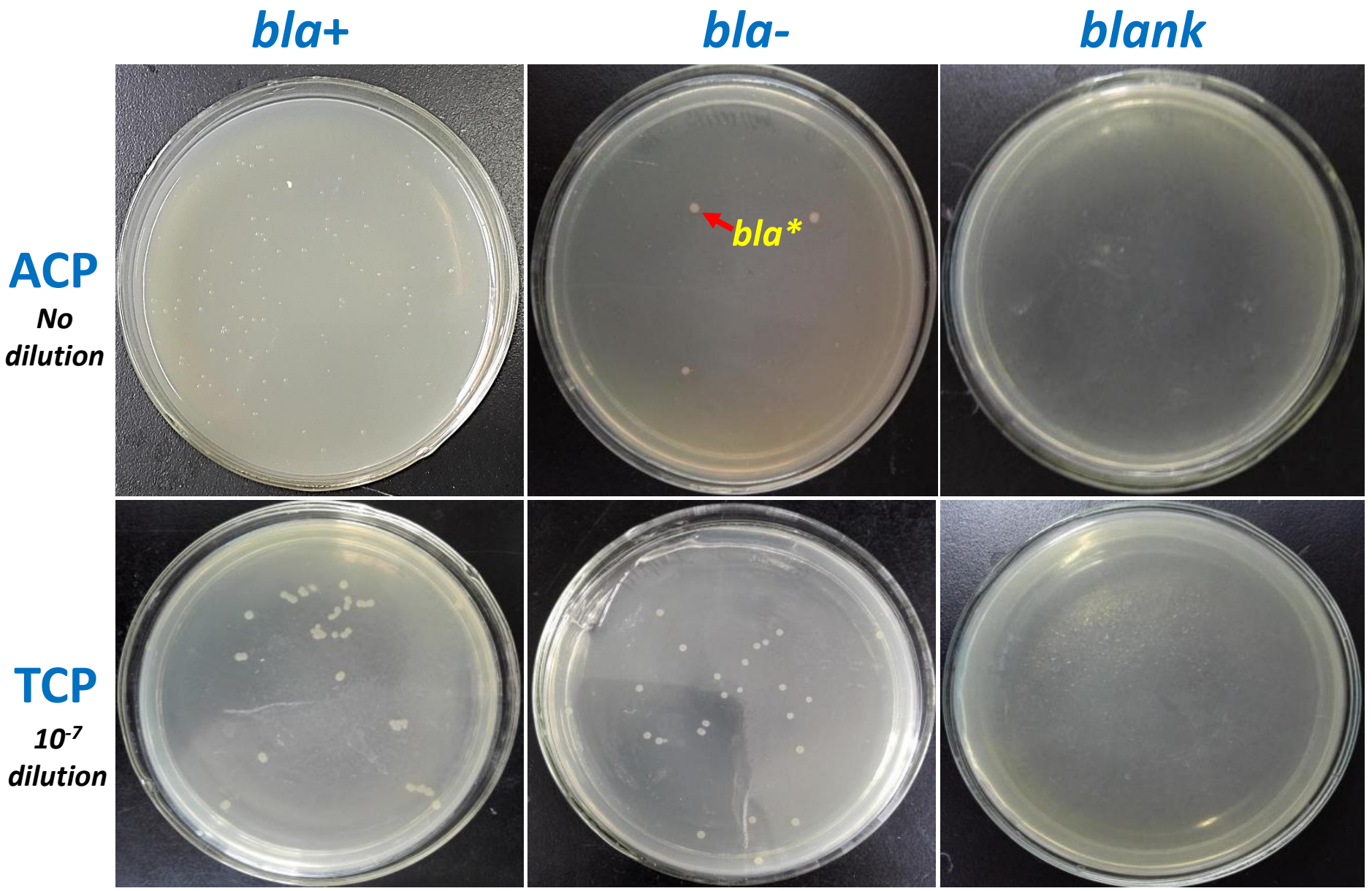
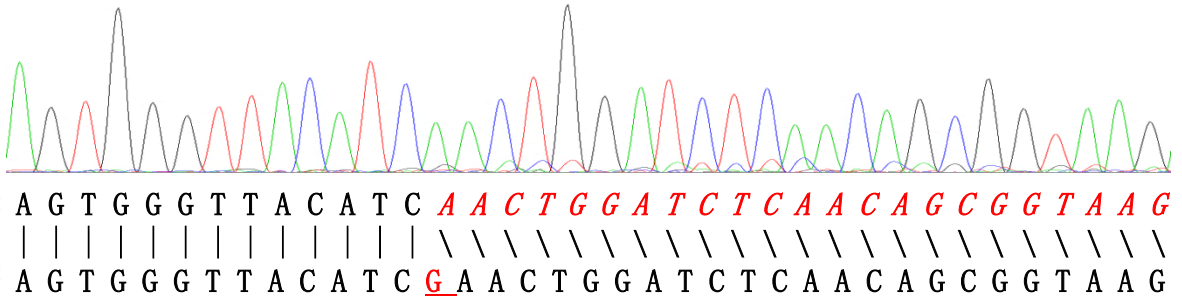
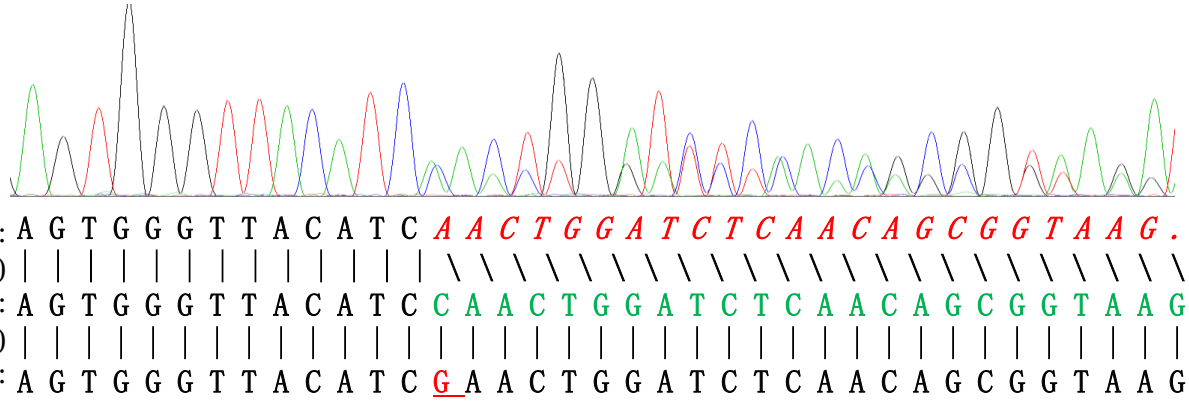


Fig 2B

Initial revertants (grow Slowly)



Later subculture (grow faster)



Final subculture (grow fast)

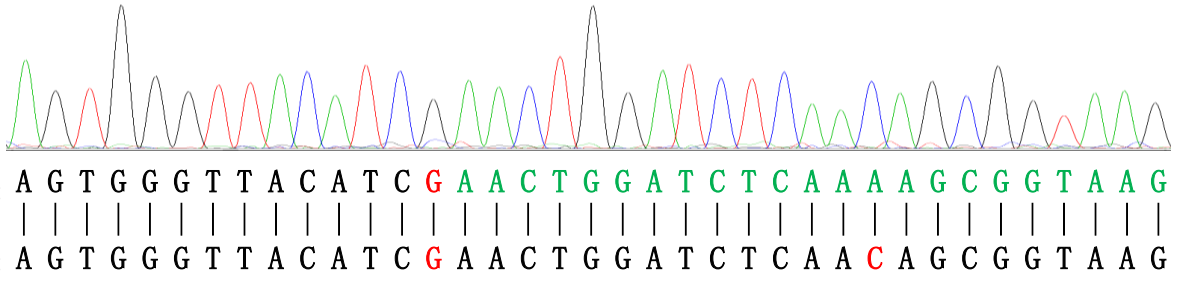
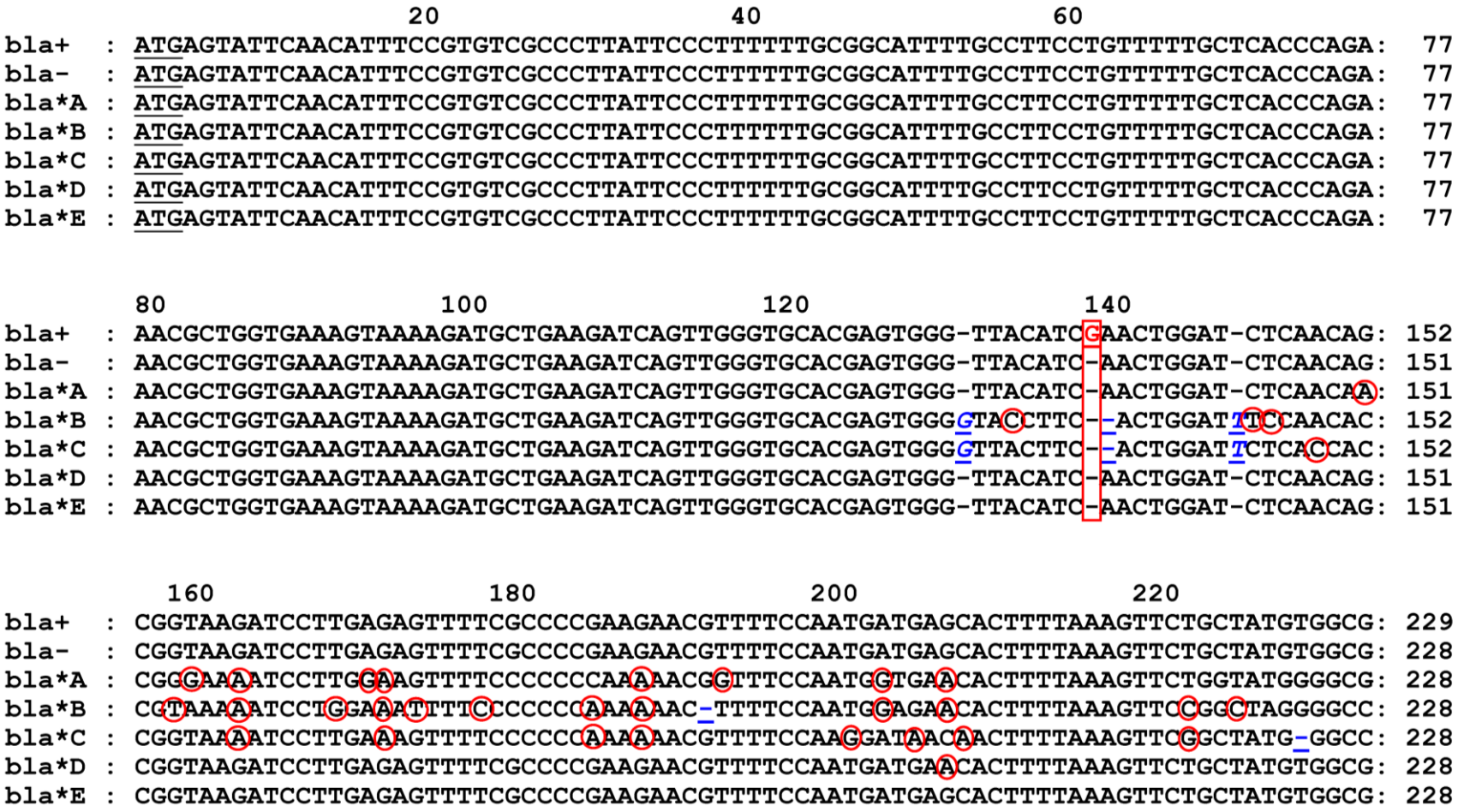


Fig 2C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bla- : ATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGA: 77
bla*A : ATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGA: 77
bla*B : ATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGA: 77
bla*C : ATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGA: 77
bla*D : ATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGA: 77
bla*E : ATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGA: 77
80 100 120 140
bla+ : AACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGG-TTACATCGAACTGGAT-CTCAACAG: 152
bla- : AACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGG-TTACATC-AACTGGAT-CTCAACAG: 151
bla*A : AACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGG-TTACATC-AACTGGAT-CTCAACAA: 151
bla*B : AACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGGTACCTTC--ACTGGATTTCCAACAC: 152
bla*C : AACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGGTTACTTC--ACTGGATTCTCACCAC: 152
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bla*E : AACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGG-TTACATC-AACTGGAT-CTCAACAG: 151
160 180 200 220
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bla*C : CGGTAAAATCCTTGAAAGTTTTCCCCCCAAAAAACGTTTTCCAAGGATAACAACTTTTAAAGTTCGGCTATG-GGCC: 228
bla*D : CGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAACACTTTTAAAGTTCTGCTATGTGGCG: 228
bla*E : CGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCG: 228

Fig 2D

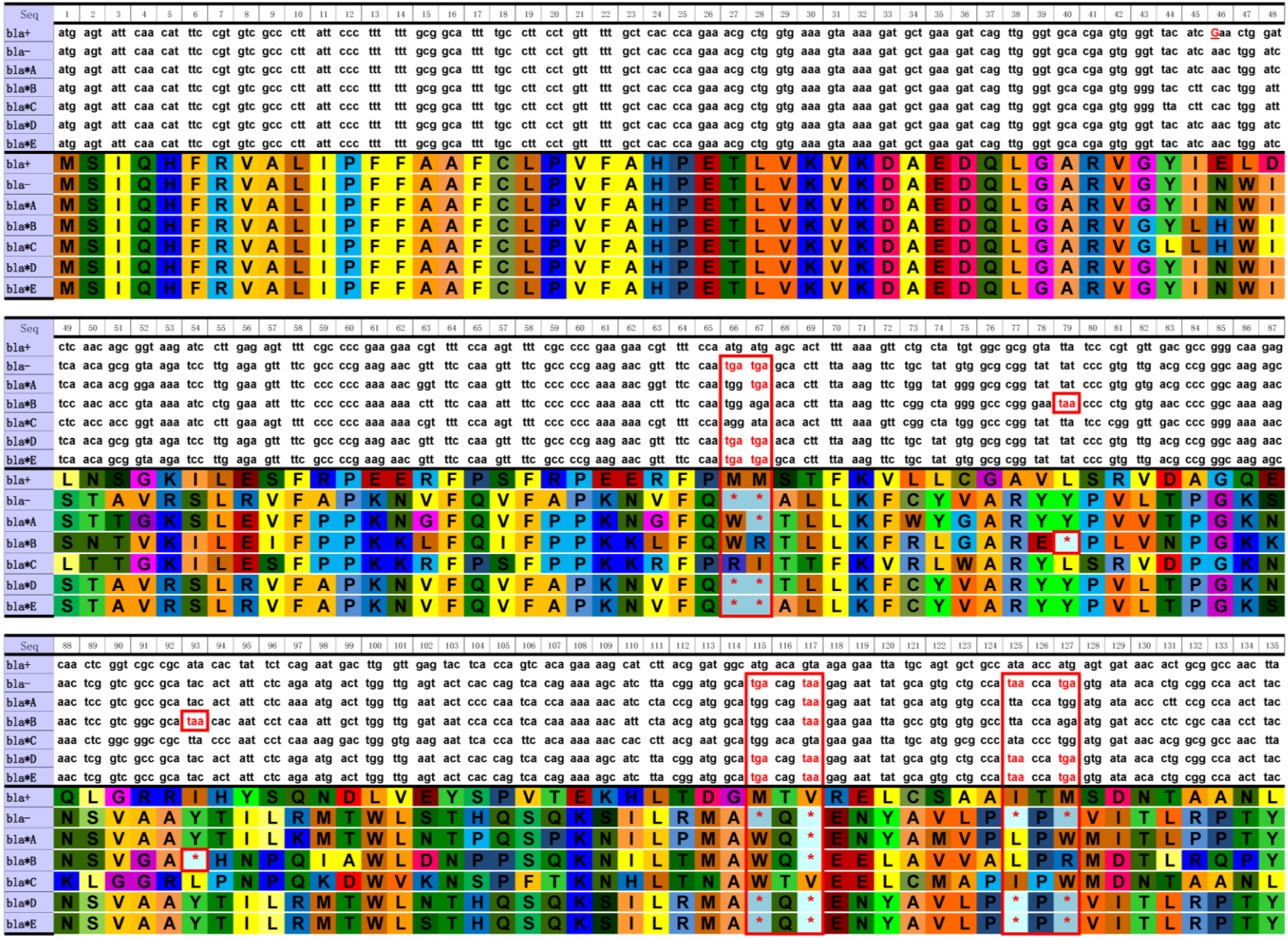
Coding sequences
Protein sequences
Coding sequences
Protein sequences
Coding sequences
Protein sequences

Fig 3 ***Random mutagenesis***

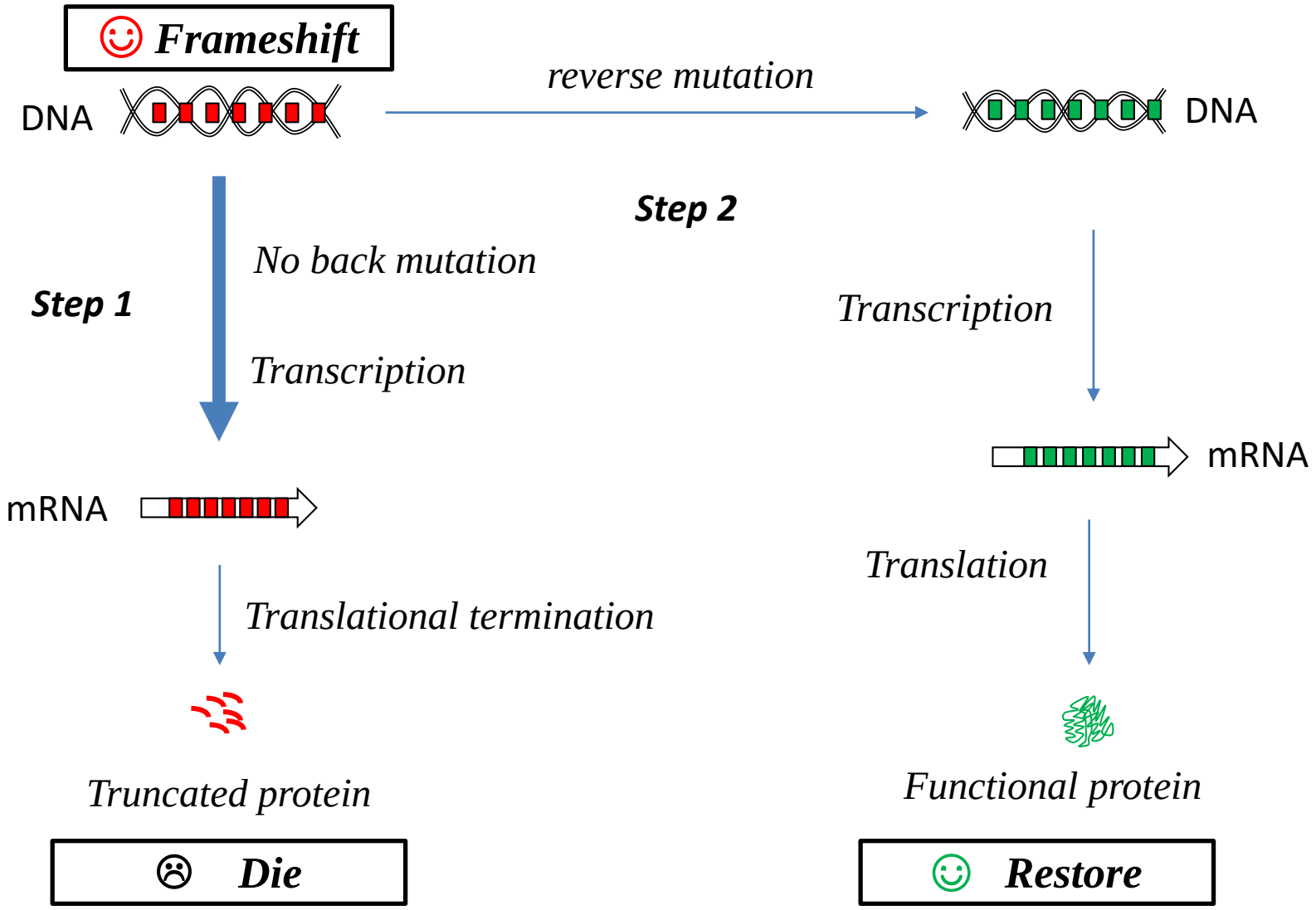

Fig 4A

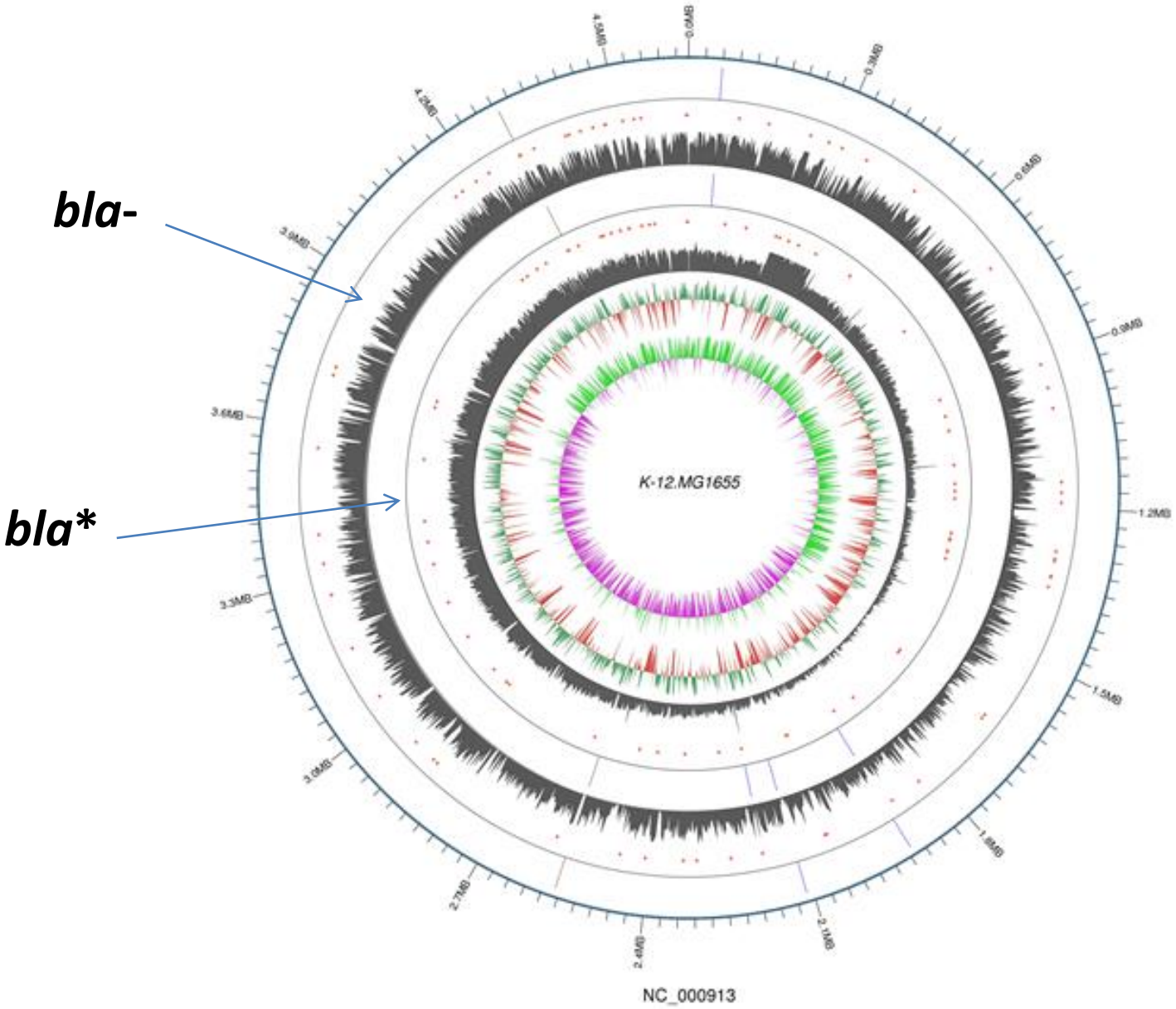

Fig 4B

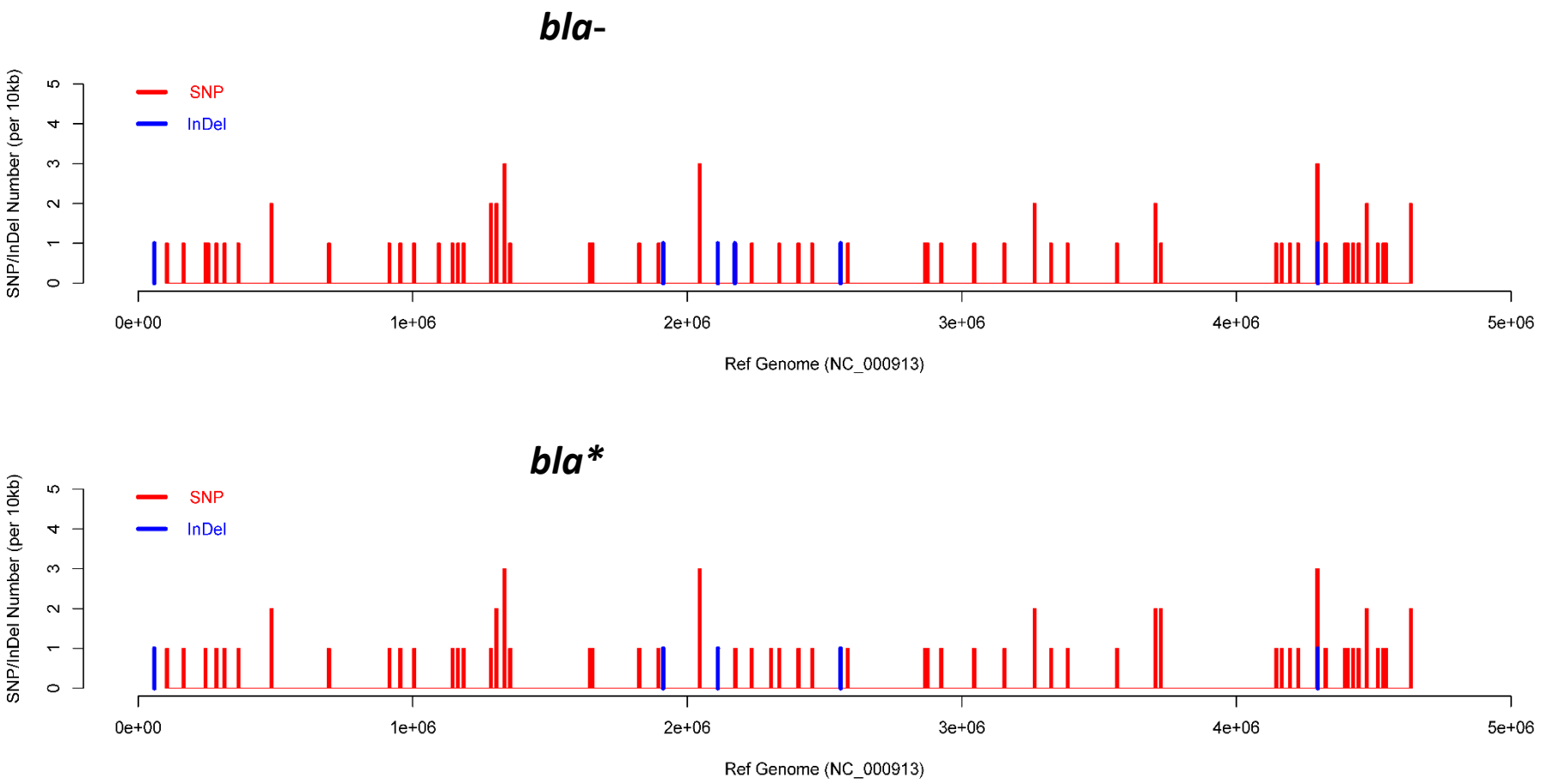
bla-
SNP
InDel
SNP/InDel Number (per 10kb)
0
1
2
3
4
5
0e+00
1e+06
2e+06
3e+06
4e+06
5e+06
Ref Genome (NC_000913)
bla*
SNP
InDel
SNP/InDel Number (per 10kb)
0
1
2
3
4
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Fig 4C

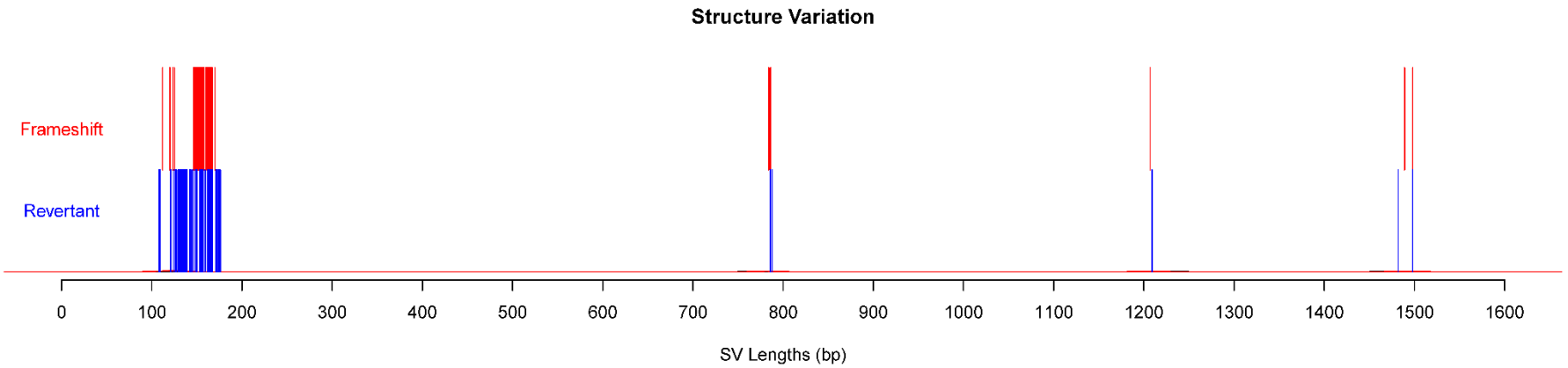

Structure Variation
Frameshift
Revertant
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
SV Lengths (bp)

Fig 5. *Expression of the PTC-free frameshifted bla (bla#)*

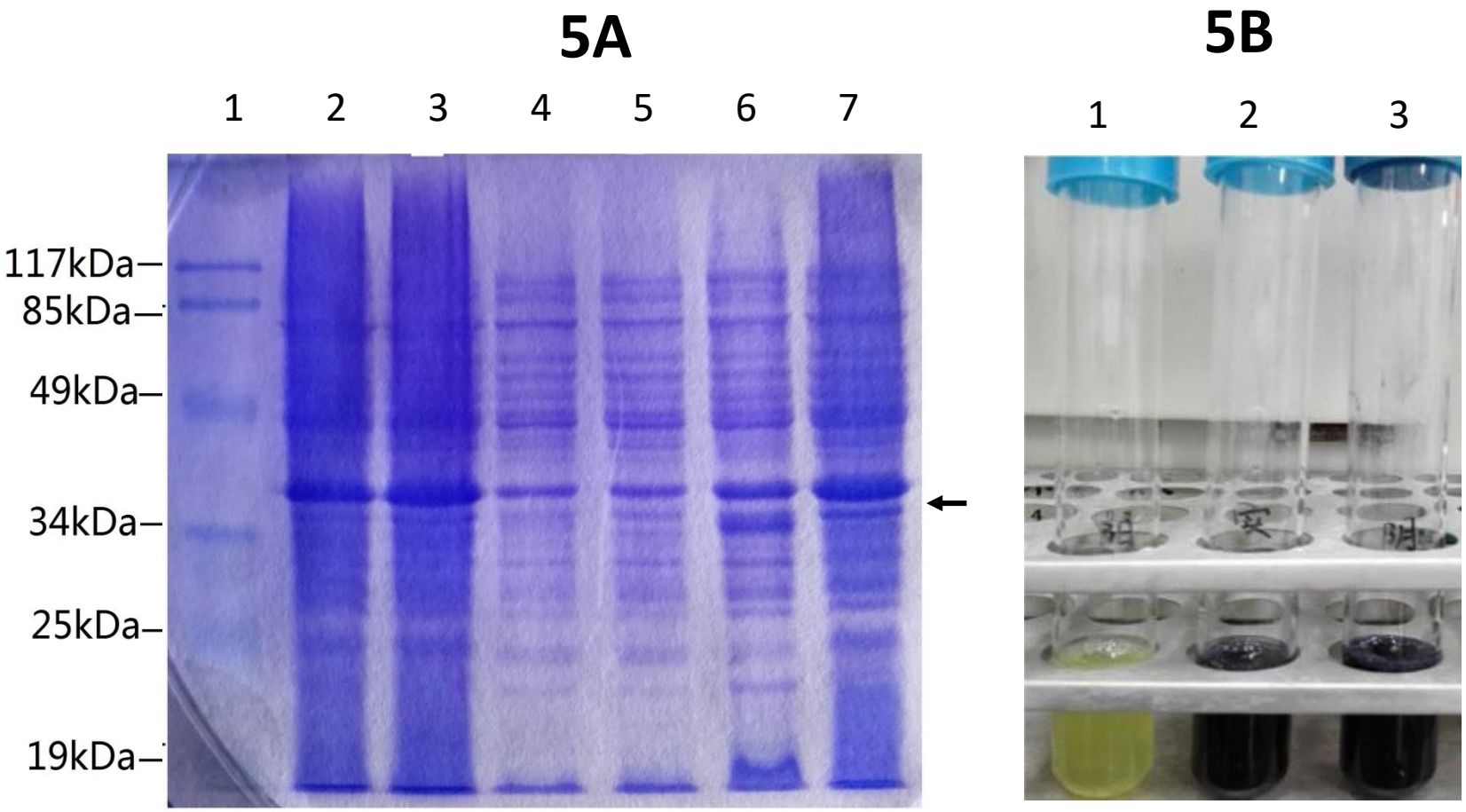
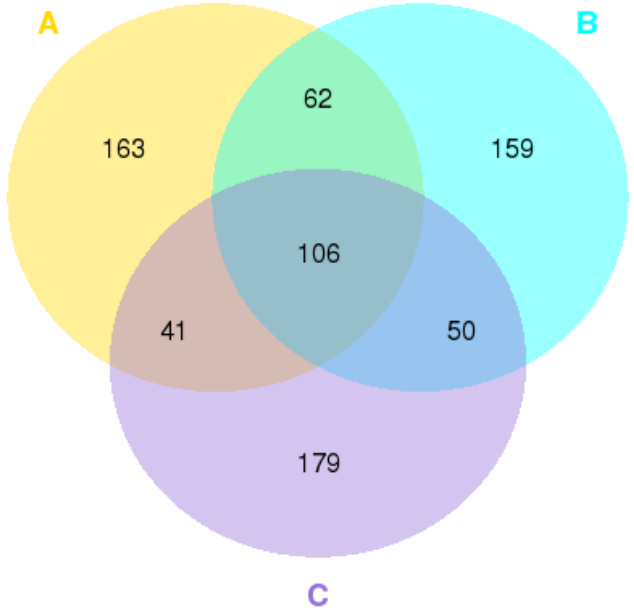
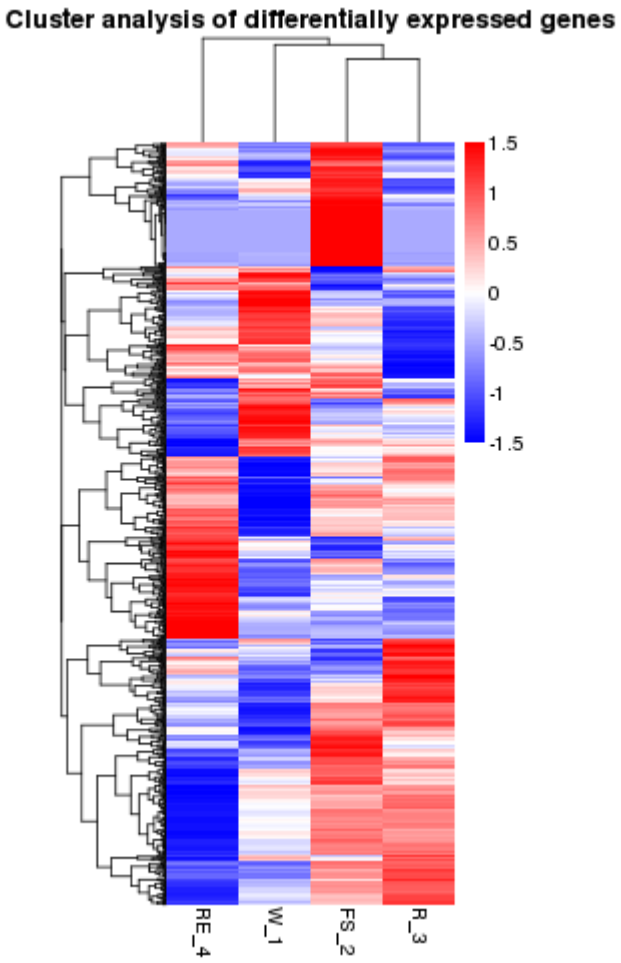


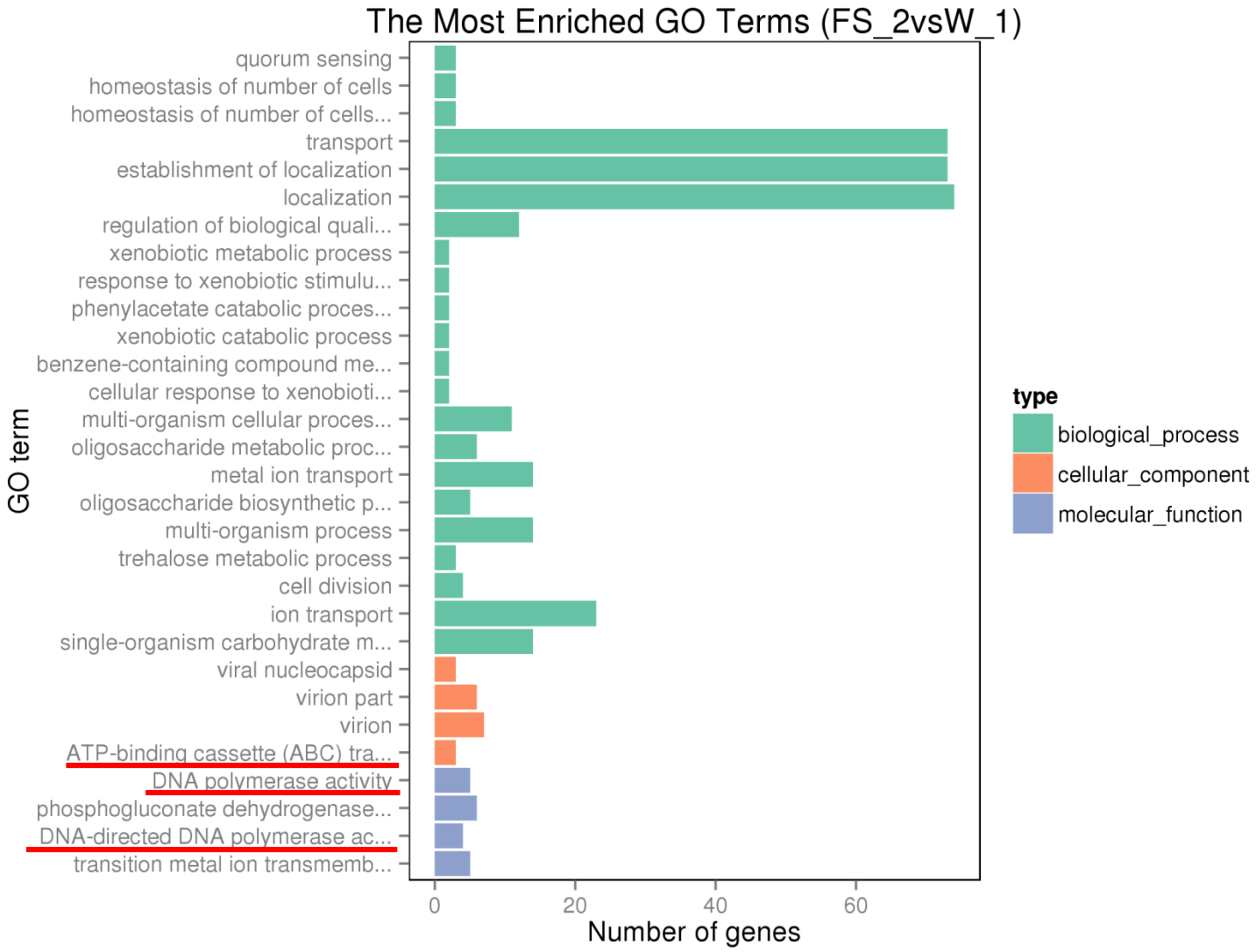
Fig 6. Transcriptome Analysis

6A Numbers of differentially expressed genes (DGE)





6B


The Most Enriched GO Terms (FS_2vsW_1)
GO term
quorum sensing
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homeostasis of number of cells...
transport
establishment of localization
localization
regulation of biological quali...
xenobiotic metabolic process
response to xenobiotic stimulu...
phenylacetate catabolic proces...
xenobiotic catabolic process
benzene-containing compound me...
cellular response to xenobioti...
multi-organism cellular proces...
oligosaccharide metabolic proc...
metal ion transport
oligosaccharide biosynthetic p...
multi-organism process
trehalose metabolic process
cell division
ion transport
single-organism carbohydrate m...
viral nucleocapsid
virion part
virion
ATP-binding cassette (ABC) tra...
DNA polymerase activity
phosphogluconate dehydrogenase...
DNA-directed DNA polymerase ac...
transition metal ion transmemb...
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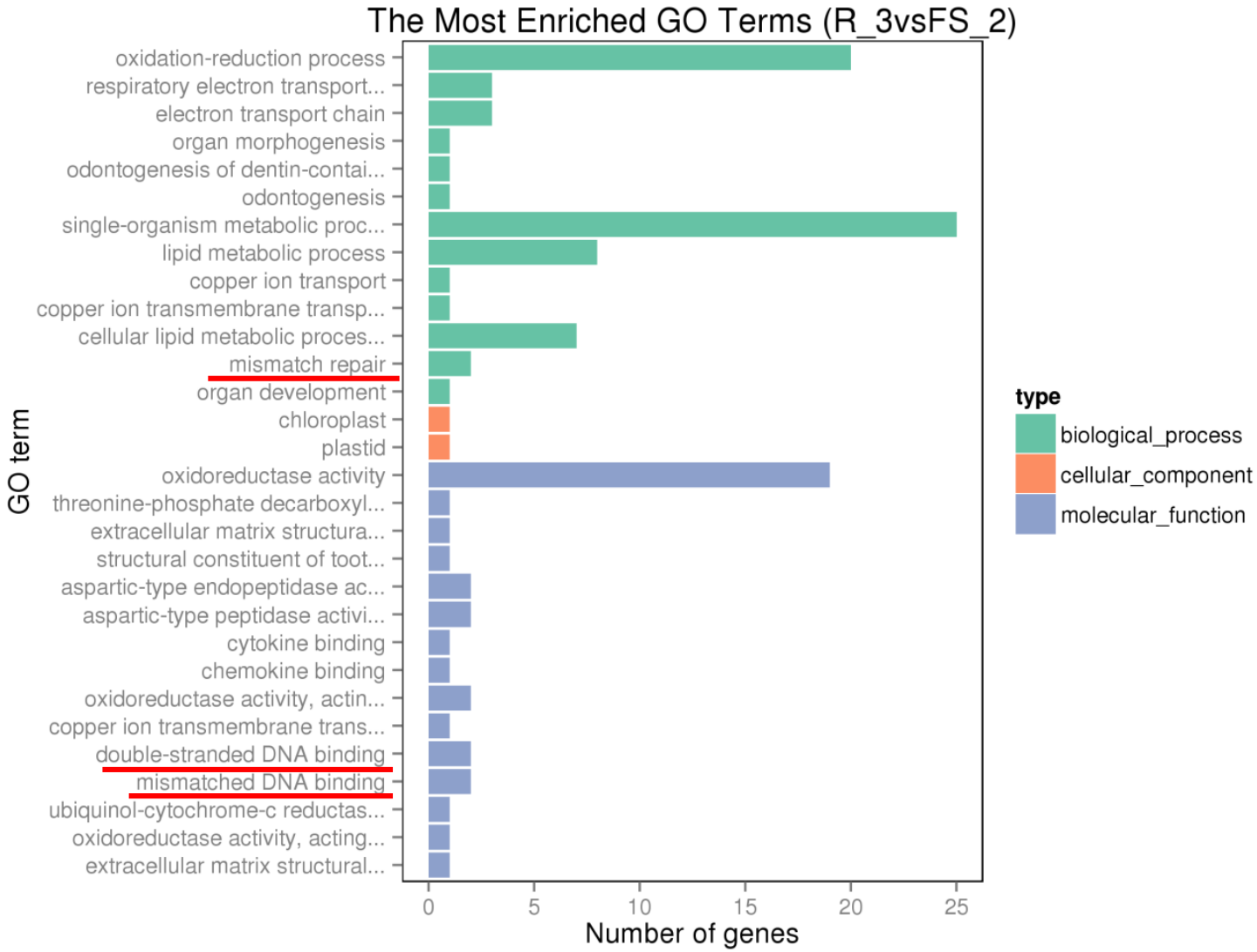
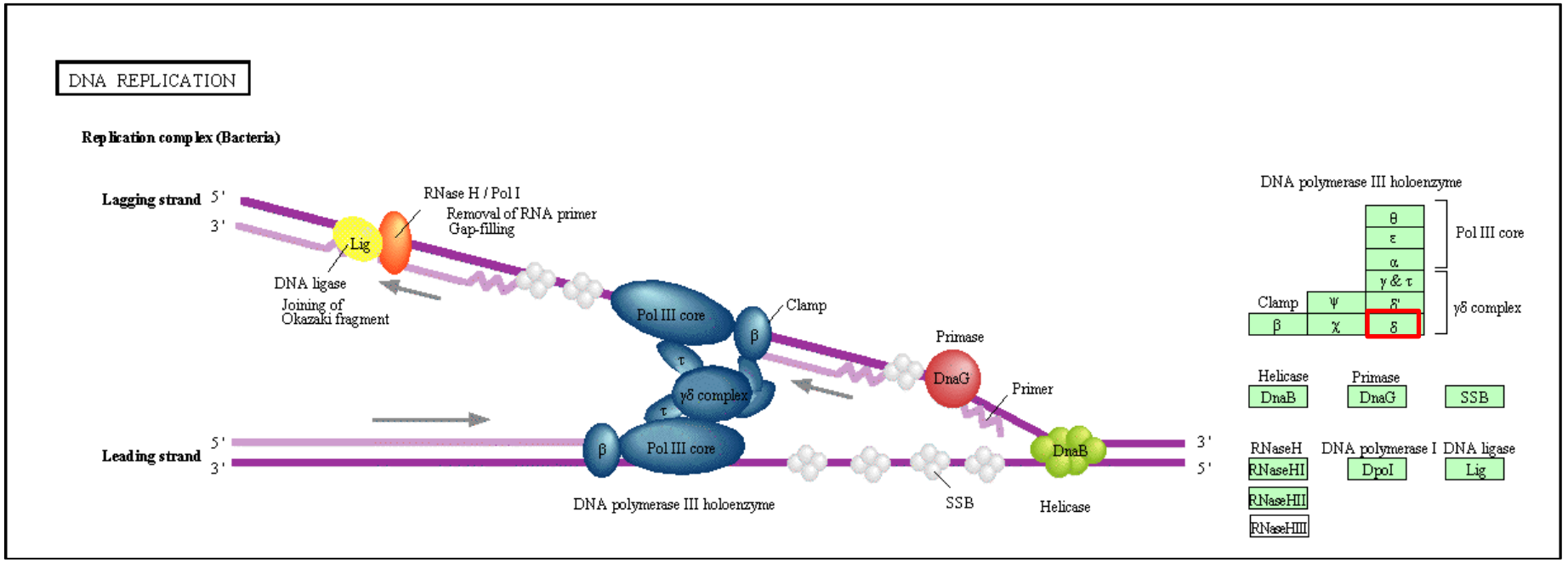
40
60
Number of genes
type
biological_process
cellular_component
molecular_function

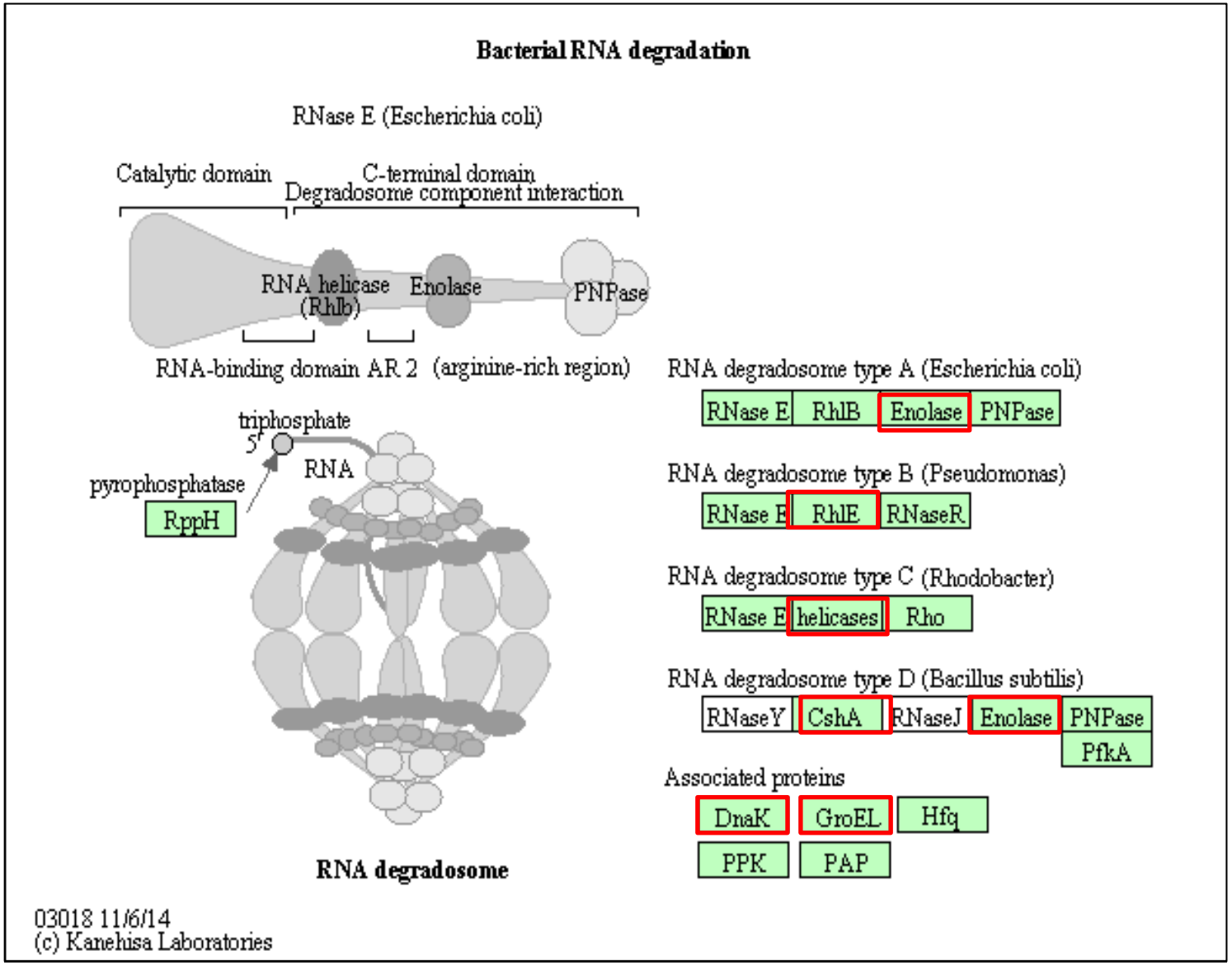
6C


The Most Enriched GO Terms (R_3vsFS_2)
GO term
oxidation-reduction process
respiratory electron transport...
electron transport chain
organ morphogenesis
odontogenesis of dentin-contai...
odontogenesis
single-organism metabolic proc...
lipid metabolic process
copper ion transport
copper ion transmembrane transp...
cellular lipid metabolic proces...
mismatch repair
organ development
chloroplast
plastid
oxidoreductase activity
threonine-phosphate decarboxyl...
extracellular matrix structura...
structural constituent of toot...
aspartic-type endopeptidase ac...
aspartic-type peptidase activi...
cytokine binding
chemokine binding
oxidoreductase activity, actin...
copper ion transmembrane trans...
double-stranded DNA binding
mismatched DNA binding
ubiquinol-cytochrome-c reductas...
oxidoreductase activity, acting...
extracellular matrix structural...
0
5
10
15
20
25
Number of genes
type
biological_process
cellular_component
molecular_function

6D genes upregulated in DNA replication



6E genes upregulated in RNA degradation/processing



6F genes upregulated in mismatch repair/homologous recombination

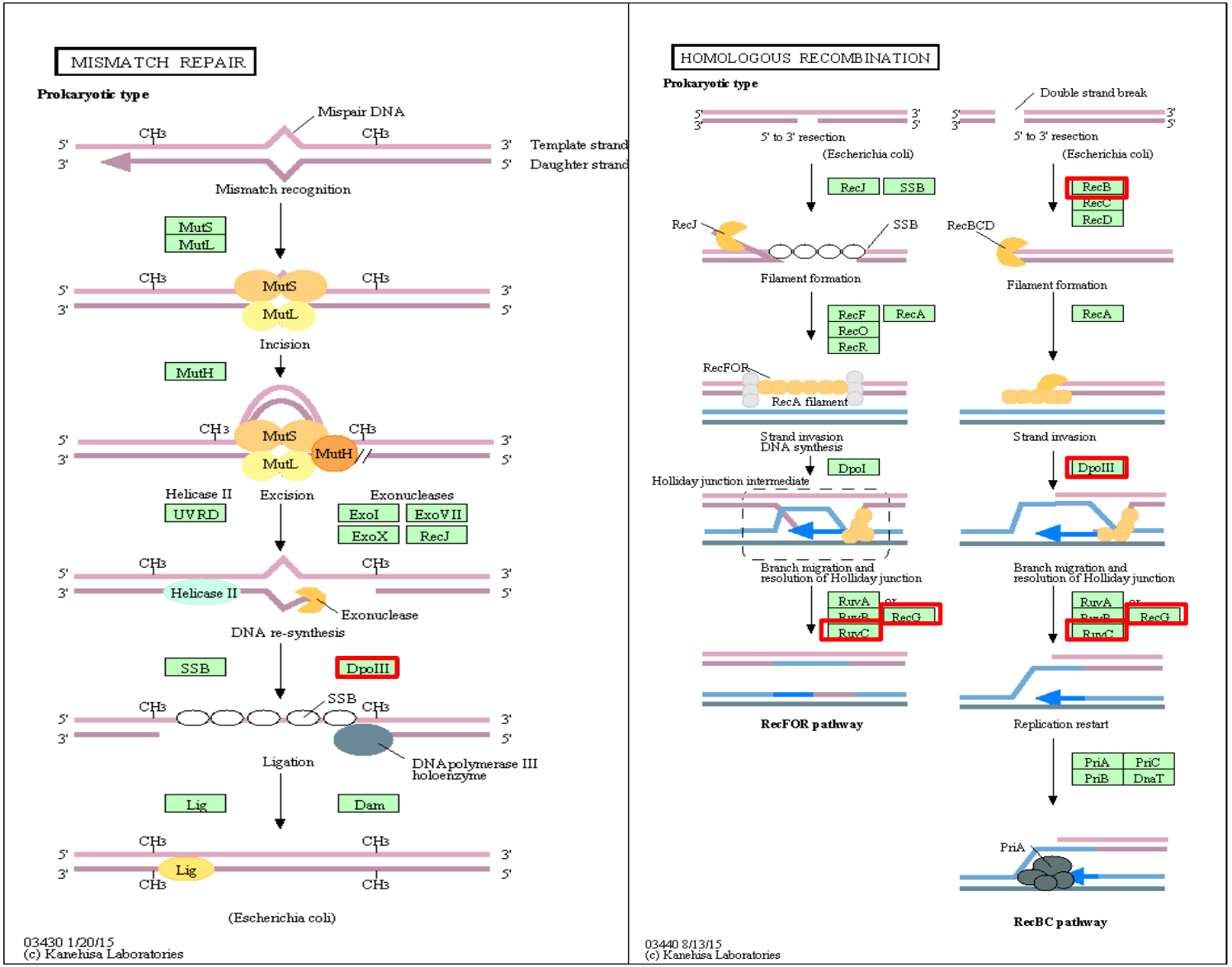


Fig 7A. Frameshift repair induced by DNA or RNA oligos (10 nM)

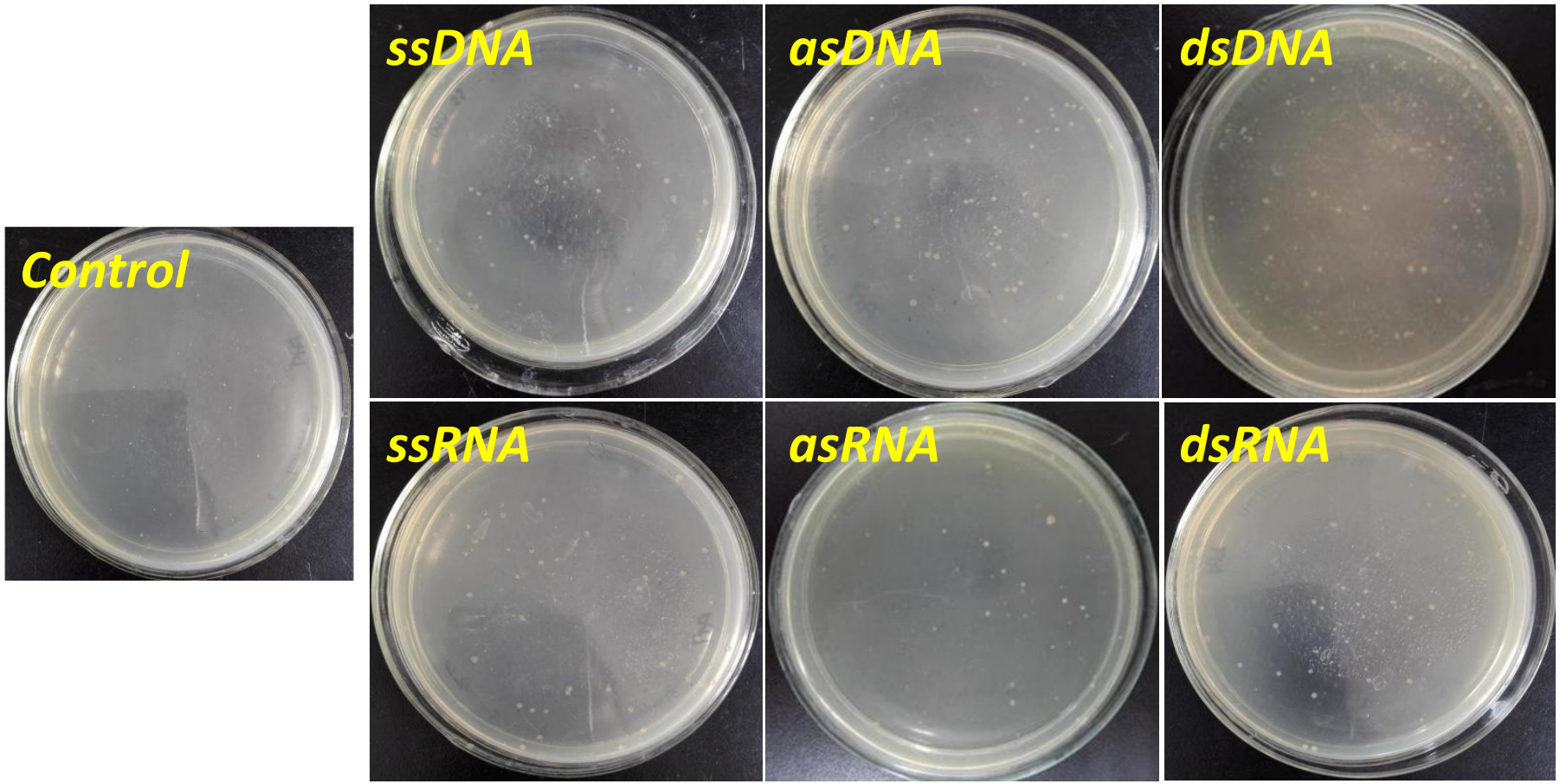


Fig 7B. The rate of reversions in different group

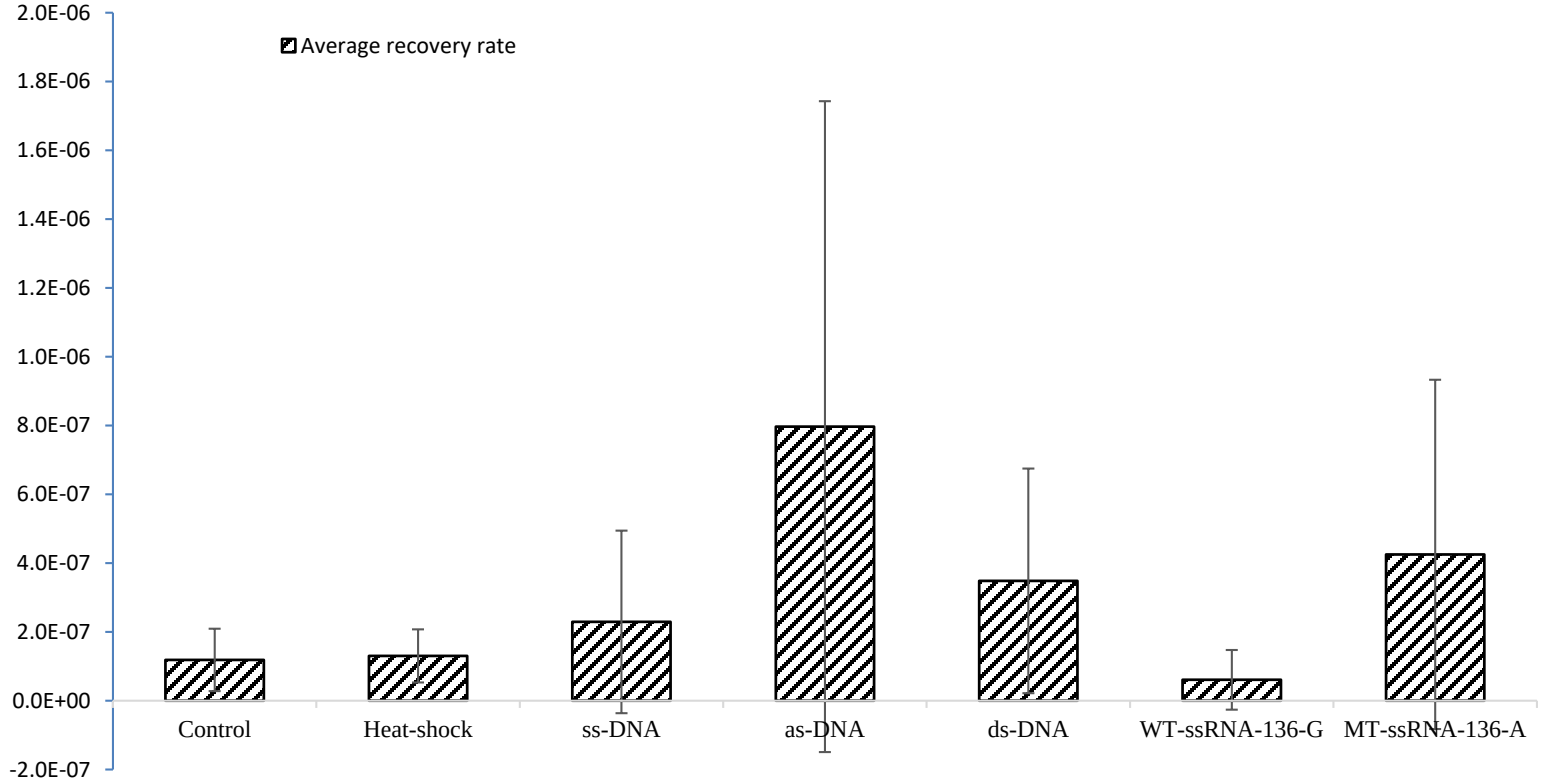
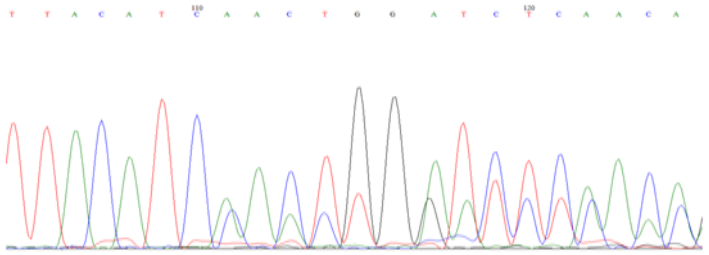


Fig 7C. Sanger sequencing diagram of the ssDNA-induced revertants



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2nd Peaks:    T G G A C G A G T G G G T T A C A T C A A A C T G G A T C T C A A C A G C G G T
              | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
WT DNA oligo: T G G A C G A G T G G G T T A C A T C G A A C T G G A T C T C A A C A G C G G T
              | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Wild-type:    T G C A C G A G T G G G T T A C A T C G A A C T G G A T C T C A A C A G C G G T
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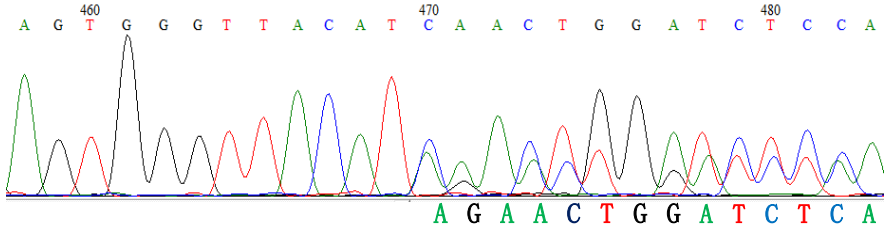

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              | | | | | | | | | | | | | | | | |   | | | | | | | | | | | | | | | | |
2nd Peaks:    T G G A C G A G T G G G T T A C A T A G A A C T G G A T C T C A A C A G C G G T
              | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
MT DNA oligo: T G G A C G A G T G G G T T A C A T A G A A C T G G A T C T C A A C A G C G G T
              | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Wild-type:    T G C A C G A G T G G G T T A C A T C G A A C T G G A T C T C A A C A G C G G T
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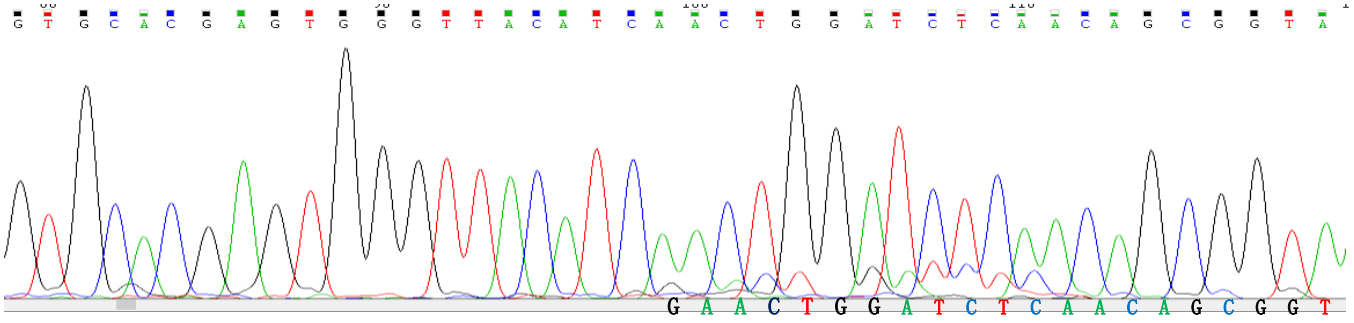
Fig 7D. Sanger sequencing diagram of the RNA-induced revertants

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Main Peaks:   T G C A C G A G T G G G T T A C A T C - A A C T G G A T C T C A A C A G C G G T
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2nd Peaks:    T G G A C G A G T G G G T T A C A T C G A A C T G G A T C T C A A C A G C G G T
              | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
WT RNA oligo: U G G A C G A G U G G G U U A C A U C G A A C U G G A U C U C A A C A G C G G U
              | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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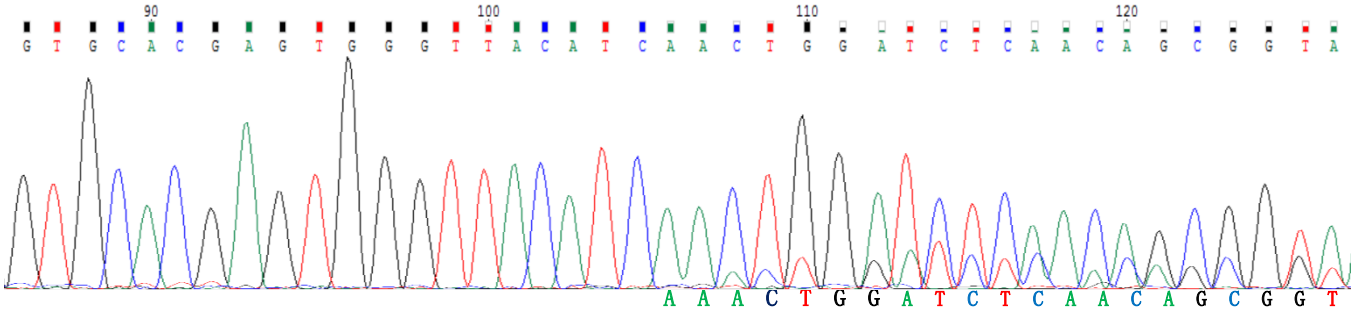


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Main Peaks:   T G C A C G A G T G G G T T A C A T C - A A C T G G A T C T C A A C A G C G G T
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2nd Peaks:    T G G A C G A G T G G G T T A C A T C A A A C T G G A T C T C A A C A G C G G T
              | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
MT RNA oligo: U G G A C G A G U G G G U U A C A U C A A A C U G G A U C U C A A C A G C G G U
              | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Wild-type:    T G C A C G A G T G G G T T A C A T C G A A C T G G A T C T C A A C A G C G G T
```

Fig 8 *Nonsense-mediated gene editing (NMGE)*