For Thursday

You must have chosen a gene of interest.

Prepare 3-4 slides on

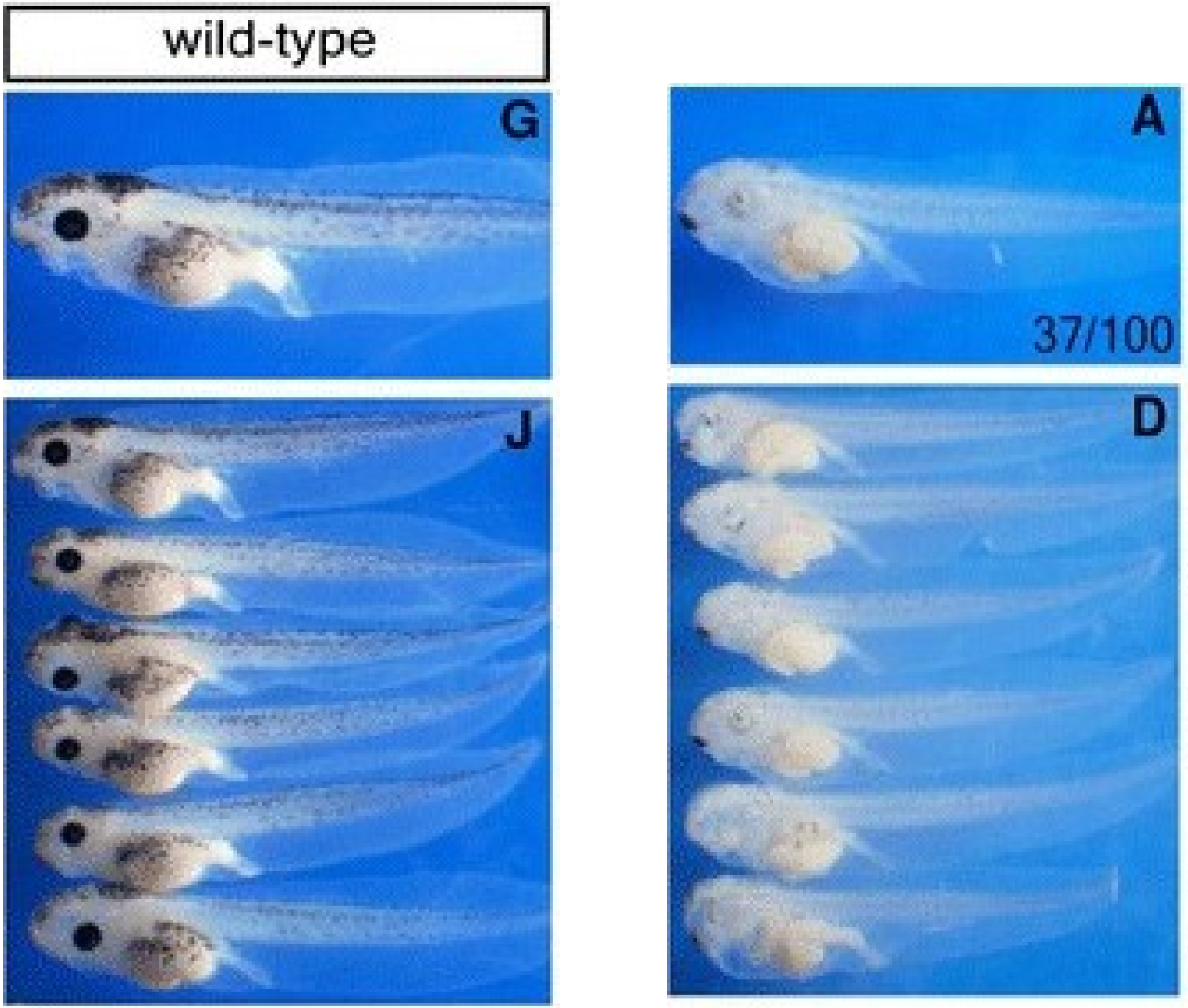
How you chose this gene

Why we should care

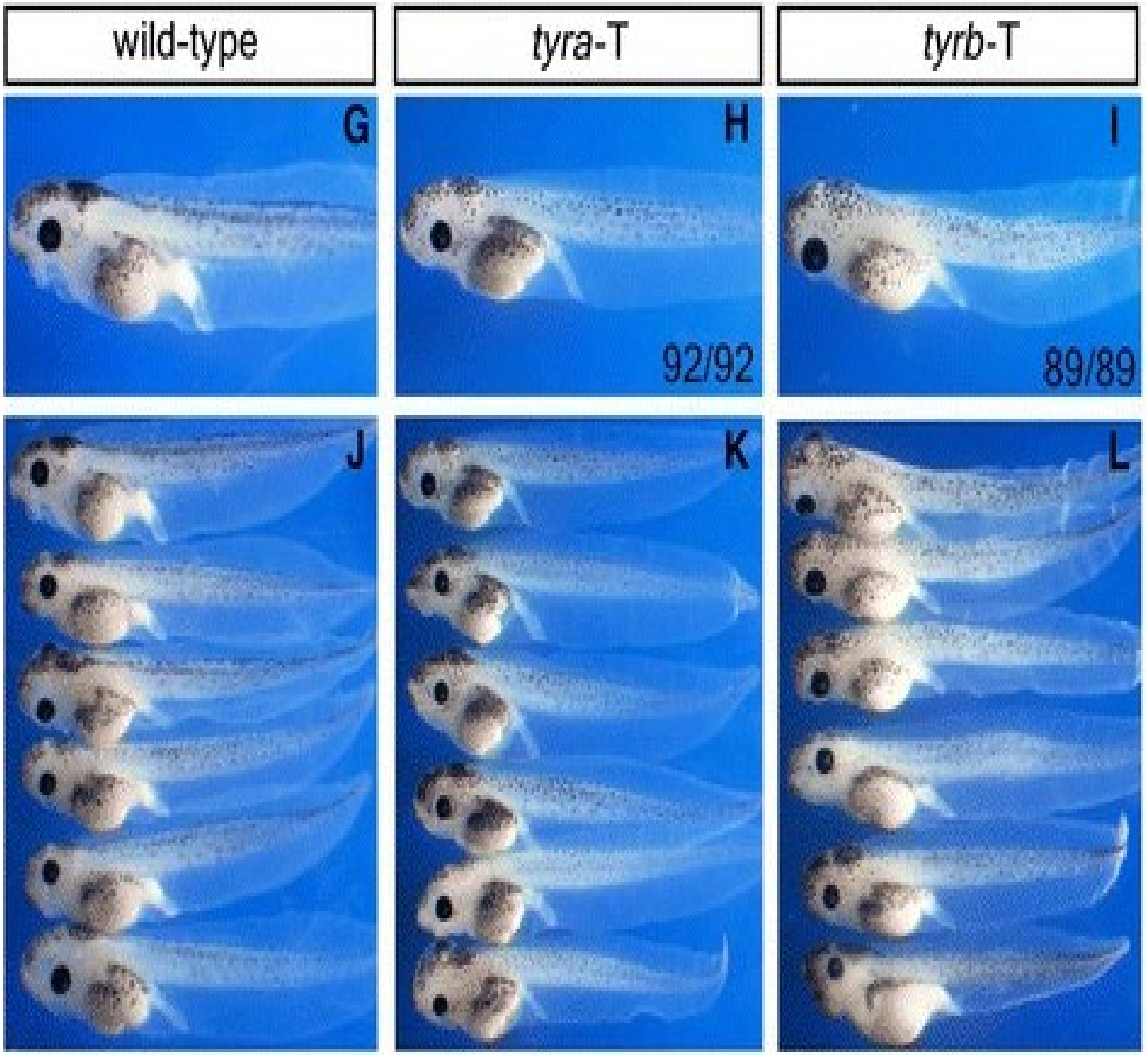
Also,install ApE and put in your gene sequence into an ApE file following instructions in the "How to design your sgRNA construct" file.

CRISPR-Cas9 oligo design

- Using Xenbase and NCBI to identify gene sequences
- Using ApE plasmid editor for cloning strategy into DR274 plasmid
- Using CRISPRdirect website for specificity check and sequence confirmation



Wang F et al 2015 Cell Biosci



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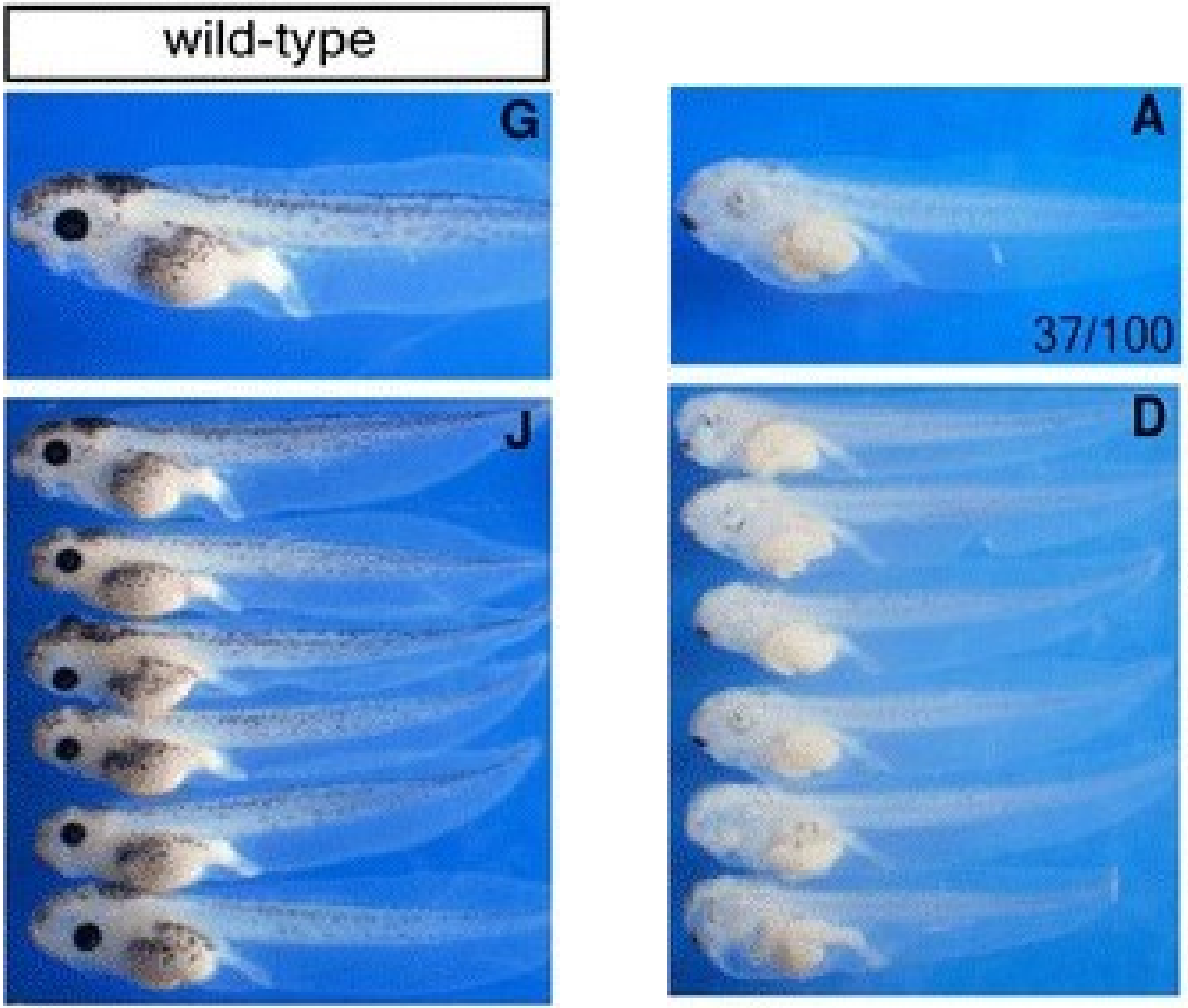

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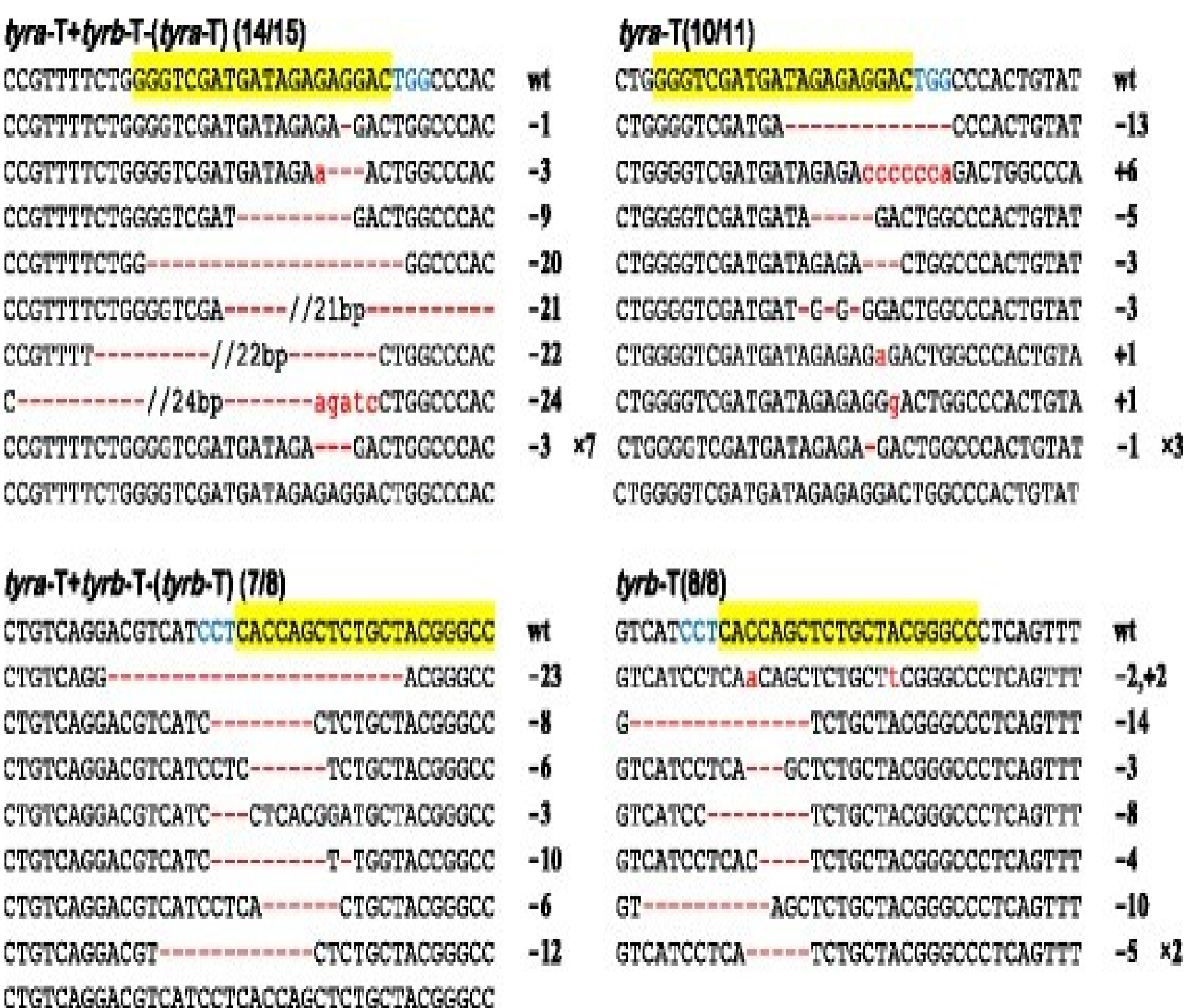
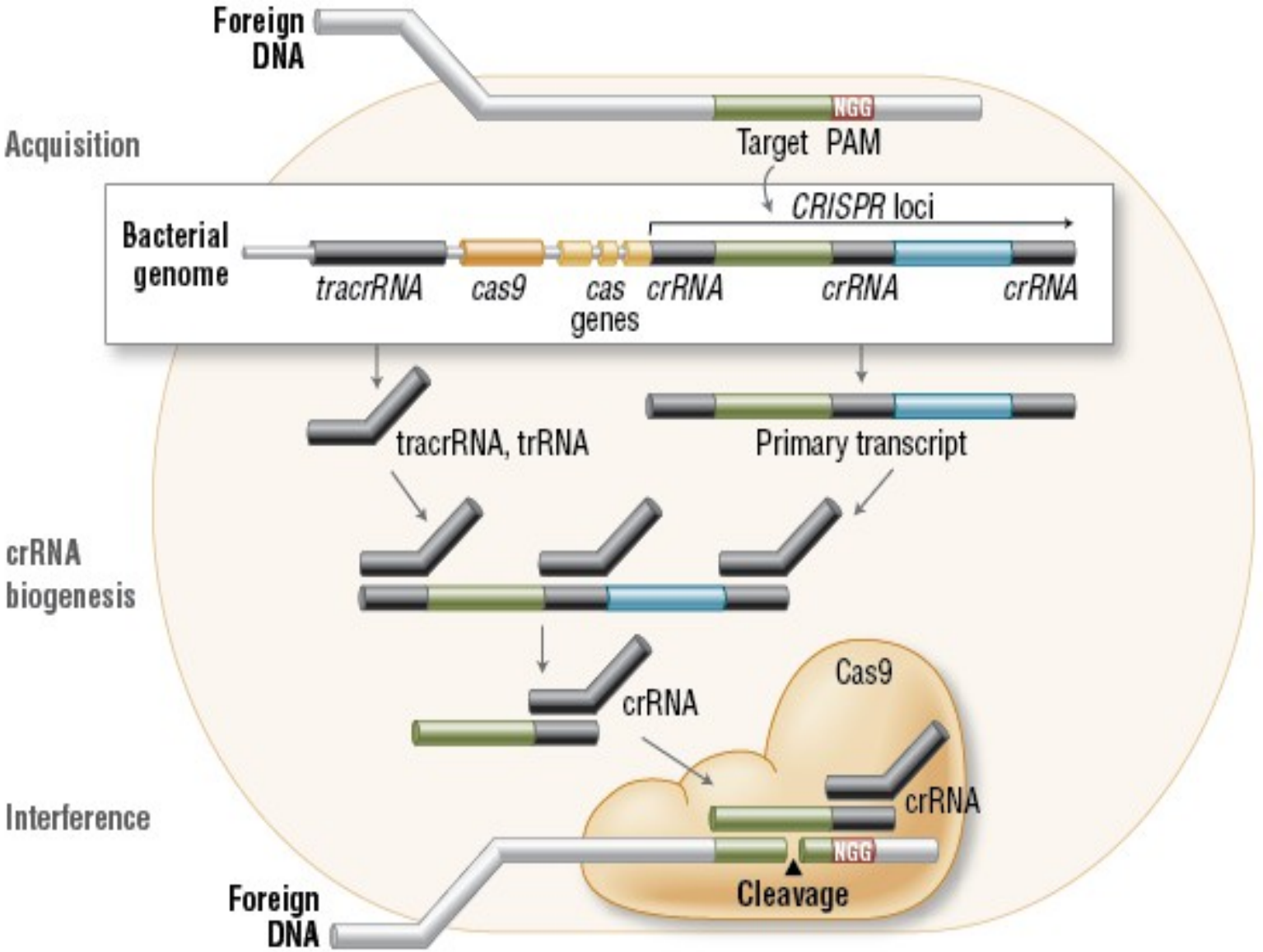
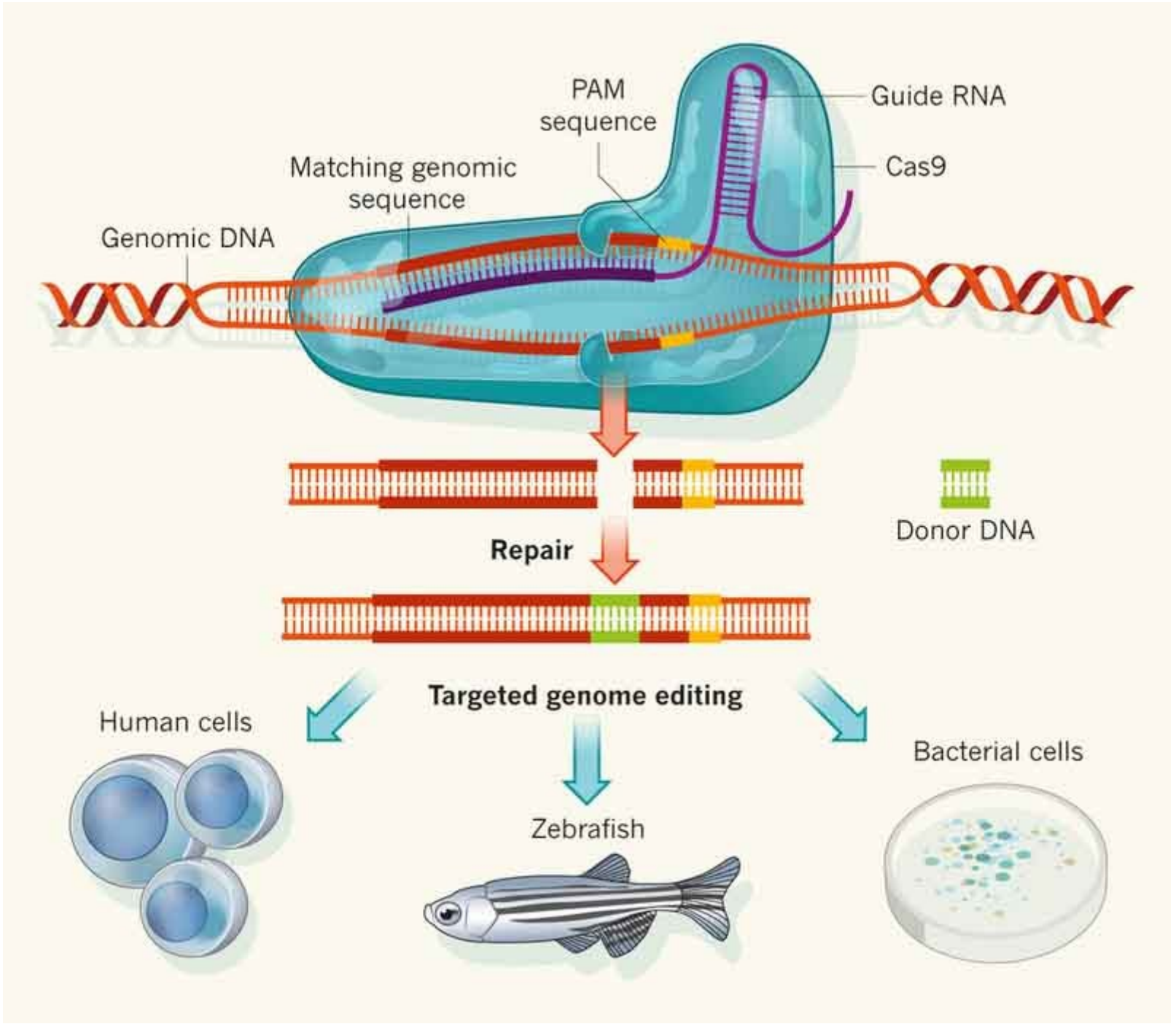

tyra-T+tyrb-T-(tyra-T) (14/15)
CCGTTTTCTGGGGTCGATGATAGAGAGGACTGGCCCAC wt
CCGTTTTCTGGGGTCGATGATAGAGA-GACTGGCCCAC -1
CCGTTTTCTGGGGTCGATGATAGAa---ACTGGCCCAC -3
CCGTTTTCTGGGGTCGAT---------GACTGGCCCAC -9
CCGTTTTCTGG--------------------GGCCCAC -20
CCGTTTTCTGGGGTCGA-----//21bp--------- -21
CCGTTTT---------//22bp-------CTGGCCCAC -22
C----------//24bp-------agatcCTGGCCCAC -24
CCGTTTTCTGGGGTCGATGATAGA---GACTGGCCCAC -3 ×7
CCGTTTTCTGGGGTCGATGATAGAGAGGACTGGCCCAC
tyra-T(10/11)
CTGGGGTCGATGATAGAGAGGACTGGCCCACTGTAT wt
CTGGGGTCGATGA------------CCCACTGTAT -13
CTGGGGTCGATGATAGAGAccccccaGACTGGCCCA +6
CTGGGGTCGATGATA-----GACTGGCCCACTGTAT -5
CTGGGGTCGATGATAGAGA---CTGGCCCACTGTAT -3
CTGGGGTCGATGAT-G-G-GGACTGGCCCACTGTAT -3
CTGGGGTCGATGATAGAGAGaGACTGGCCCACTGTA +1
CTGGGGTCGATGATAGAGAGGgACTGGCCCACTGTA +1
CTGGGGTCGATGATAGAGA-GACTGGCCCACTGTAT -1 ×3
CTGGGGTCGATGATAGAGAGGACTGGCCCACTGTAT
tyra-T+tyrb-T-(tyrb-T) (7/8)
CTGTCAGGACGTCATCCTCACCAGCTCTGCTACGGGCC wt
CTGTCAGG----------------------ACGGGCC -23
CTGTCAGGACGTCATC--------CTCTGCTACGGGCC -8
CTGTCAGGACGTCATCCTC------TCTGCTACGGGCC -6
CTGTCAGGACGTCATC---CTCACGGATGCTACGGGCC -3
CTGTCAGGACGTCATC---------T-TGGTACCGGCC -10
CTGTCAGGACGTCATCCTCA------CTGCTACGGGCC -6
CTGTCAGGACGT-----------CTCTGCTACGGGCC -12
CTGTCAGGACGTCATCCTCACCAGCTCTGCTACGGGCC
tyrb-T(8/8)
GTCATCCTCACCAGCTCTGCTACGGGCCCTCAGTTT wt
GTCATCCTCAaCAGCTCTGCTtCGGGCCCTCAGTTT -2,+2
G-------------TCTGCTACGGGCCCTCAGTTT -14
GTCATCCTCA---GCTCTGCTACGGGCCCTCAGTTT -3
GTCATCC--------TCTGCTACGGGCCCTCAGTTT -8
GTCATCCTCAC----TCTGCTACGGGCCCTCAGTTT -4
GT----------AGCTCTGCTACGGGCCCTCAGTTT -10
GTCATCCTCA-----TCTGCTACGGGCCCTCAGTTT -5 ×2

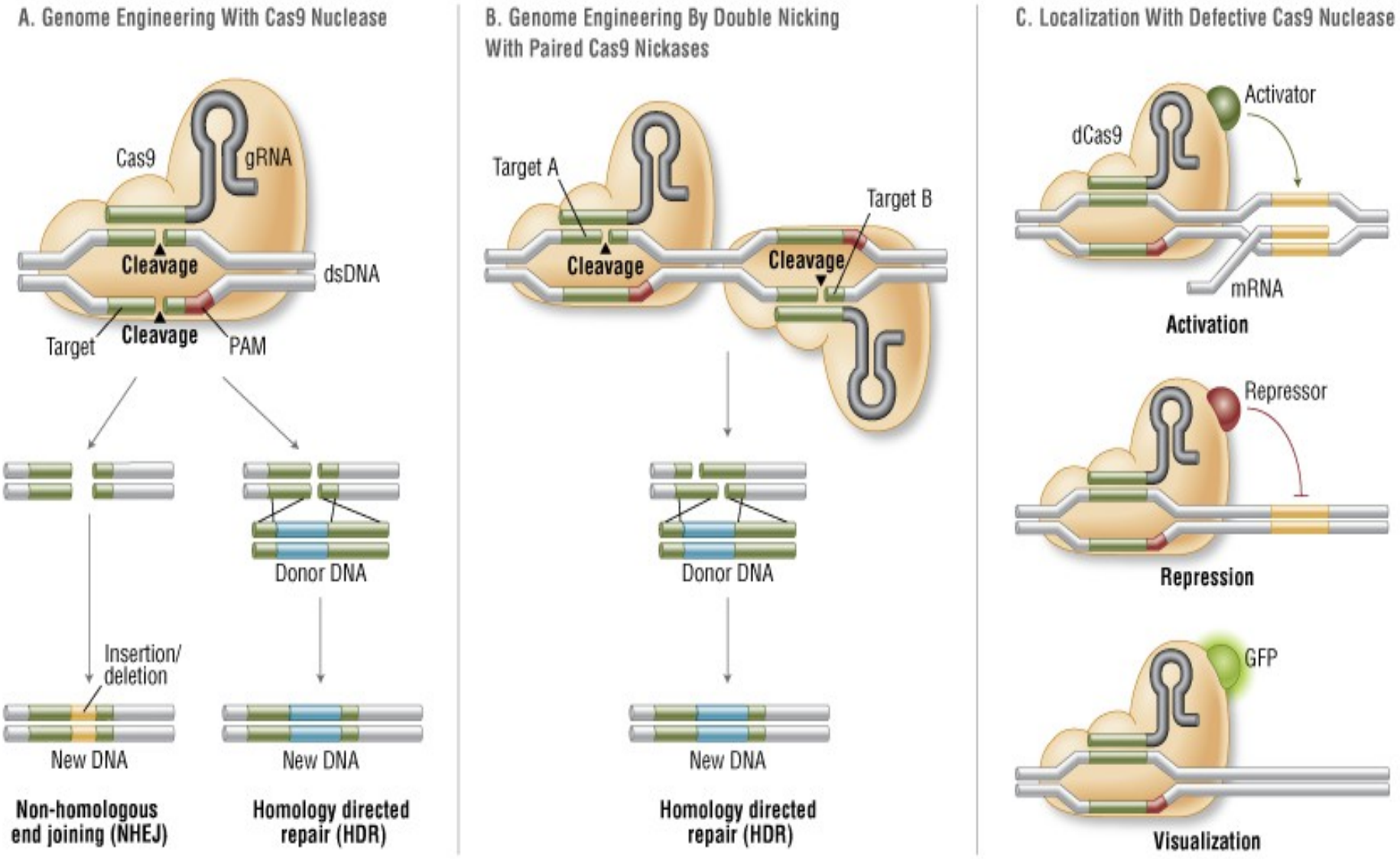
CRISPR-Cas9

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)

CRISPR-associated (Cas) enzyme


Foreign DNA
NGG
Target PAM
Acquisition
CRISPR loci
Bacterial genome
tracrRNA
cas9
cas genes
crRNA
crRNA
crRNA
tracrRNA, trRNA
Primary transcript
crRNA biogenesis
Cas9
crRNA
crRNA
Interference
NGG
Cleavage
Foreign DNA

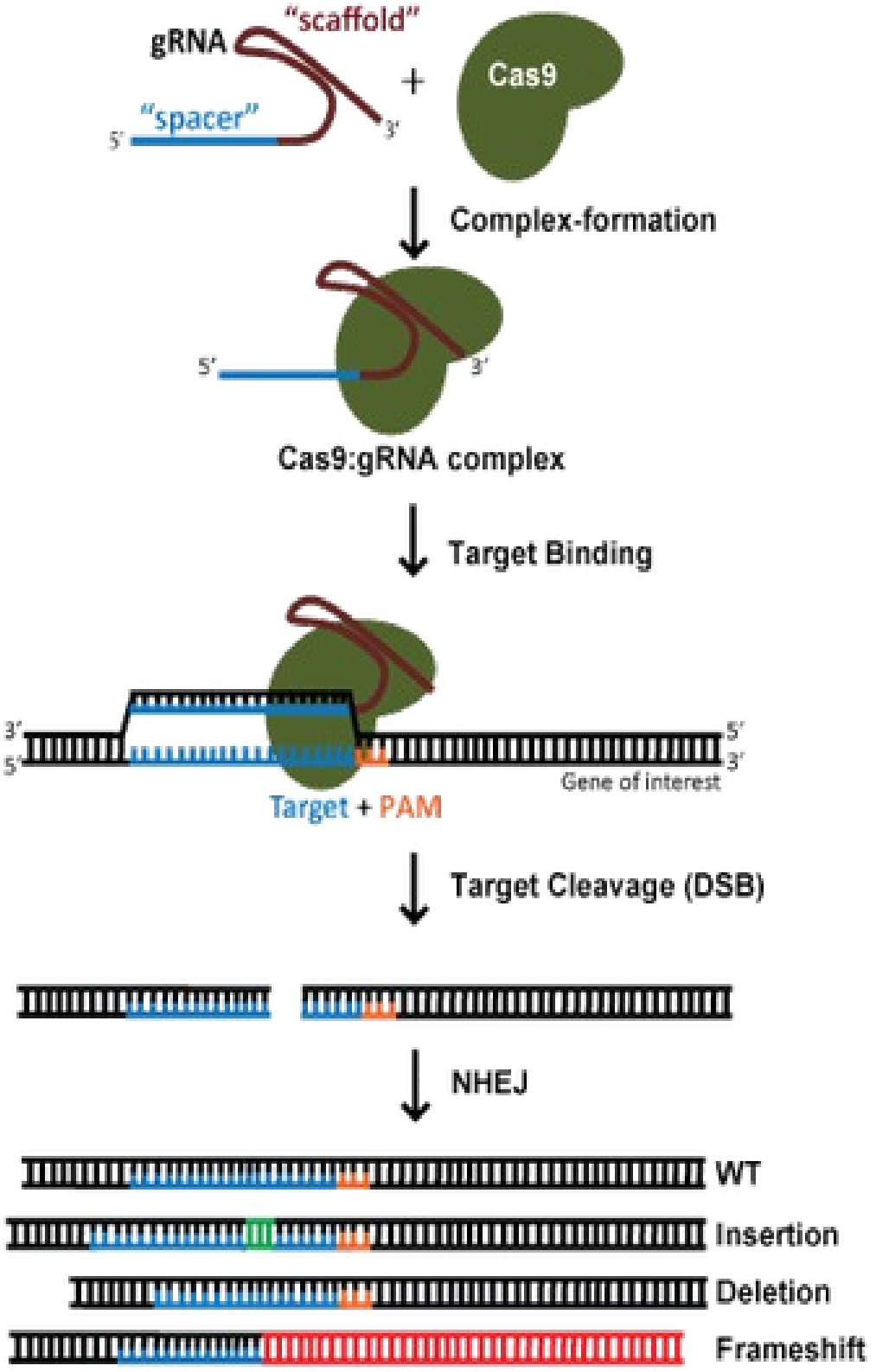

PAM sequence
Guide RNA
Matching genomic sequence
Cas9
Genomic DNA
Donor DNA
Repair
Targeted genome editing
Human cells
Zebrafish
Bacterial cells


A. Genome Engineering With Cas9 Nuclease
Cas9
gRNA
Cleavage
dsDNA
Target
Cleavage
PAM
Donor DNA
Insertion/
deletion
New DNA
New DNA
Non-homologous
end joining (NHEJ)
Homology directed
repair (HDR)
B. Genome Engineering By Double Nicking
With Paired Cas9 Nickases
Target A
Target B
Cleavage
Cleavage
Donor DNA
New DNA
Homology directed
repair (HDR)
C. Localization With Defective Cas9 Nuclease
dCas9
Activator
mRNA
Activation
Repressor
Repression
GFP
Visualization

CRISPR

Why is this technology so exciting and with such great potential?

What are the main concerns with the technology and its application to human biology?


gRNA
"scaffold"
"spacer"
5′
3′
+
Cas9
Complex-formation
5′
3′
Cas9:gRNA complex
Target Binding
3′
5′
5′
3′
Gene of interest
Target + PAM
Target Cleavage (DSB)
NHEJ
WT
Insertion
Deletion
Frameshift

T7 RNA pol promoter site

→ txn initiation site

5'-**TAATACGACTCACTATA**GGAGAGACCGAGAGAGGGTCTCAGT
coding strand

3'-**ATTATGCTGAGTGATAT**CCTCTCTGGCTCTCTCCCAGAGTCA
template strand

5'-GGAGAGACCGAGAGAGGGUCUCAGU RNA

Oligos will always look like this

F1 5' TAGGN18
R1 5' AAACN18

Install ApE

http://biologylabs.utah.edu/jorgensen/wayned/ape/

For CRISPR-Cas9 targeting

Prefer a protein coding exon at the beginning of the sequence hoping to get a frameshift mutation

Avoid exon-intron junctions

Avoid within introns since intronic mutations may have no effect on your gene of interest and these same introns may contain other genes such as miRs that would mislead you in genotype and phenotype analysis.