



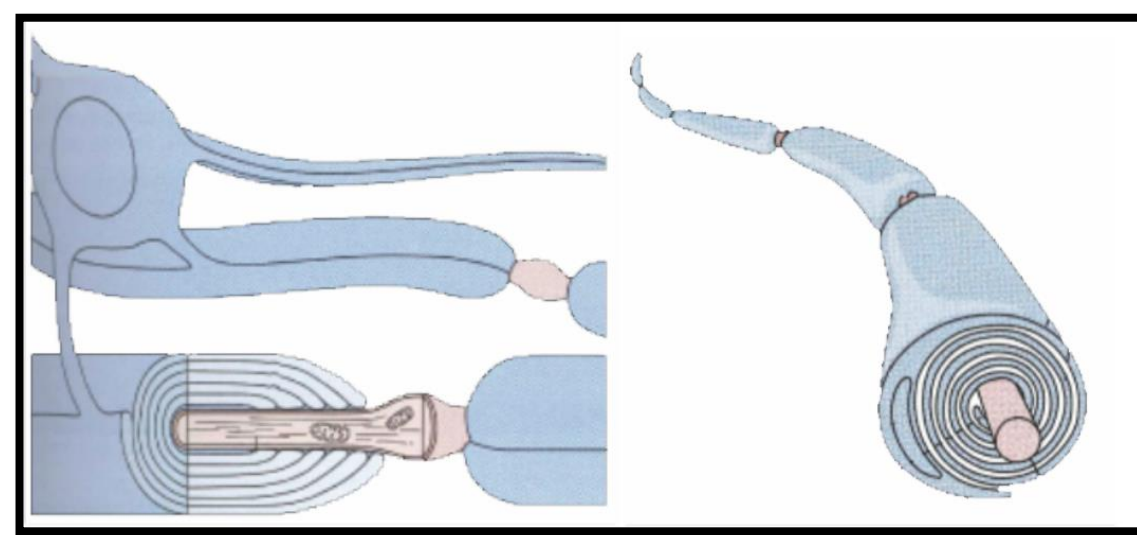
Testing a candidate muscle gene, *tcf15*, in a neural patterning mutant, *stl159*, in *Danio rerio*

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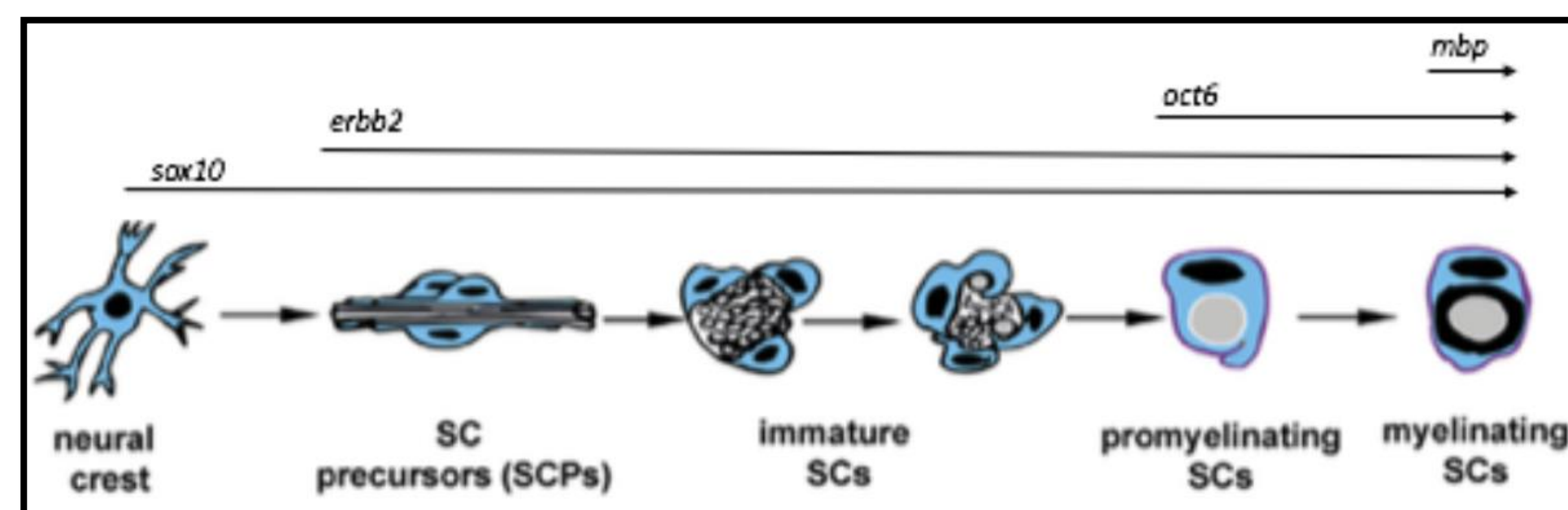
Zebrafish are the simplest vertebrate system for studying the development of myelinating glia

- Myelin is a sheath of lipid and protein that allows for effective propagation of action potentials in vertebrate nervous systems
- Myelin is produced in the CNS by Oligodendrocytes and in the PNS by Schwann Cells



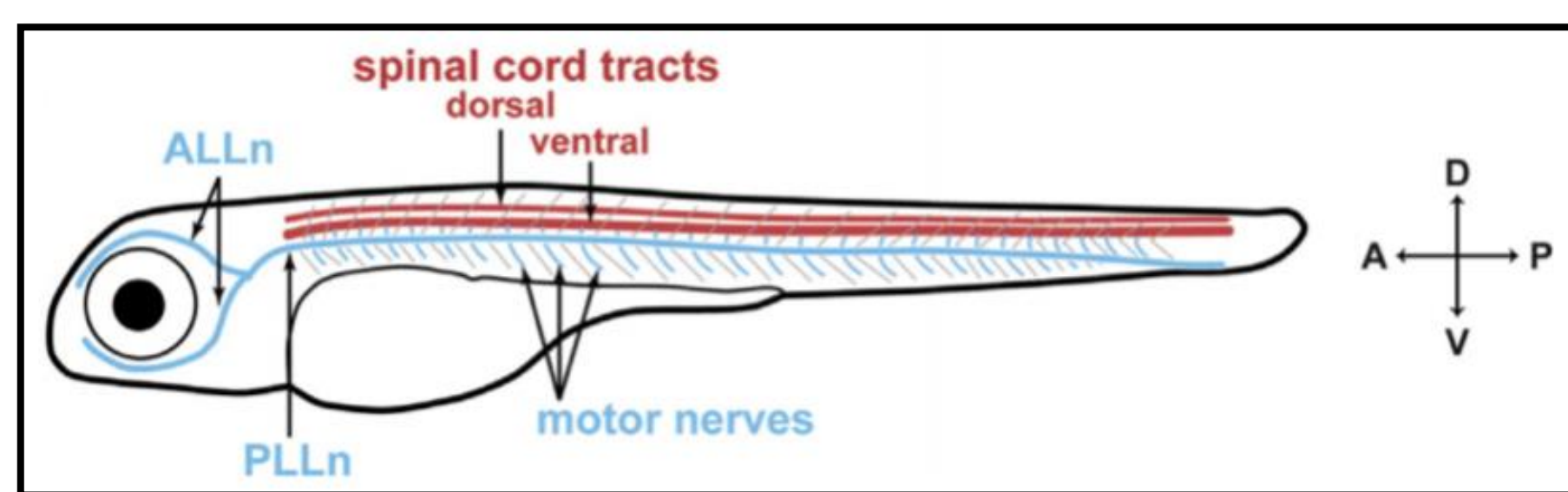
Kandel et al. 2006

- Schwann cell development is genetically tractable via specific developmental markers



D'Rozario et al. 2017

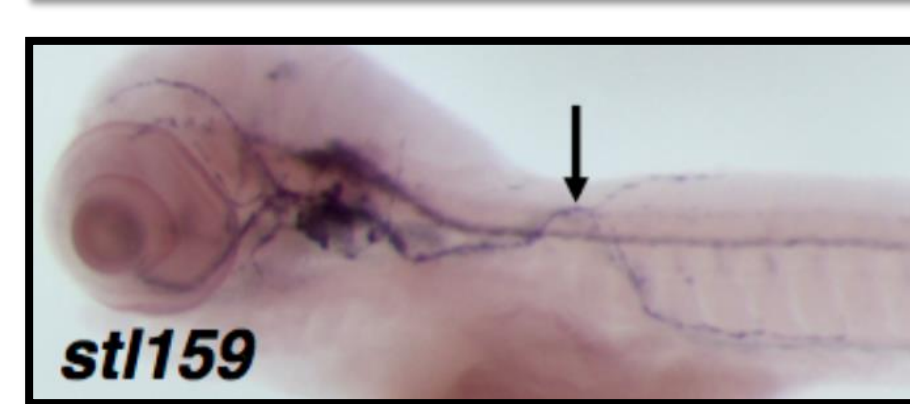
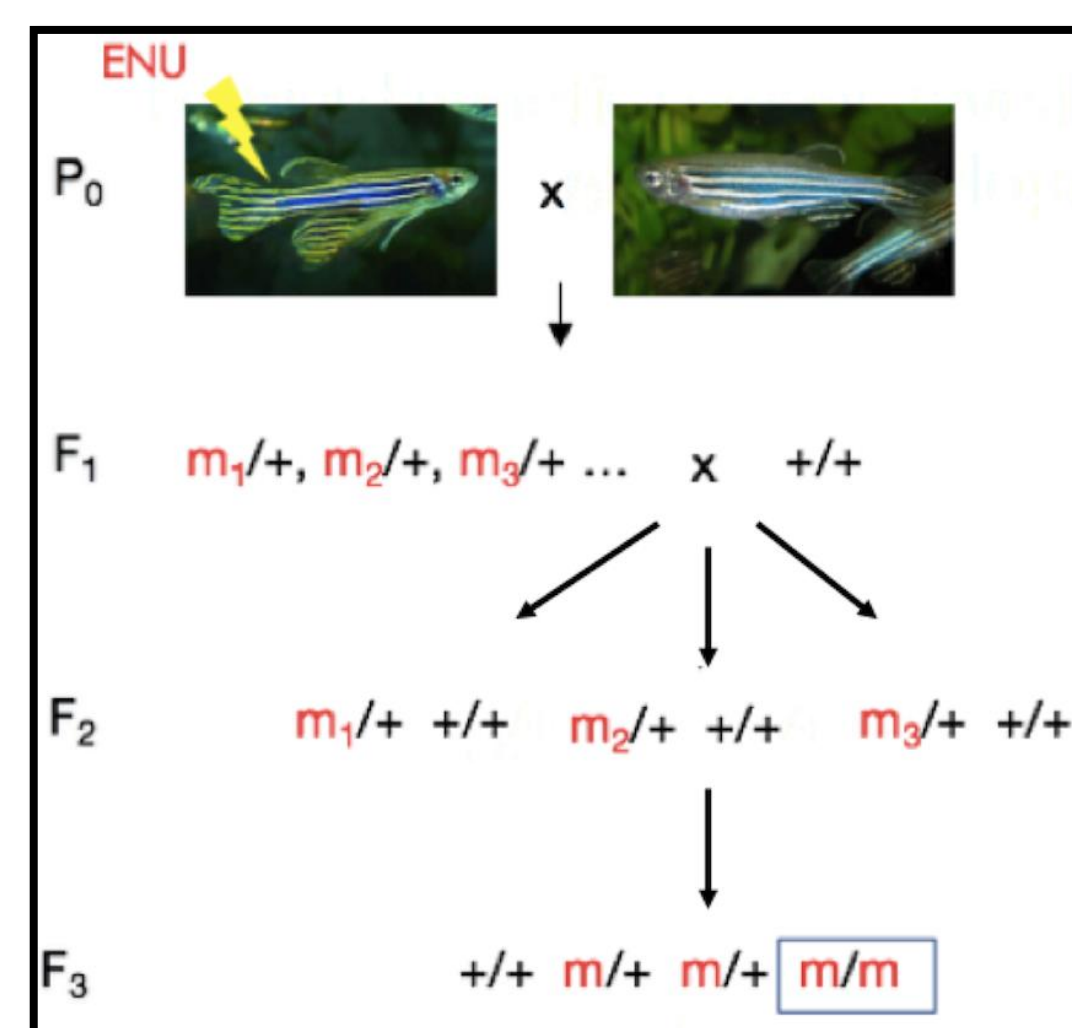
- Genome and mechanism of myelination is highly conserved in zebrafish



D'Rozario et al. 2017

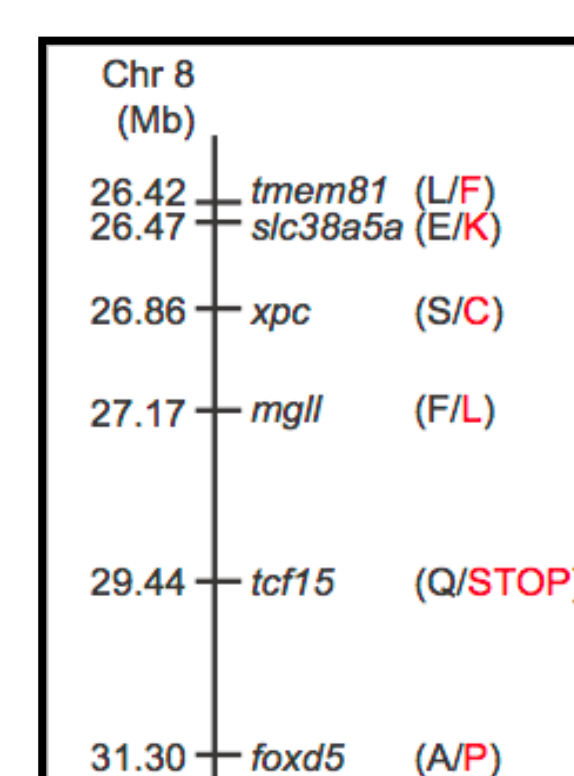
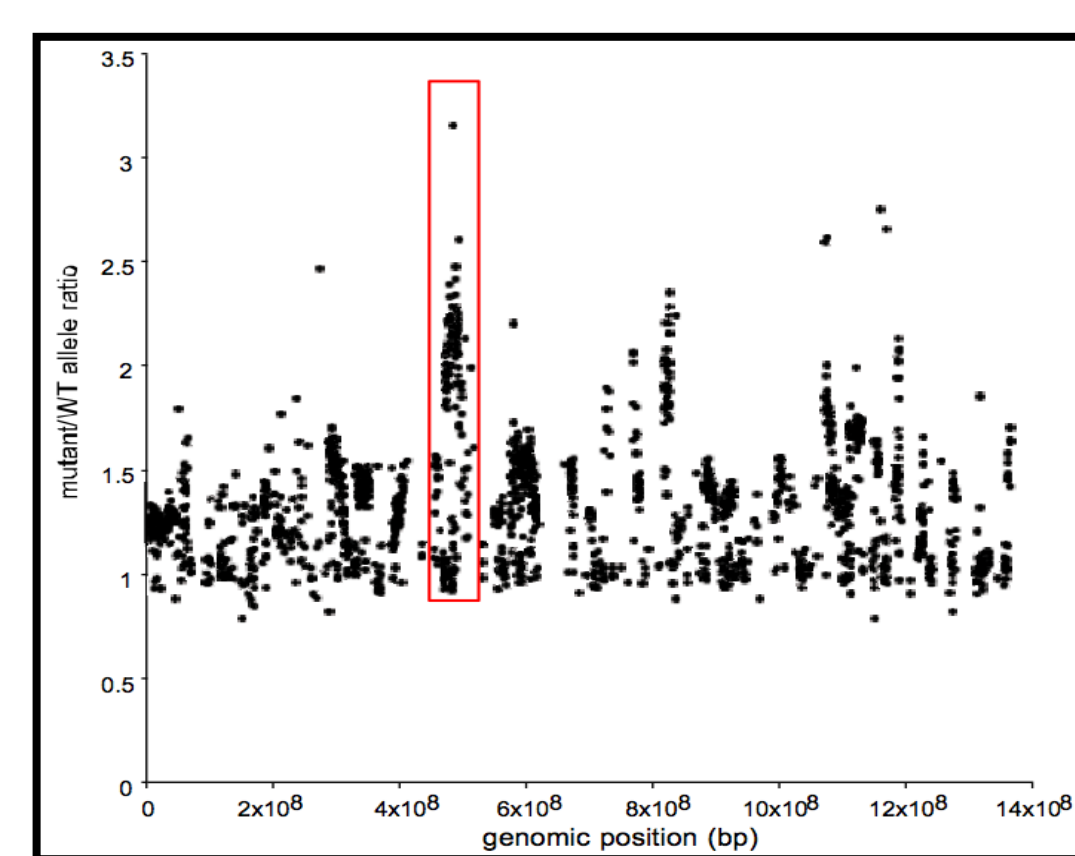
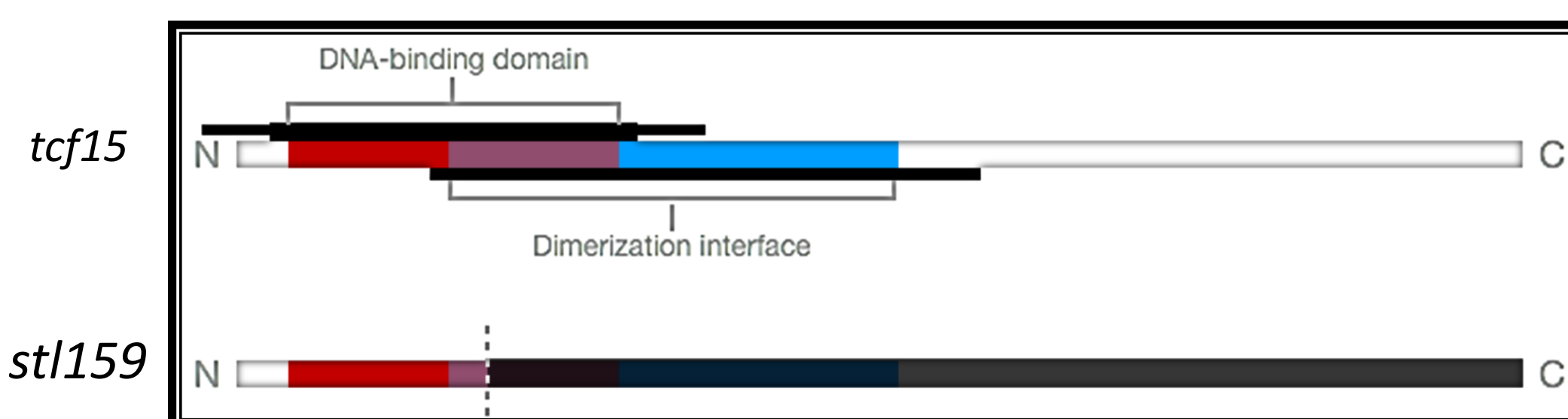
Forward Genetic Screen reveals myelin mutant, *stl159*, and Whole Genome Sequencing (WGS) links mutant to *tcf15* gene

- 30+ myelin mutants were revealed from a screen at Washington University, St. Louis



Petersen, 2016

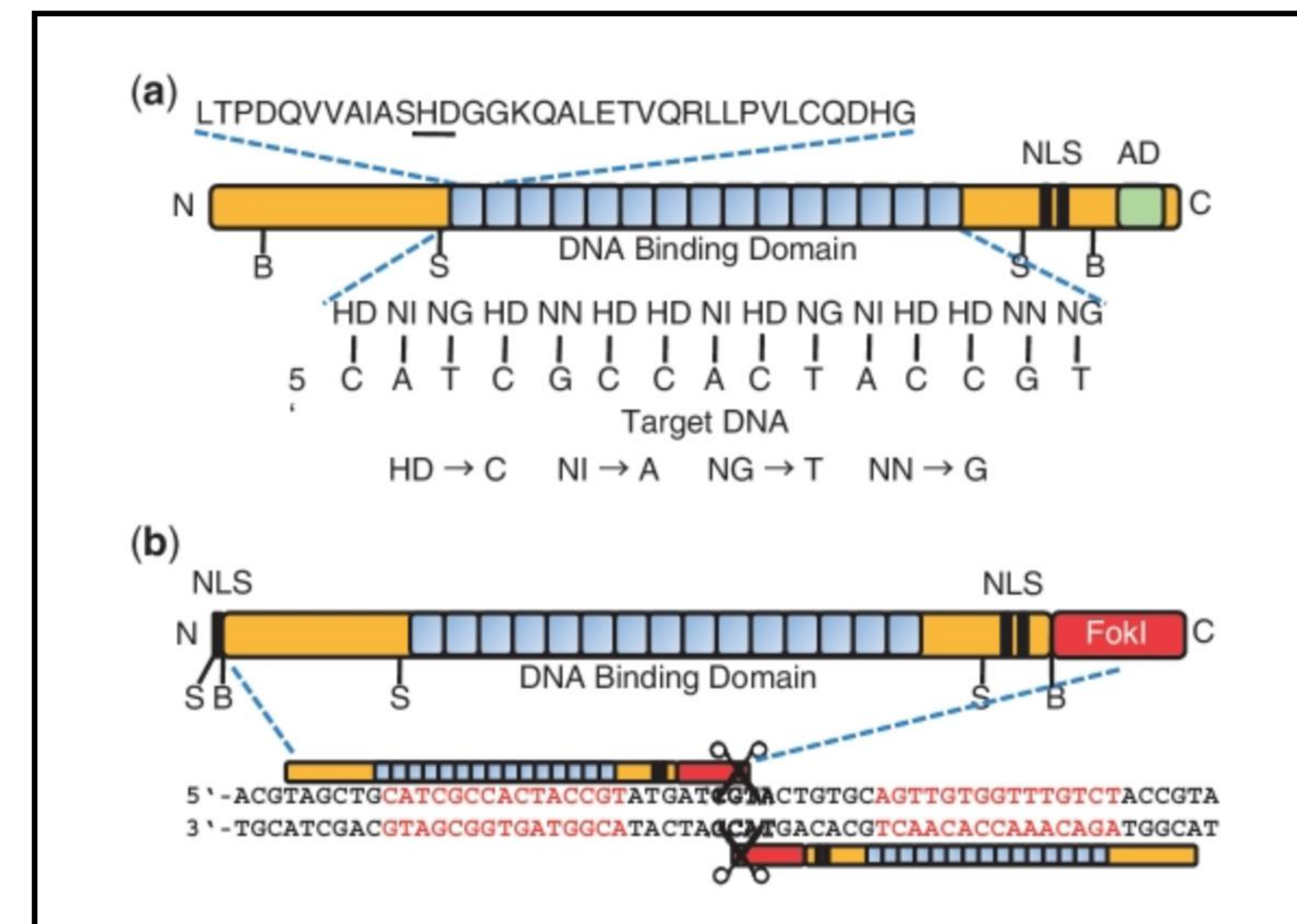
- Tcf15* is a good candidate gene for the *stl159* mutant because:
 - WGS revealed a genomic lesion that potentially falls within the *tcf15* gene region that codes for an early STOP codon
 - Tcf15* is present in most vertebrate organisms
 - Tcf15* encodes a transcription factor that is involved in the proper patterning of the mesoderm and in normal somite formation during the gastrulation phase of embryogenesis
 - In other models, mutated *tcf15* has shown defects in paraxial mesoderm development, somitogenesis and skeletal system morphogenesis, among other anatomic abnormalities.



Monk Lab, Wash U St. Louis

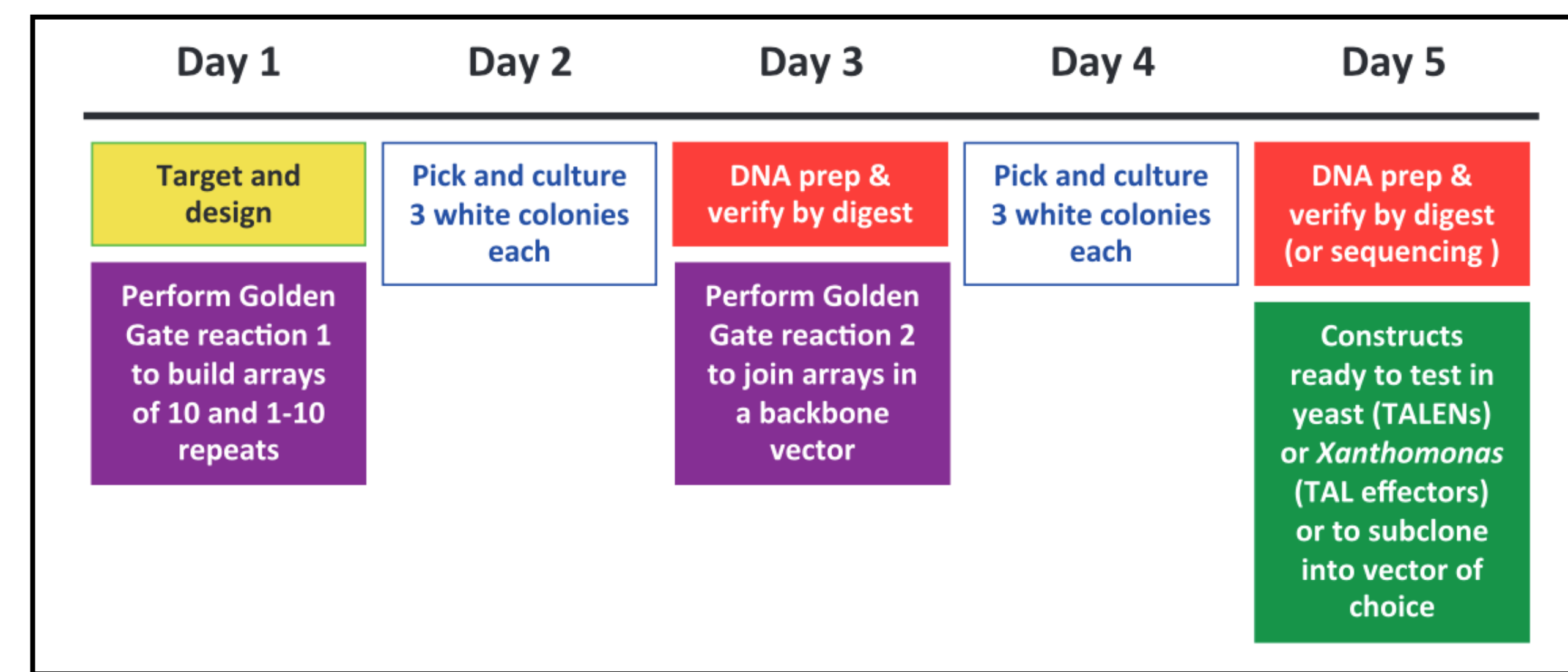
Loss-of-Function experiment utilizes Transcription-Activator Like Nucleases (TALENs) as method of gene modification to determine if *tcf15* is necessary for proper neural development

- Using TALENs to induce a mutation in the *tcf15* gene will reveal if the gene is necessary for proper neural development, and reveal whether the mutation is linked to the *stl159* phenotype
- TALENs consist of TALE proteins attached to a nuclease engineered to cut specific sequences of DNA
- TALENs are composed of Repeated Variable Di-residues (RVD) that are specific and bind to a single nucleotide.
- A typical TALEN will consist of 20 RVD's with a spacer region of ~12 nucleotides. Two TALENs will specifically bind flanking the gene of interest, and cause a double stranded break (DSB) by the FokI nuclease.
- TALENs are cloned into pFUS vectors which are zebrafish expression vectors



Cermak et al. 2011

- Golden Gate TALEN Assembly Protocol shows constructing final TALENs require two separate Golden Gate reactions to create 4 intermediate TALENs with 10 RVD repeats prior to assembling the final two constructs both consisting of 20 RVD repeats.



Cermak et al. 2011

- Day 1: Target and design

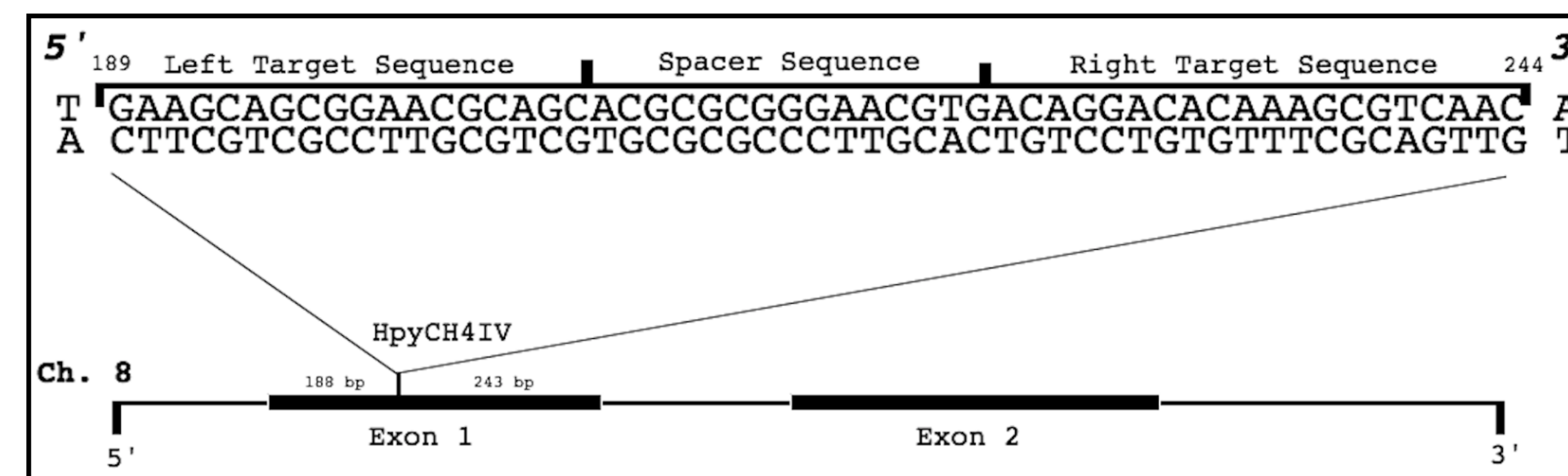


Figure 1. TALEN construct designed through TALEN Targeter Program on Cornell University's website.

Genotyping wild-type reveals proper design of primers which allows future genotyping of TALEN induced mutated zebrafish

- Primers were selected via ApE program to produce a product of 364 bp length
- Digest with HpyCH4IV cuts product into 130 and 234 bp bands
- TALEN induced mutated zebrafish should not be able to cut and digest with these specific primers and enzyme

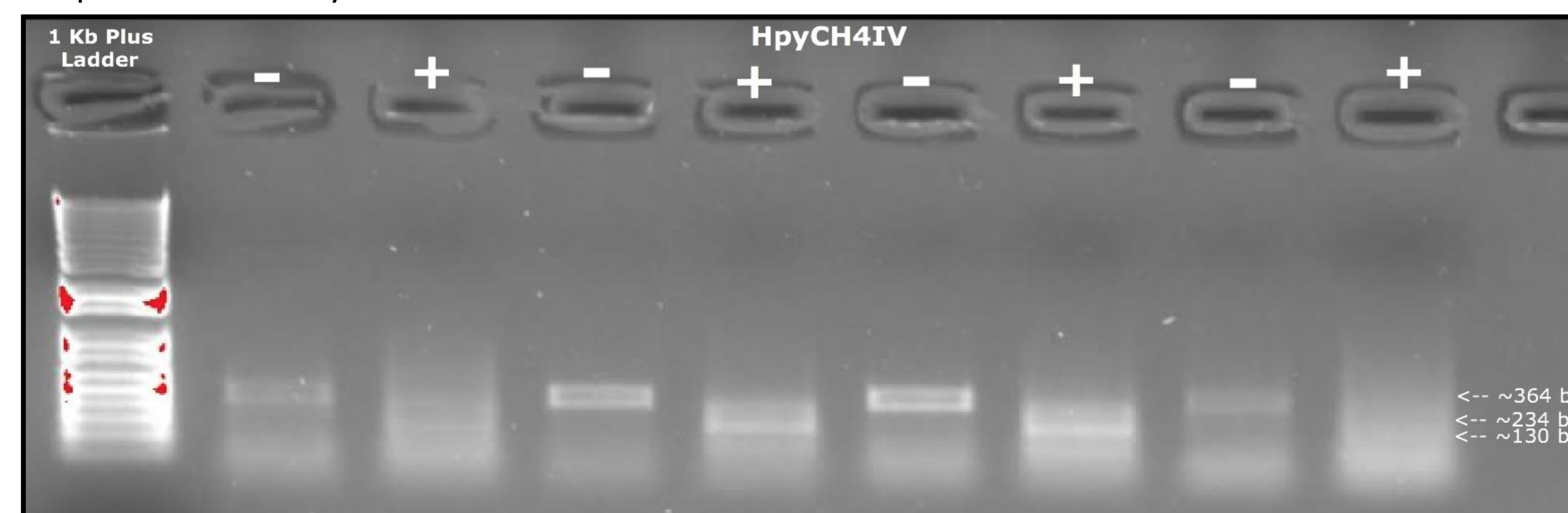
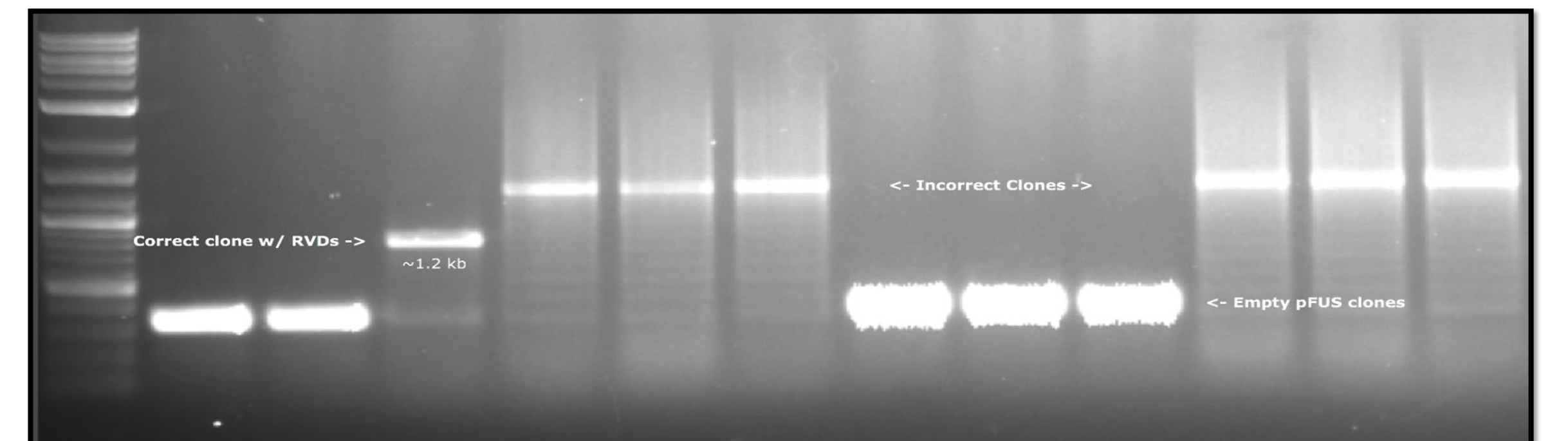


Figure 2. Primers for genotyping wild-type were designed through ApE, and were digested with HpyCH4IV restriction enzyme (RE). Gel indicated primers and digest worked.

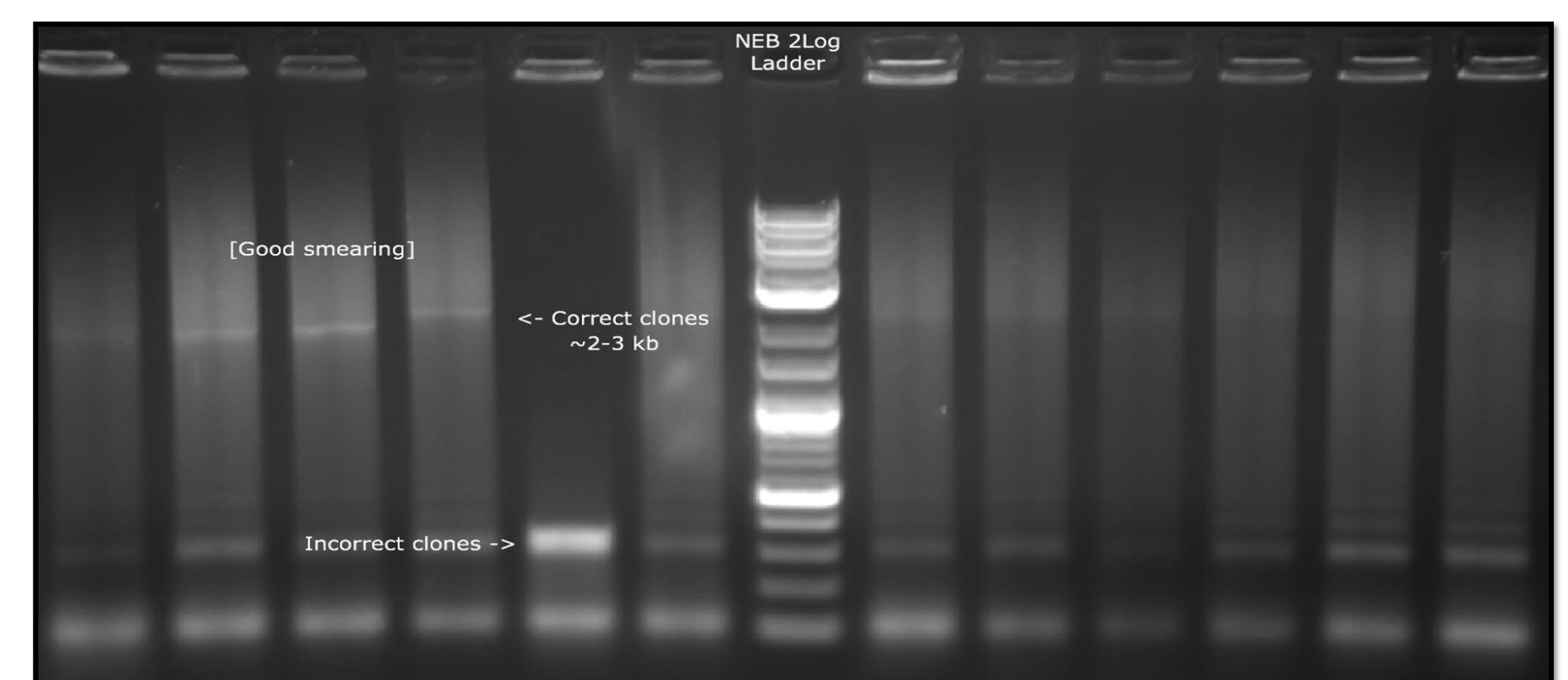
Expected Results of constructing TALENs from Golden Gate Assembly Protocol

- The first Golden Gate reaction yielding correct clones is shown in lane 4. This clone consists of the pFUS vector with 10 RVD repeats.



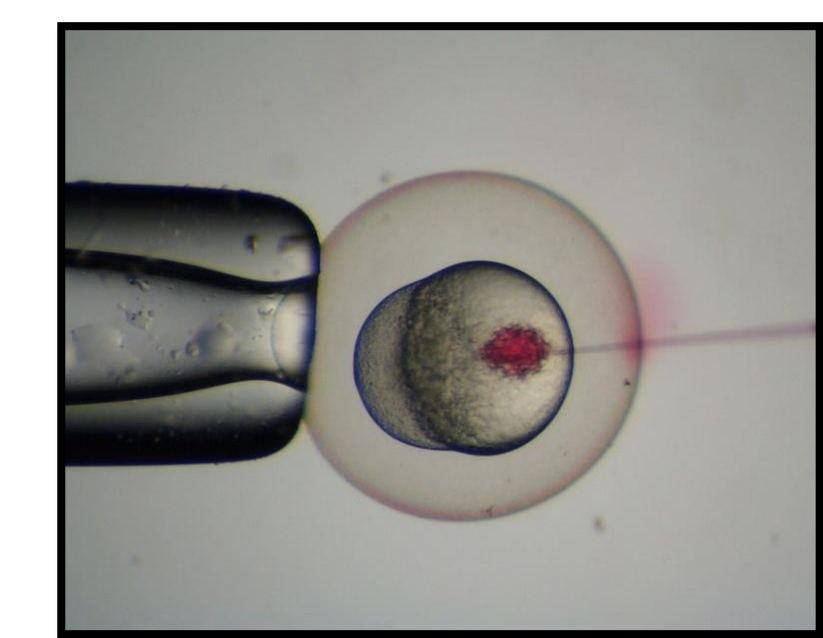
Golden Gate Assembly Protocol

- The second Golden Gate reaction with the correct clones consisting of 20 RVD repeats are shown in lanes 2-4.



Golden Gate Assembly Protocol

- Upon successful assembly of the final TALENs, linearization followed by transcription, will form a mRNA product that will be microinjected in wild-type *Danio rerio* embryos.



David Mawdsley, Heath Lab

Future Direction: Rescue experiment can reveal whether *tcf15* is sufficient enough to rescue phenotype

- Can human *tcf15* rescue the phenotype?
 - Tcf15* is highly conserved across species
 - A rescue experiment of cloning human *tcf15* into a pCS2+ zebrafish expression vector that is transcribed into mRNA for microinjections into *stl159* embryos can reveal whether *tcf15* is sufficient enough to rescue the phenotype.



Petersen

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