

Targeted Deletion of Zebrafish *IncRNAis18* with TALENs

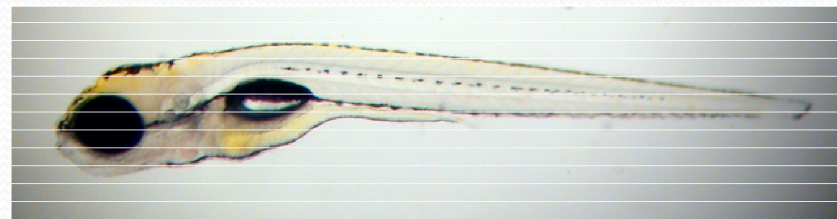
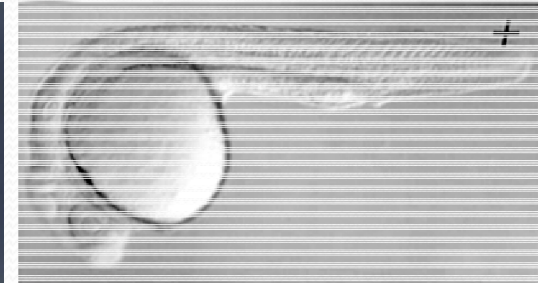
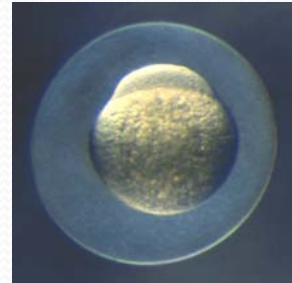
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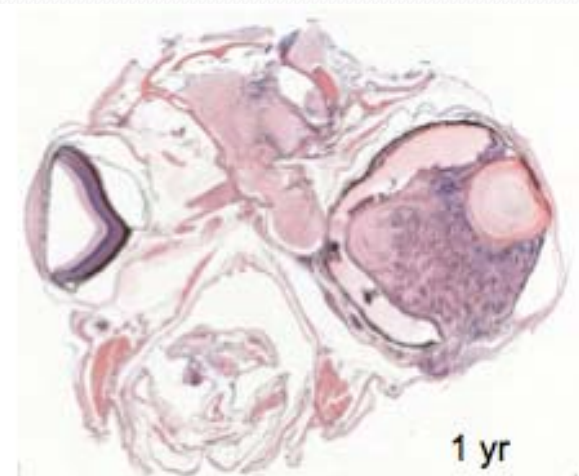
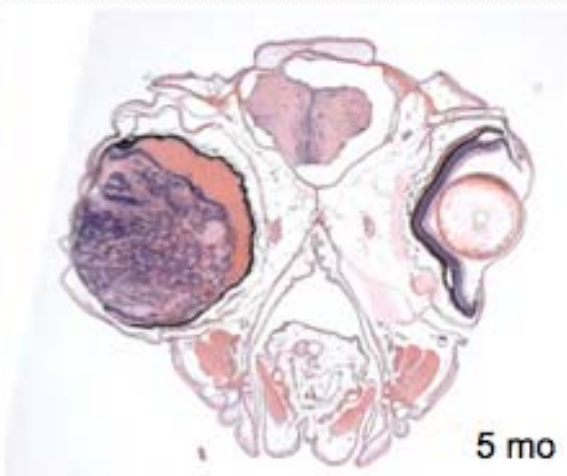
Why Zebrafish?

- Transparent embryos for *in vivo* microscopic studies of development
- Genome sequenced and annotated
- Classical genetics/mutants
- Molecular genetic tools for functional genomic studies -
 - Transposons
 - TALENs
 - CRISPRs
 - Transgenic reporters
- Inexpensive and Fecund!



Glioma tumors in zebrafish line is18

- Zebrafish line is18 contains a large transgene array integrated in a novel lnc-RNA gene.
- Phenotype
 - Heterozygotes develop glioma tumors as adults
 - Homozygotes are lethal at 5 dpf



Why is this important?

- **Glioma:**

- 30% of brain cancers
- 80% of malignant brain cancers in adults

- **Treatments:**

- Surgery
- Radiation
- Chemotherapy
 - temozolomide

GBM

- **Outcome and Survival:**

- 12-14 months post diagnosis
- Treatment has no effect on overall survival

WHO grading of Glioma

Glioma/Astrocytoma

Circumscribed

- **Grade I** - Pilocytic astrocytoma (**benign**)

Diffusely infiltrating

- **Grade II** - Diffuse astrocytoma
- **Grade III** - Anaplastic astrocytoma
- **Grade IV** - Glioblastoma (GBM)

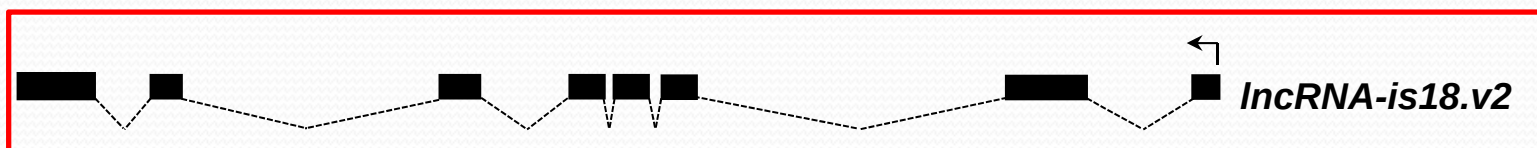
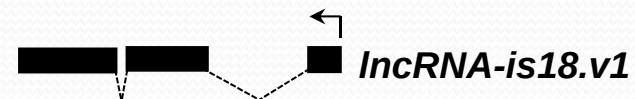
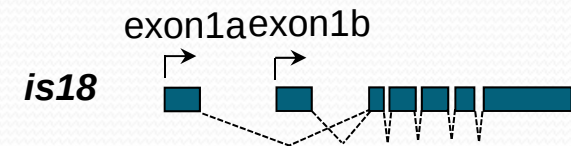
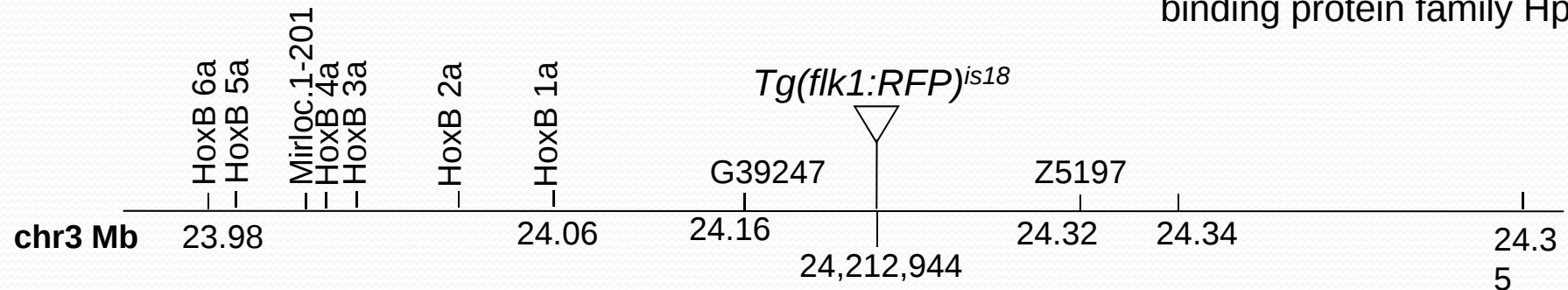
} **malignant**

Project Goal

- The goal is to isolate a 2nd mutant allele of *lnc-RNAis18*.
 - A transgene that lies within *lnc-RNAis18* is linked to the tumor phenotype.

← ----- *HoxB1-13a* ----- →

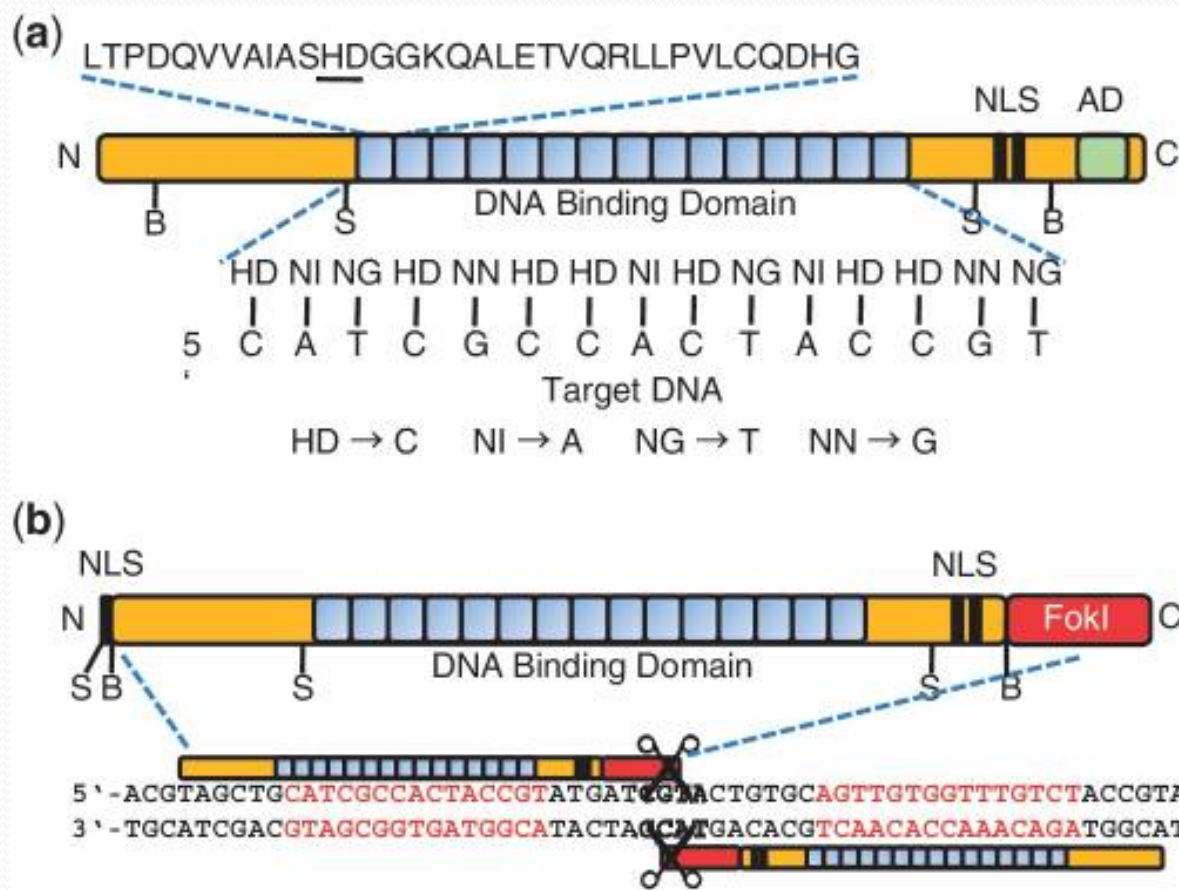
is18, heterochromatin binding protein family Hp1



Methods

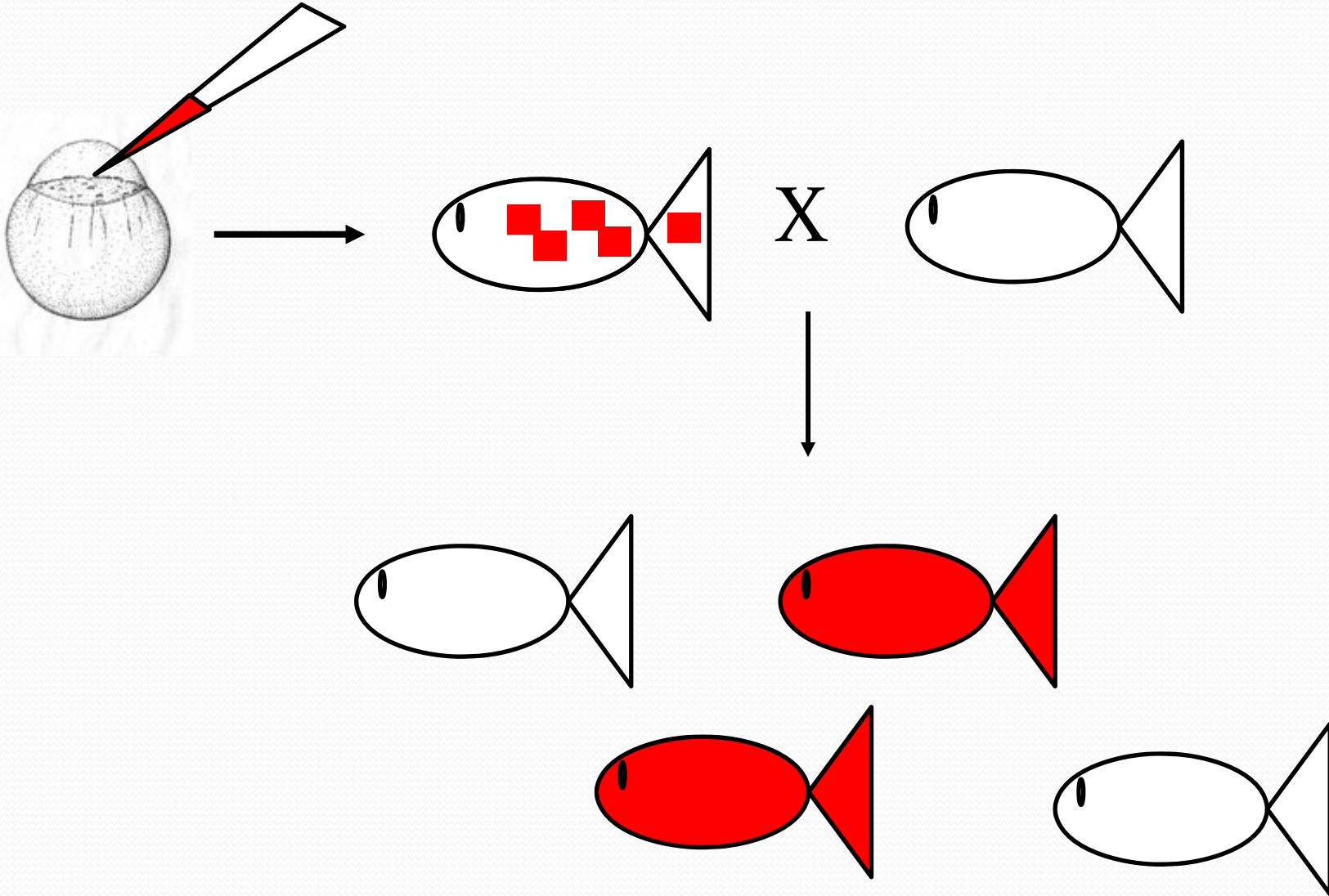
- TALENs
 - Golden Gate method
 - Creation of two TALEN pairs
 - Exon 2 and exon 5 for the lnc-RNA is18
- mRNA synthesis of TALEN pairs
- Injection of each TALEN pair individually
- Injection of both TALEN pairs
 - Injection into WIK x WIK
- Genomic DNA extraction
 - Verification of deletion through PCR and gel electrophoresis
 - Sequence verification
- Screen for germline transmission
 - Raise F₁ generation
 - Screen F₁ generation for mutant zebrafish
- Use F₁ mutants to create homozygous mutants
 - Watch for phenotype
- Cross F₁ to is18line
 - Watch for phenotype

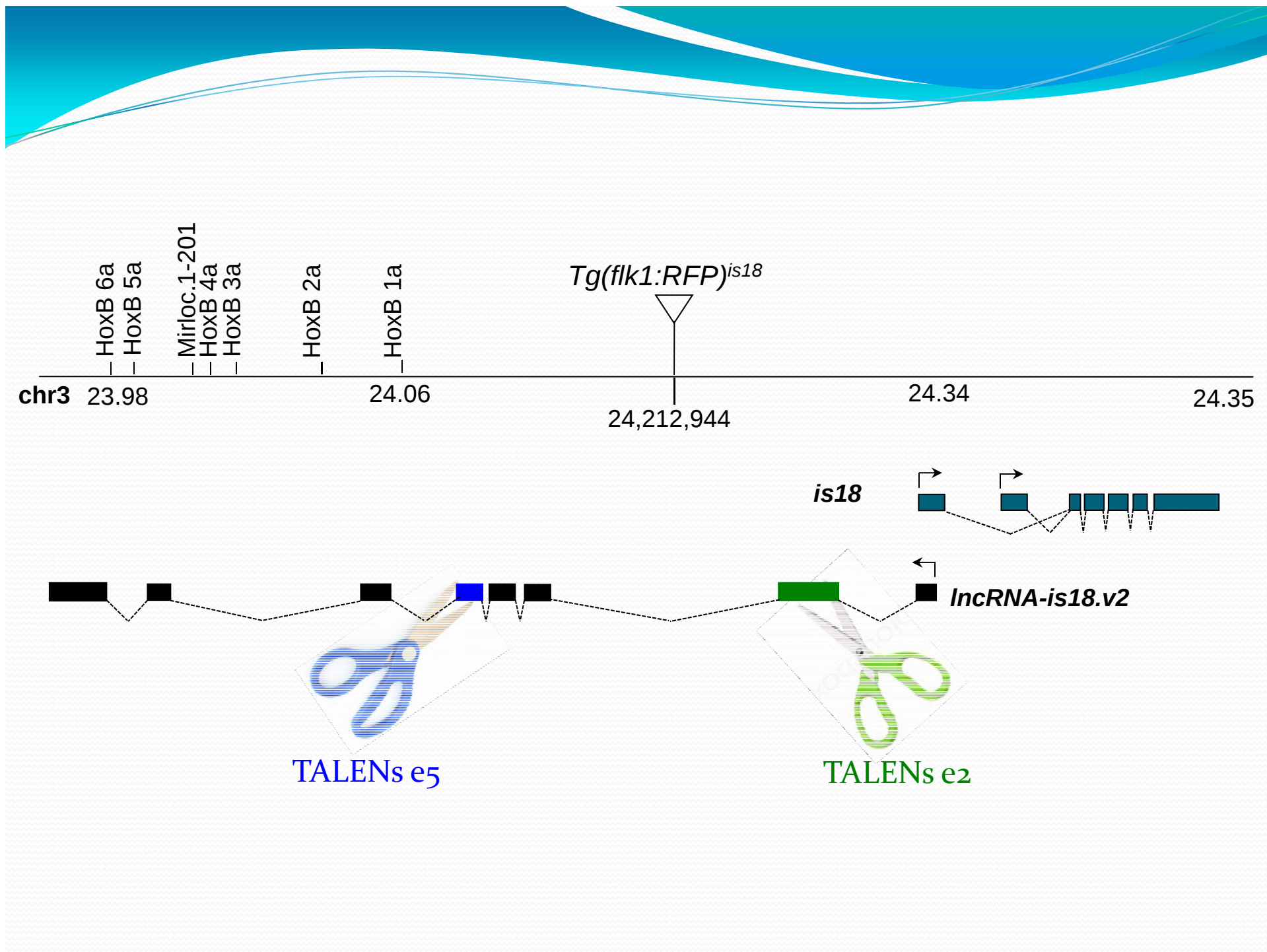
Making target specific double stranded breaks with TALENs



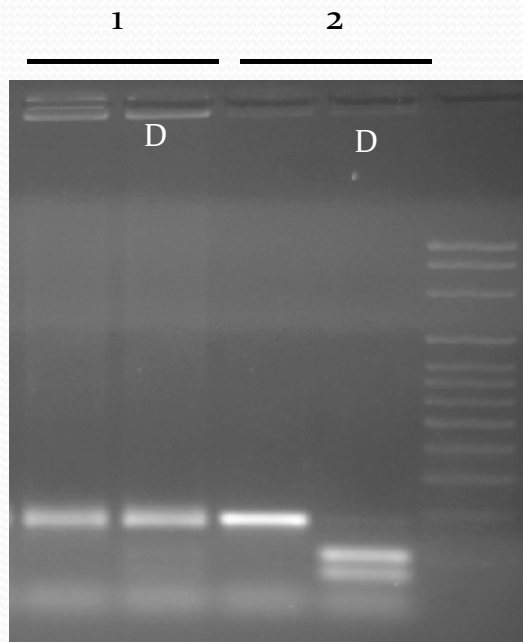
- TALENs consist of a fusion of a Transcription Activator-Like Effector with a Fok I Nuclease
- They contain a repeat variable region which can be edited to target specific areas of DNA.
 - HD binds to C
 - NG binds to T
 - NI binds to A
 - NH (not shown) binds to G

Injection of TALENs

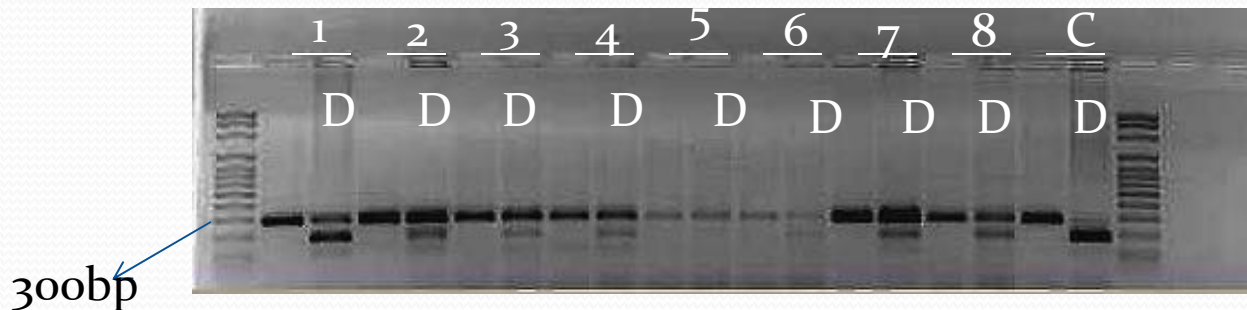




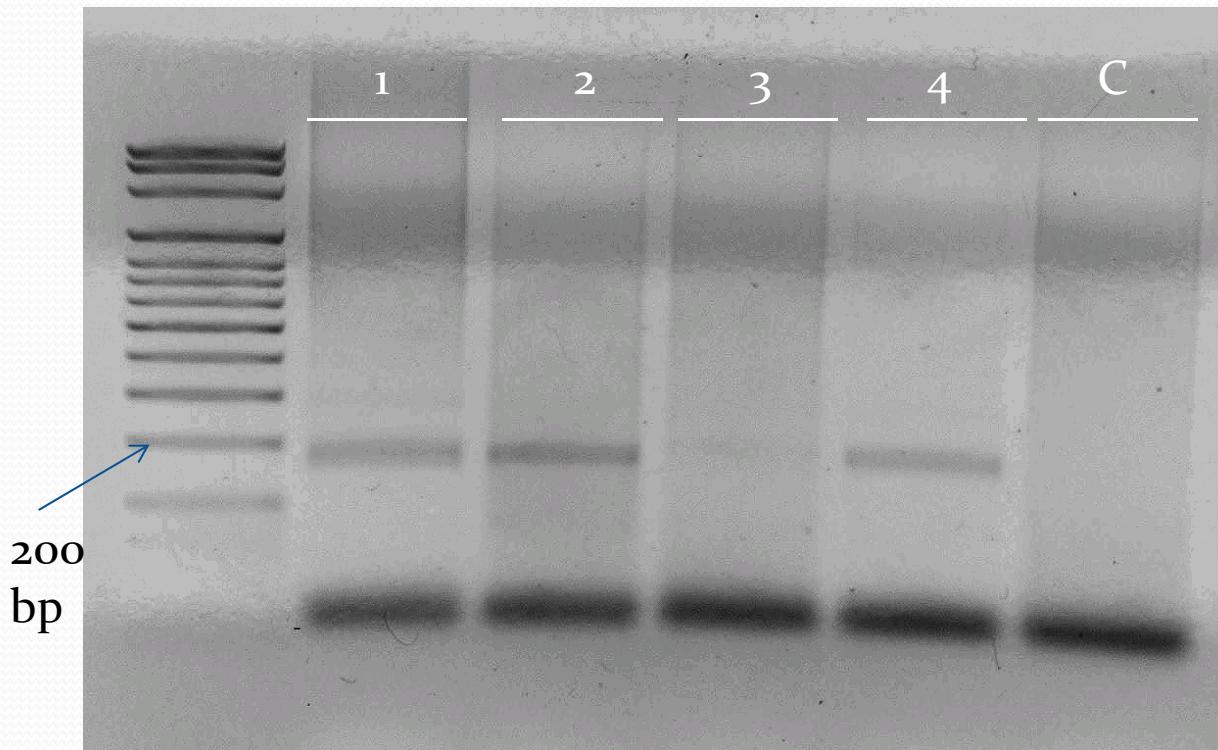
Targeted mutation of *Inc-RNAis18* Exon 2 and 5 with TALENs



- Exon 2 TALEN test
 - Injected 25pg of the TALEN pair and pooled the embryos and digested with enzyme NsiI for determination of mutation.
 - D = digested
 - 1. 25 pg injected- pooled 5 larvae
 - 2. Uninjected(10/25 pg)- pooled 4 larvae
- Exon 5 TALEN test
 - TALEN E5 40pg injection
 - Size expected for TALEN function approximately 287 bp
 - C is for control
 - D is for digested
 - TALENs mutated the targeted site in all embryos

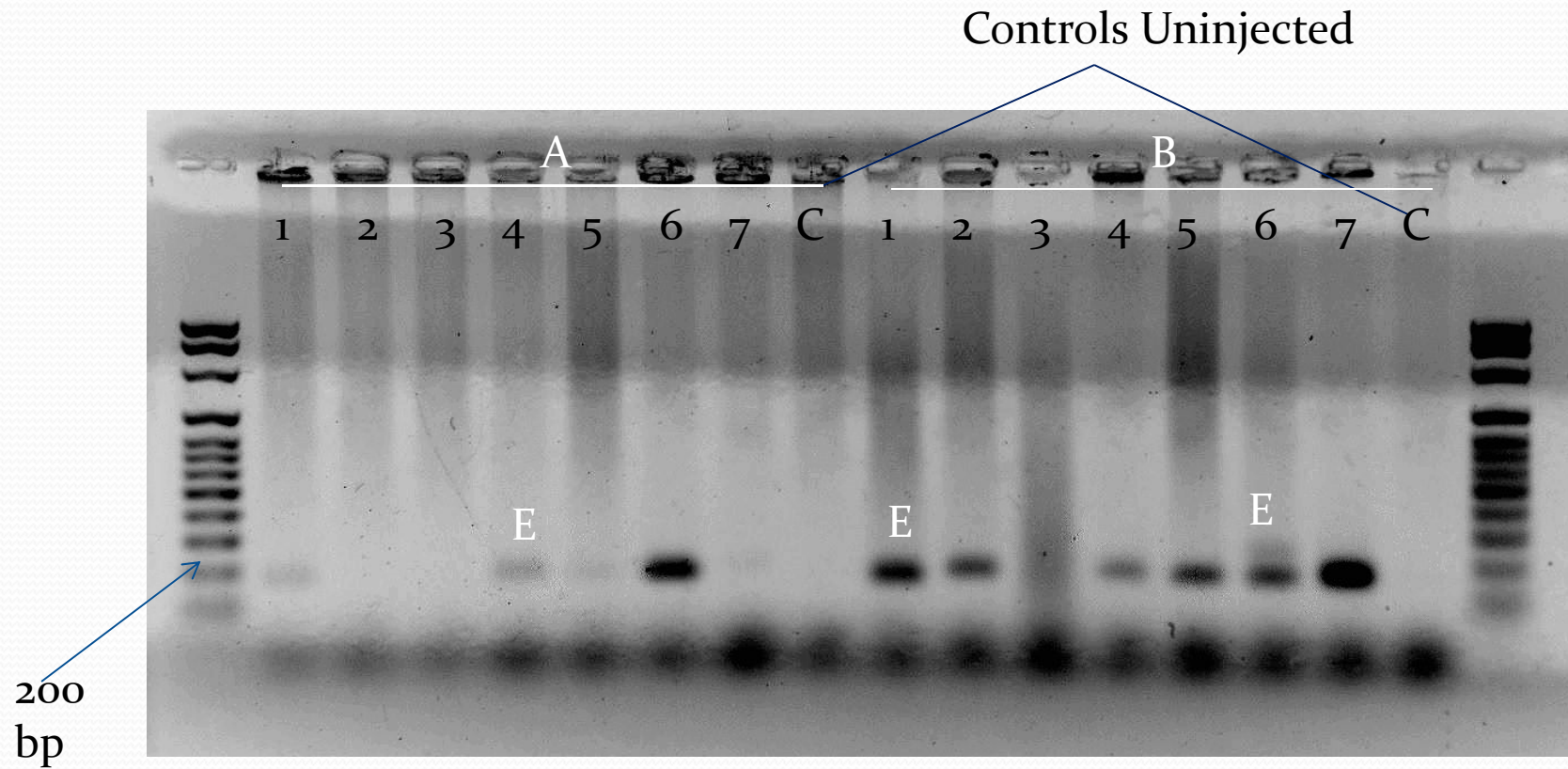


PCR detection of a deletion allele after co-injection of *Inc-RNAis18* Exon 2 and Exon 5 TALENs



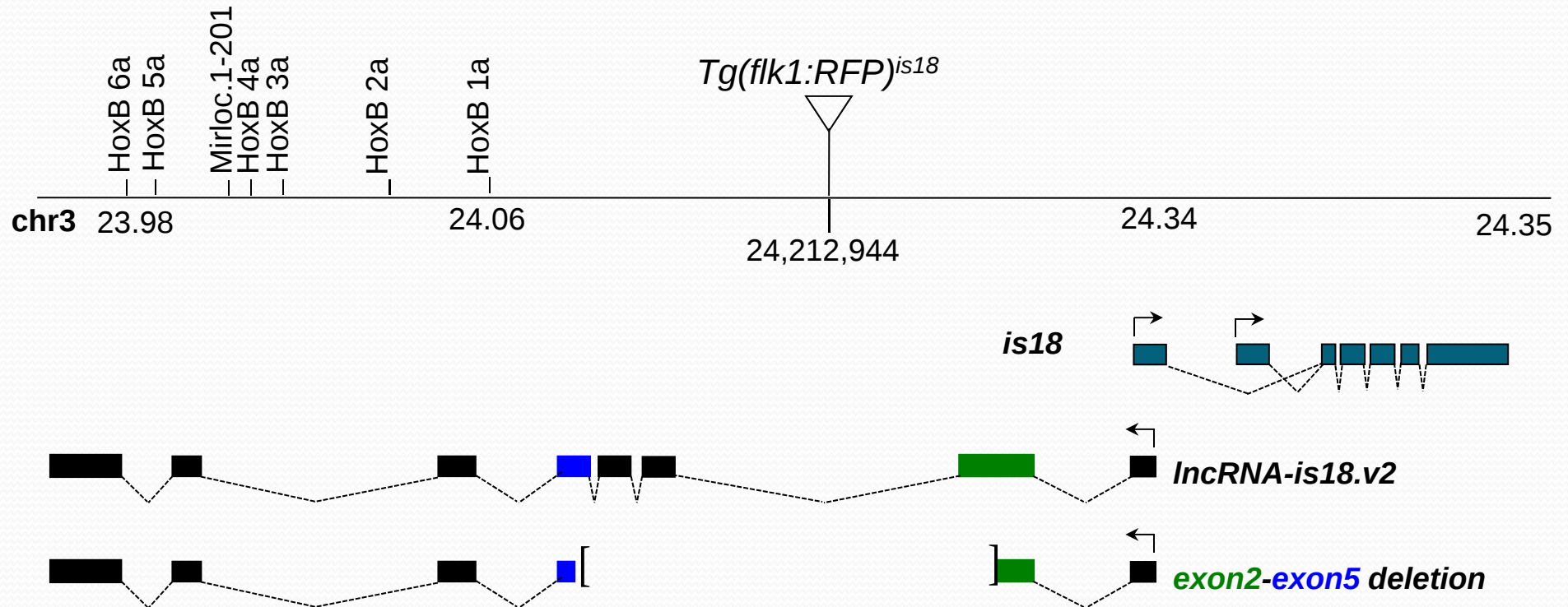
- Expected band size: 174 bp.
- C is for uninjected control
- Band indicates deletion of 147 kb in *Inc-RNAis18* gene.

30 pg injection



- E = excised bands, cloned and sequenced

Isolation of a *lncRNA-is18* deletion allele by TALEN-mediated mutagenesis



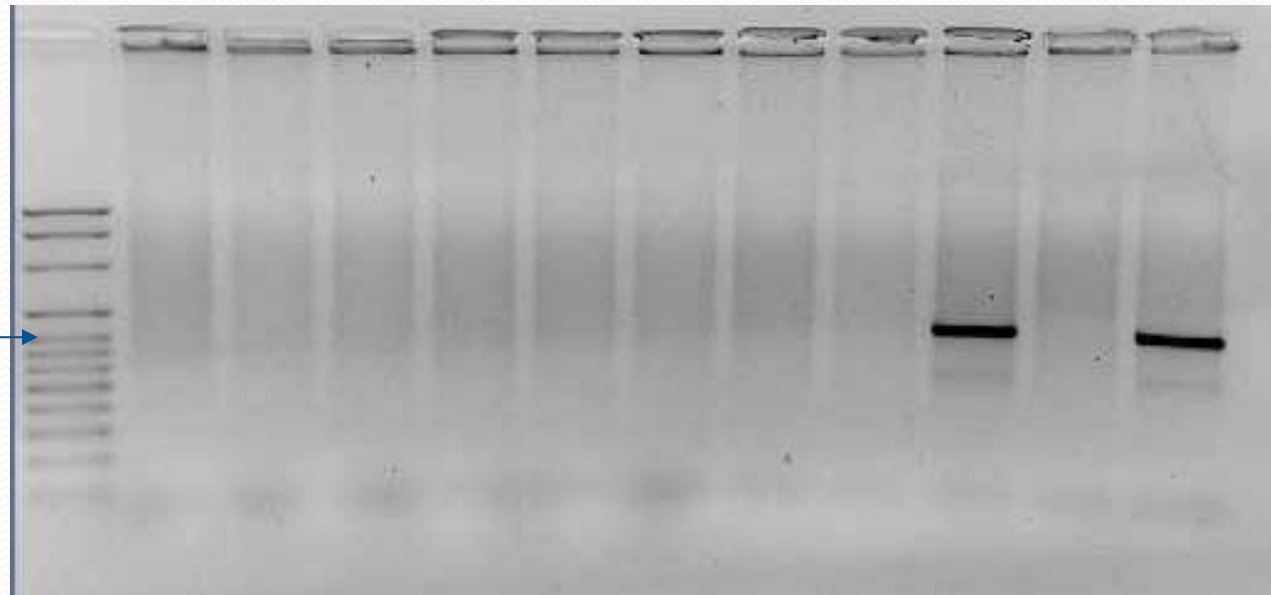
1A: ...aatctcacCTCTCAACGGAGTGCTGGATCAACACCATATctgaaa...

4B: ...aatctcacCTCTGCCAGGATTCTGTCAACGGAGTGCTGGATCAACACCATATctgaaa...

M₁BT:...aatctcacCTCTGCCAGGATAACATAACCCCTTTTTTTTTTTTAAAGTTTCAGGTGACTTTATTAAAGAAATATTGCTGATCAAATCACCCTAATACCTCTCATCAACGGAGTGCTGGATCAACACCATATctgaaa...

Passing of deletion allele through the germline

800 bp



Sequence:

#9:

...CAAACACATGCACAATTAATCTCACCTCTGCCAGGAGATGATGAGNCNNNATGAGAT
GGAGTGCTGGATCAACACCATATCTGAAATAAAGTTGCAAAATTAAAATGCTG...

#11:

...ACATGCACNATTAATCTCNCCTCTGCCAGGAGATGATNNNNC>NNANGANANGGAGTG
CTGGATCAACACCATATCTGAAATAAAGTTGCAAAATTAAAATGCTGACTTCATATGG
AAGGTAACTCAAACACTGAACGTCTTTCC...



Current and future work

- Current:
 - Genotyping the F_1 deletion positive founders
 - Watching the F_2 incross for a phenotype
 - Observing the is18deletion line crossed with the is18 line for a phenotype
- Future:
 - Understand how the lncRNAis18 plays a role in tumor formation
 - Discover other mechanisms that have roles in tumor progression from glioma to glioblastoma



Summary

- One can use TALENs to make a double stranded cut at any designated location in the zebrafish genome.
- By the co-injection of two TALEN pairs we have shown that it is possible to delete 147 kb of genomic DNA.
- Displays the usefulness of the technology used for creating deletion alleles of non-coding RNAs for genetic analysis.
- TALENs allow us to create mutant alleles to discover and observe new phenotypes.



Acknowledgements

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