

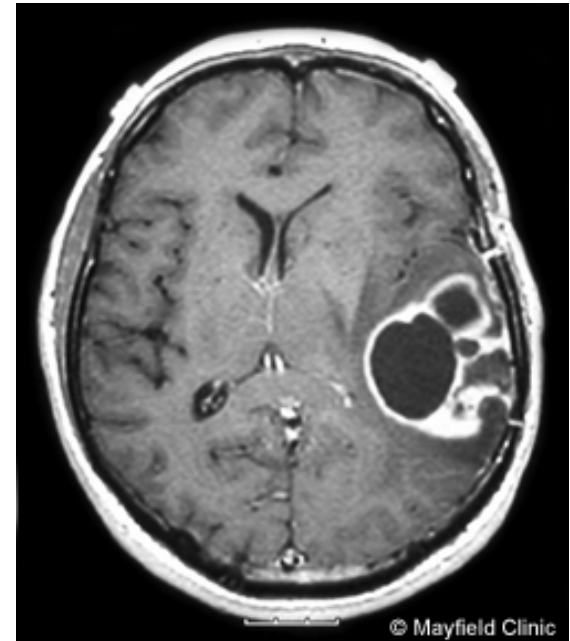
Twisting Our Understanding of Glioblastomas

Jeanette Schwensen

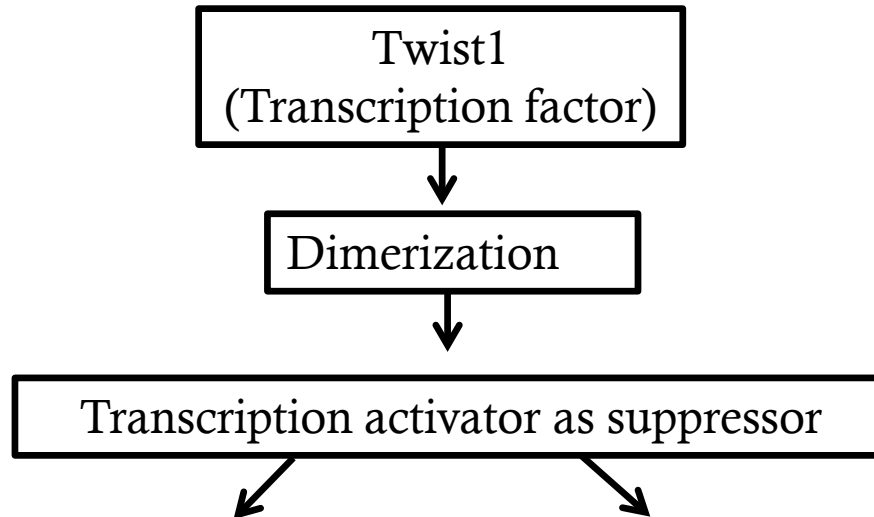
UW Medicine South Lake Union
Institute for Stem Cells and Regenerative Medicine
Dr. Robert Rostomily Lab

Gliomas

- Glioma – tumor from glia brain tissue
- Glioblastomas (GBM) - Grade IV astrocytoma
- Highly malignant
- Deadly
- Glioma Stem Cells
 - Responsible for invasive potential and recurrence of GBMs



What is Twist1?



Development:

- Regulates mesodermal development

Tumor:

- Regulator of Epithelial Mesenchymal Transition (EMT)
- Promotes tumor cell metastasis

Experimental Plan

Literature: Decrease in Twist1 decreases invasive properties of malignant cells

Rostomily Lab: Twist1 also affects the ability to form a tumor

Hypothesis:

If Twist1 is deleted from human glioma cells then it will decrease tumorigenicity

How to Determine Twist1 Function in Glioma

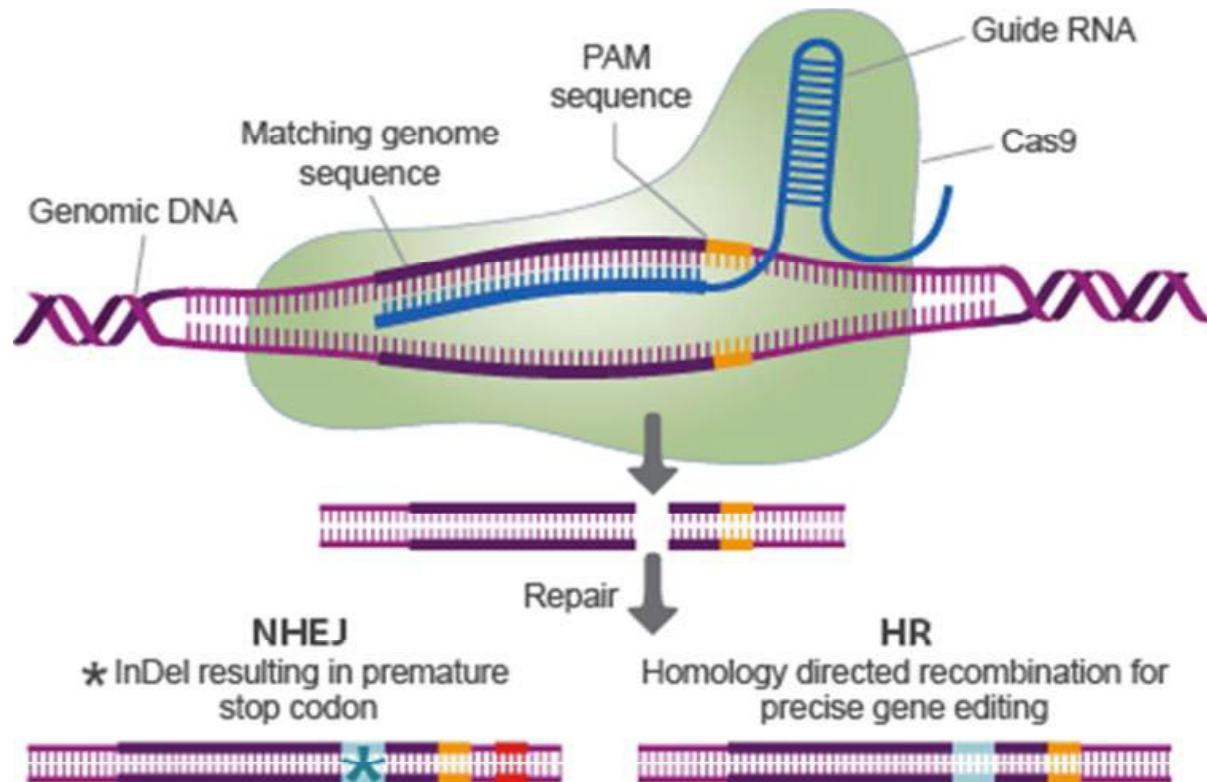
Loss of Function:

- Cre/Lox Technology
- siRNA
- **CRISPR/Cas9**

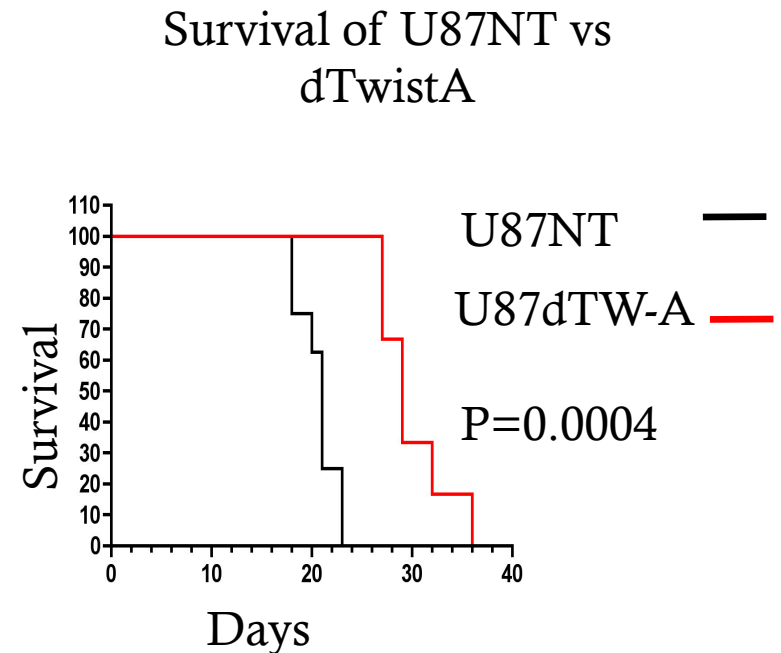
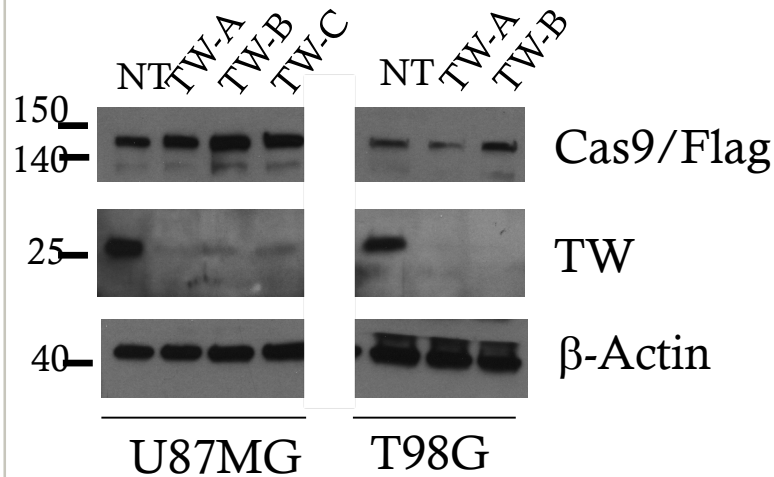
Gain of Function

- Gene overexpression

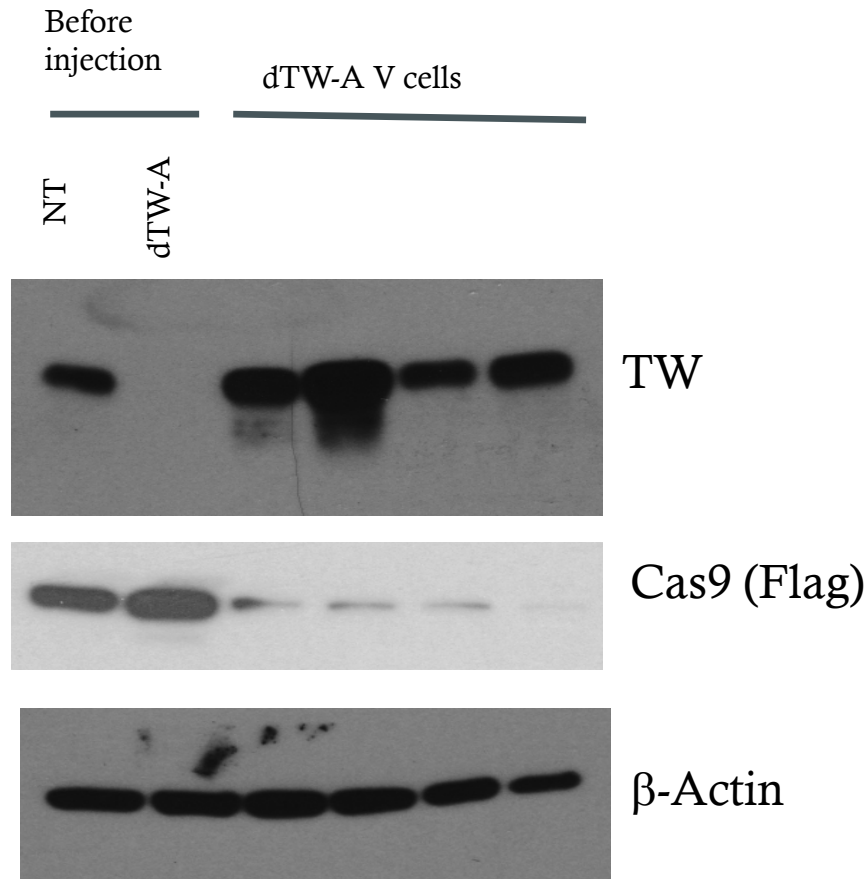
Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)



Twist deletion may reduce tumor malignancy

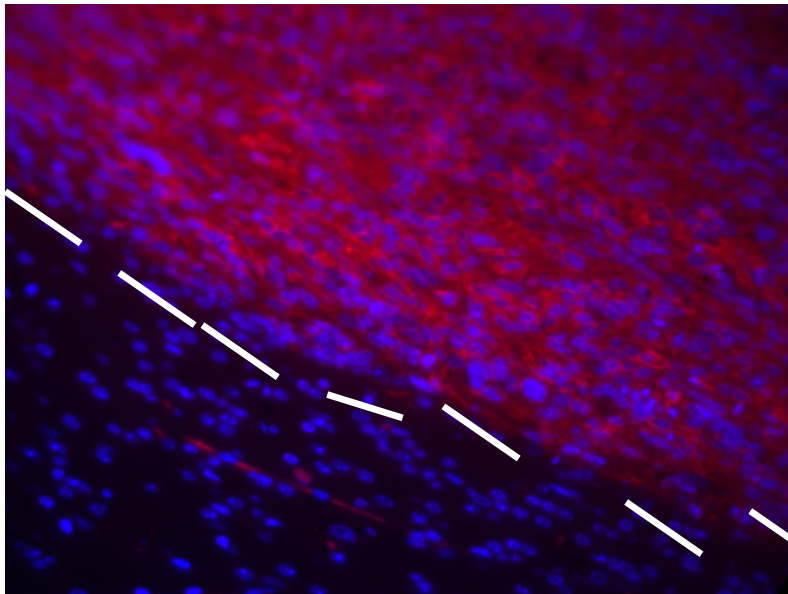


Twist+ uninfected cells are selected for in mouse tumor formation



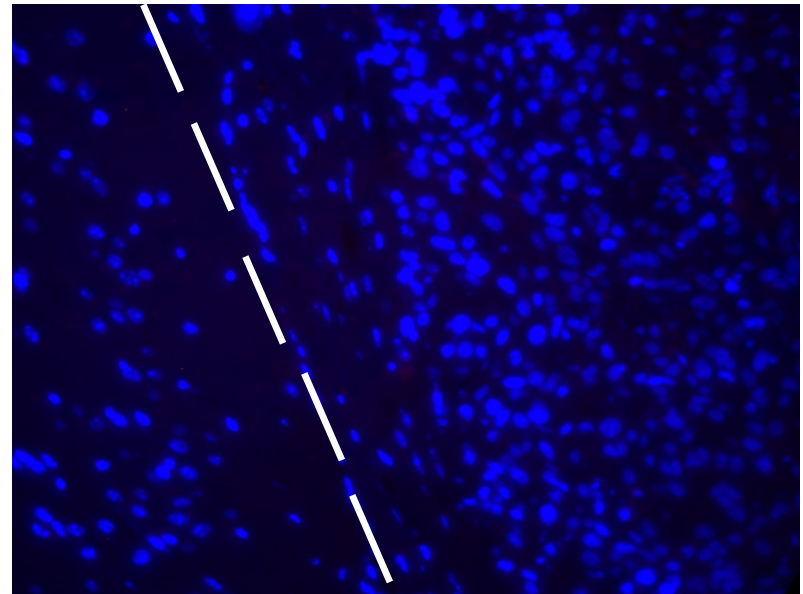
Detection of Cas9 Expression in U87MG Derived Tumors Growing in Mouse Brain

Control
U87NTF_{lag}



Red = positive for Cas9

U87dTwistA



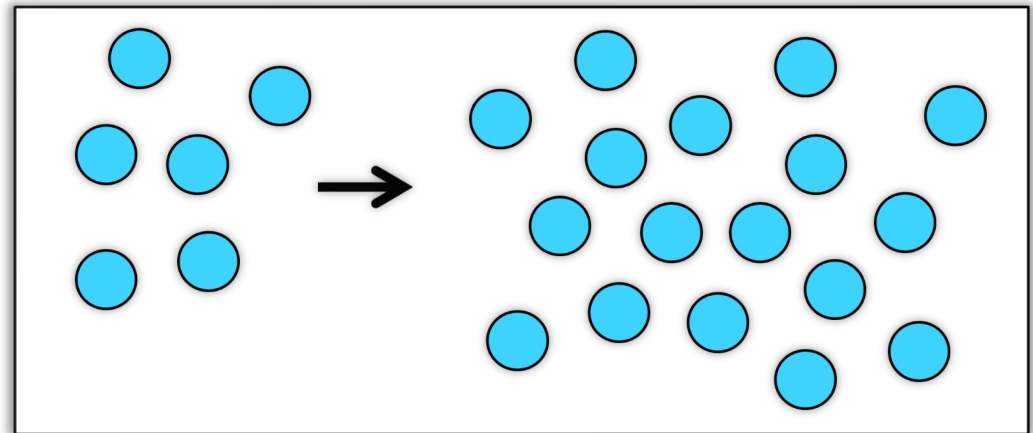
No expression of Cas9

What happened?

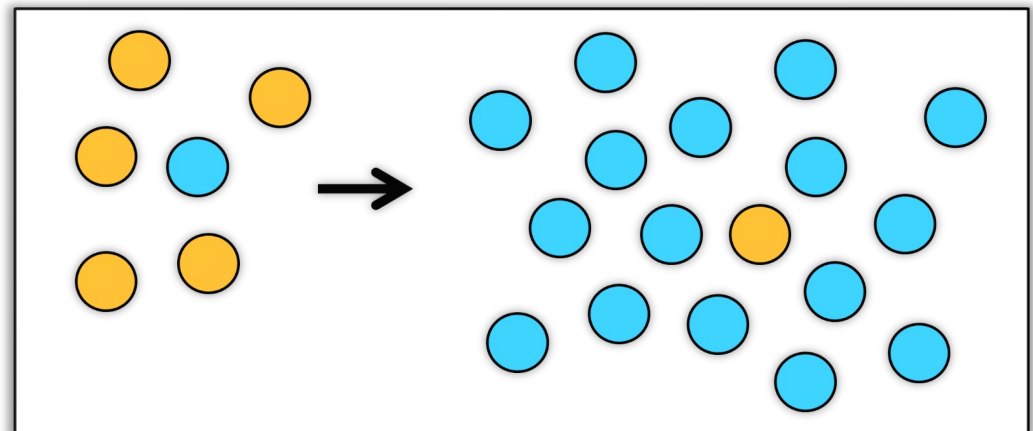
Injected Cells

Resulting Tumor

Expected
Results



Experimental
Results



Key

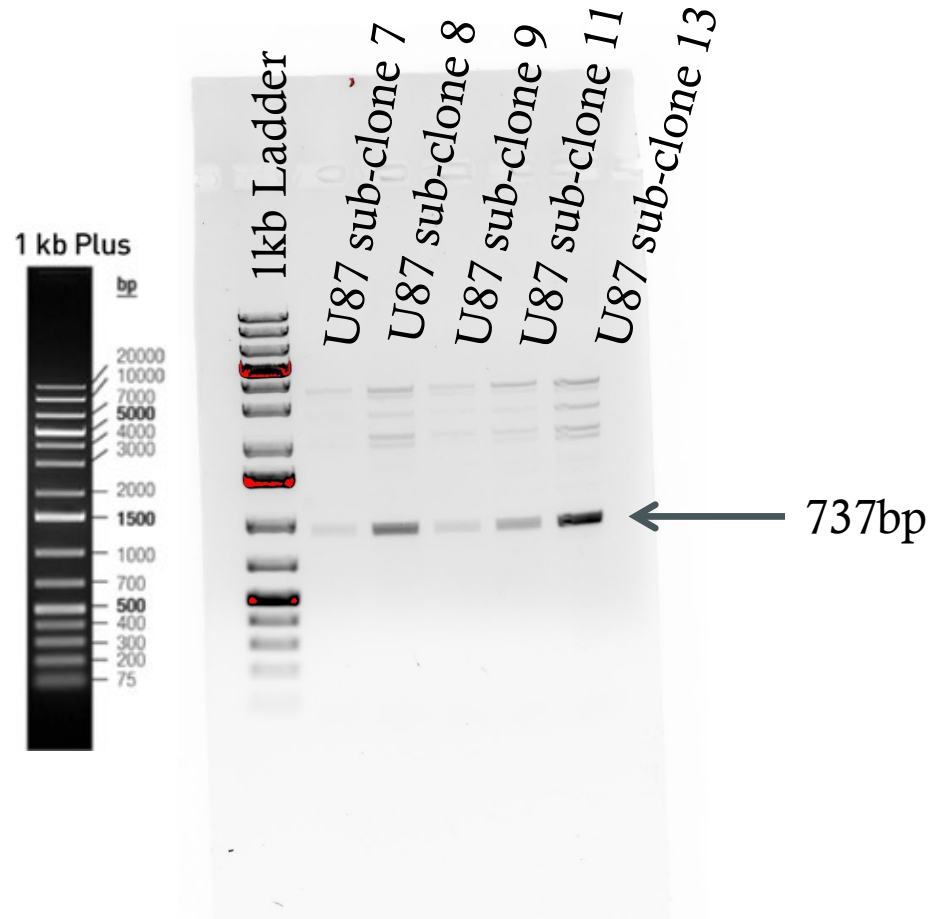
● Twist +

● Twist -

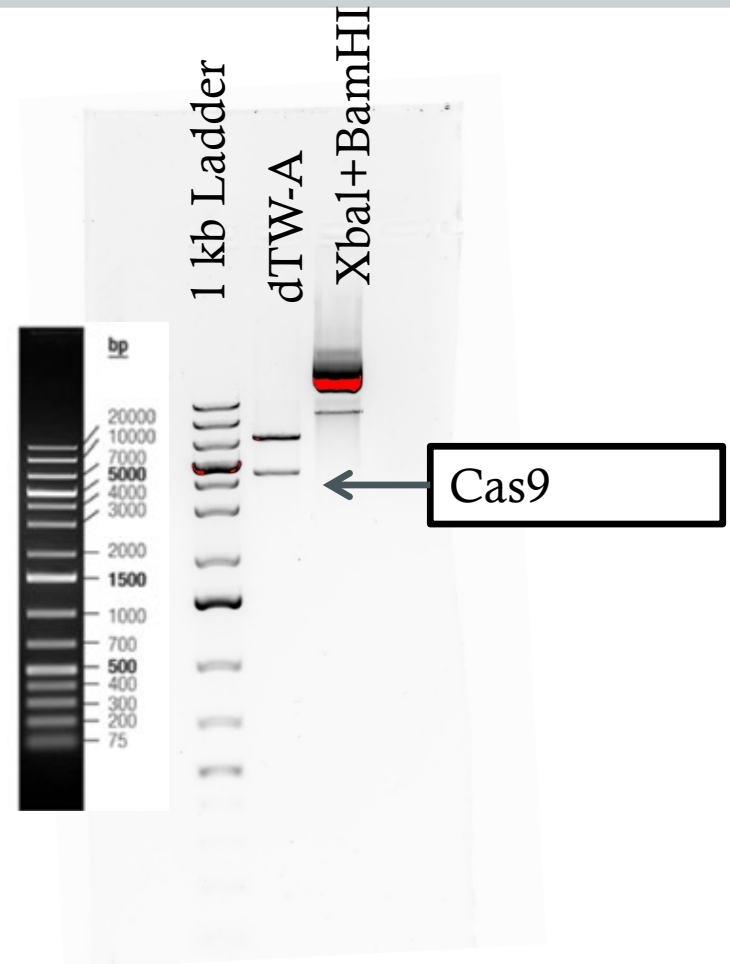
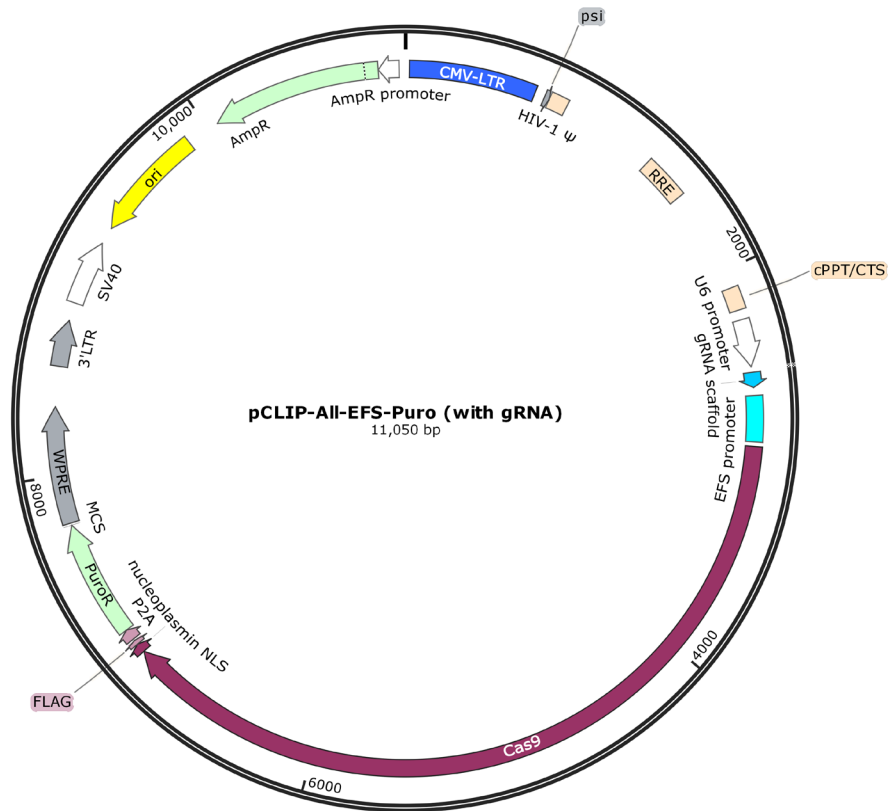
Conclusion

- U87 cells have significantly reduced ability to form tumors (significantly decreased tumorigenicity)
- Next Experiment: Isolate U87 dTwistA single cell sub-clones that do not have contaminants (absolutely no twist expression)
 - Isolate single cell clones
 - Perform screening for presence of mutations
 - Surveyor assay and direct sequencing
 - Determine which InDels generate pre-mature stop codon and remove any WT or twist revertant cells

U87 subclone PCR



Deletion of Cas9



Out of Lab Activities

- Tumor Board Meetings at UWMC
- Neurosurgery Conference at UWMC
- Gamma Knife at HMC
- Neurological Surgery Resident Shadowing
- Awake Craniotomy
- Bifrontal Craniotomy
- Pediatric Neurosurgery

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