Beth Israel Deaconess Medical Center

A teaching hospital of Harvard Medical School

BOSTONIVF

Inactivation of Latent Herpes Simplex Virus (HSV) Using CRISPR/Cas9-based Antivirals

Lauren Albrecht Murphy, MD

May 7, 2016

Study Purpose

- Further elaborate the capabilities of a the specific and relatively new gene editing tool, CRISPR/Cas9

- The point of the research is not modification of HSV itself (although a worthy goal), but to show how CRISPR can interact with an invading virus and change the course of it's lifecycle within a cell and ultimately within an organism.

Overview

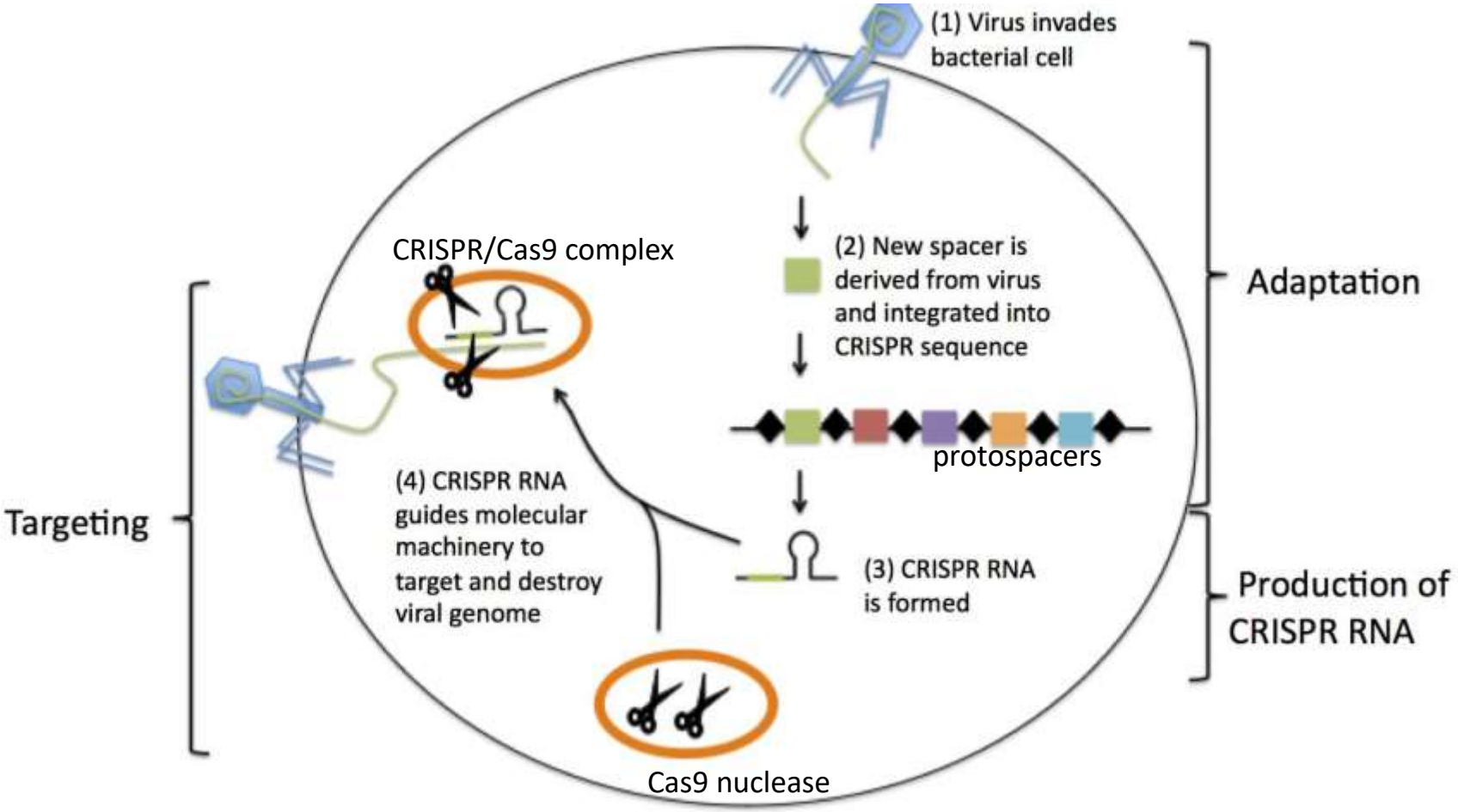
- Brief introduction to CRISPR/Cas-9
- Discussion of HSV as a target
- Project Aims
- Findings so far
- Future steps/goals

CRISPR/Cas9

- CRISPR: clustered regularly interspaced short pallendromic repeats
- First reported in 1987 in Japan (Ishino)
- Discovered as part of bacterial adaptive immunity in 2005 (Mojica)
- 2011 system can function in species other than bacteria
- 2012 cleaving/cutting system was fully characterized
- 2013 genomic editing first harnessed

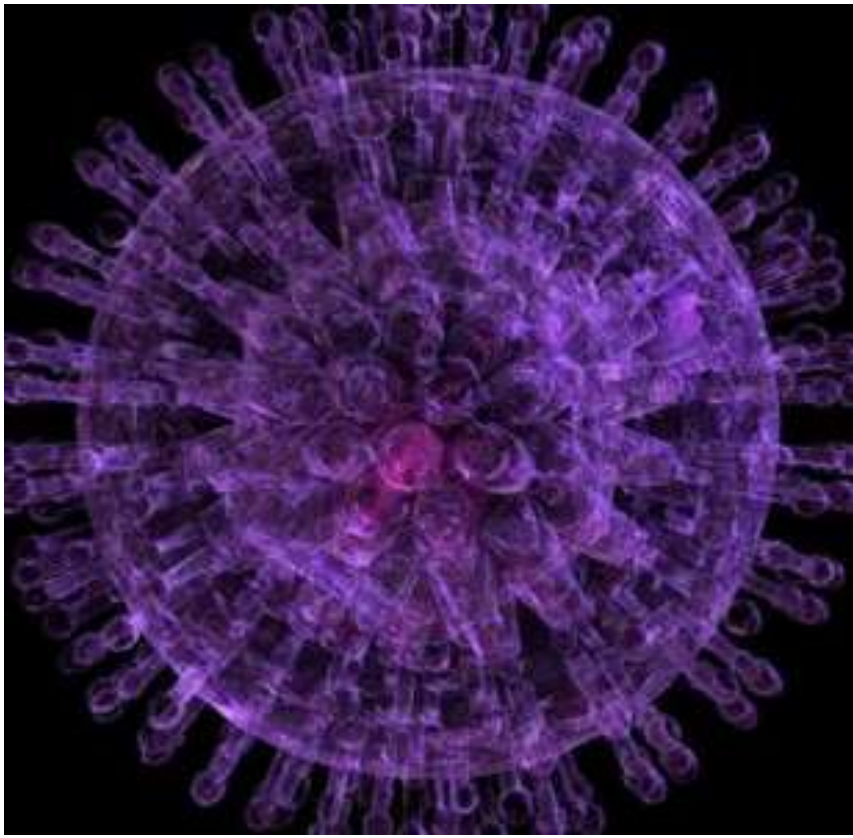
(Zhang, MIT)

CRISPR Immune System

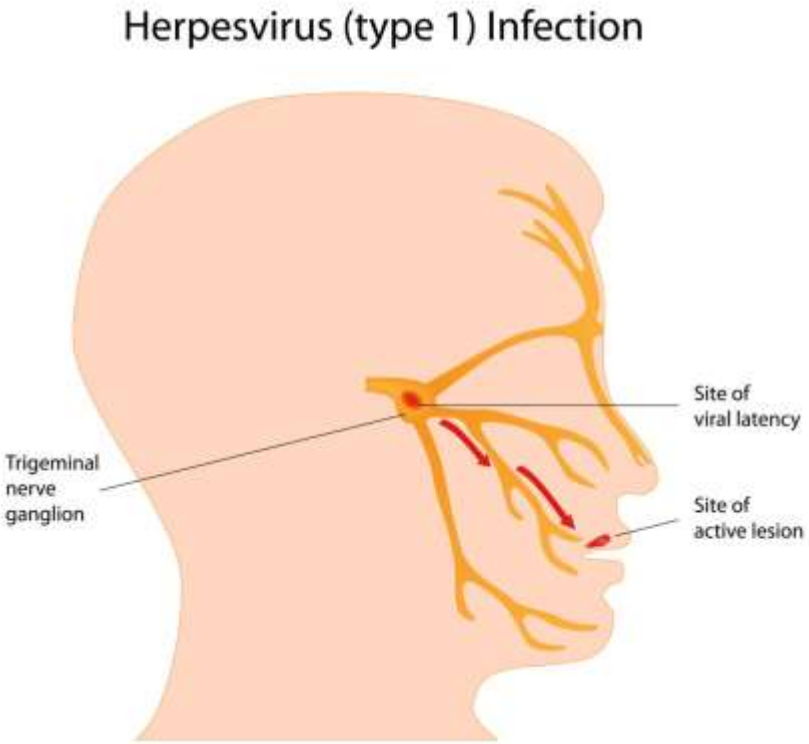


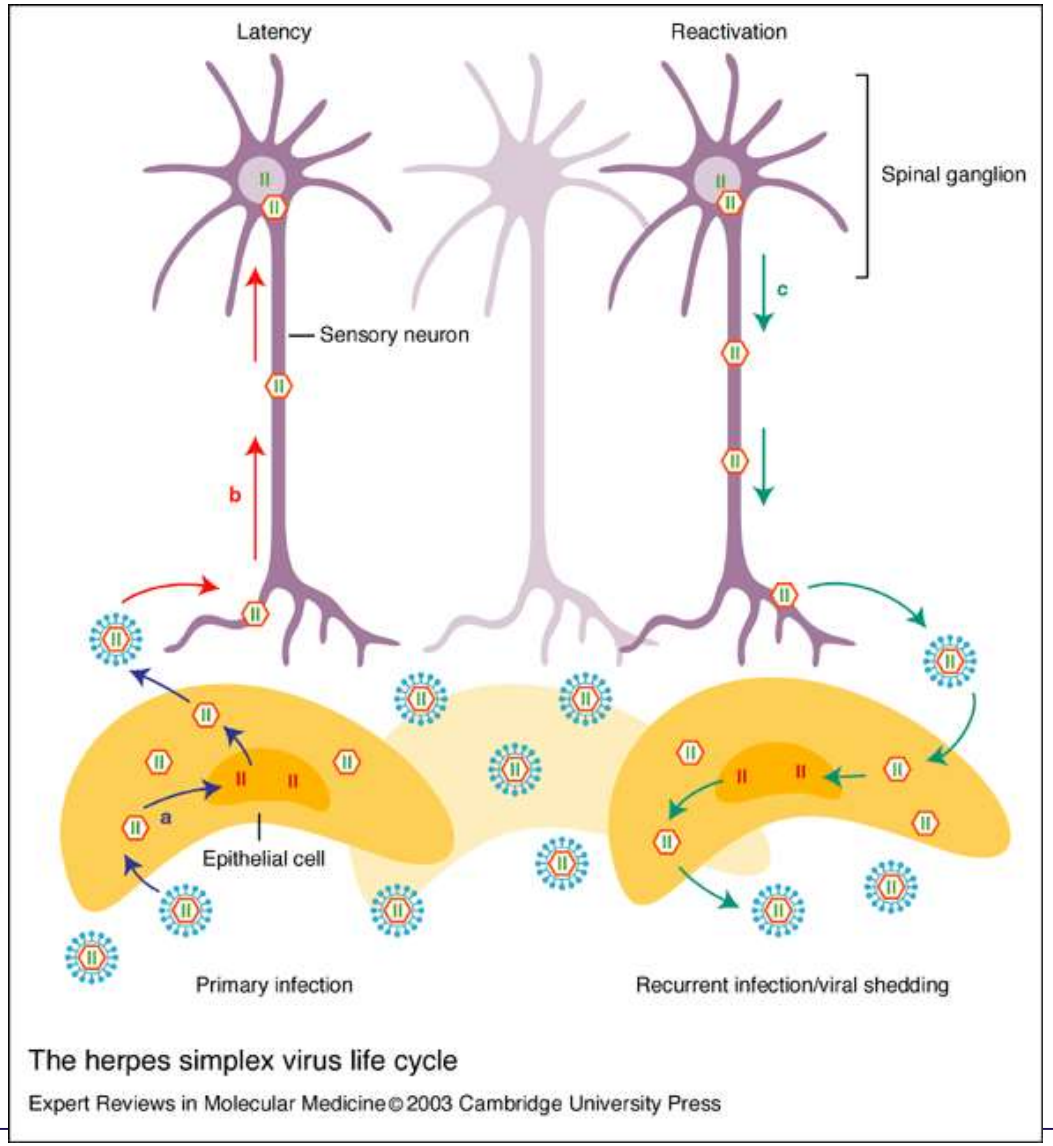
Why Target Herpes Simplex Virus

- Ideal CRISPR target…
  - Defined reservoir
  - Clinically relevant
  - Latent stage
  - Animal models and human disease models
- Herpesviridae family:
  - HSV-1, HSV-2
  - VZV
  - EBV
  - CMV
- Ubiquitous, life long disease, increases HIV transmission, significant neonatal impact, extensive morbidity



Pathophysiology - HSV





The herpes simplex virus life cycle

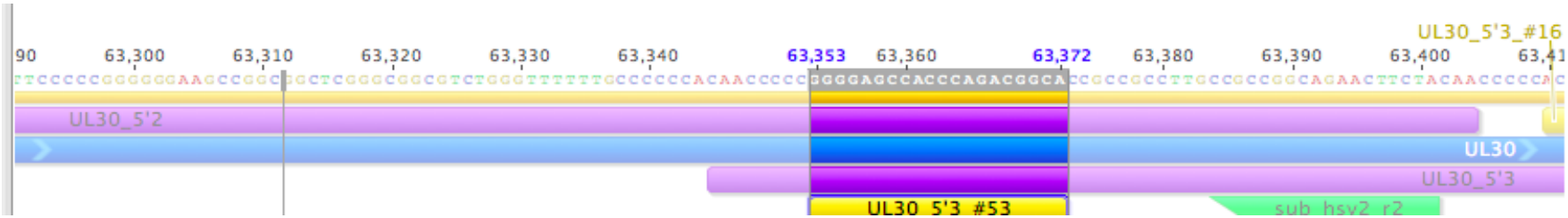
Expert Reviews in Molecular Medicine © 2003 Cambridge University Press

Study Aims

- Identify CRISPR/Cas9 guide RNAs targeting essential HSV genes
- Utilize CRISPR/Cas9 in vitro in human sensory neurons to cut HSV genes and demonstrate the efficacy of the system
- Trial in vivo in mouse model

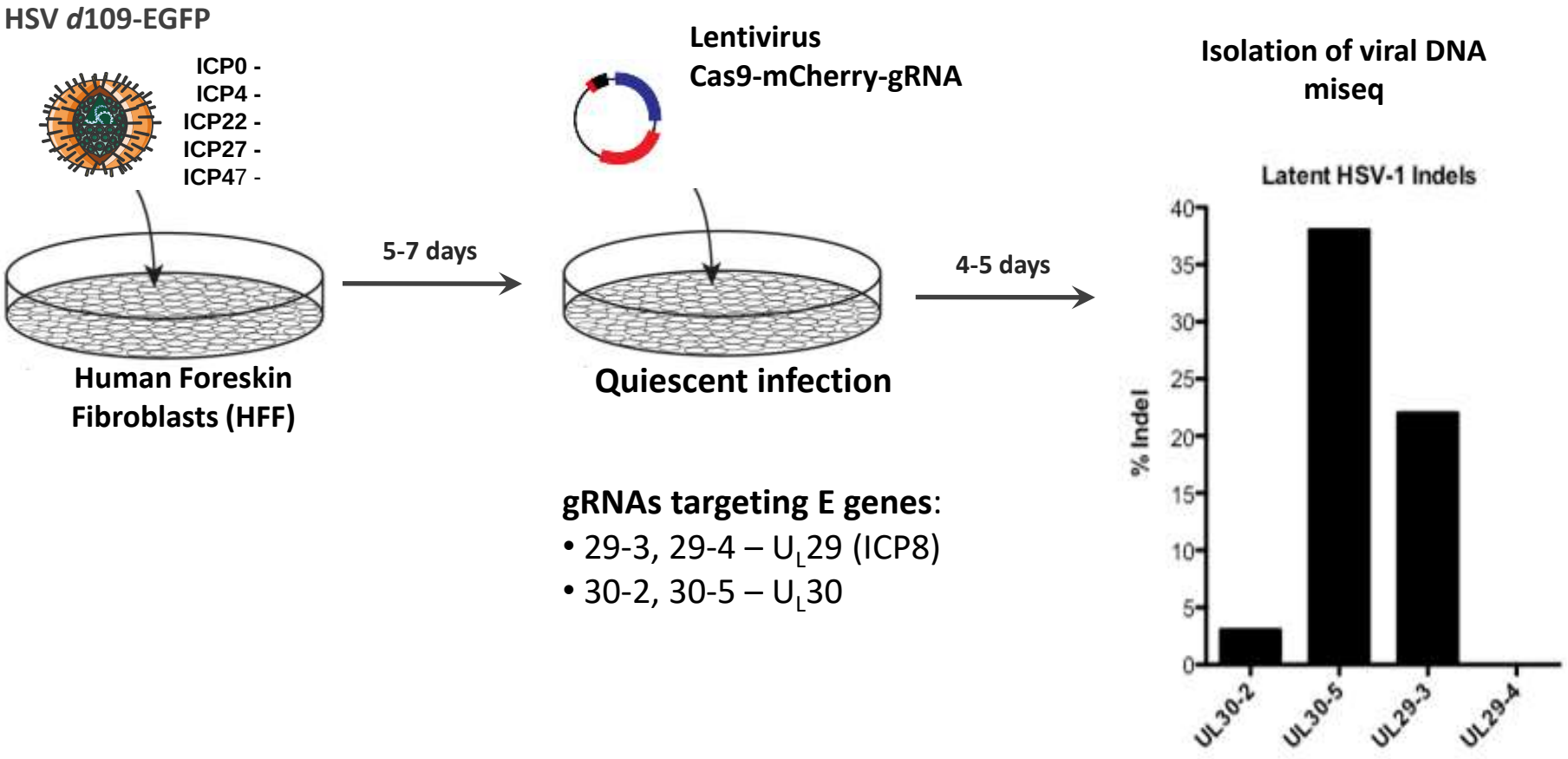
Experimental Steps

1. Develop invitro cutting assay
   - Used Geneious software and Crispr.mit.edu crispr design tool



1. Package developed CRISPR/Cas9 cutting assay into vectors: Lenti virus and AAV
2. AAV small vector that lacks pathogenicity
   - Lenti vector: less expensive, more toxic to cells
   - Cas 9: s.auerus smaller than more common Cas 9 from streptoccocus species

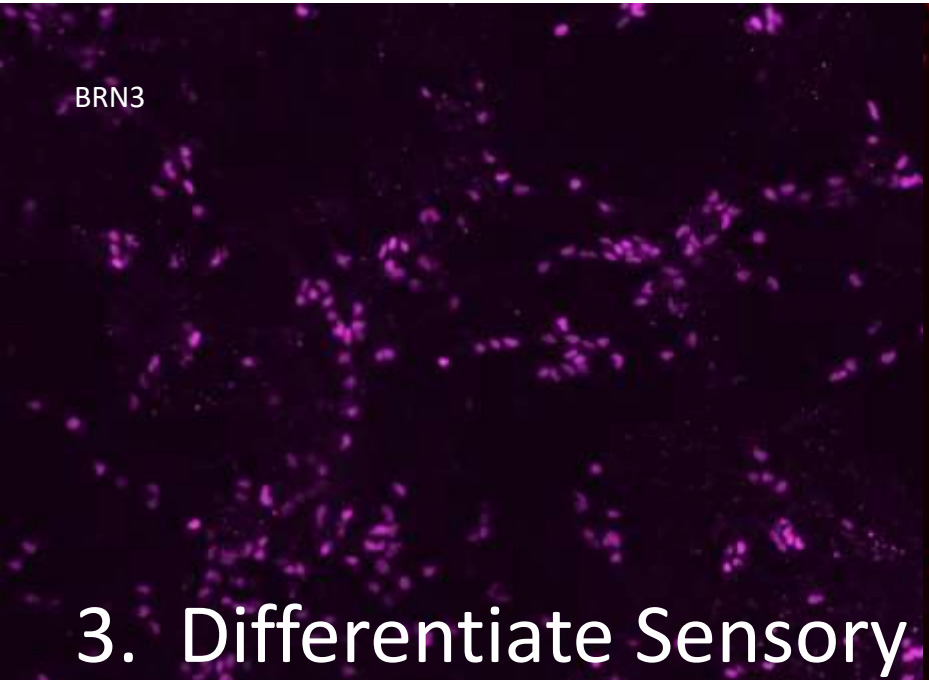
Screening for SaCas9-gRNAs targeting latent viral genomes

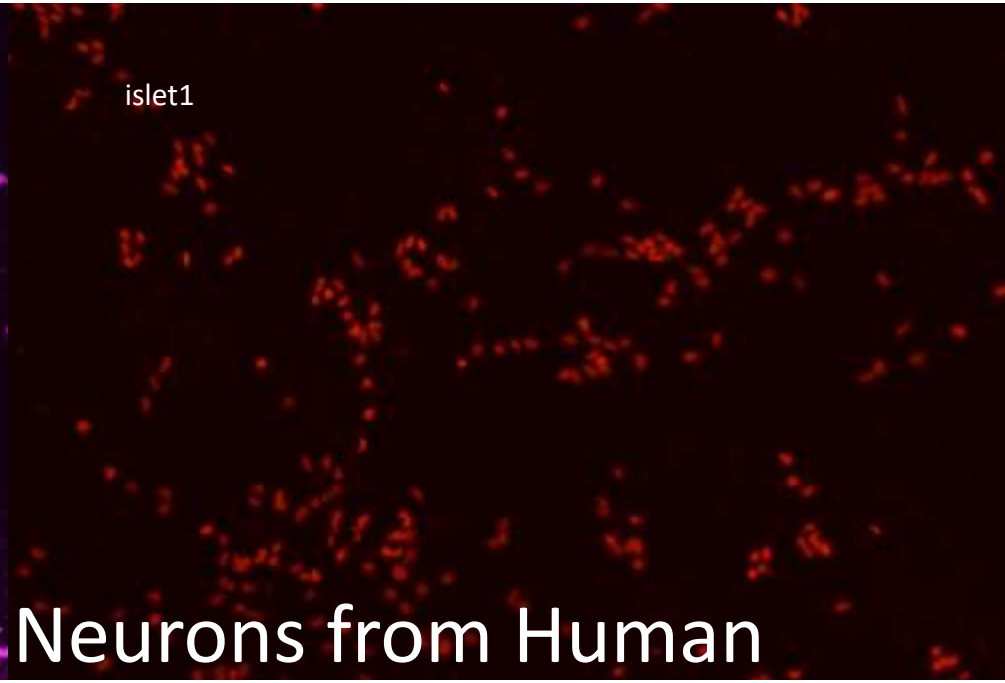


gRNAs targeting E genes:

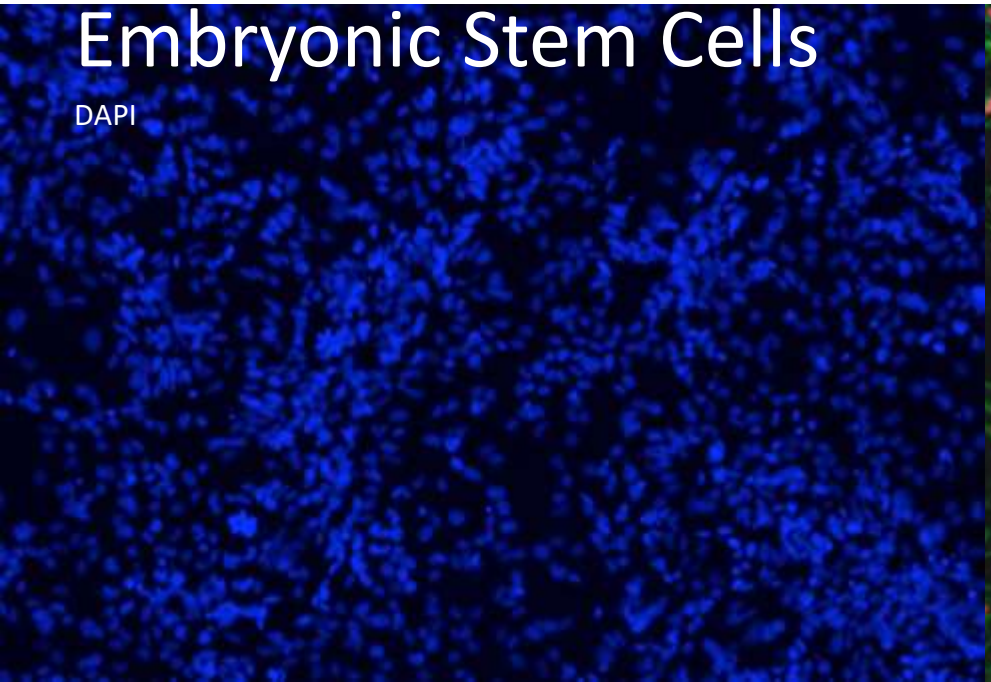
- 29-3, 29-4 – U_L29 (ICP8)
- 30-2, 30-5 – U_L30

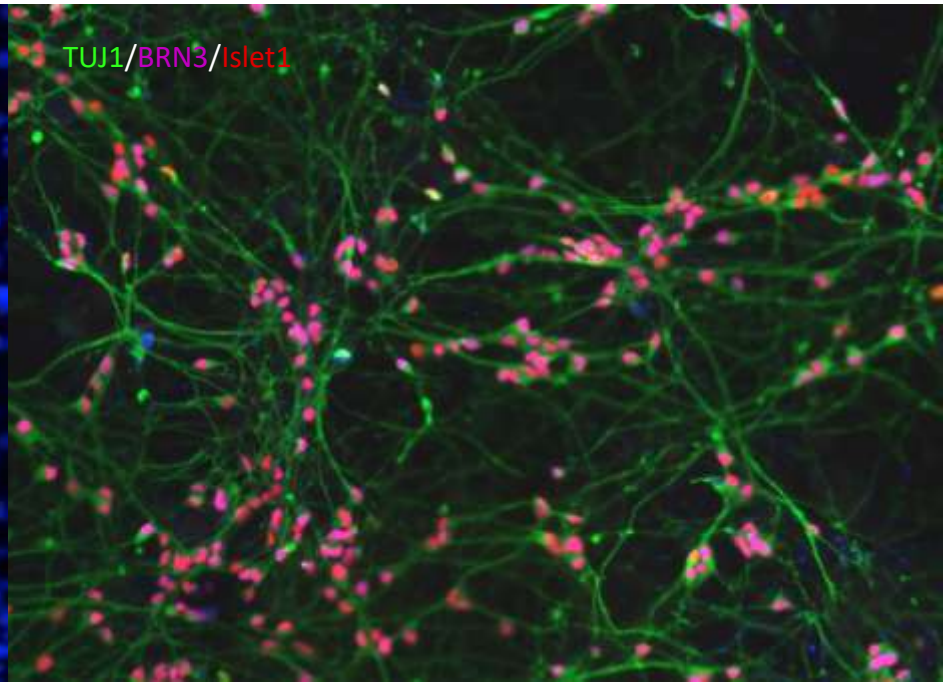
50K well of Sensory Neurons, fixed at Day 18 of differentiation from HuES3. Merged BRN3 (647) Islet (555) TUJ1 (488)





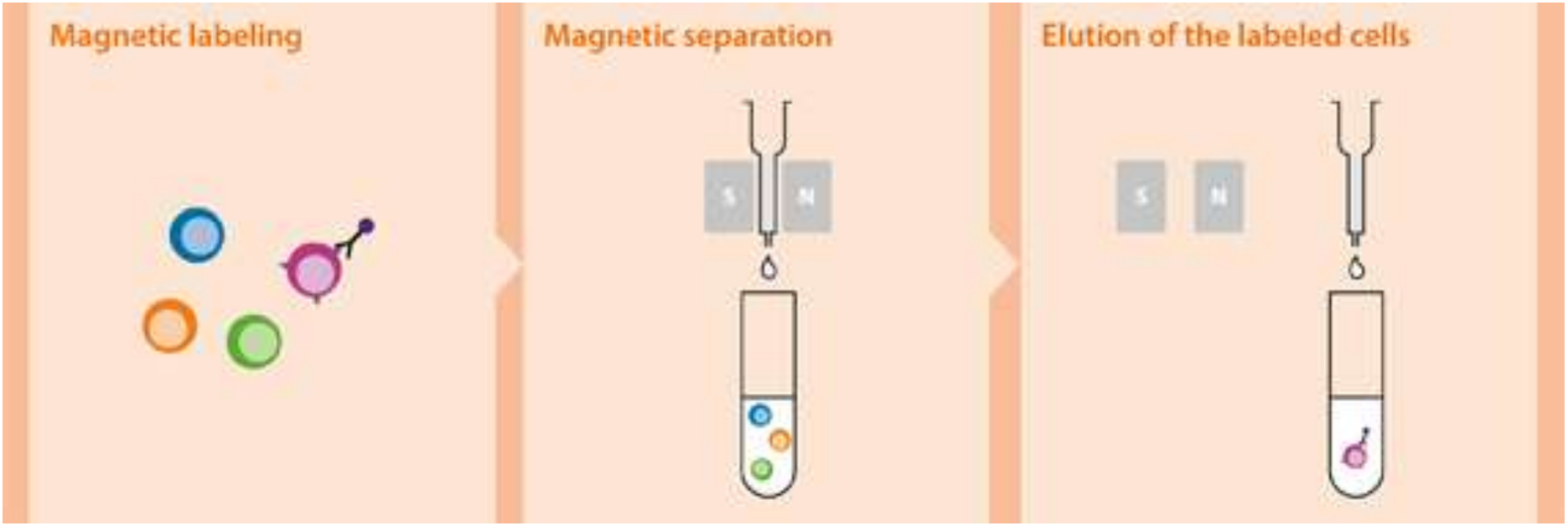
3. Differentiate Sensory Neurons from Human Embryonic Stem Cells



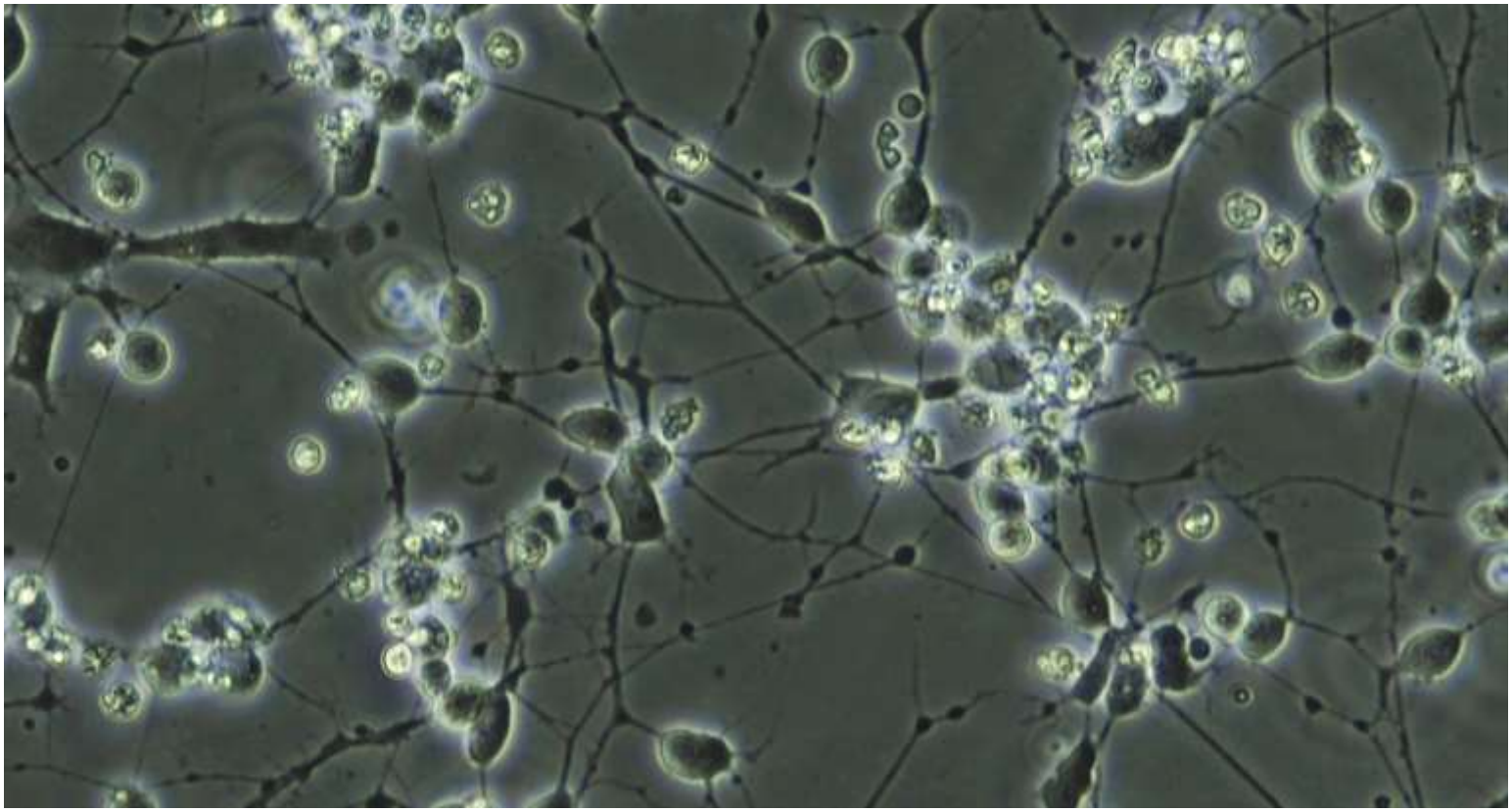


4. Purify population for sensory neurons

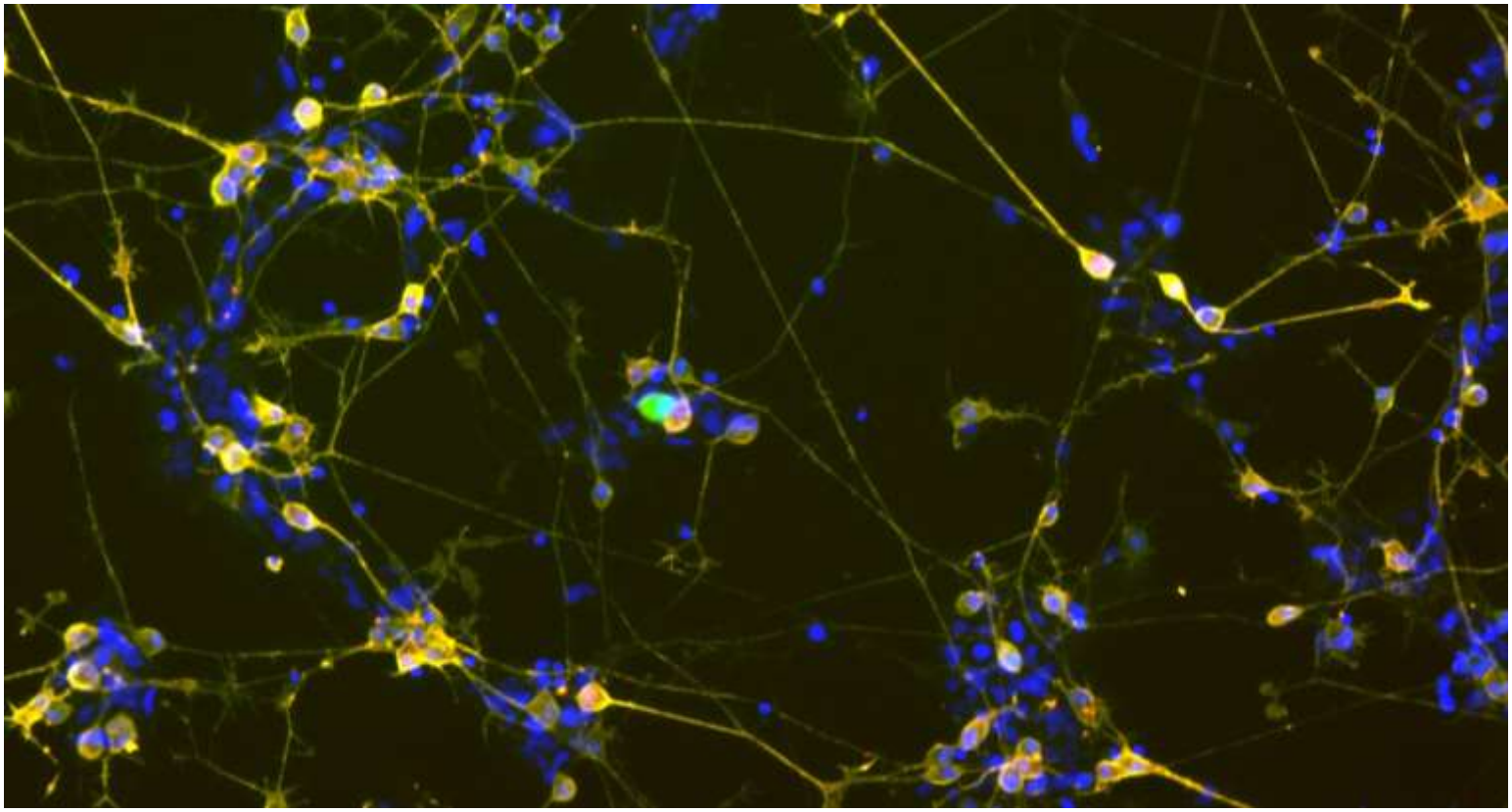
- Use MACS to sort for a mature neuronal marker, NCAM



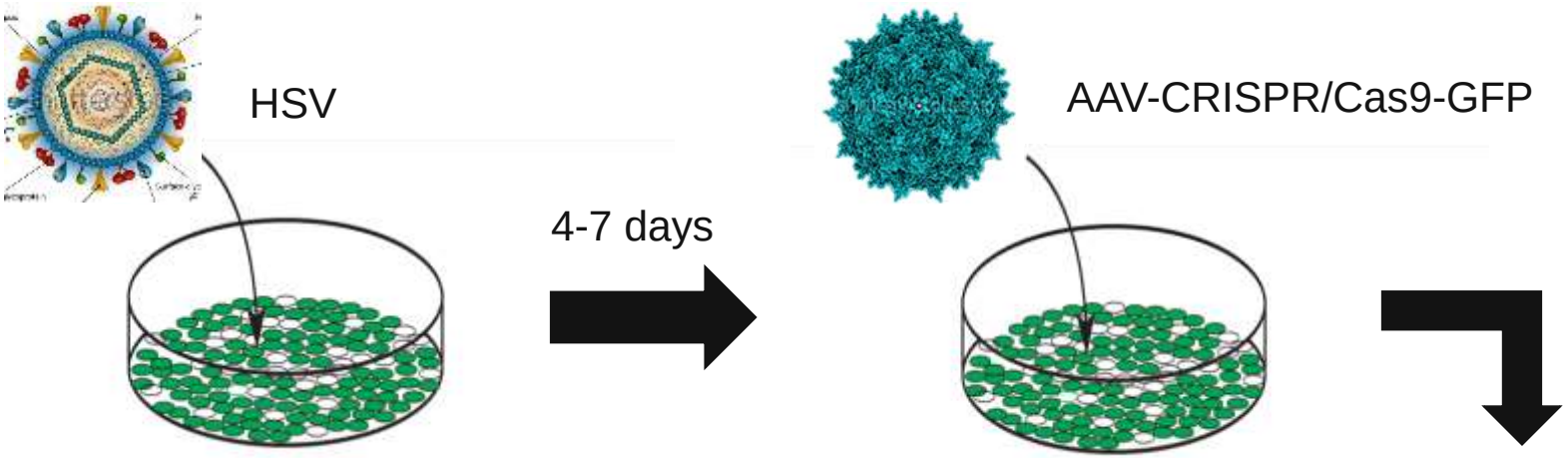
BF 1.3.17 NCAM+ sort (Abx 1:100), plated on MG at 330K/well. 20x magnification



NCAM+ sort with MACS, antibody 1:100 dilution. Stain for BRN3A (C-20) (goat IgG 488), NCAM (mouse IgM 555). Fresh D.13 SND, MACS sorted, then plated on MG at 400K (2.21.17) for 3 days, fixed on 2.24.17. Well#3

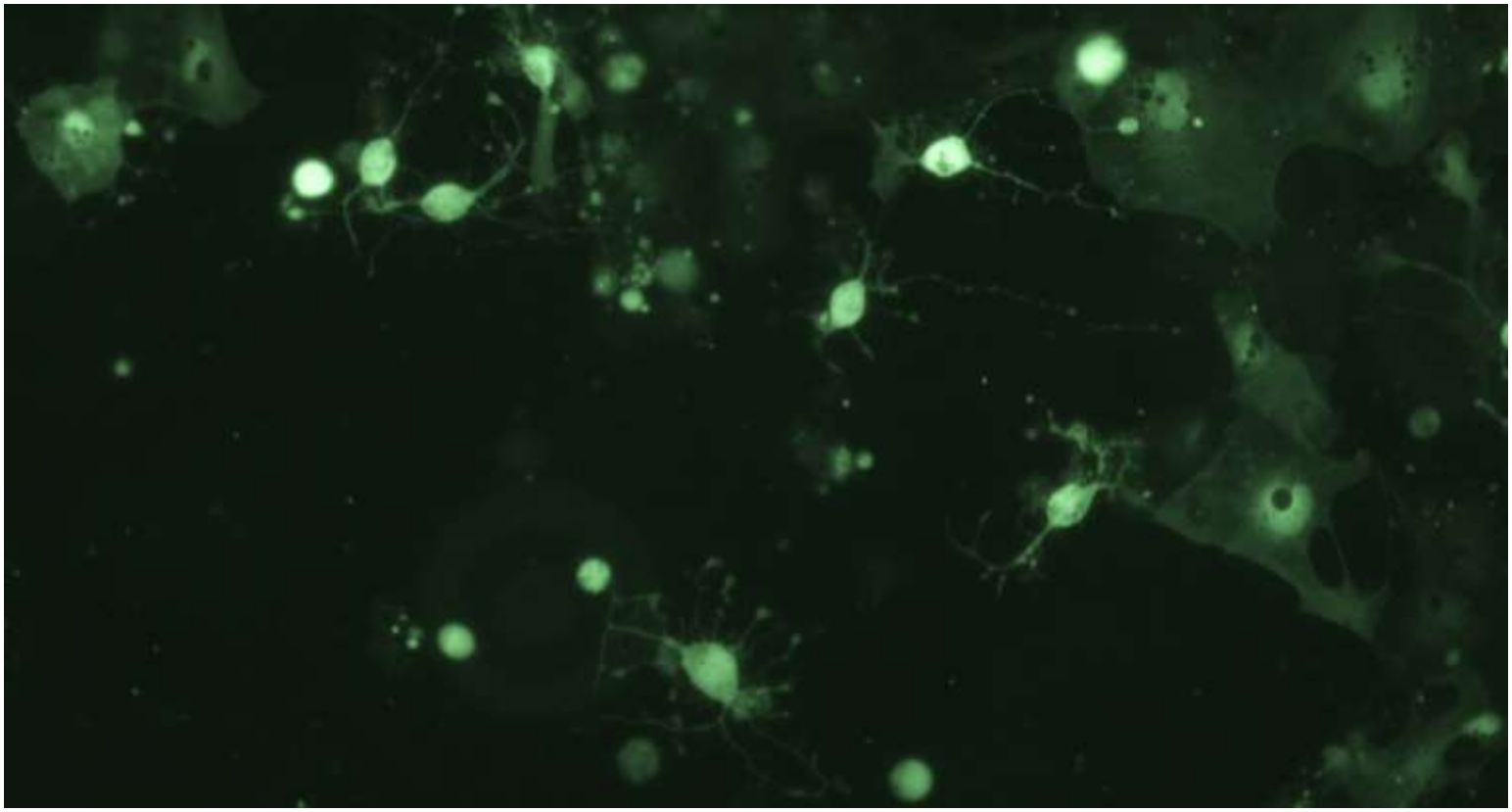


5. Infect Sensory Neurons with HSV-d109 and AAV-CRISPR-GFP virus



Harvest the cells and sequence the DNA

NCAM+ cells at 20K magnification, infected with AAV-CRISPR-GFP



Final Experimental Step

Evaluate for cutting of viral genome with genetic sequencing

Significance

- Expands the proven scope of the CRISPR/Cas9 system
  - S.aureus derived Cas9, AAV vector
- Opens up therapeutic options for HSV, Herpesveridans Family, and other viral infections
- Further illuminates CRISPR/Cas9 system in human cells and ultimately in human genome modification with far reaching impact on human reproduction

Beth Israel Deaconess Medical Center

A teaching hospital of Harvard Medical School

BOSTONIVF

THANK YOU