

# **Induction of Retinal Ganglion Cells through Transcription Factor Mediated Reprogramming**

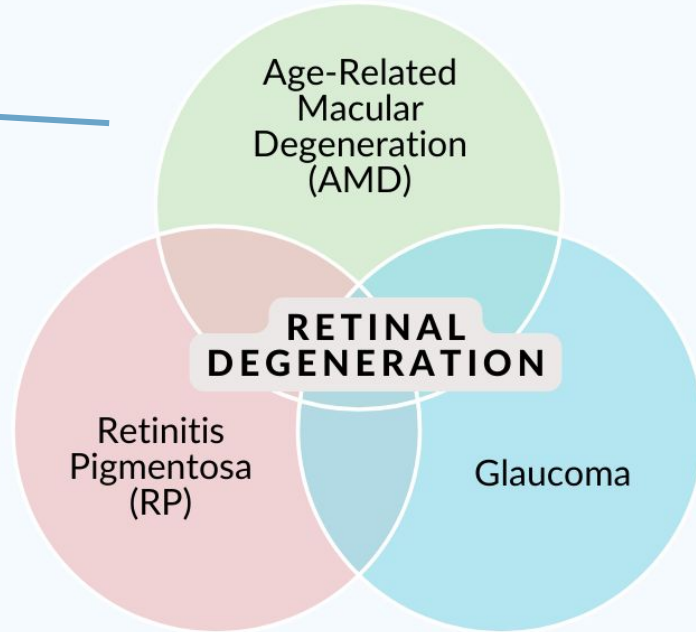
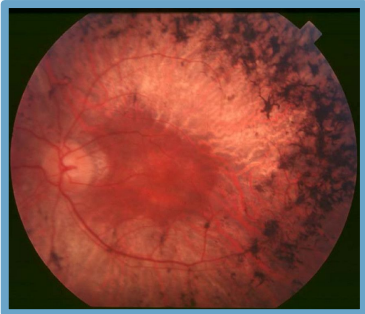
**Manan Chopra**  
Department of Biological Sciences

Mentor: Dr. Karl Wahlin  
Department of Ophthalmology

35th Annual URC Conference (Spring 2022)

# 285 MILLION

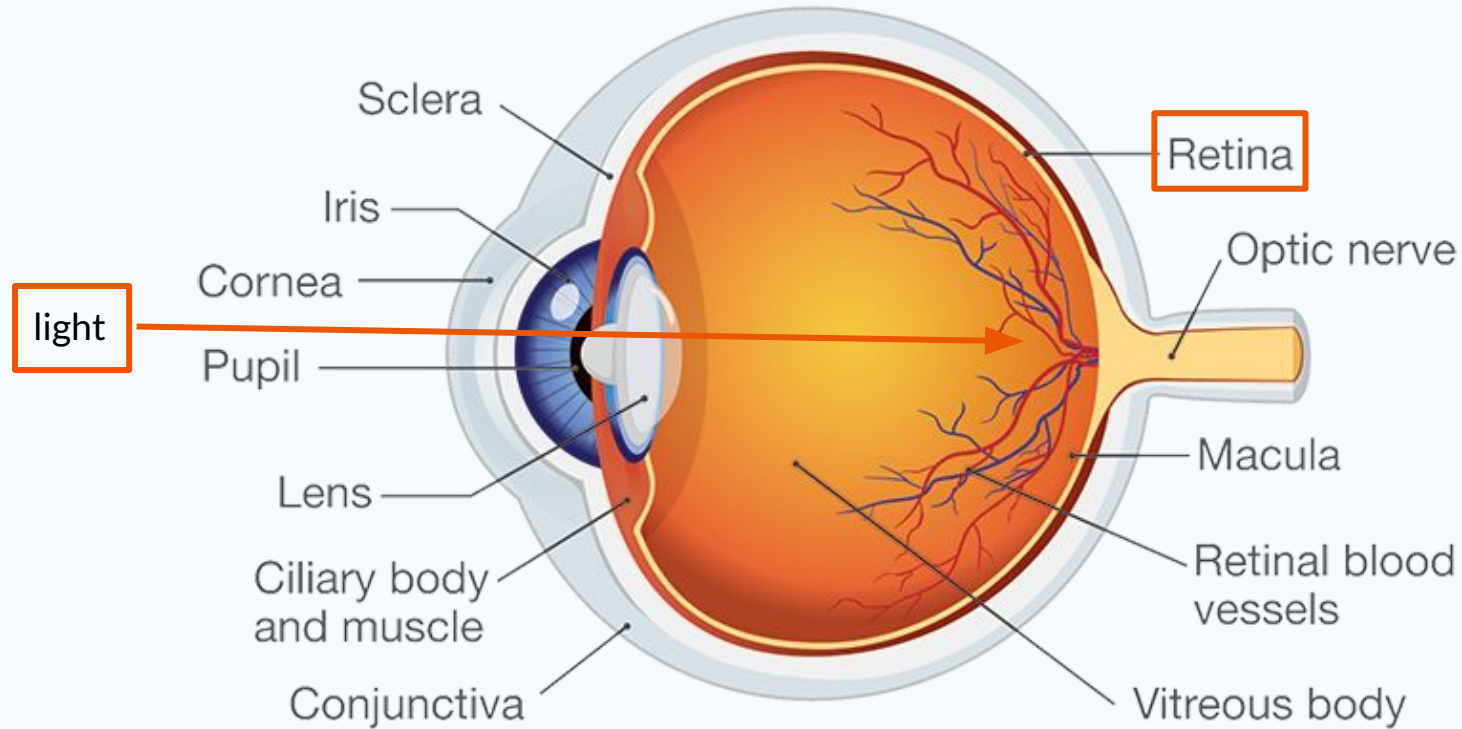
## AFFECTED WORLDWIDE (2022)

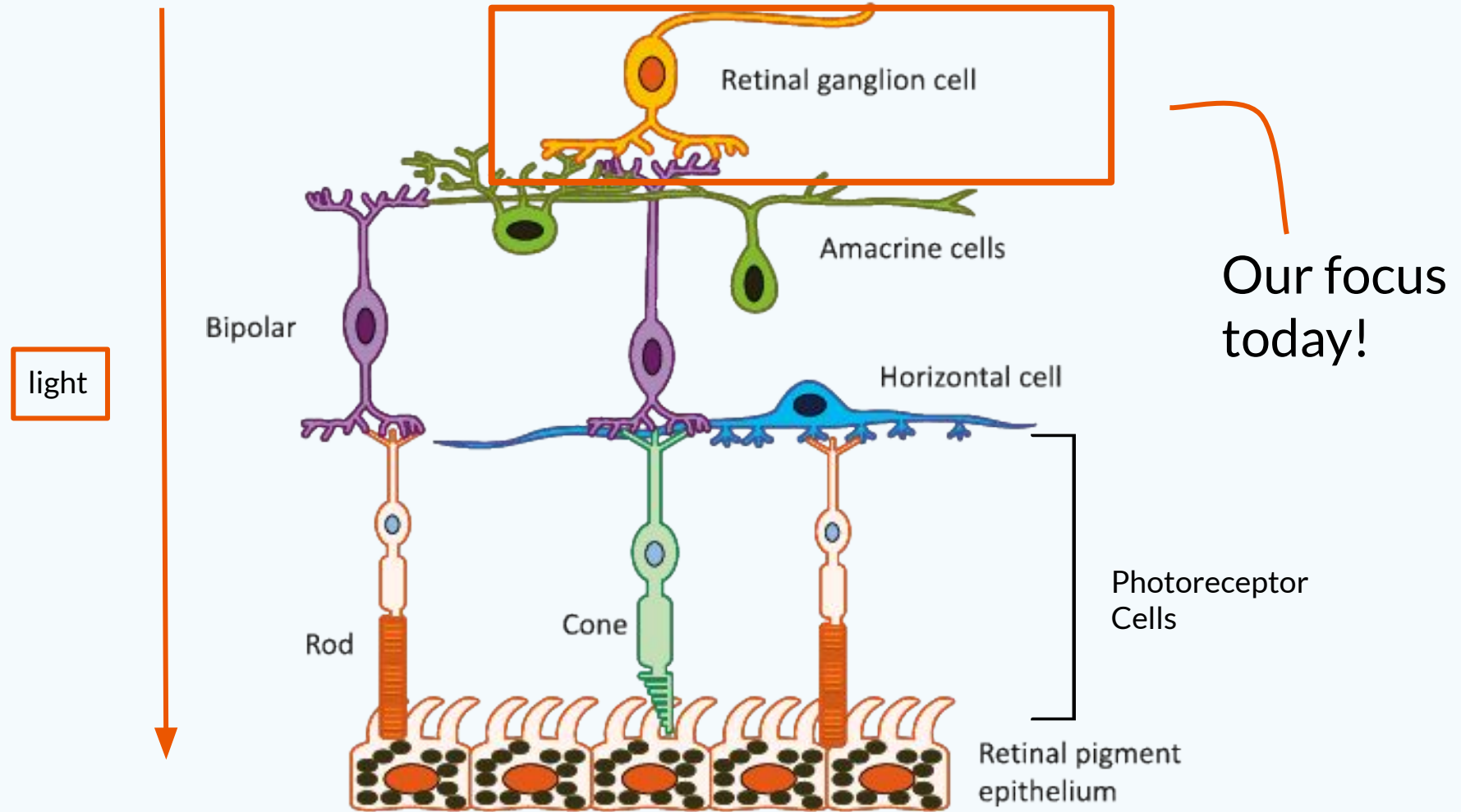


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# The Retina & Retinal Ganglion Cells (RGCs)

# Human Eye Anatomy





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# Transcription Factors (TFs)

# transcription factors

of eukaryotic cells

**1** Activator proteins bind to pieces of DNA called **enhancers**. Their binding causes the DNA to bend, bringing them near a **gene promoter**, even though they may be thousands of base pairs away.

## note

This diagram simplifies the DNA greatly—promoters, enhancers, and insulators can be dozens or even hundreds of base pairs long.

Enhancers

Activator proteins

Other transcription factor proteins

**2** Other transcription factor proteins join the activator proteins, forming a protein complex which binds to the **gene promoter**.

Gene

Promoter

**3** This protein complex makes it easier for **RNA polymerase** to attach to the promoter and start transcribing a gene.

RNA polymerase

**4** An **insulator** can stop the enhancers from binding to the promoter, if a protein called **CTCF** (named for the sequence CCCTC, which occurs in all insulators) binds to it.

Methyl groups

Insulator

**5** **Methylation**, the addition of a **methyl group** to the **C** nucleotides, prevents **CTCF** from attaching to the insulator, turning it off, allowing the enhancers to bind to the promoter.

CTCF  
(CCCTC-binding factor)

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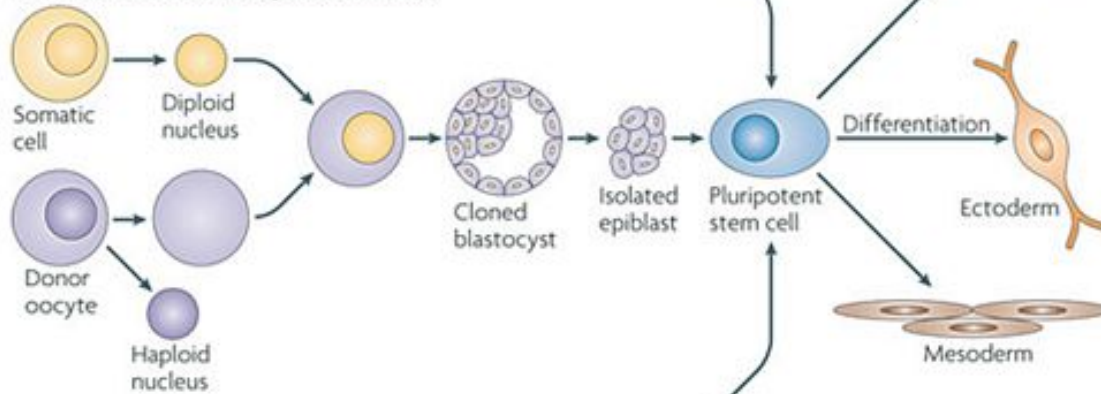
# Pluripotent Stem Cells (PSCs) & 'Reprogramming'



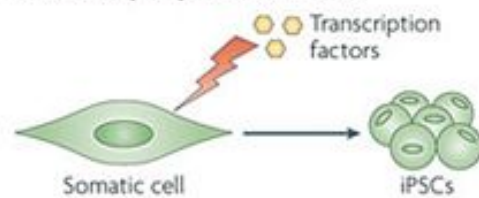
**a Human embryonic stem cells**



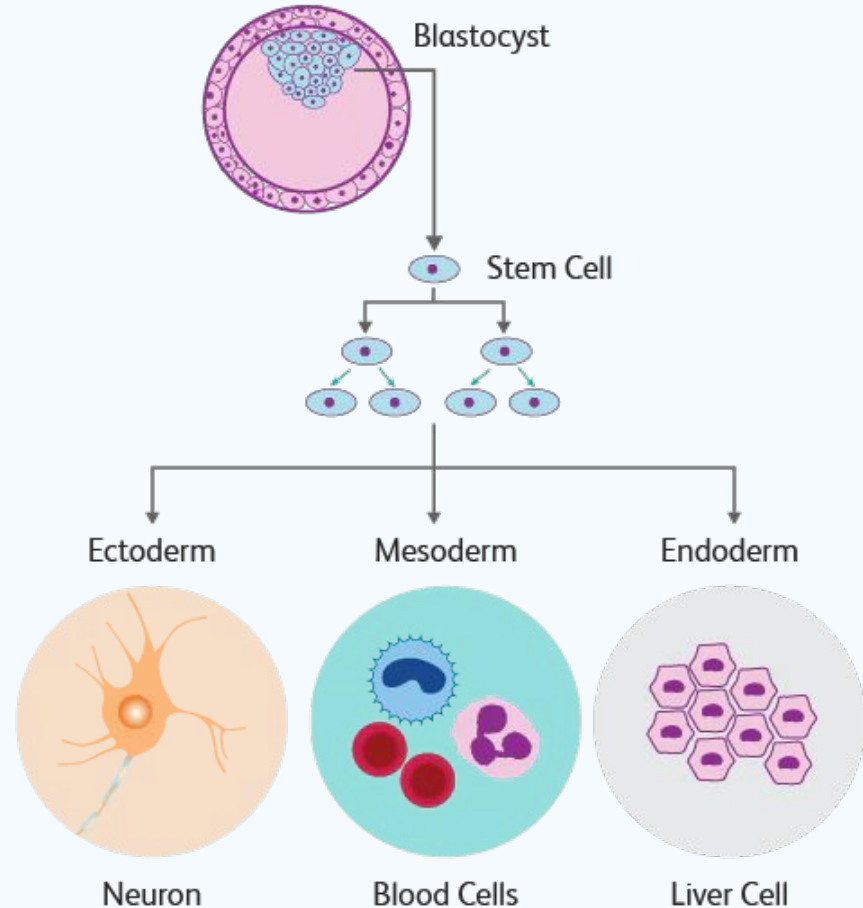
**b Nuclear transfer embryonic stem cells**



**c Induced pluripotent stem cells**

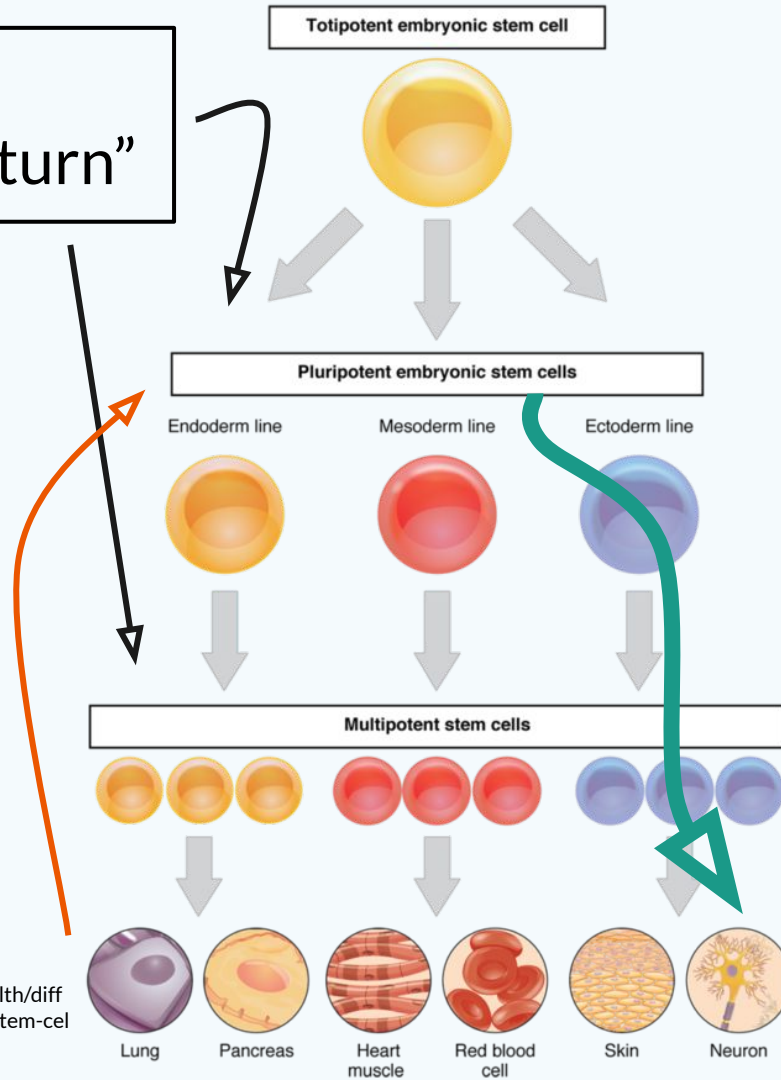


# STEM CELLS IN VIVO



IN VIVO:  
“points of no return”

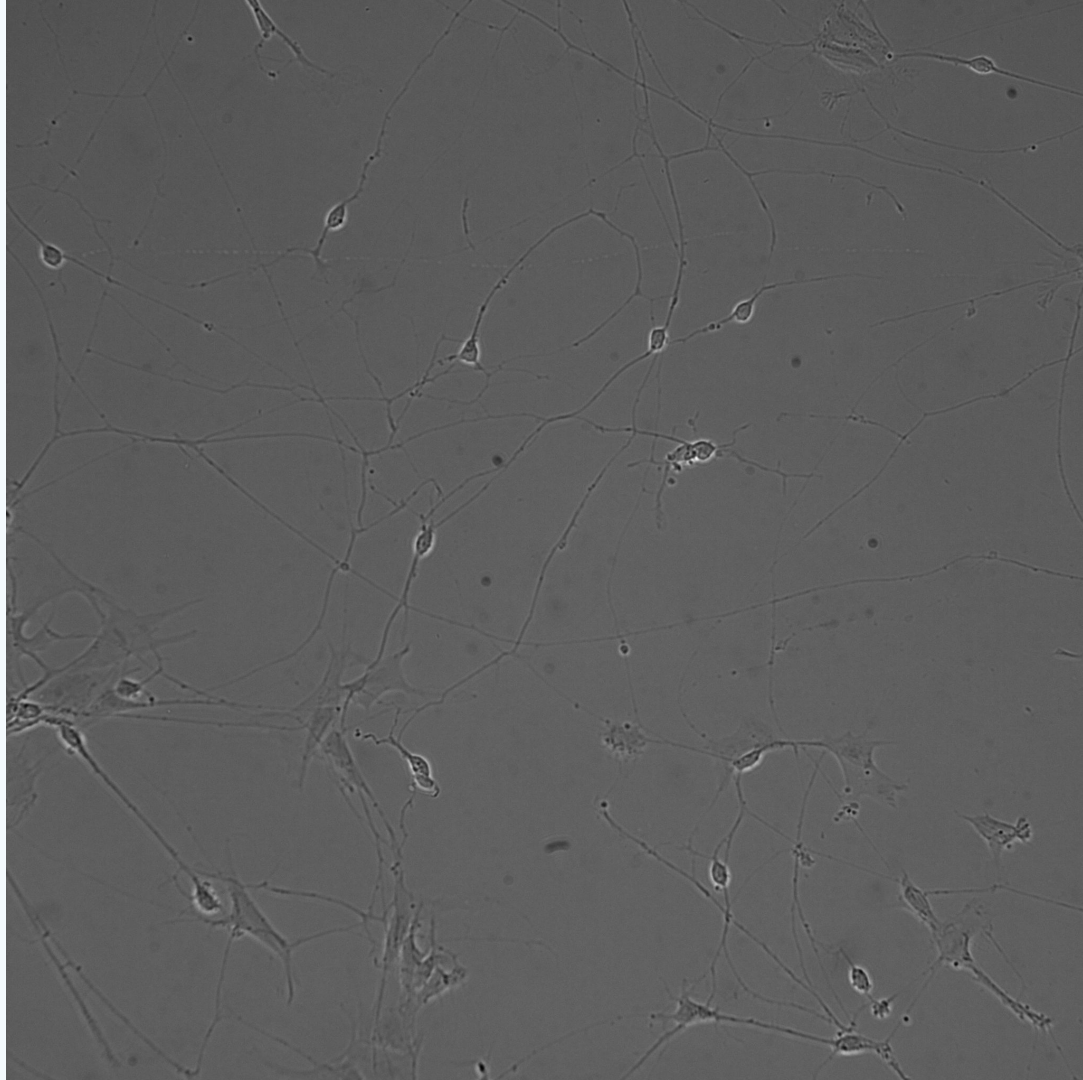
IN VITRO:  
“induced”  
PSCs using  
TFs

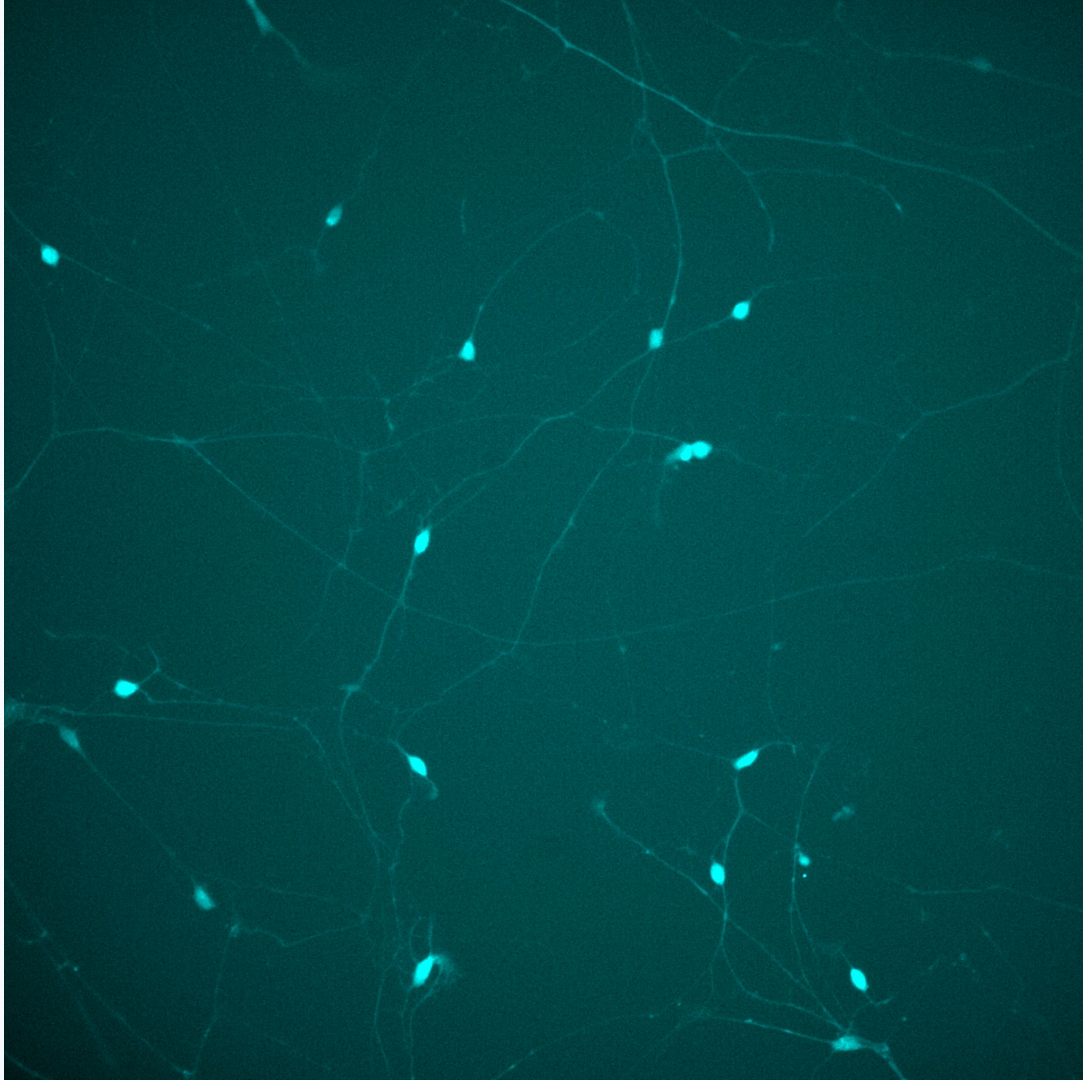


We want to  
take iPSCs and  
create  
“induced”  
RGCs

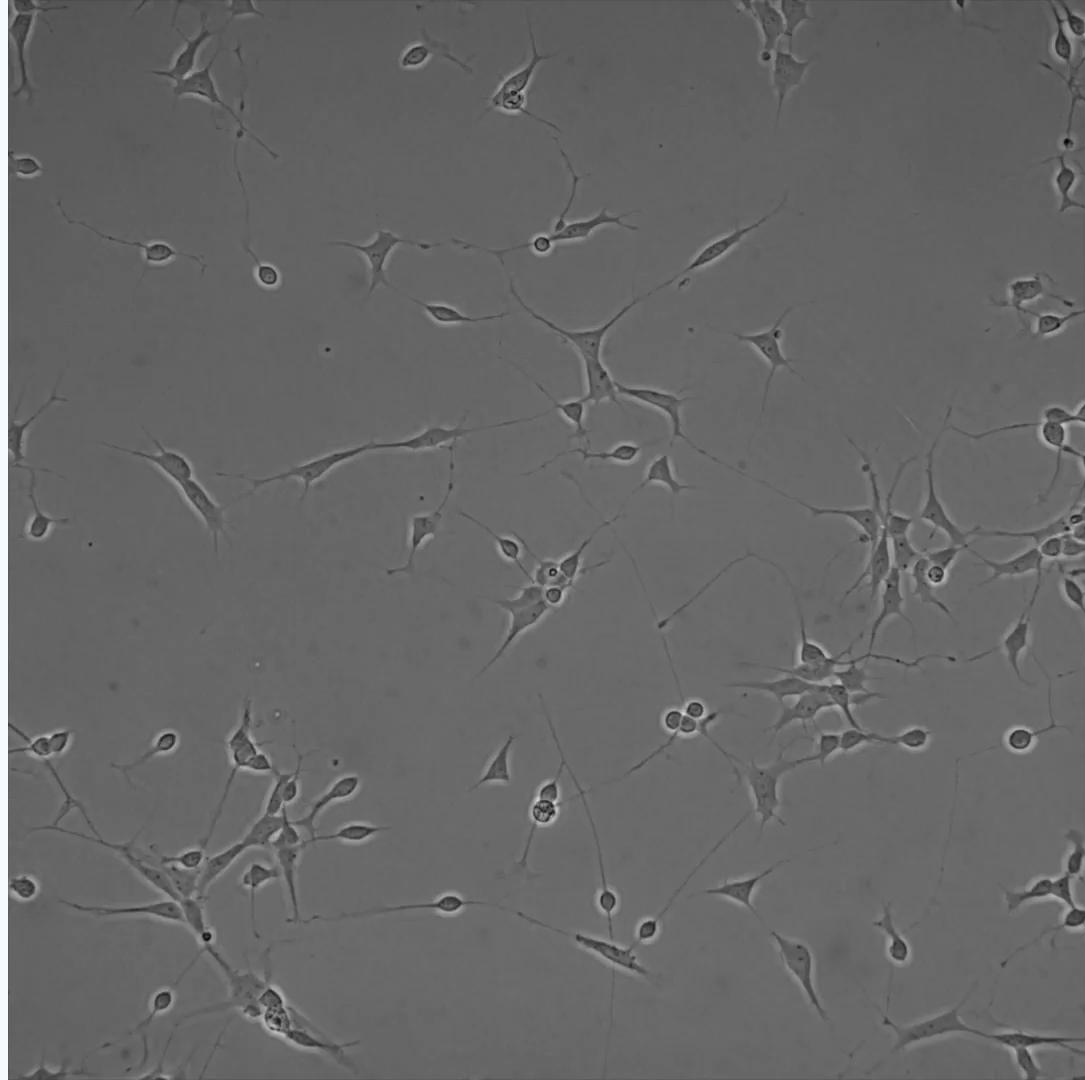
**We can use certain transcription factors to direct pluripotent stem cells into desired cell types (in our case, RGCs).**

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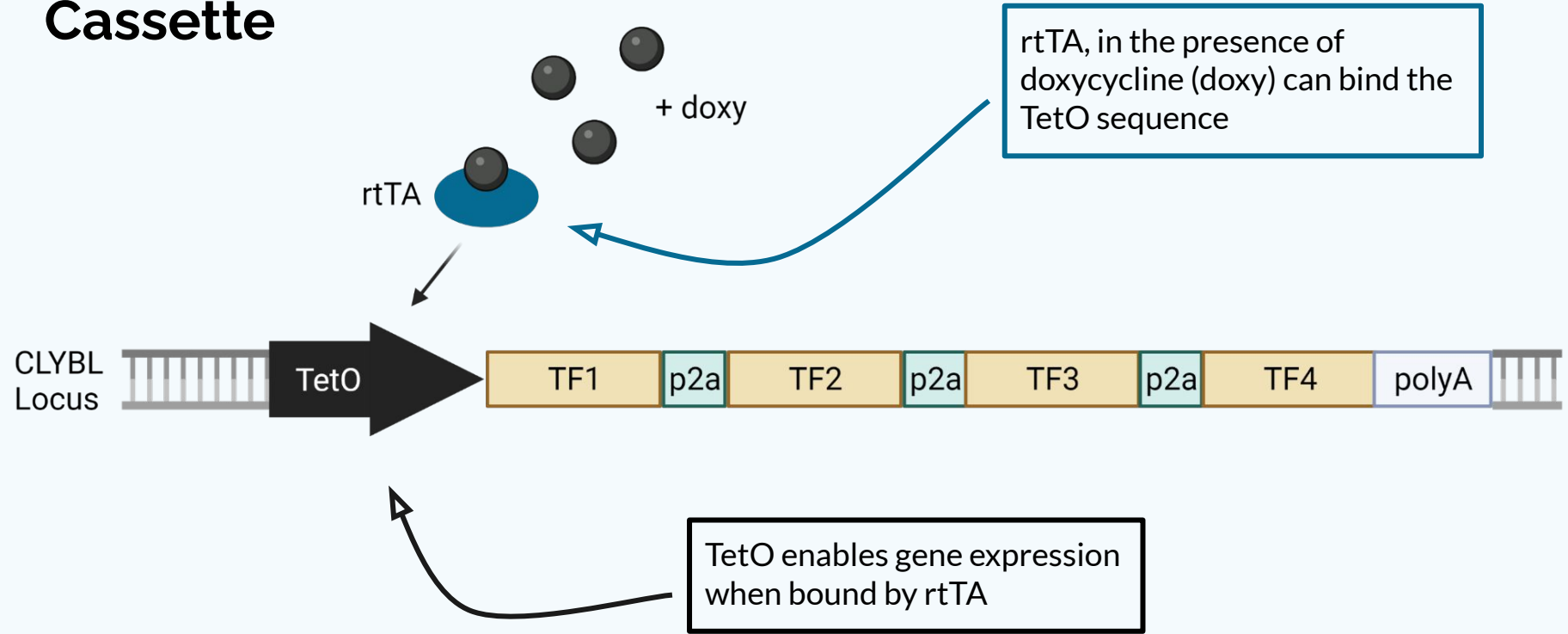








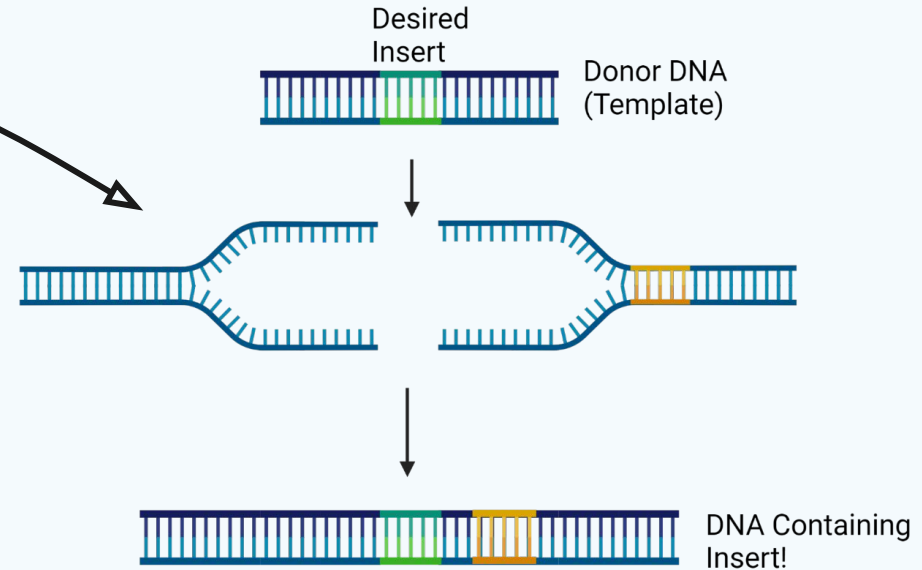
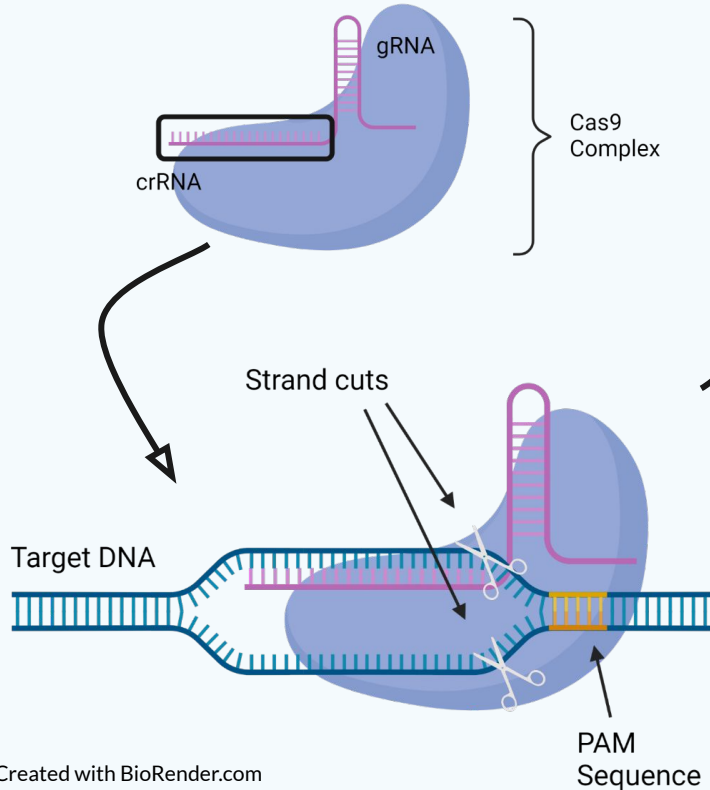
# The Doxycycline Inducible Cassette

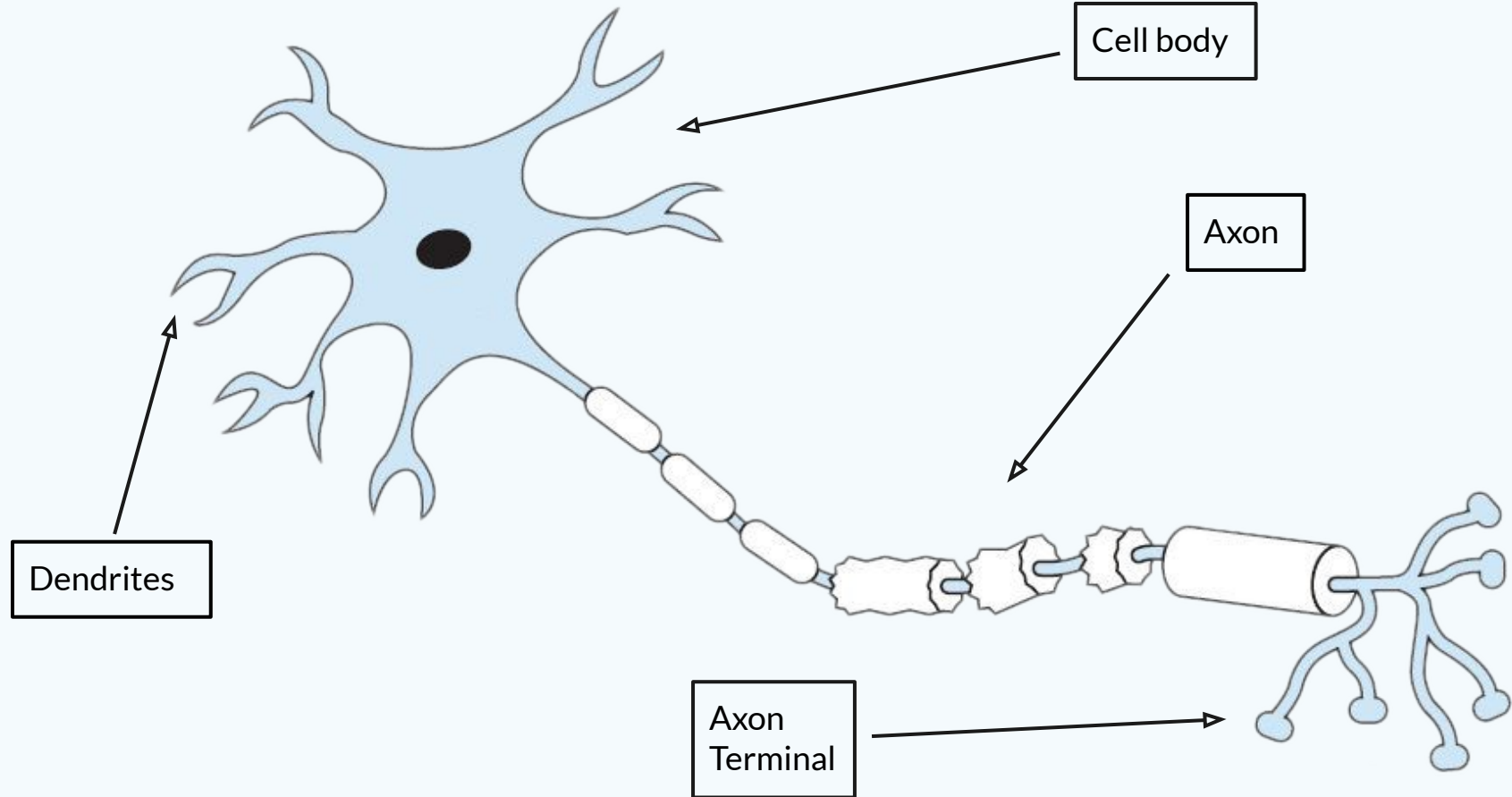




# Insertion of the Cassette using CRISPR-Cas9 Editing

- Allows for very specific insertion of a desired insert
- Must provide a donor sequence which the cell will use to repair breaks induced by Cas9





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# Bioinformatics Analysis

## Sequencing & Data Visualization

# Summary of Transcriptomic Analysis

1



## RNA Extraction

Take cells with the NAIP2 expression cassette (experimental group) and cells without NAIP2 expression cassette (CTL) and extract RNA.

2



## RNAseq

Send RNA samples out to perform sequencing, obtain reads.

3



## Galaxy Workflow

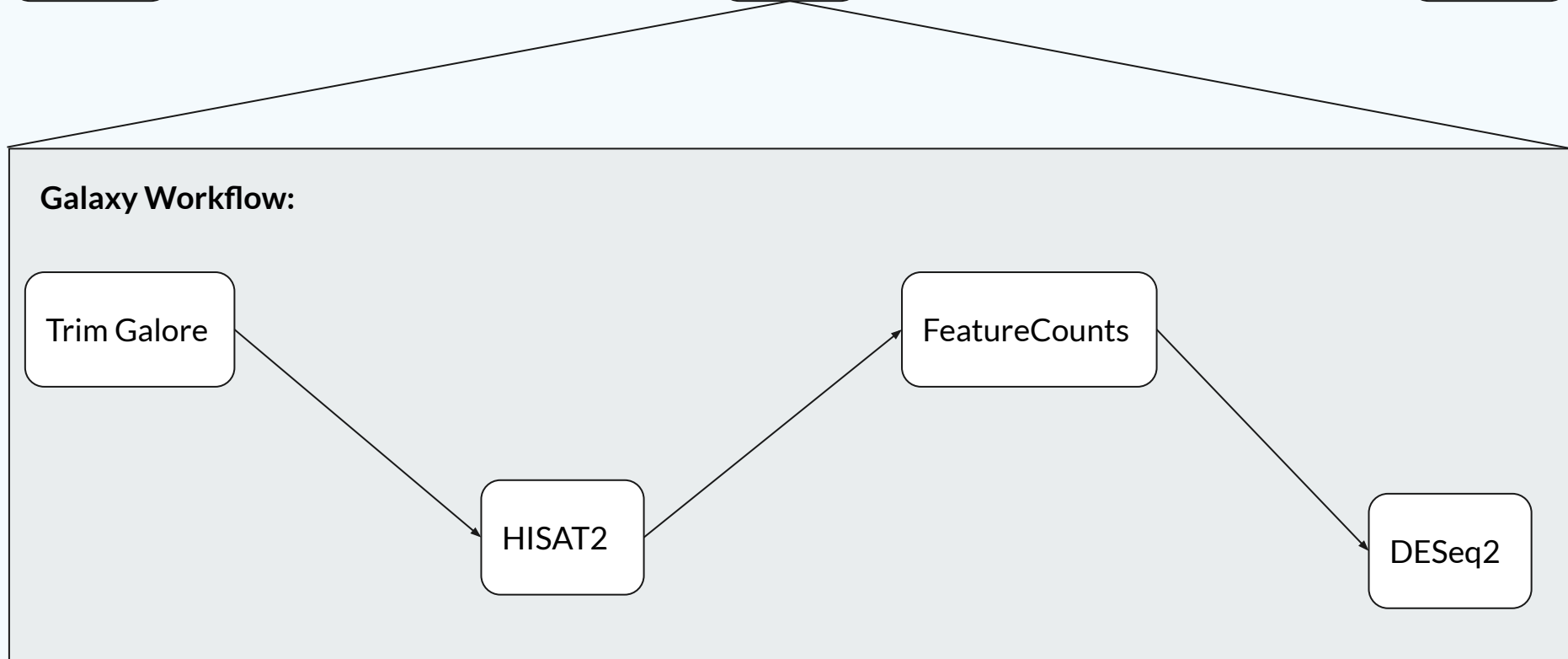
Process raw data from RNAseq so that we can interpret our results. Additionally, perform quality control checks on the data.

4

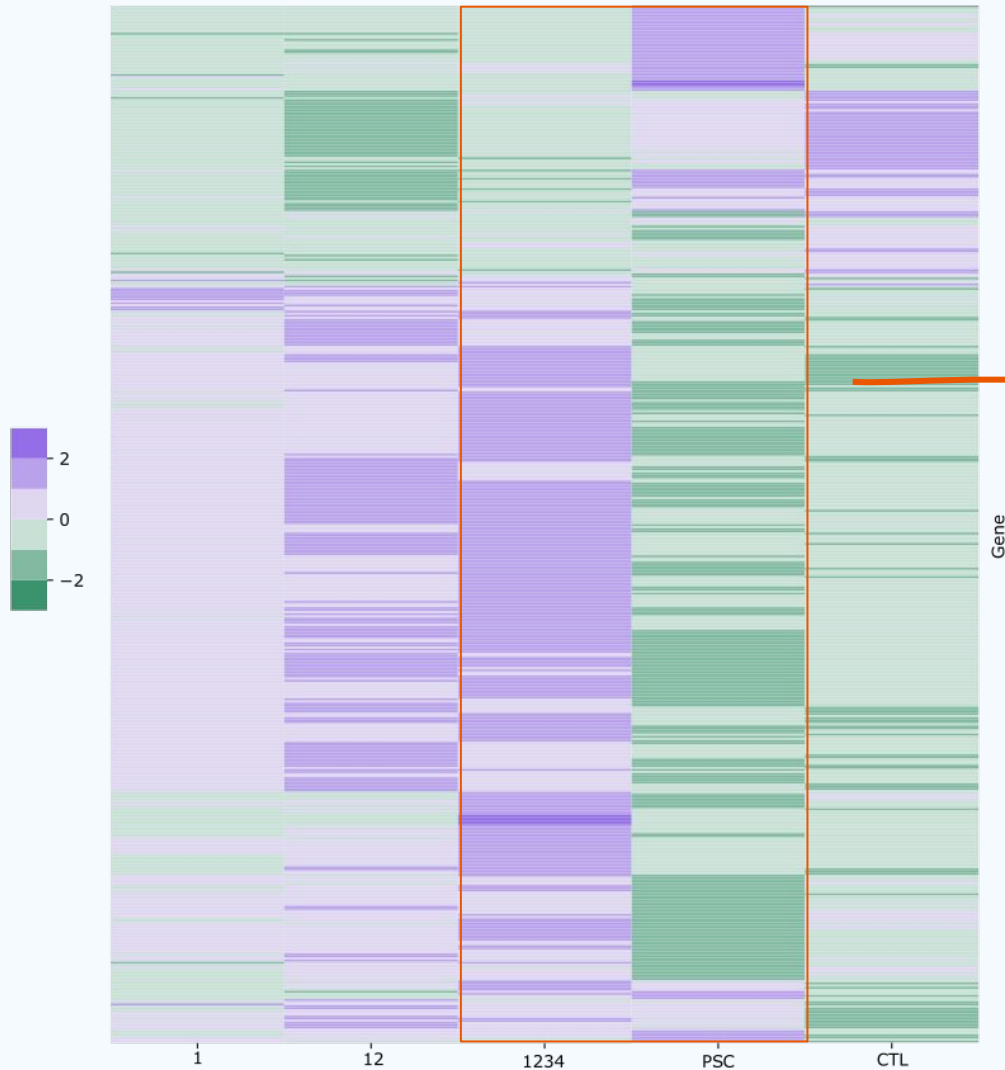


## Data Visualization & Analysis

Using expression values outputted by the Galaxy workflow (specifically DESeq2), plot data with various methods to show trends and show an RGC-like expression profile in our experimental group.

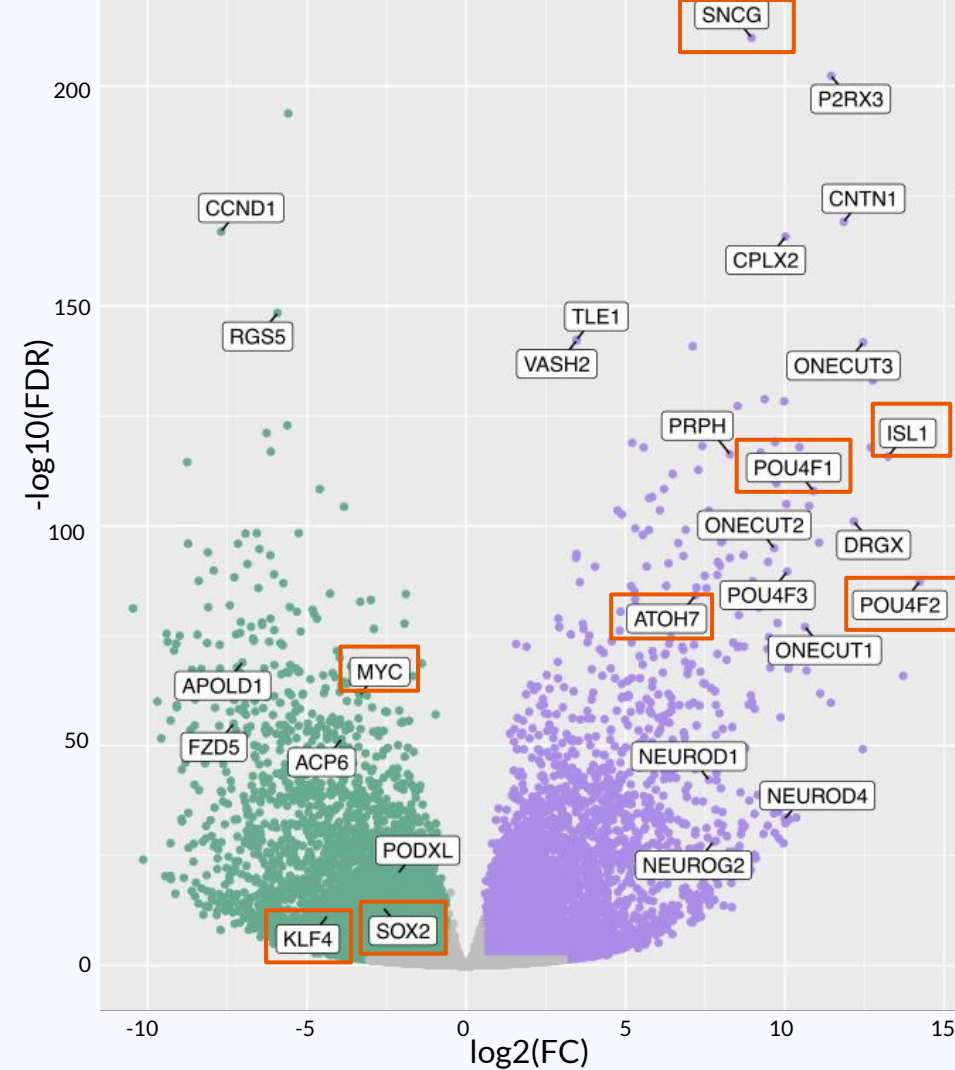


# Expression Levels of Top 500 Differentially Expressed Genes in Different Samples



Showing significant difference between exp. group and PSCs

# Relative Expression Levels of Genes Expressed in CTL sample vs exp. sample



Showing upregulation of various RGC marker genes

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# Future Directions



- Redo bioinformatic analysis, this time comparing our induced RGCs to the transcriptome of known RGC cells
- Photoreceptor Cells (PRs) are another big area of cell death leading to retinal disorders
  - induced PRs
- Other areas of the brain
  - Regeneration of dopaminergic neurons for Parkinson's

# Special Thanks!

- Dr Karl Wahlin
- Dr David Artis
- Jason Avalos, Brenda Cruz, and the URH Team
- Nick Dash, Shawna Jurlina
- Devansh Agrawal, Kevin Mazo, Hope Do, Ali Dragozova
- Grace Kim, Daphne Pham
- Samuel Levine