

Do engineered **iPS** and **ES** cells have similar molecular signatures?

Comparing expression and **epigenetics** in stem cells

George Bell, Ph.D.

Bioinformatics and Research Computing

2012 Spring Lecture Series for High School Students



What we'll do today

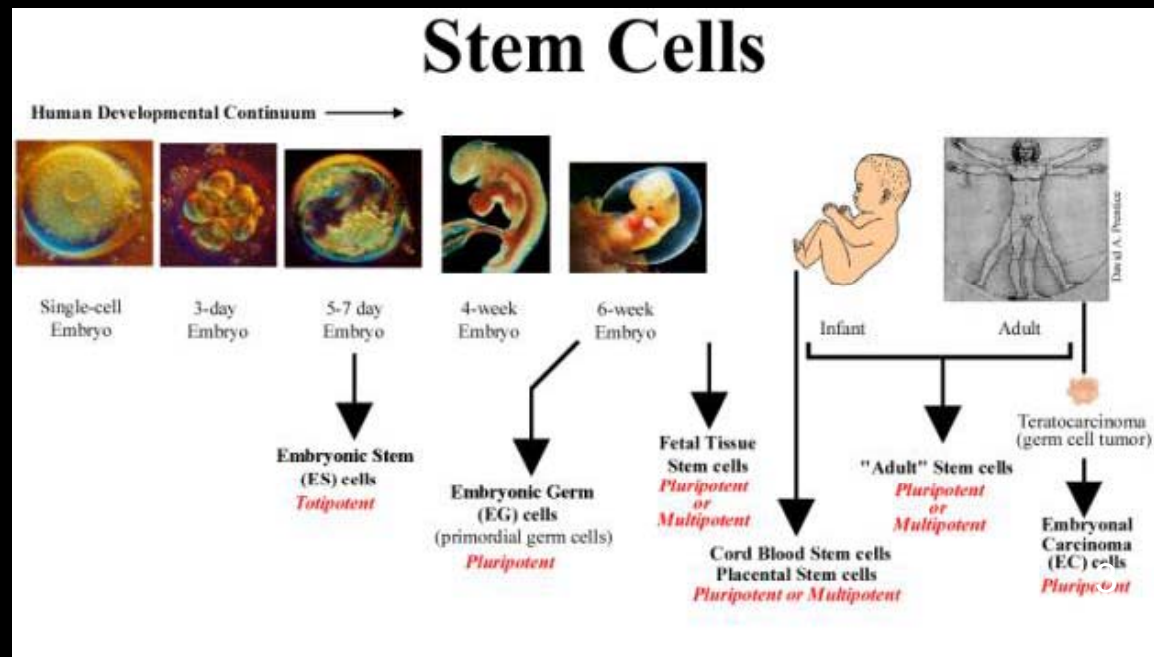
- Research questions in stem cell biology
- Measuring gene expression levels
- Compare gene levels in fibroblasts, ES, and iPS cells
- Structure and function of histones
- Assaying histone modifications
- Compare histone modifications in fibroblasts, ES, and iPS cells

Types of stem cells

- What is a stem cell, anyway?
 - ability to self-renew (and produce more stem cells)
 - ability to differentiate into different/any cell types
- Embryonic stem (ES) cells

www.stemcellresearch.org

- Adult stem cells
- Induced pluripotent stem (iPS) cells

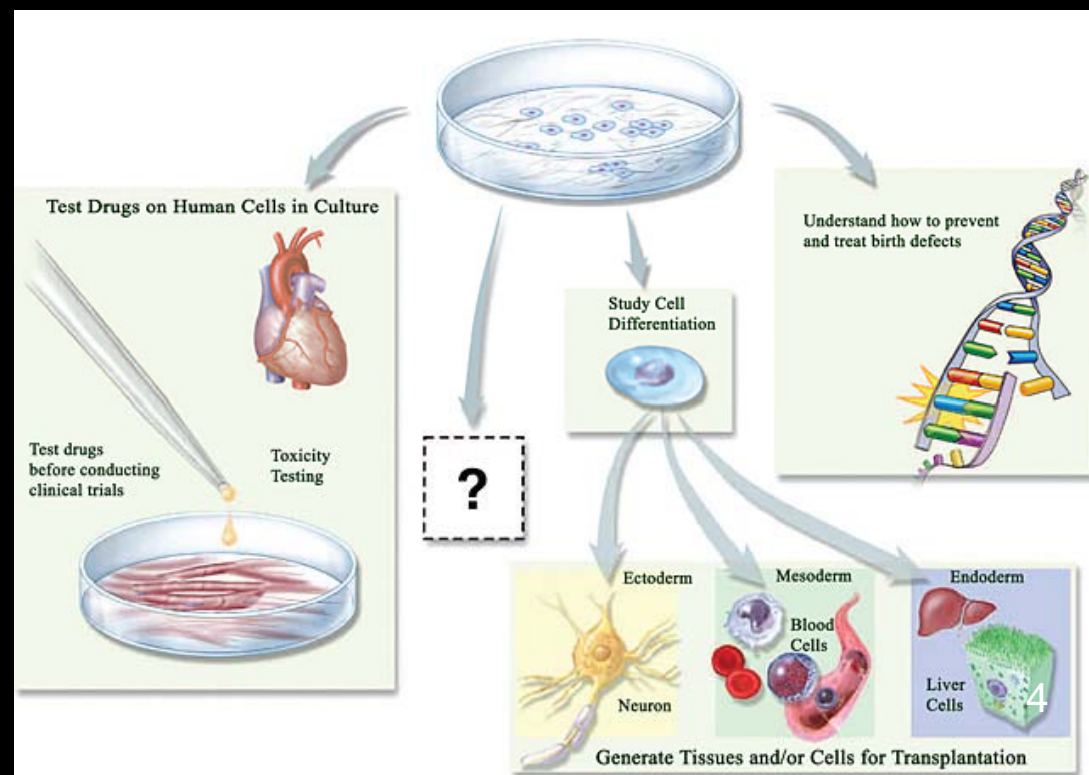


What genes are special in stem cells?

- Given that stem cells can self-renew and differentiate into many or all types of cells,
 - What genes are responsible for this behavior?

stemcells.nih.gov

- Can these genes teach us about
 - Human development?
 - Cell division?
 - Differentiation?
 - Regenerating damaged tissue?



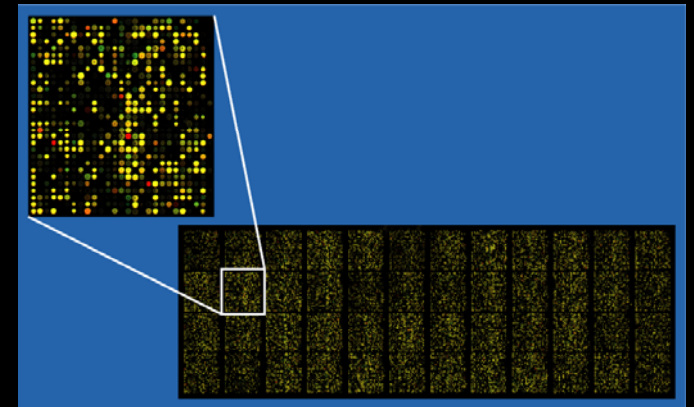
Bioinformatics

- **Bioinformatics = the application of computational methods to the field of molecular biology**
 - Also called Computational Biology
- **More and more biology experiments include lots and lots of measurements so many biologists need to**
 - Use computers to analyze data
 - Use statistics to help determine the confidence of any conclusions

Measuring levels of each gene

- DNA microarrays

- Glass slides with up to millions of spots of short DNA sequences
- When a solution of DNA (often converted from RNA) is added, genes stick to spots which are found in their sequence



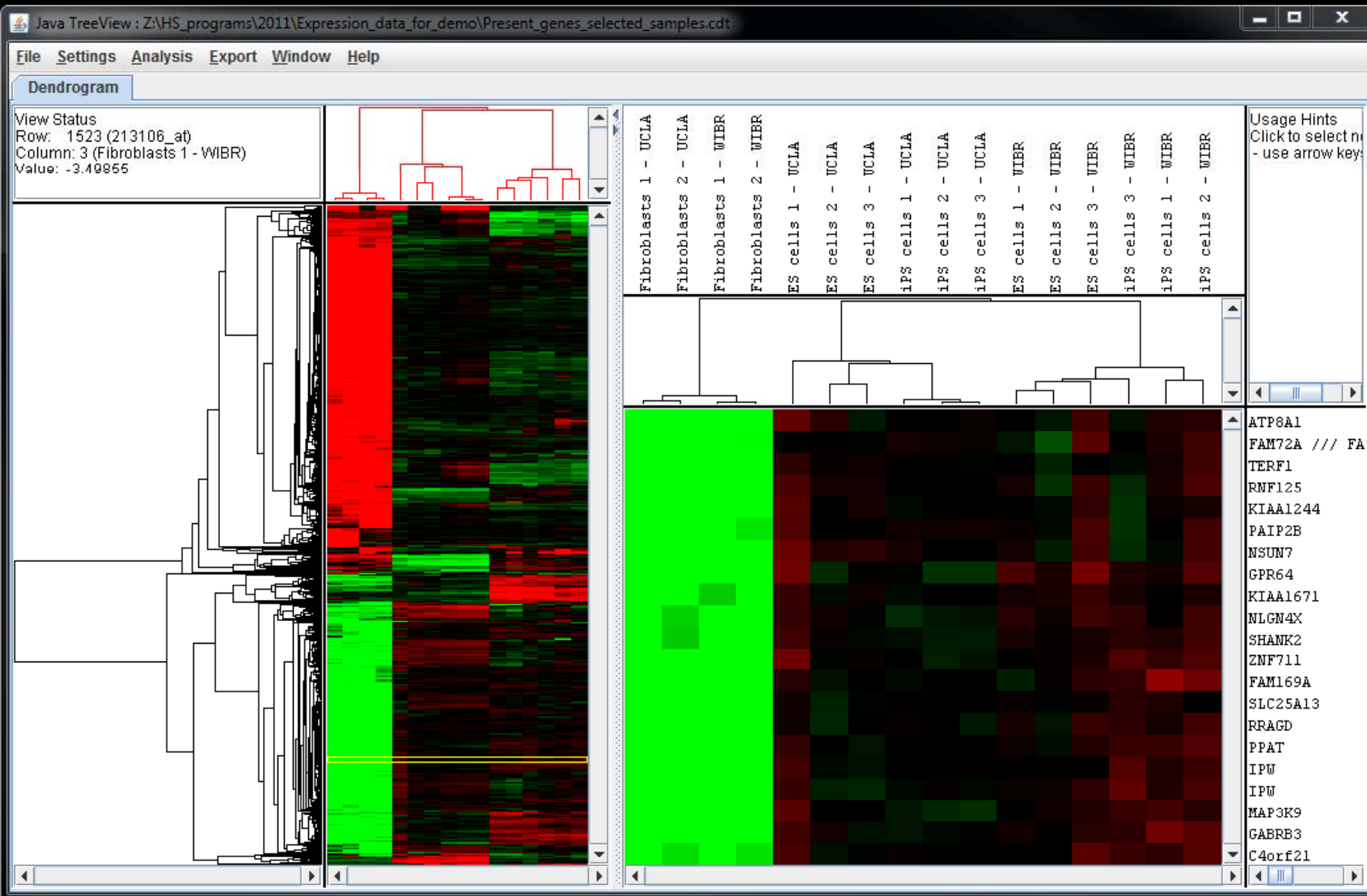
- High-throughput sequencing

- Convert RNA to DNA and break into small pieces
- Read beginning of DNA sequence

To do – open your clustered expression matrix

1. Open the program Java Treeview by double-clicking on it
2. Open your clustered expression matrix
 - File => Open
 - Select the cdt file (containing relative mRNA levels) on the Desktop in HS_Program_2012/Expression/
3. [Click on “Dismiss” if necessary]
4. With your mouse, select any interesting region of the colored panel at left.
5. What are you looking at?

To do – examine your heatmap



By convention:

Red =
Higher
than the
average
for this
gene

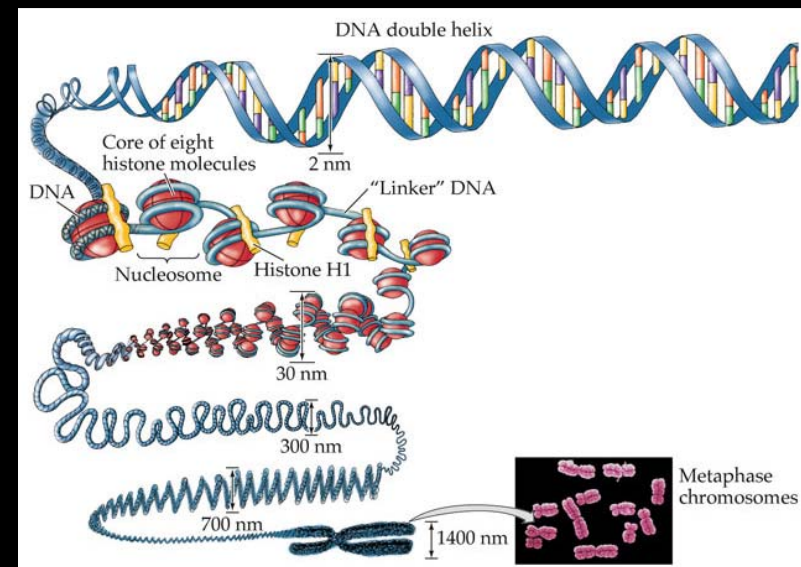
Green =
Lower
than the
average
for this
gene

Genes modified to make iPS cells

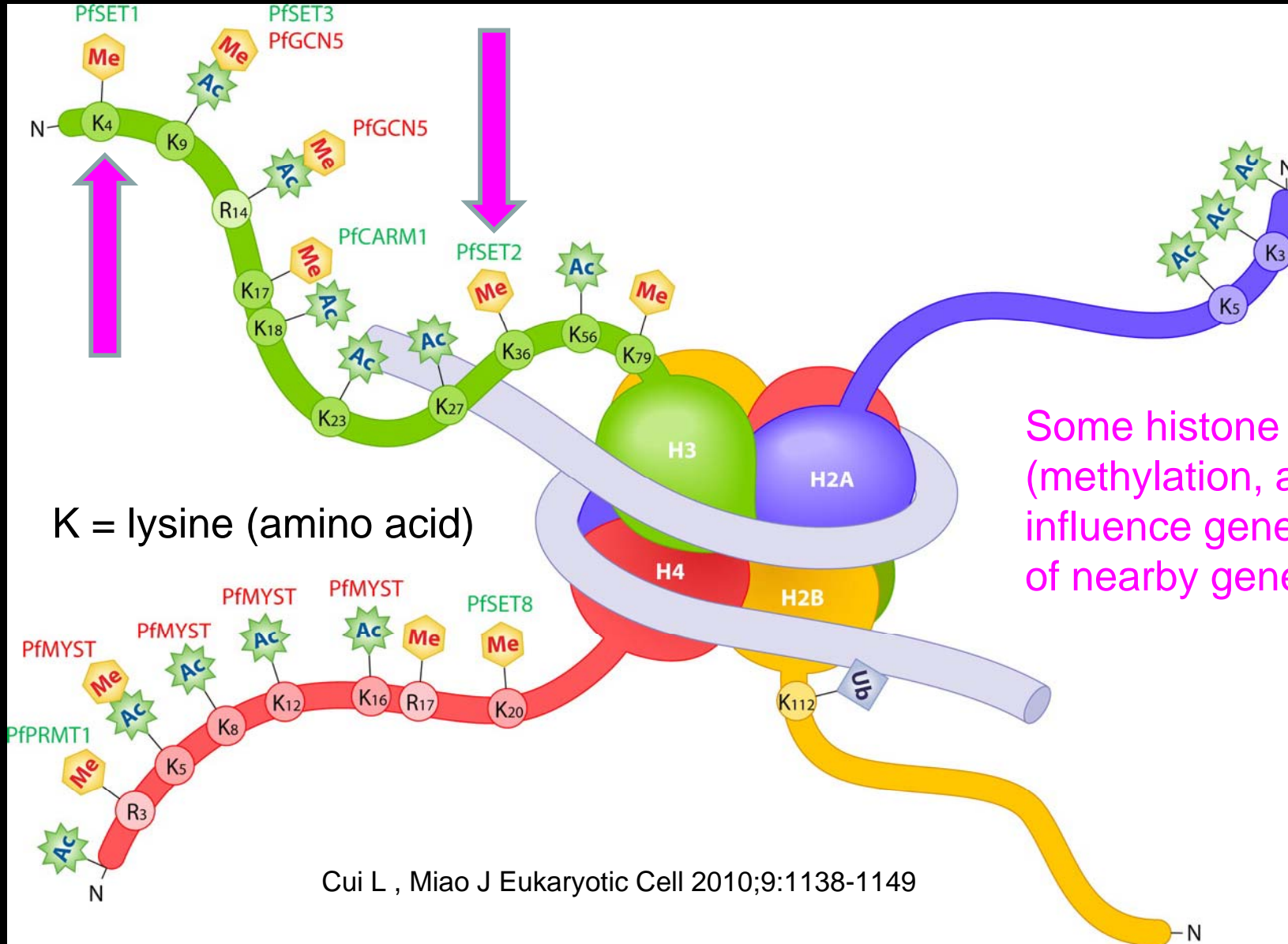
- To turn fibroblasts into iPS cells, several stem cell genes were turned on, like Pou5f1, Sox2, Nanog, and Myc
- Compared to fibroblasts, do the levels of these genes actually change?
- To answer this question, go to Analysis > Find Genes
 - Pou5f1
 - Sox2 (multiple probes)
 - Nanog
 - Myc

Epigenetics

- The study of heritable changes that involve changes other than DNA sequence
- Common epigenetic changes include
 - DNA methylation: a methyl group is added to a nucleotide
 - Example: Cytosine => 5-methylcytosine (which may turn off nearby genes)
 - **Histone** modifications: Amino acids in proteins that make up nucleosomes can be chemically modified



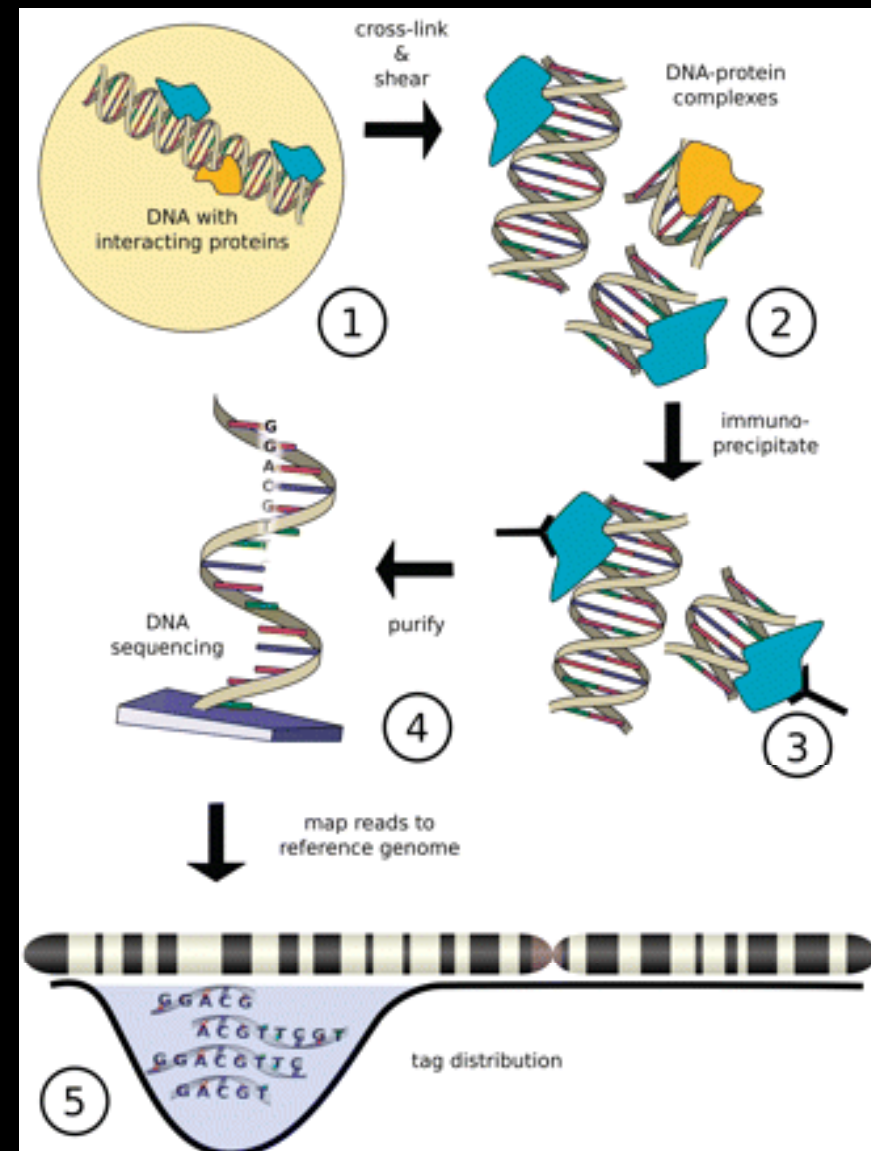
A nucleosome is made of 2 copies of 4 different histone proteins



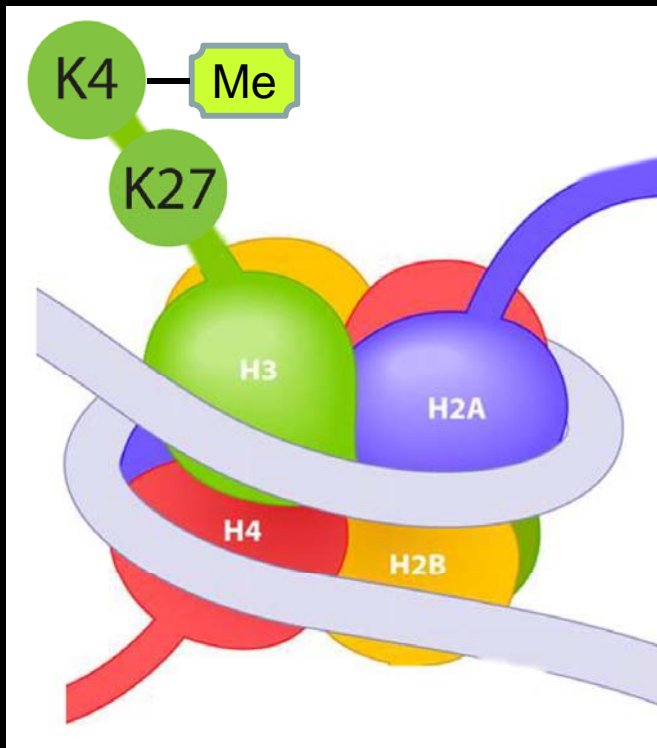
Identifying histone modifications

- Mix DNA from your cells with a specific histone antibody
- Let antibody stick to the specific modified histone
- Get DNA that wraps around this histone
- Sequence DNA
- Map DNA to genome

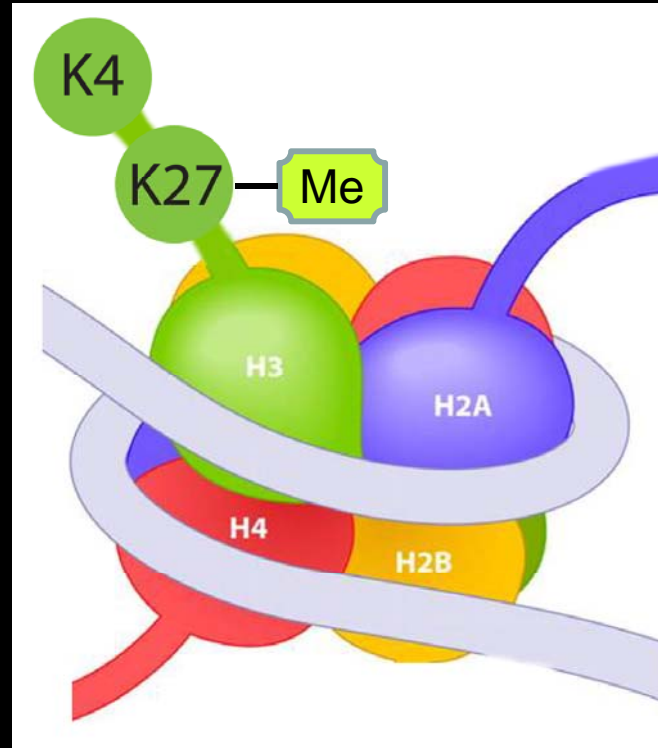
Szalkowski AM and Schmid CD (2010)



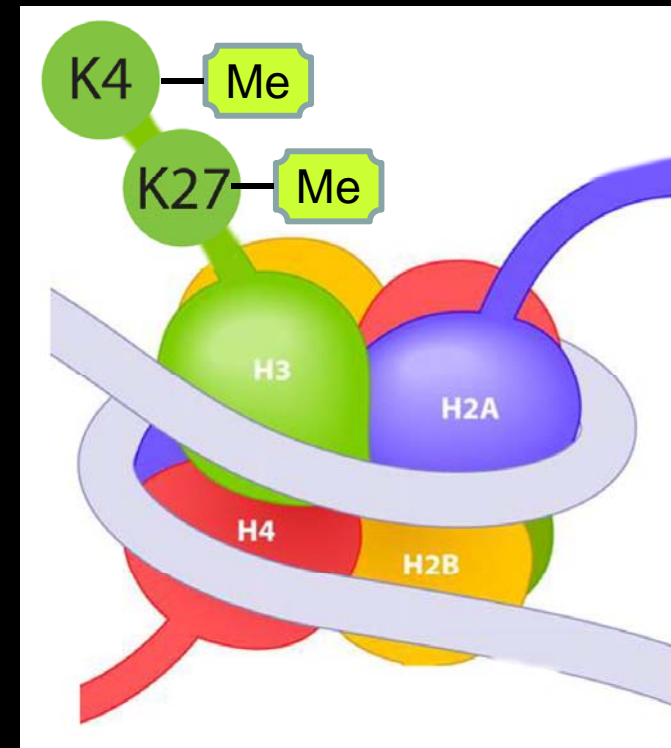
Significance of histone marks



Gene is
active

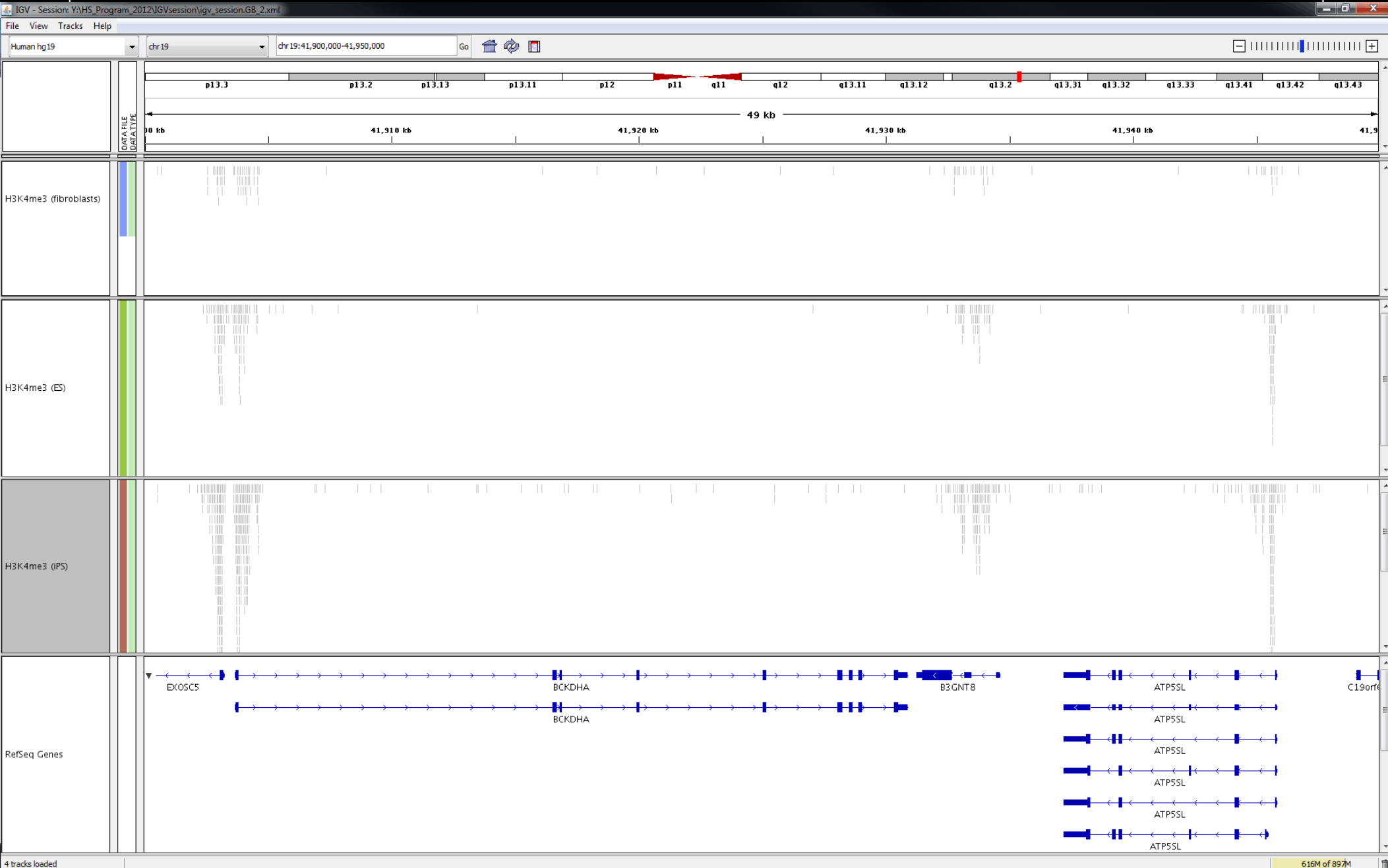


Gene is
NOT active



Gene is
NOT active
but ready for activity

Finding locations of histone modifications with a genome browser



Loading the histone modification data into the genome browser

- Open IGV (the Integrative Genomics Viewer)
- Load the short DNA reads from the histone modification analysis by
 - Go to File >> Open Session
 - Select a file on you Desktop:
HS_Program_2012/IGV/session/igv_session.xml
- Note that the names on the left indicate the histone modification (H3K4me3 or H3K27me3) and cell type

Examining the epigenetics of stem cell genes

- Where IGV shows the chromosome location (in the white box), type one at a time (and then click "Go")
 - Sox2
 - Nanog
 - Myc
 - Pou5f1 (chr6:31,130,000-31,140,000)
- For each of these, do you see a bunch of H3K4me3 and/or H3K27me3 tags (reads)?
- In what cell types?
- What does this tell you?

Other exercises

Summary

- Gene expression profiles can be used to examine gene activity
 - Microarrays
 - High-throughput sequencing
- Many genes are expressed at a different level in stem cells compared to differentiated cells
 - Almost all genes are turned on in a similar level in ES and iPS cells
- Histone-specific antibodies can identify which histone modifications occur throughout the genome.
- Many epigenetic marks are different between stem cells and differentiated cells
 - Most histone marks appear in the same places in the genomes of ES and iPS cells
- So are engineered iPS cells and embryonic stem cells the same?