

# What we'll do today

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

### Comparing expression and epigenetics in stem cells

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2012 Spring Lecture Series for High School Students



- 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

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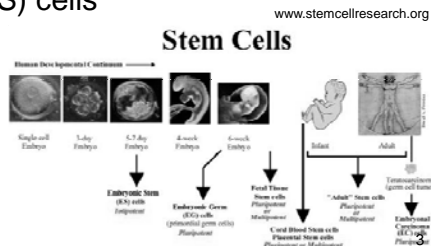
## Types of stem cells

- 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

### Adult stem cells

### Induced pluripotent stem (iPS) cells

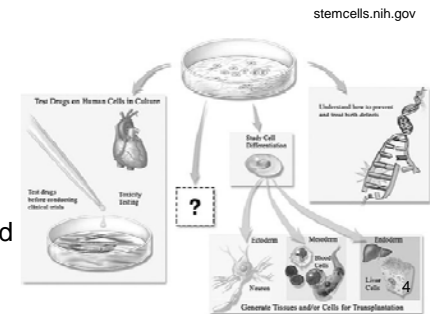


## What genes are special in stem cells?

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

### Can these genes teach us about

- Human development?
- Cell division?
- Differentiation?
- Regenerating damaged tissue?



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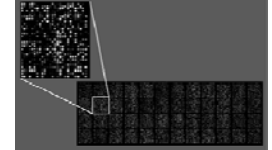
# Bioinformatics

- 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

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# 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



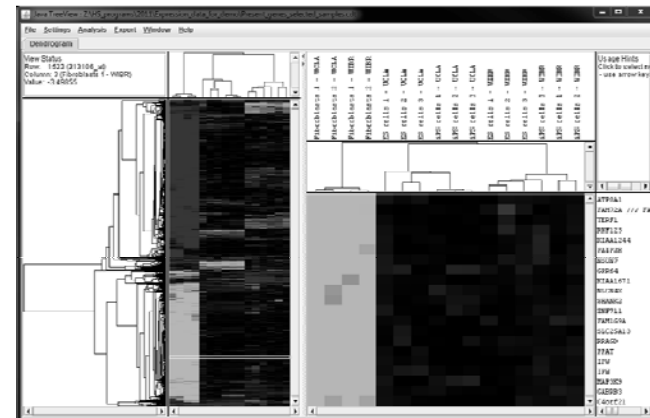
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## To do – open your clustered expression matrix

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?

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## To do – examine your heatmap



By convention:

Red = Higher than the average for this gene

Green = Lower than the average for this gene

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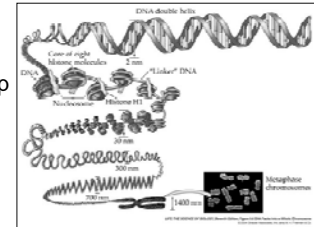
## Genes modified to make iPS cells

- 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

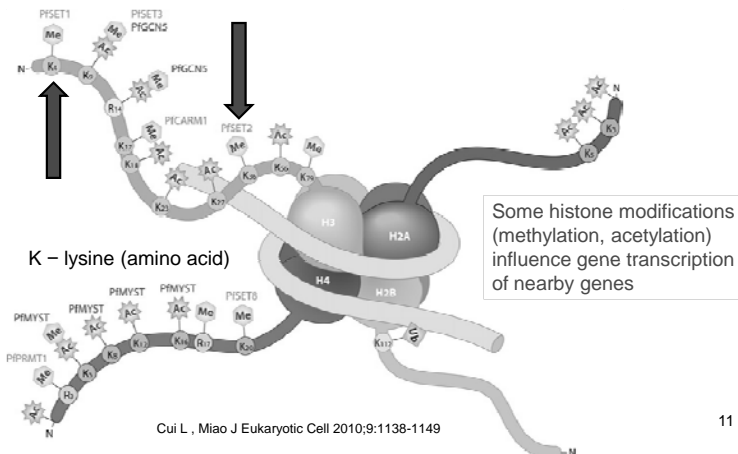
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## Epigenetics

- 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



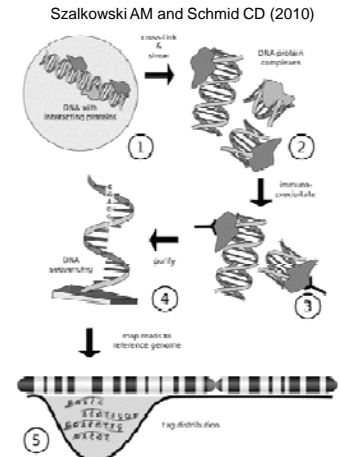
## A nucleosome is made of 2 copies of 4 different histone proteins



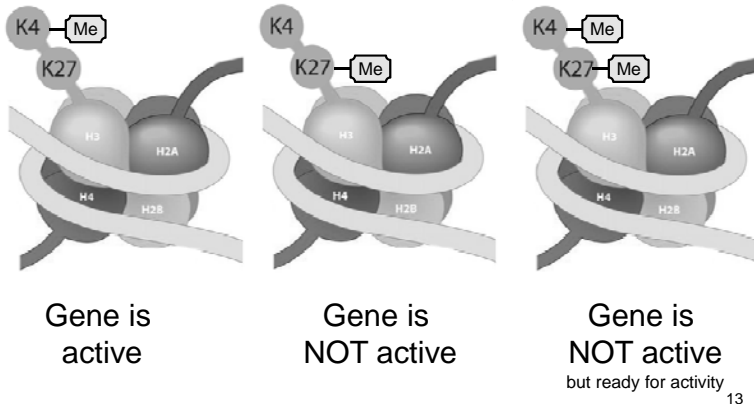
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## Identifying histone modifications

- 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



## Significance of histone marks



## 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/IGVsession/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

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## 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?

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