

What's special about stem cells?

Using molecular profiling
to look at gene activity

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Bioinformatics and Research Computing

2011 Spring Lecture Series for High School Students



What we'll do today

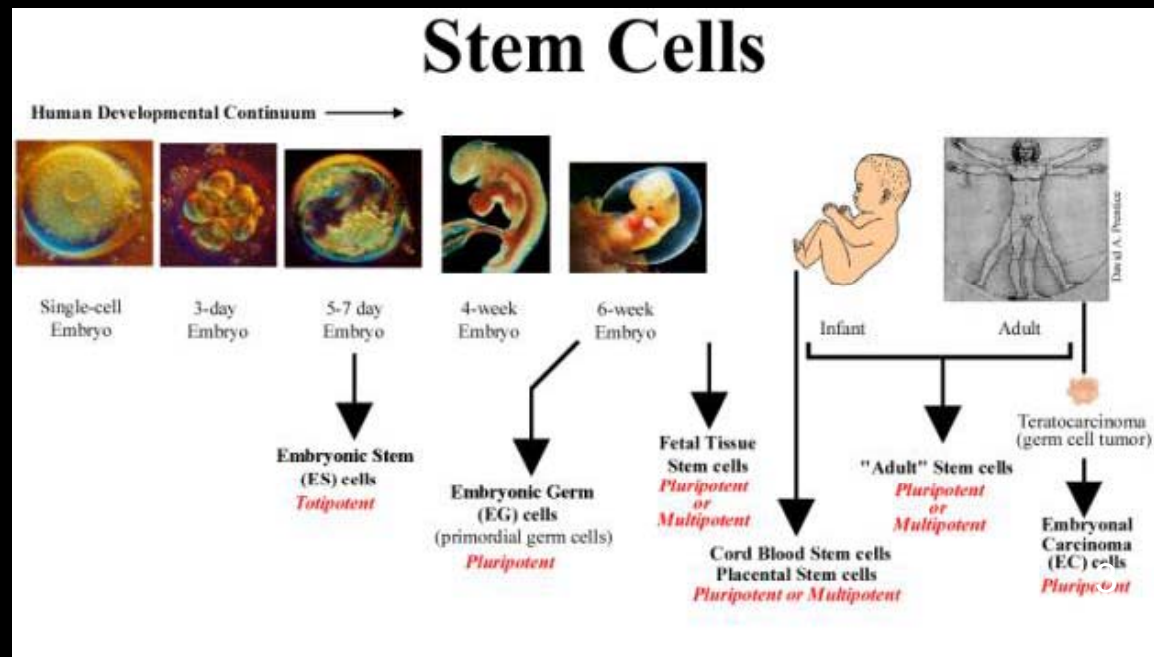
- Research questions in stem cell biology
- Measuring gene expression levels
- Starting with gene levels in different stem cells and other cells
 - Identify most variable genes
 - Get relative gene levels
 - Cluster to group most similar genes and most similar cell types
- Compared to differentiated cells
 - What genes are changed in all stem cells?
 - What genes are changed in some types of stem 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 cells

www.stemcellresearch.org

- Adult stem cells
- Induced pluripotent stem cells

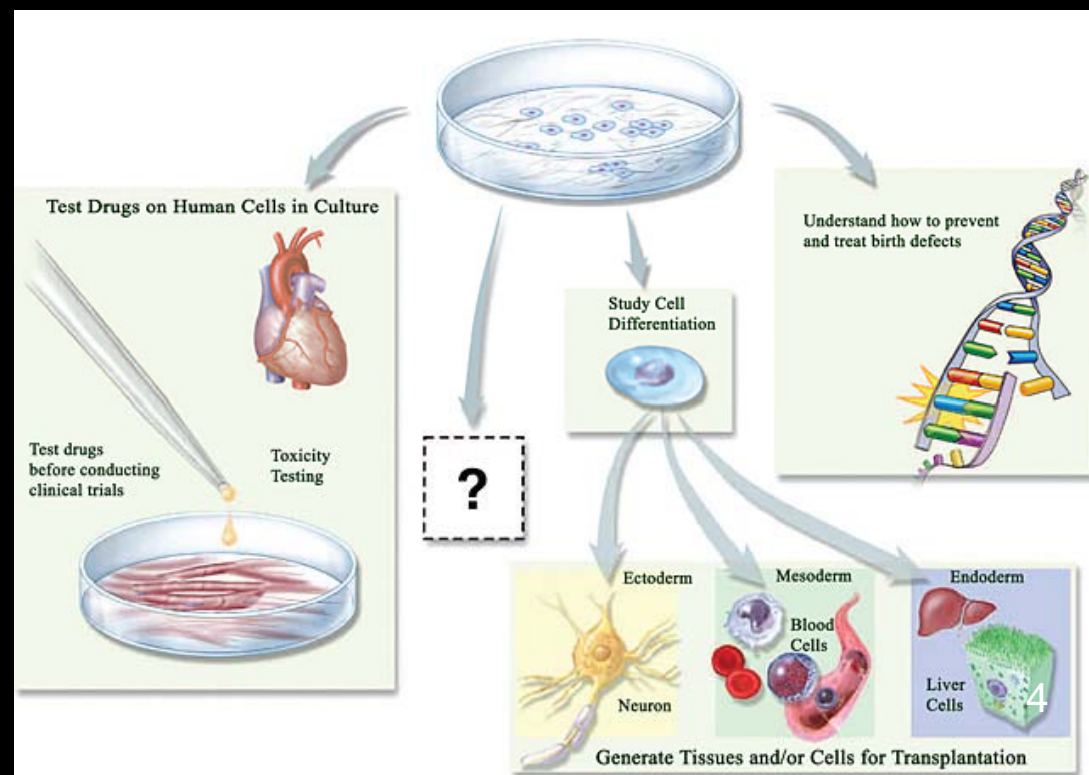


What genes are special in stem cells?

- 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

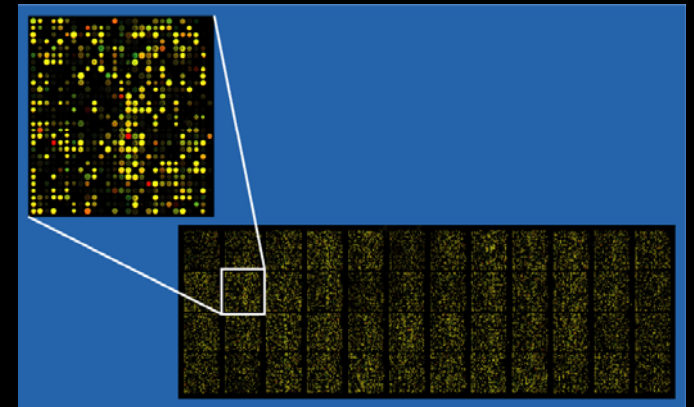
- Can these genes teach us about
 - Human development?
 - Cell division?
 - Differentiation?
 - Regenerating damaged tissue?



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 short DNA sequence from one or both ends

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

Using math to understand biology

- Log transformations (bases) $\log_2 8 =$
- Median $\text{median } \{5, 8, 10, 12\} =$
- Standard deviation (to measure variability)
- Mean/median centering
- $\text{Log } (A/B) = \log A - \log B$

values	32	256	1024	4096
log2	5	8	10	12
Median centered	$5 - 9 = -4$	$8 - 9 = -1$	$10 - 9 = 1$	$12 - 9 = 3$

Sources of expression data

- The final step of an experiment is usually publishing the project in a journal
- When a project is published, all of the data may be made public so
 - Others can verify the findings
 - Others can use the data to help with their research
- The National Center for Biotechnology Information (NCBI) hosts much of this data on their web sites
- Today's data is from:
 - Whitehead Institute (Guenther et al., 2010)
 - UCLA (Lowry et al., 2008; Chin et al., 2009)

Matrix of gene “expression levels”

- Each column represents a cell type
- Each row represents a gene
 - The levels of some genes is measuring from more than one spot (probe) on the microarray

Probe	Gene symbol	Fibroblasts	ES cells	iPS cells
220184_at	NANOG	5	11	12
208286_x_at	POU5F1	7	13	12
228038_at	SOX2	4	12	13

Matrix of gene “expression levels” (details)

- Open Expression_log2_values_HS_2011.xlsx and look at
 - The first row
 - The first two columns
 - What information is shown?
- The numbers represent
 - Levels of mRNA
 - Measured by the amount of dye-bound DNA that binds to a DNA probe (a spot containing a gene tag)
 - Log2-transformed
 - Since $2^{10} = 1024$,
 - A RNA level of 1024 has been converted to 10

To do – Select most variable genes

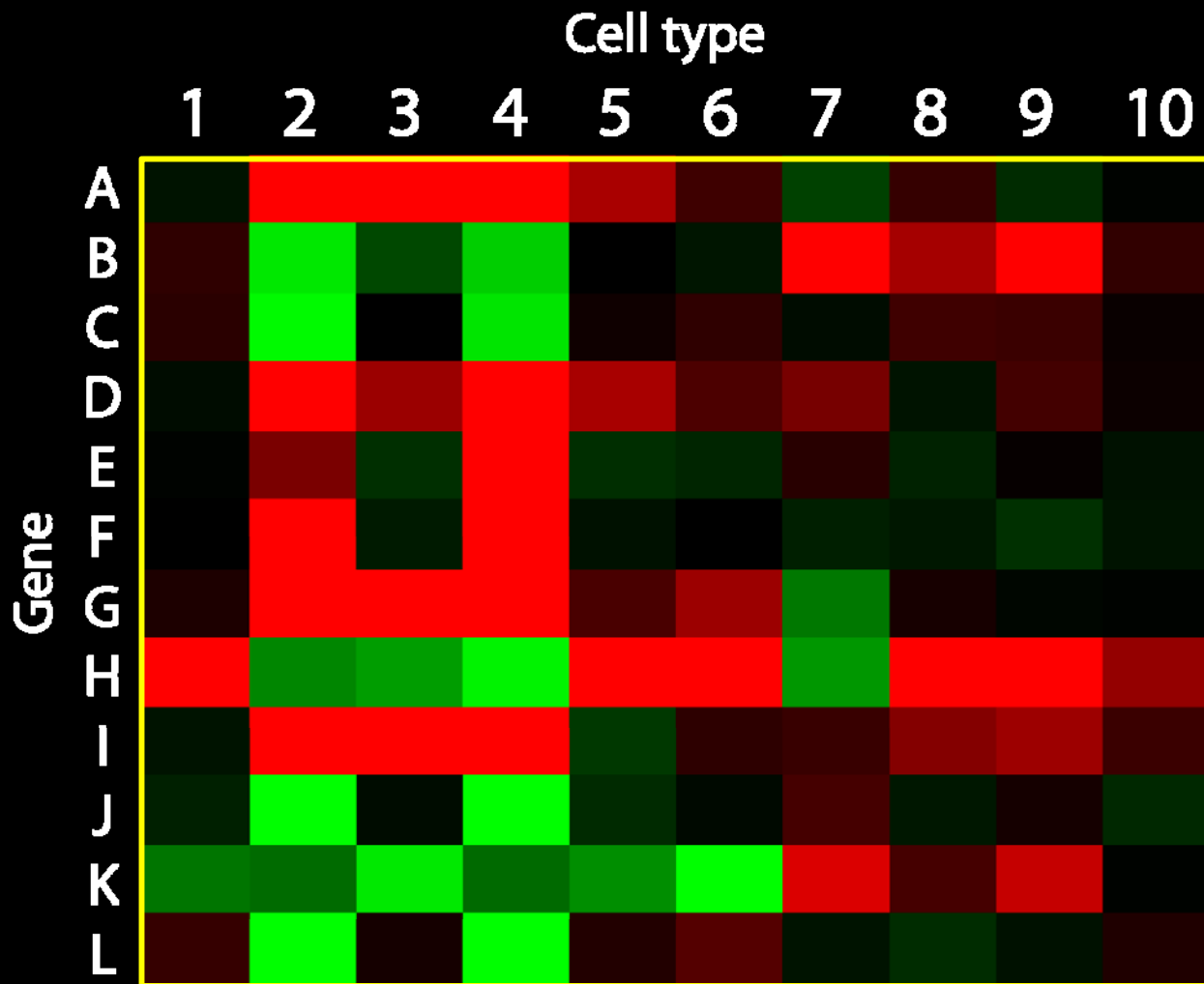
1. Open the matrix of log₂ expression values (Expression_log2_values_HS_2011.txt) in Cluster.
 - File => Open data file
 - How many genes are you starting with?
2. Go to “Filter Data” tab (to remove genes with relatively constant levels)
 - Check “SD (Gene Vector)”
 - Enter 1.5 in following box (to filter out genes with a standard deviation < 1.5)
 - Click on “Apply Filter”
 - How many genes remain after filtering?
 - When complete, click on “Accept [Filter]”

To do – get relative gene levels

While in Cluster

- Click on the “Adjust Data” tab
- Check “Center genes” and select “Median” which will
 - Find the median gene level of each gene in all cell types
 - Subtract the median from each gene level which will set it
 - to 0 if its level is the median level
 - to a positive value x (if 2^x -fold above the median)
 - to a negative value y (if 2^{-y} -fold below the median)
 - Help us identify genes that increase or decrease their levels
- Click on “Apply” to center your expression matrix
- Look at the bottom of the program to make sure it says “Done adjusting data”

Displaying a matrix as a heatmap



$\log_2(\text{level} / \text{median})$

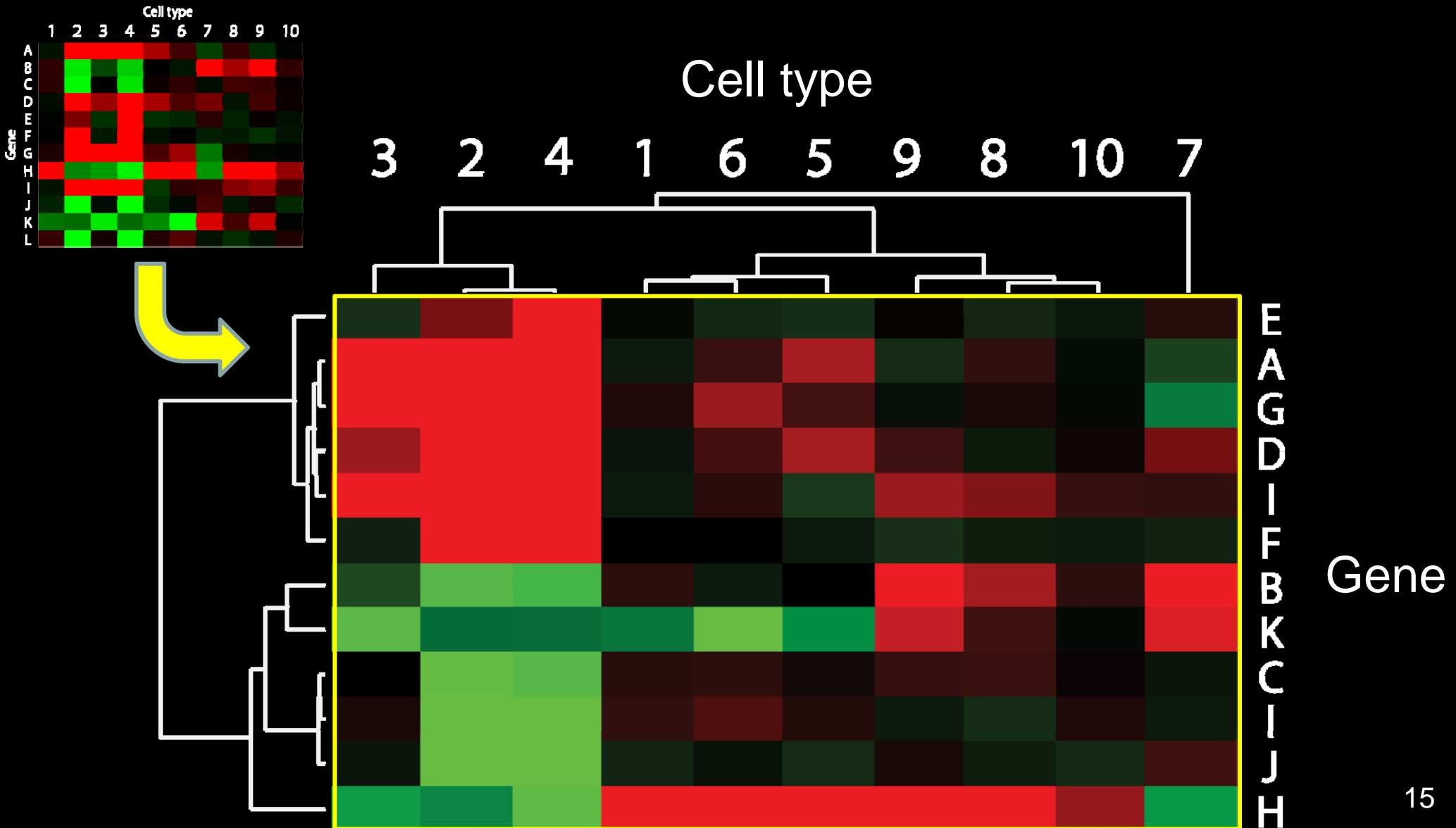


How can we re-order cell types and genes so similar ones are nearby?

Clustering an expression matrix

- To be able to better see changes in the levels of genes, we want to
 - Re-order genes so similar ones are closer
 - Re-order cell types so similar ones are closer
- For genes, our program is going to
 - compare every gene (row of numbers) to every other gene
 - Draw a tree showing how close each gene's "profile" is to every other gene's profile
 - Use the tree to order the genes in a new matrix
- The same thing will be done with cell types (columns)

Hierarchical clustering output



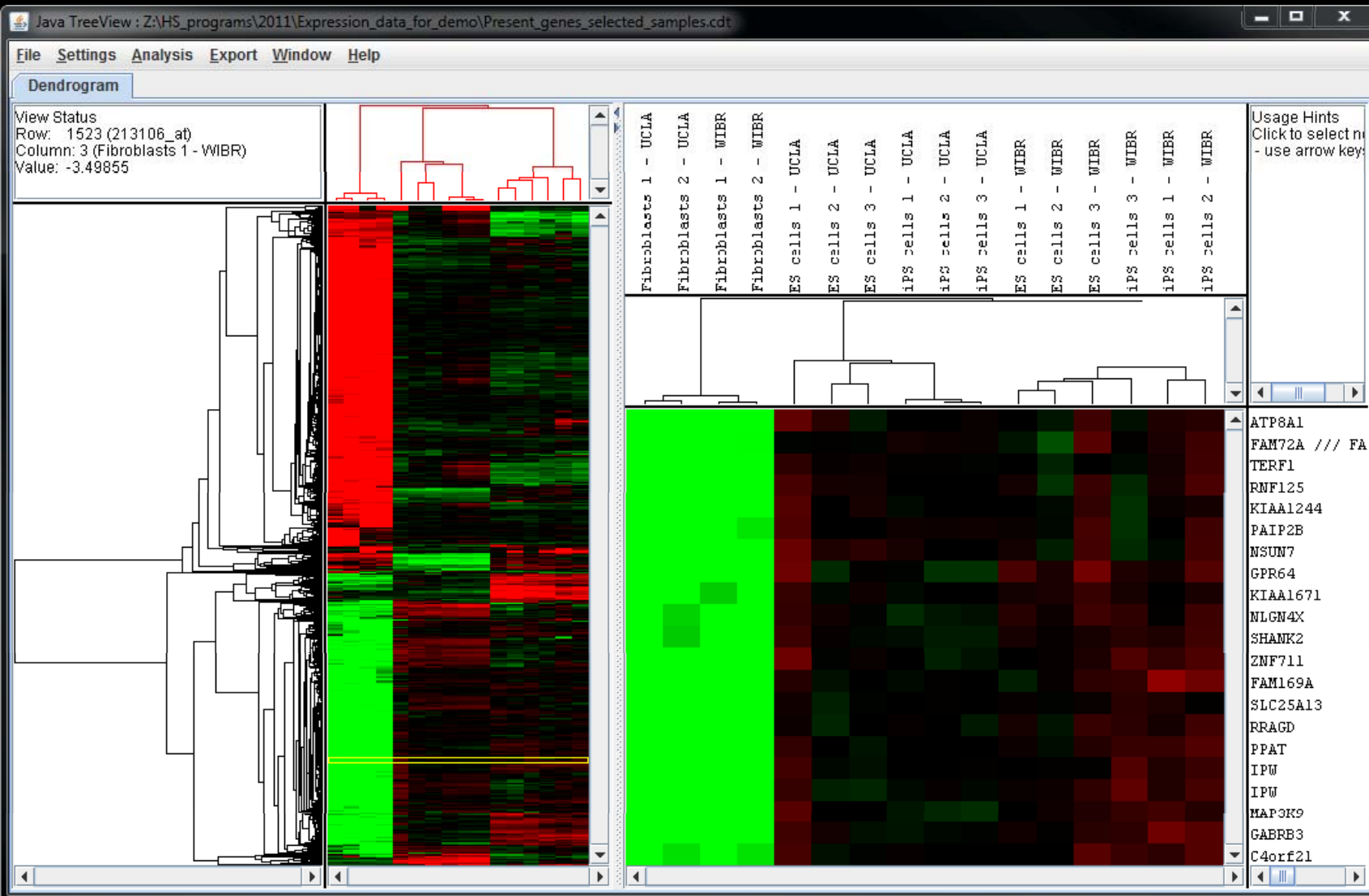
To do – cluster by gene and cell type

- While in Cluster
 - Click on the “Hierarchical” tab
 - In the Genes square, check “Cluster”
 - In the Arrays (cell types) square, check “Cluster”
 - To perform the actual clustering to re-order rows and columns,
 - Click on the “Average linkage” box
 - Look at the bottom of the program to make sure it says “Done Clustering”

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 that you created with Gene Cluster
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
median
for this
gene

Green =
Lower
than the
median
for this
gene

What genes are changed in stem cells?

- Compared to fibroblasts, how do the levels of certain genes change?
- Go to Analysis > Find Genes
 - Pou5f1
 - Sox2 (multiple probes)
 - Nanog
 - Xist (multiple probes)
 - Runx1
 - Fgf7 (multiple probes)
 - Twist2 (multiple probes)

How do ES and iPS cells compare?

- Compared to embryonic stem cells, are any genes
 - Higher in iPS cells?
 - Lower in iPS cells?
- Which genes seem to be laboratory-specific?
- Any other interesting expression patterns?

Getting information about genes

Using the gene symbol

- Method 1: Search the Web with the gene symbol
- Method 2: NCBI Gene
 - <http://www.ncbi.nlm.nih.gov/gene/>
- Method 3: GeneCards
 - <http://www.genecards.org/>
- Access these resources via Java Treeview
 - Go to Settings > URL Settings
 - In the box, type
 - <http://www.ncbi.nlm.nih.gov/gene?term=HEADER>
 - <http://www.genecards.org/cgi-bin/carddisp.pl?gene=HEADER>
 - Select NAME
 - Check “Enable” and click on “Close”
 - Back on the detailed heatmap, click on a gene symbol

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
 - Some genes are consistent in all stem cells
 - Some genes seem to be different in some types of stem cells
- Current research addresses:
 - Which of these “stem cell genes” are biologically important?
 - Exactly what do these important genes do?