

Transcriptomics: measuring across the genome

BIO311 01/31/2017

Preview

- Experimental design
- Microarrays
- RNA-Sequencing (RNA-seq)
- Example: yeast cell cycle

What is a genome?

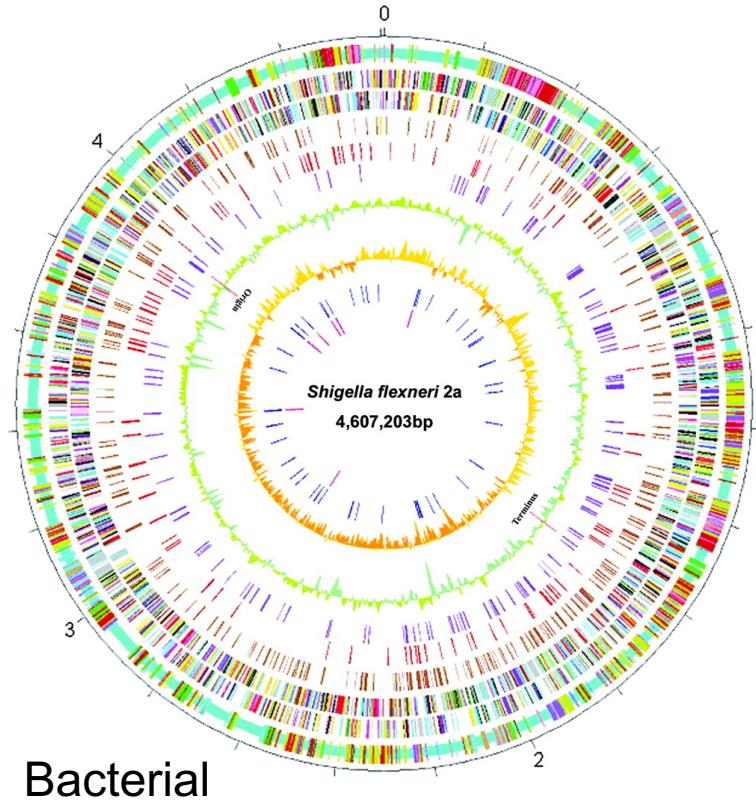


Image from Nie et al., 2006, 7:173

What is a genome?

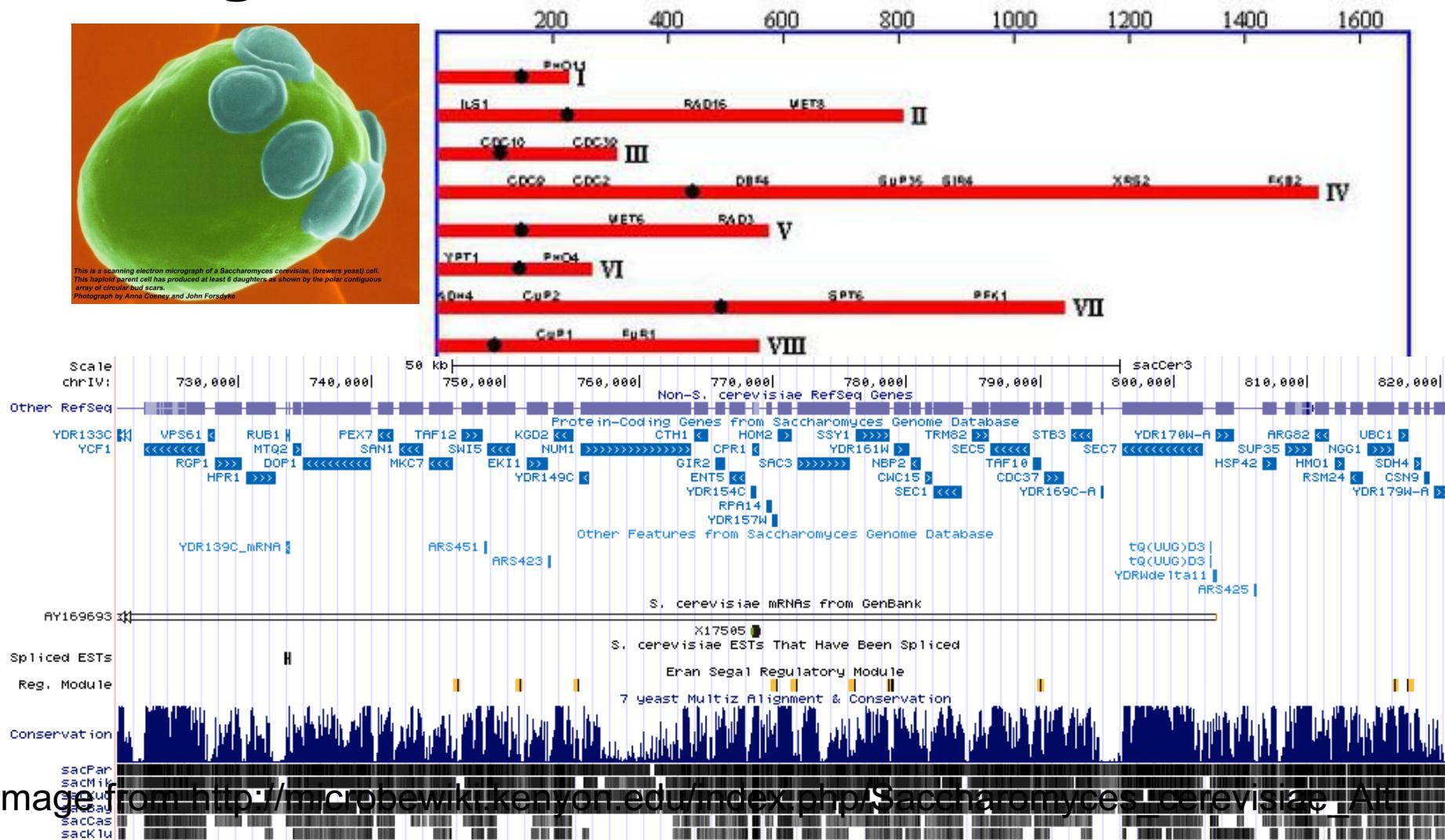
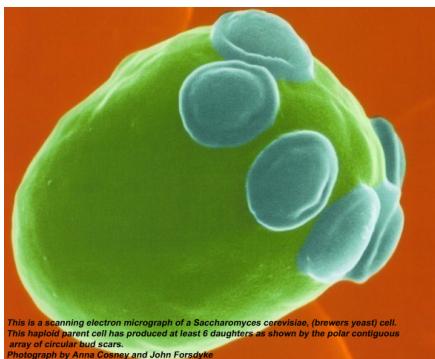


Image from UCSC genome browser, genome.ucsc.edu

Genome wide expression analysis

- Goal: to measure _____ all genes in genome
- RNA levels vary with the following:
 - Cell _____
 - Developmental stage
 - _____
- _____ of expression under various growth conditions provide useful information as to _____.

Experimental design

- A CRUCIAL component for getting biological meaningful results (otherwise, _____).
- Understanding the _____ is required for _____.
- Example: Yeast cell cycle (synchronization, mutants vs wild type)

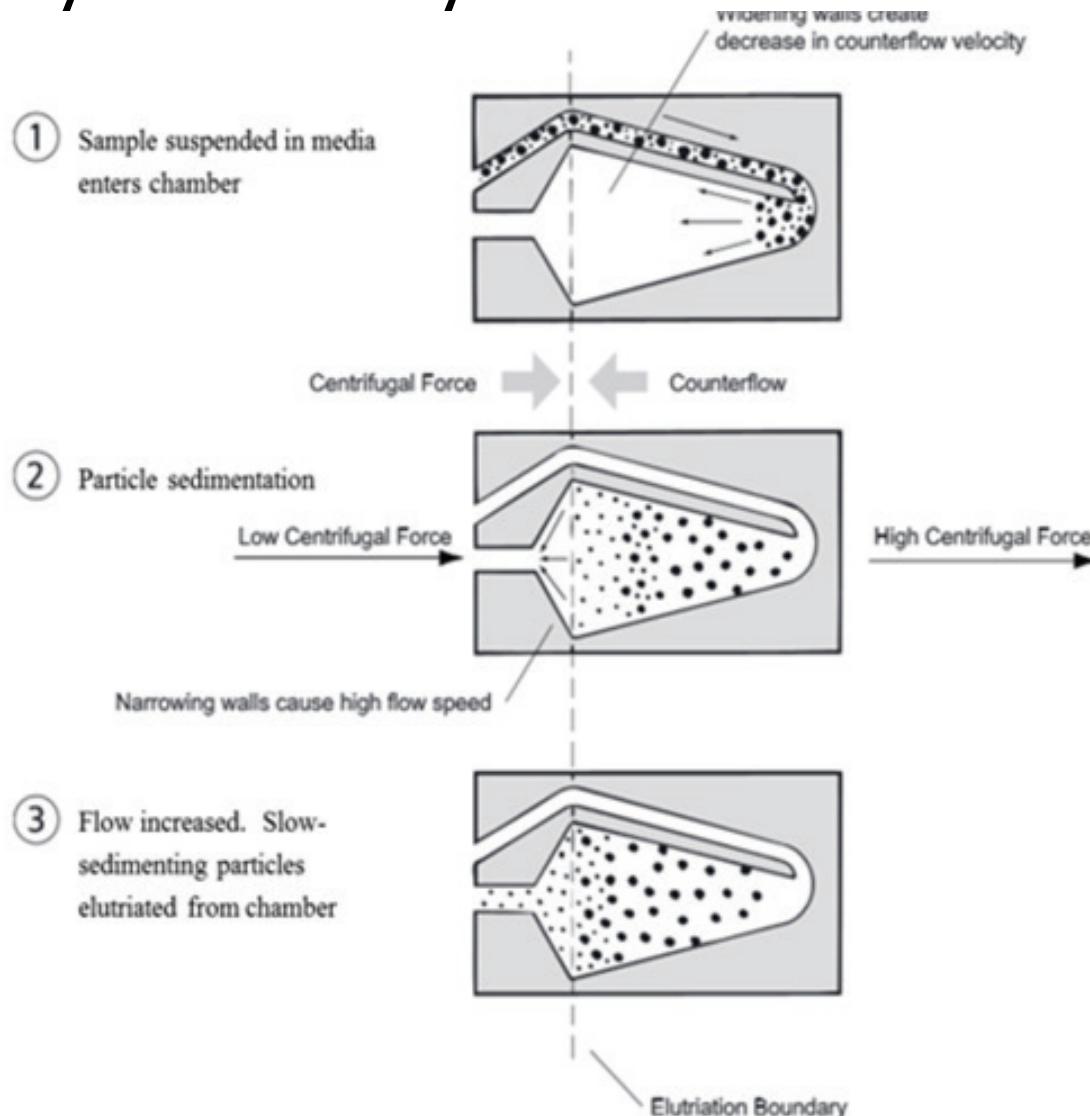
Spellman et al., experimental design

1. _____ cells in the cell cycle
2. _____ cells from _____
3. Measure _____ over time

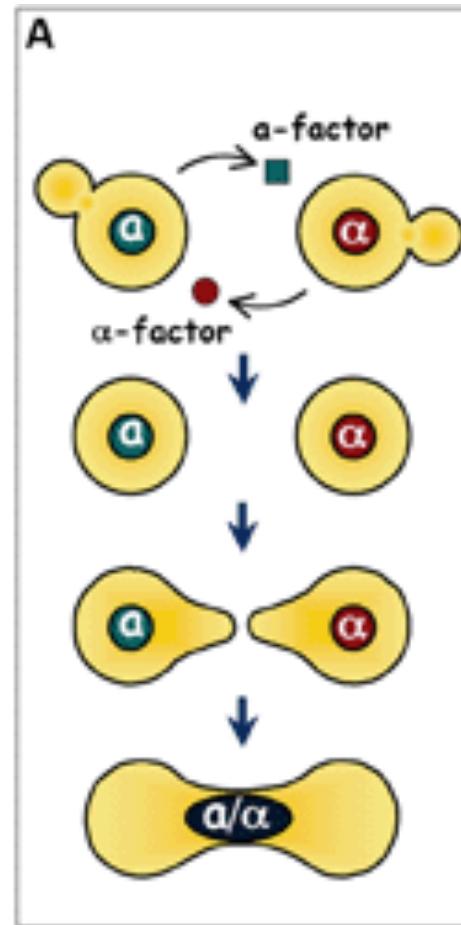
Synchronization

- Elutriation
- Alpha factor
- cdc15

Mechanical synchrony



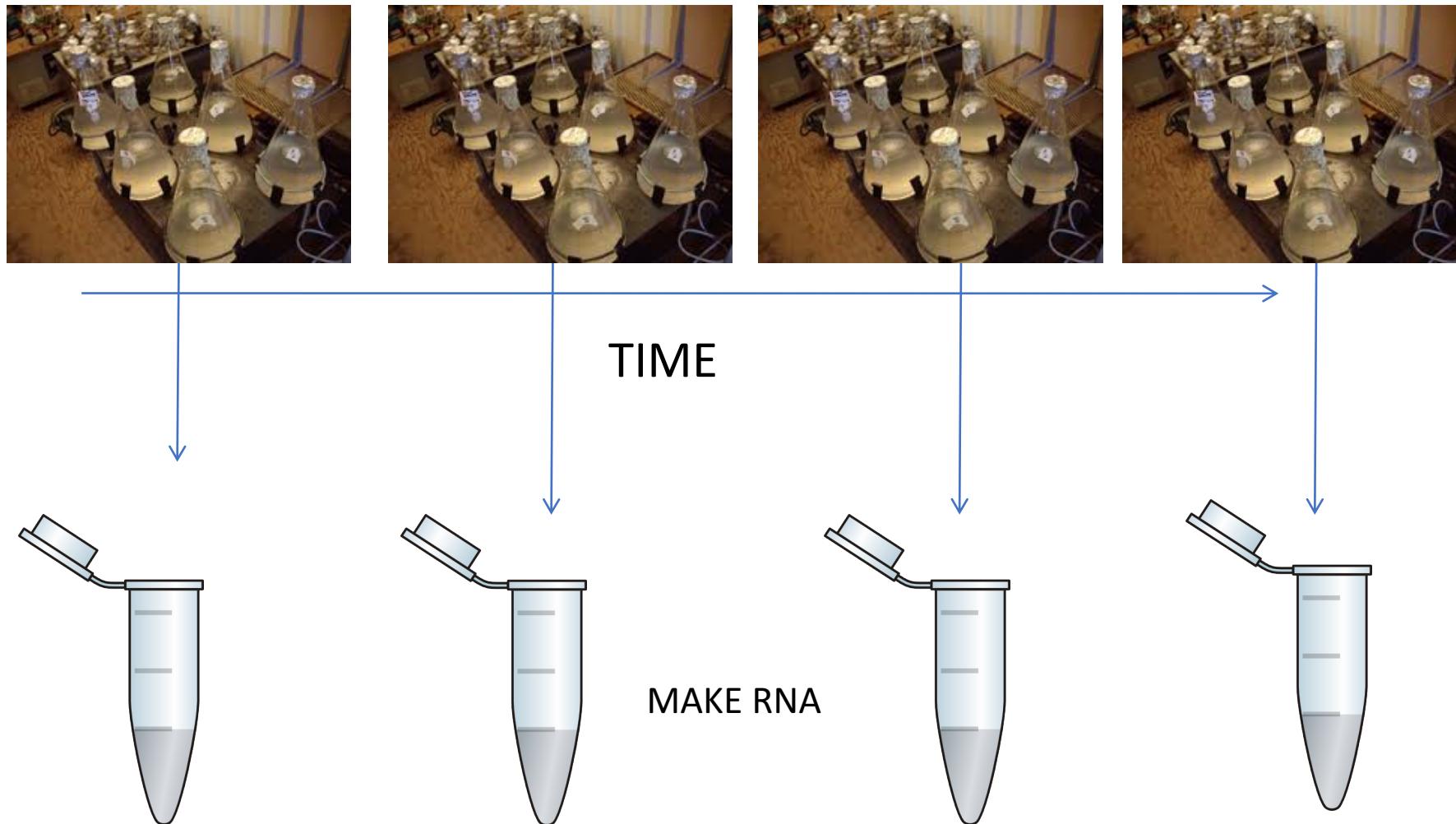
Chemical synchrony



Genetic synchrony

- Cdc15 – temperature sensitive mutant.
- 30C – cells go through cell cycle normally
- 37C – cells arrest.
- Back to 30C – release arrest and measure transcription over time.

Experimental design: yeast cell cycle (cont'd)



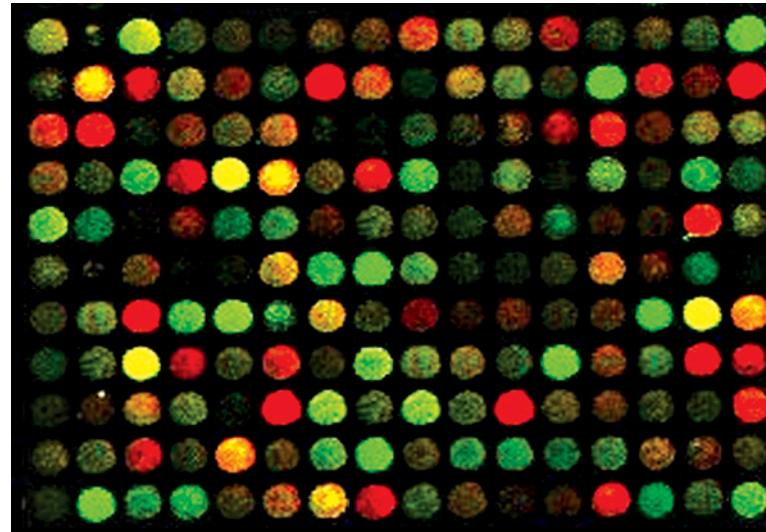
Basics of microarrays

- DNA attached to

 - Glass or plastic
- RNA is

 - Usually indirectly
- Bound DNA is the

 - Labeled RNA is the “target”



Hybridization

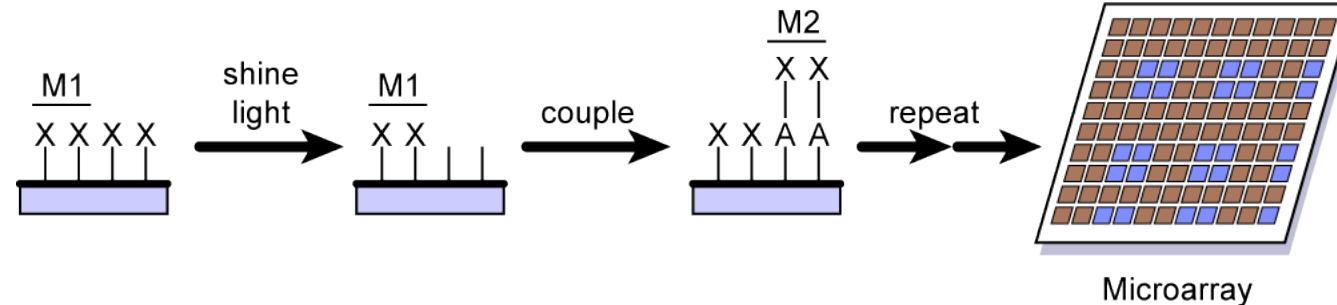
- Measurements of RNA abundance by microarrays based on

 - Between _____ strands of RNA and DNA
 - Or two complementary DNA strands
- Movie: [microarray animation*](#)

*Thanks to Dr. Malcom Campbell, Davidson College!

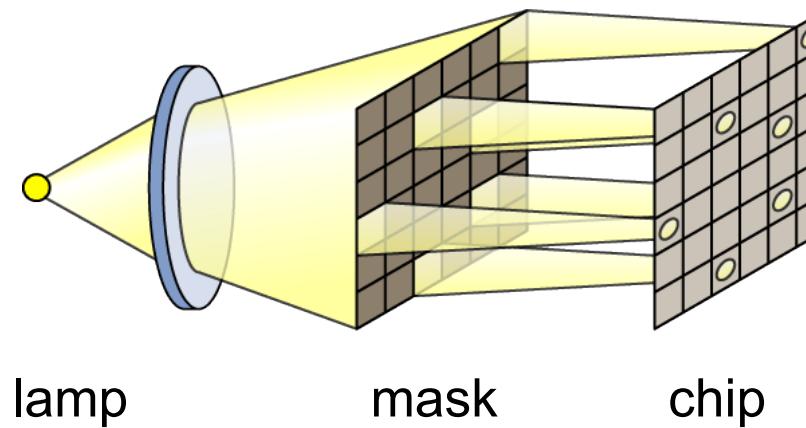
How microarrays are made: Affymetrix GeneChips

- _____ synthesized on silicon chip
 - One base at a time
- Uses process of _____
 - Developed for printing computer circuits



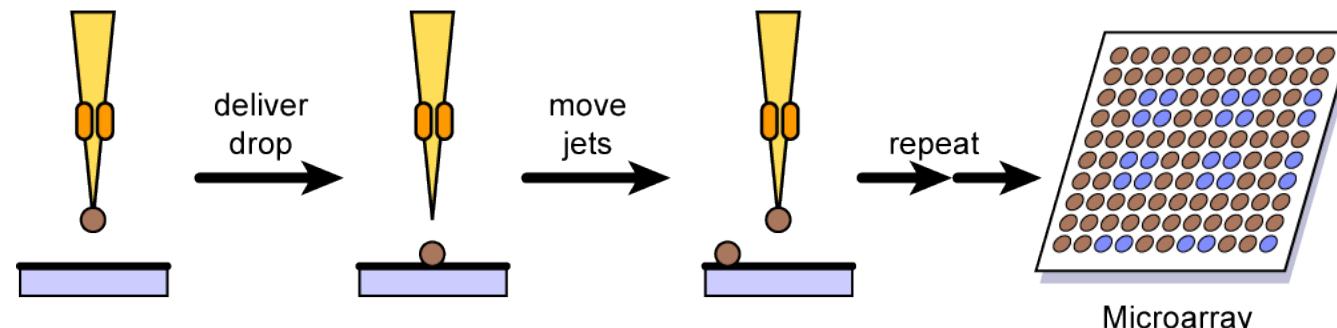
Photolithography

- Light-activated _____ reaction
 - For addition of bases to growing oligonucleotide
- Custom _____
 - Prevent light from reaching spots where bases not wanted
- Mirrors also used
 - NimbleGen™ uses this approach



Ink-jet printer microarrays

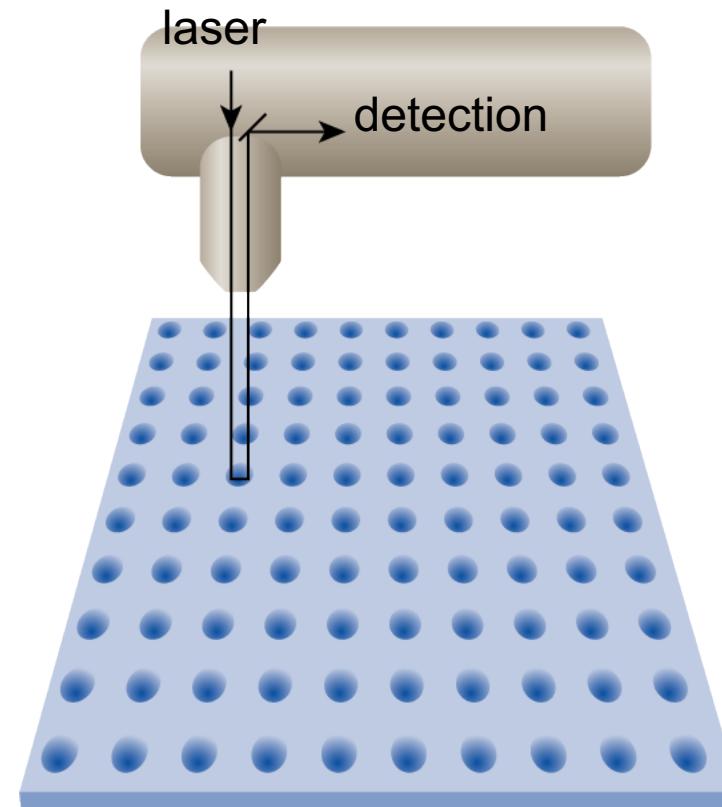
- Ink-jet printhead draws up DNA
 - Printhead moves to specific location on solid support
 - _____ ejected through small hole
 - Used to spot
-
- on
glass slide
- Use pioneered by Agilent Technologies, Inc.



3.34

Scanning of microarrays

- scanning microscopy
 - beam excites each spot of DNA
 - Amount of detected
 - Different lasers used for different wavelengths
 - Cy3
 - Cy5



RNA-seq

- Illumina
- [seq movie](#)
- Ion torrent
- <http://vimeo.com/68069581>

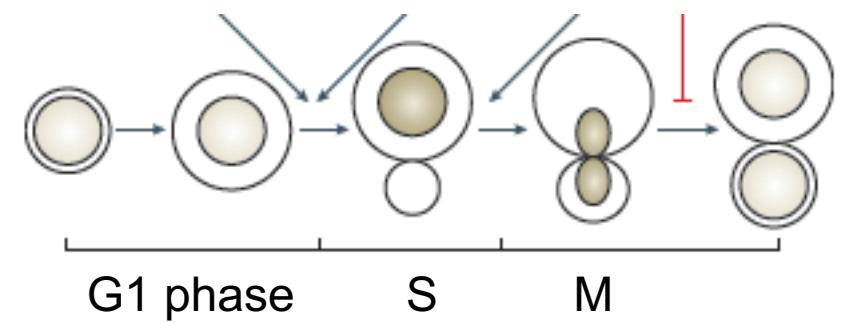
Data analysis preview

- From scanner: _____ per spot
-
- Ratio calculation: _____ vs _____
- Analysis: _____ expressed genes, _____, _____.

Transcriptome results

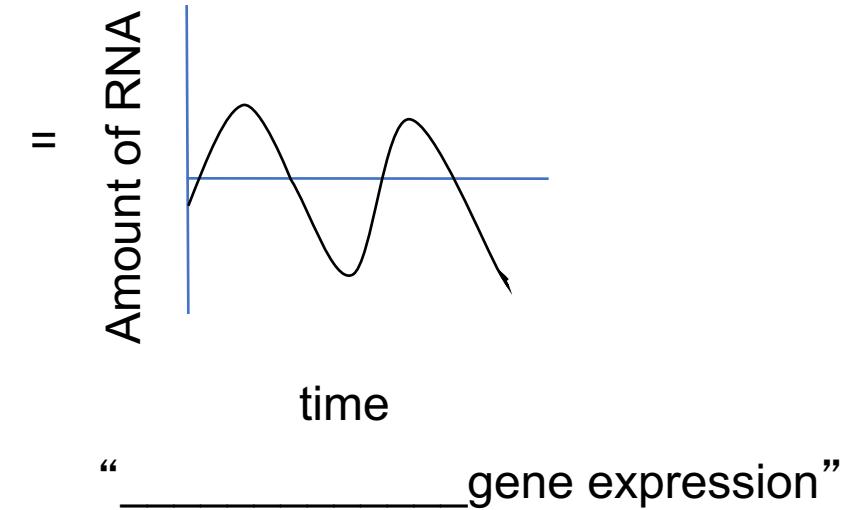
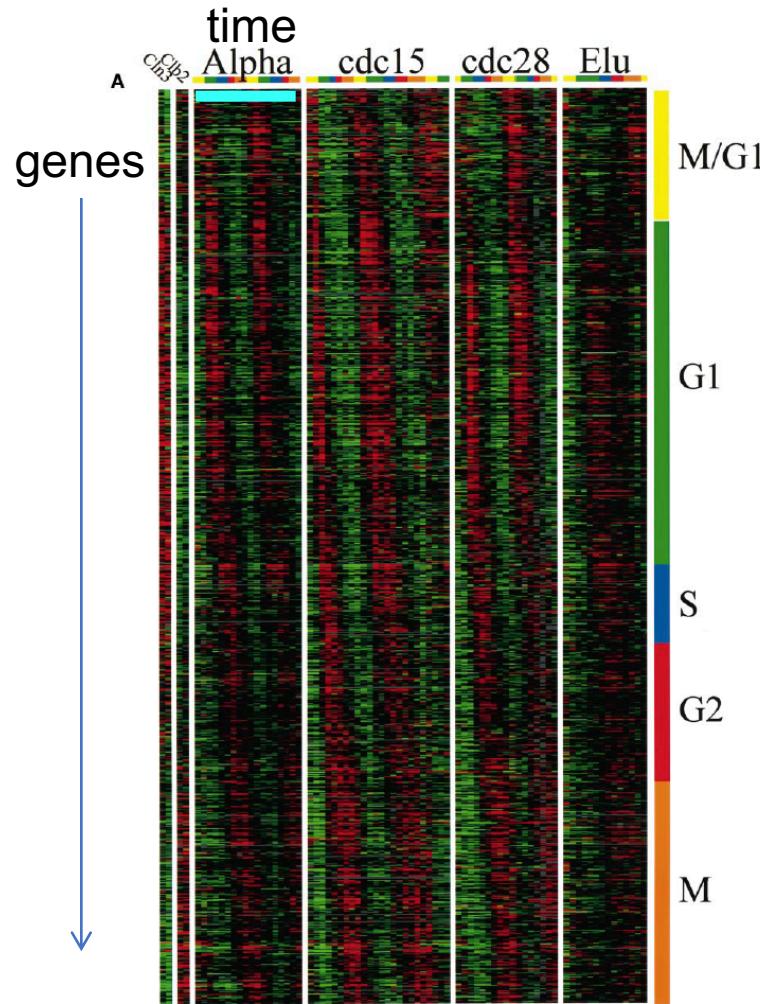
- How do we interpret the data?
- How did Spellman and colleagues interpret their data?

Cell cycle

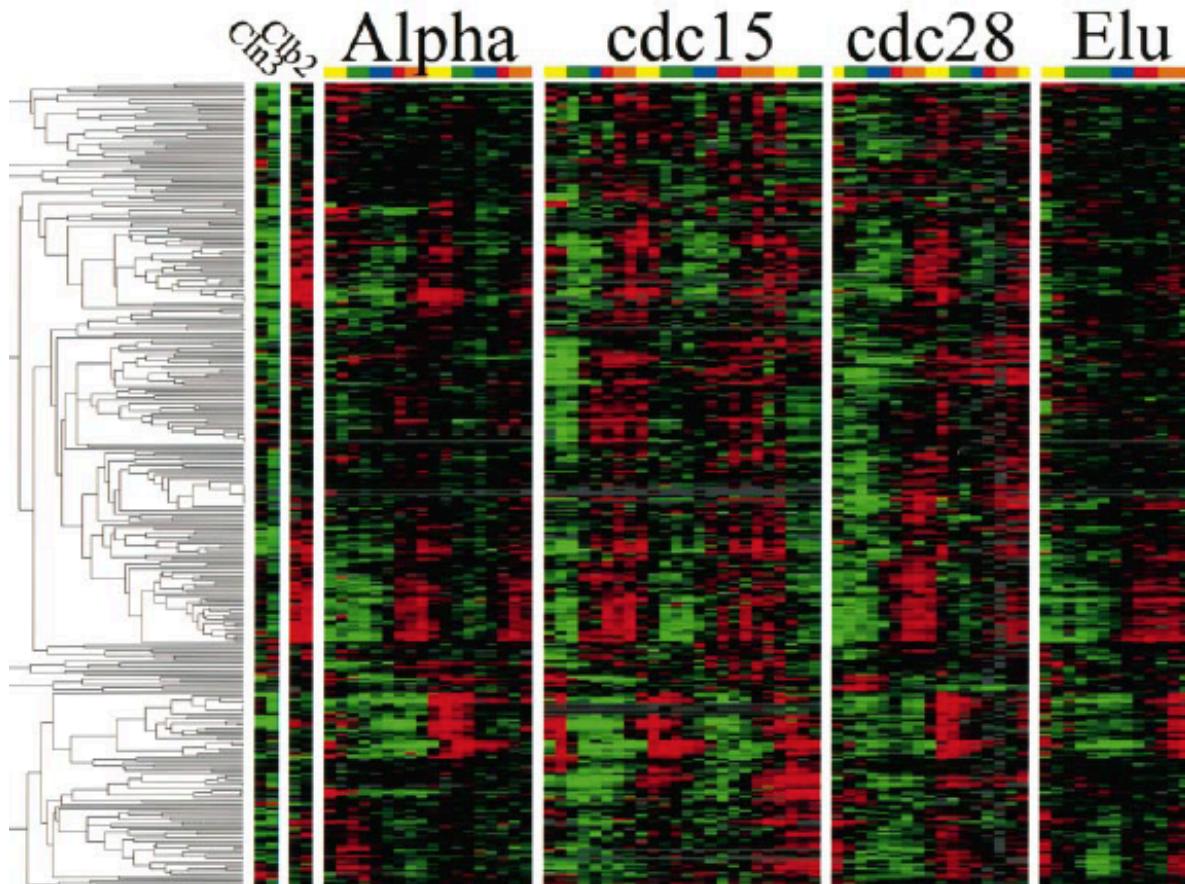


from Bloom and Cross 2007

What Spellman and colleagues observed



How are the genes turning on and off?



Conclusions

- Genes are expressed according to the _____.
- A set of _____ regulates these genes (turns them on and off).
- The _____ themselves respond to the cell cycle (phosphorylation by _____ -)
- ***Groups of genes with _____ tend to be expressed in _____ (Clusters).