Investigate RNA-seq data using public databases

M2D5

Your samples for qPCR

Remember:

- Parental cell line, DLD-1
  - +/- etoposide

- Mutant cell line, BRCA -/-
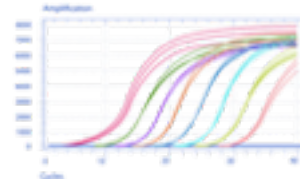 - +/- etoposide

Seed cells

Induce DNA damage (etoposide)

ACAUGGAUUA

TGTACCTAAT

Purify RNA and prepare cDNA

Measure transcript levels

Your samples for qPCR

Remember:

- Parental cell line, DLD-1
  - +/- etoposide

- Mutant cell line, BRCA -/-
  - +/- etoposide
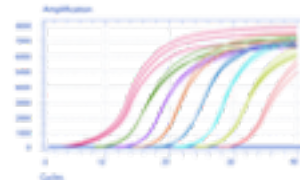
Seed cells

Induce DNA damage (etoposide)

ACAUGGAUUA

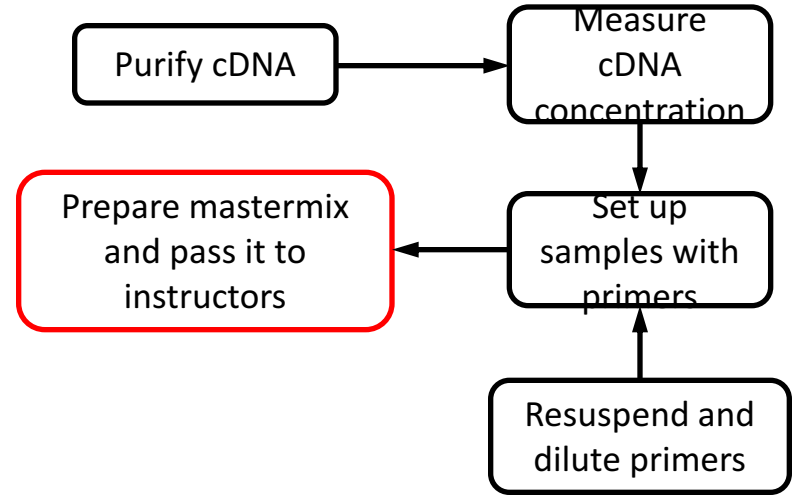TGTACCTAAT

Purify RNA and prepare cDNA

Measure transcript levels

Perform qPCR

Remember:

Or CDKN1A ↙

- p21:
    - Critical gene involved in replication
    We use it to see if cells replicate normally

- GAPDH:
    - Reference gene. Our control

Purify cDNA → Measure cDNA concentration

Prepare mastermix and pass it to instructors ← Set up samples with primers

Resuspend and dilute primers

|	Primers					
	GAPDH		**p21 (instr.)**		**p21 (yours)**	
Etoposide	-	+	-	+	-	+
DLD-1	A	B	E	F	I	J
BRCA2 -/-	C	D	G	H	K	L

Clean up cDNA: remove all enzyme, buffers + dNTPs
- Qiagen QIAquick PCR purification kit

steps	contents	purpose
prepare	5X Buffer PB to cDNA	Contains guanidinium thiocyanate. Ensures DNA can bind to silica membrane
bind	silica membrane in column	
wash	Buffer PE	Mostly ethanol. Wash away salts/buffers/impurities ** then, get rid of *all* ethanol
elute	water	Compete for binding to membrane. Elutes DNA

Perform qPCR

- Like a regular PCR:
  - Template DNA (cDNA)
  - Fw and Rv primer
  - dNTPs
  - DNA Polymerase
  - Buffer

- ... *except*:
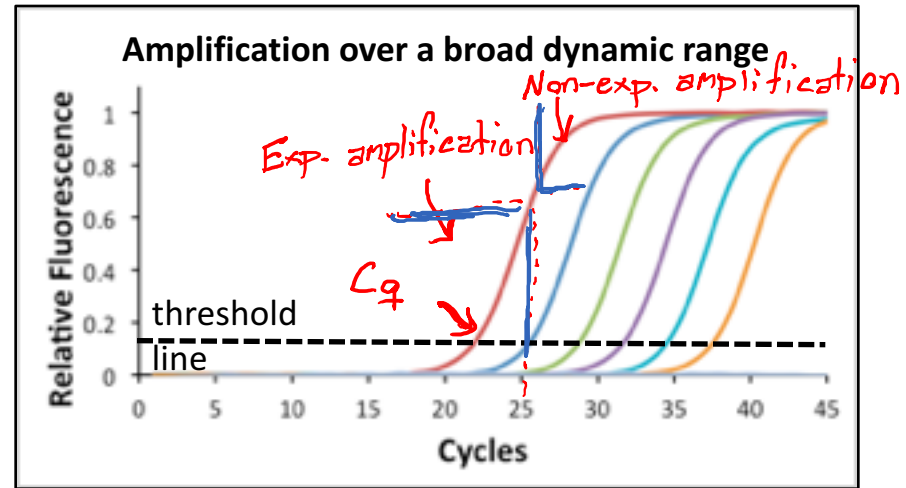 - Contains dye that binds dsDNA
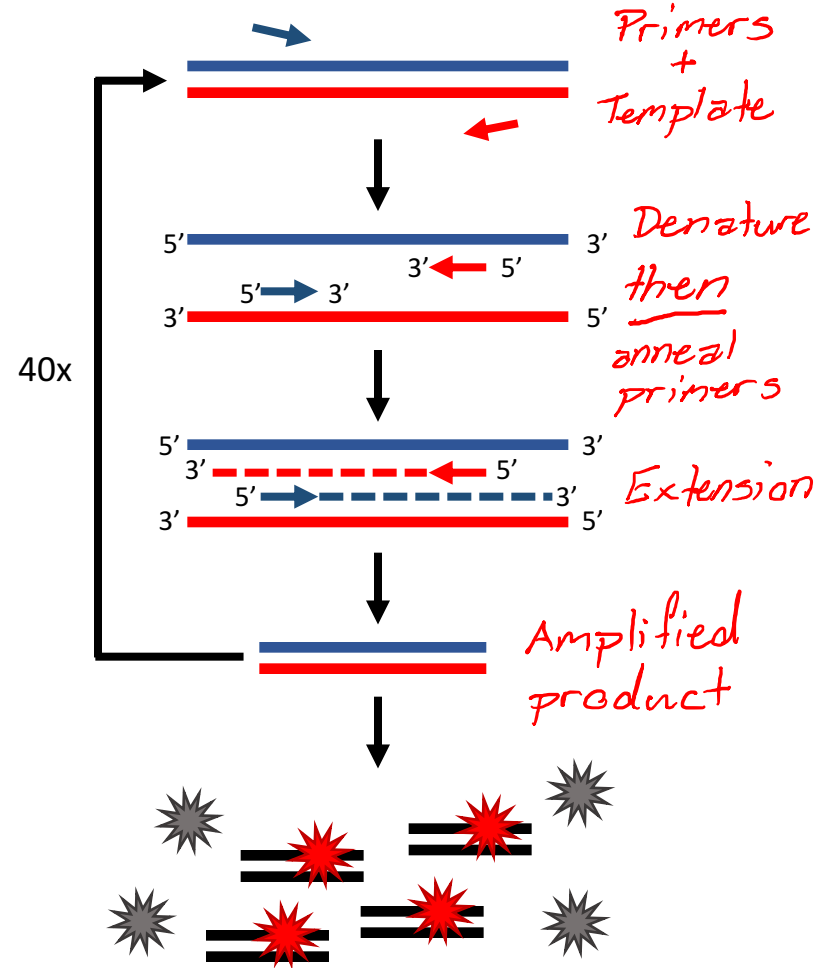
Perform qPCR

- Dye binds dsDNA
  - Is quenched if unbound

- Fluorescence is a function of [DNA]
  - Remember, DNA is exponentially amplified

- We use controls with a known DNA concentration to calculate [DNA] in our sample

- C_q value = cycle # where the fluorescent signal is significantly above background

On publishing qPCR data:
http://clinchem.aaccjnls.org/content/55/4/611

Amplification over a broad dynamic range

Non-exp. amplification
Exp. amplification
C_q
threshold line

Using controls to calculate [DNA] in sample

$y = -3.3729x + 10.39$
$R^2 = 0.9983$

Your DNA conc. must be in this range
Standards with known DNA conc.

Best practices
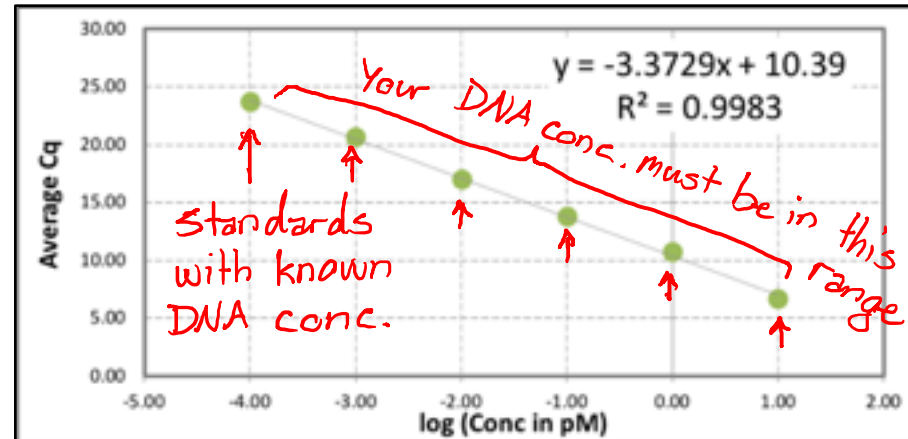
Using public databases

The Cancer Genome Atlas – TCGA
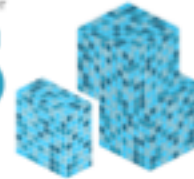
- Sequencing data
- Healthy – diseased comparison
- Clinical data

Ensembl

- Genome annotations
- Comparative genomics
- Variation
- Regulatory data

Exploring the TCGA database

KEGG to look at pathways

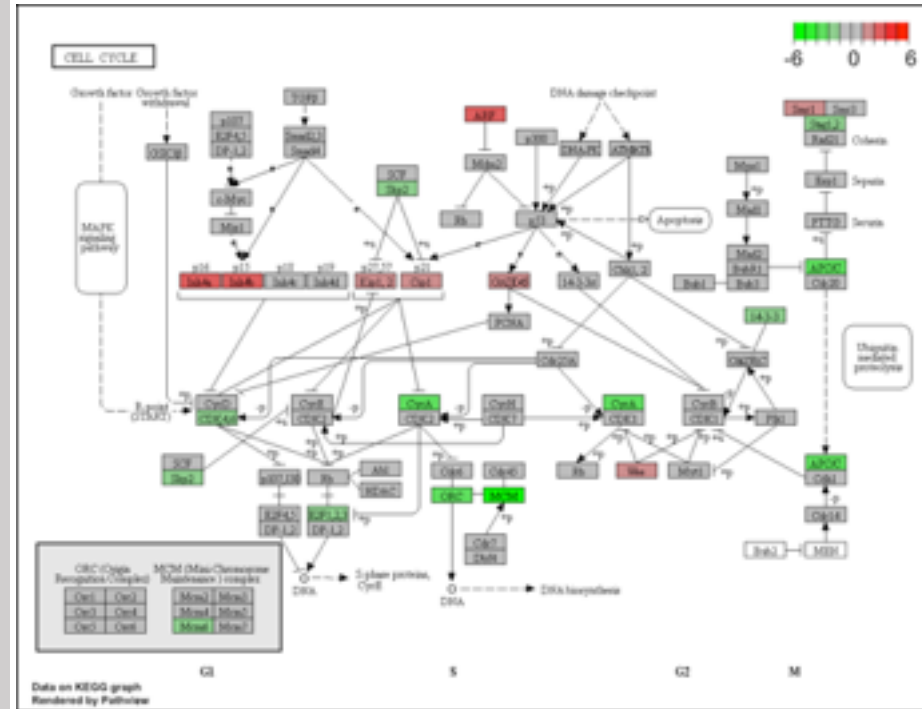- Comparison between our sample and sample from TCGA

- Are certain pathways different?
  - What is a tissue sample?
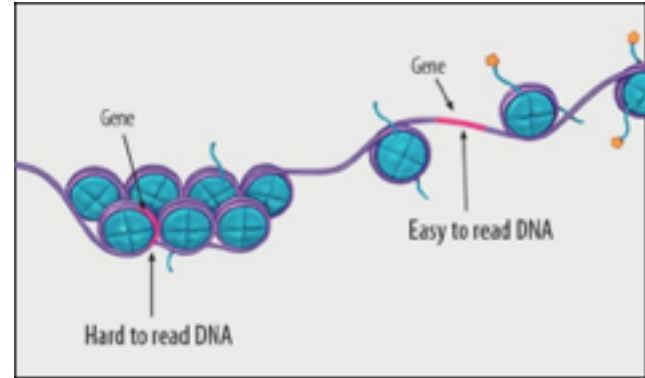
From patient. Can be healthy or diseased.

  - What is a cell line?

A cell, i.e. a fibroblast (skin cell), that has been immortalized such that it can be passaged indefinitely
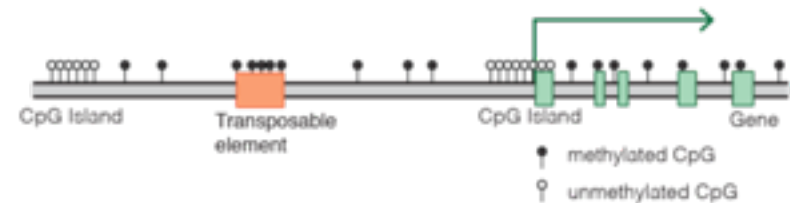
There is more to DNA than just the sequence

- Epigenetic data:

  - **Histones** and post-translational modifications of their tails

  - **DNA methylation**
    - Unmethylated =

    - Methylated =

Typical mammalian DNA methylation landscape

SPRING BREAK 2018!

- M1 Data Summary revision due Monday, March 26th 10pm
- Journal Club Presentations, April 3rd and 5th