

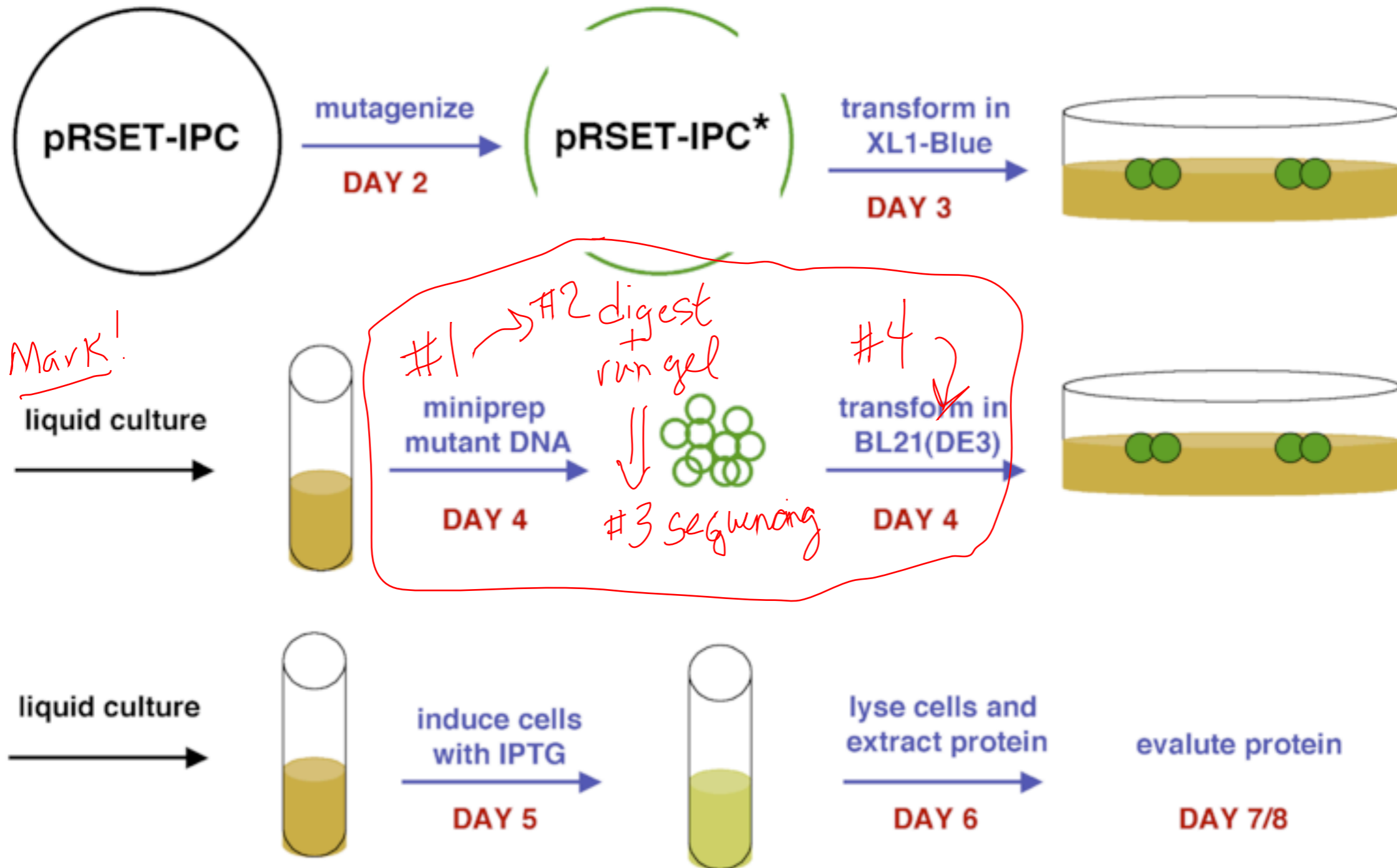
M2D3: Bacterial Amplification of DNA

3/20/13

1. Lab Treat
2. Turn in MID2 FNT up front in folder
3. Plan for today:
 - Quiz
 - Load gels: Do NOT add loading dye directly to PCR reaction!
Tag team the loading process (see wiki for order)
 - Pre-lab lecture (~ 45 min)
 - Transform your mutant + controls into XL-1 e.coli
4. M2D4 usually runs long -- therefore, please start on your FNT today!
5. Lab statistics!



Protein Engineering -- Experimental Overview



Restriction Enzyme Screening for Mutant

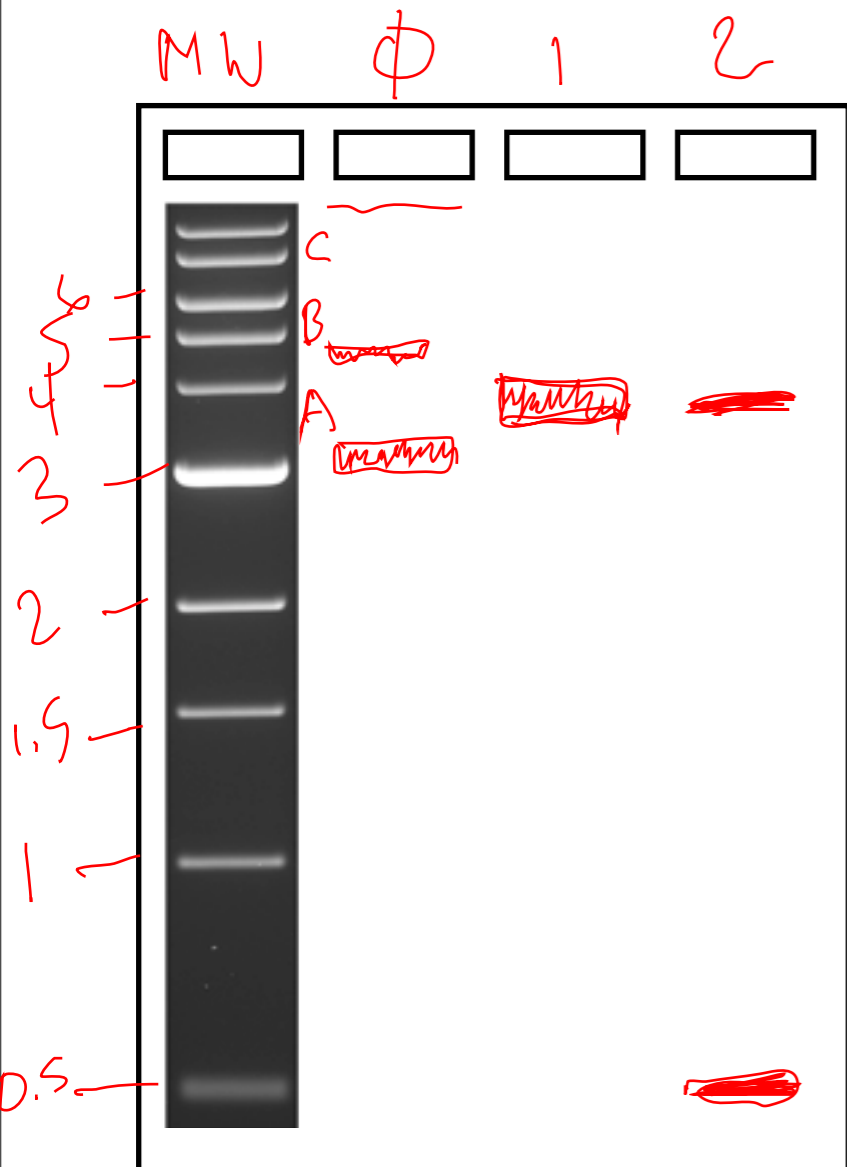
★ If we know that DpnI removed all parental DNA why do we need to screen with RE (and sequencing)?

Tag $1 \cdot 10^5$ bp
 pfu $1 \cdot 10^6$ bp

★ How can we tell that our vector has been cut?

PRSET }
 IPC } 4Kb

Plasmid vs. cut, linear DNA



Number of cuts:

- 0) A) Supercoiled - fast
- 1) B) Nicked/Incomplete digest - slow
- 2) C) Higher order structure - v. slow/freeze
- 1) - RE
- 2) sum to 4Kbp

Restriction Enzyme Screening for Mutant: Diagnostic Gel

Two digests — control → parental
 — your mutation

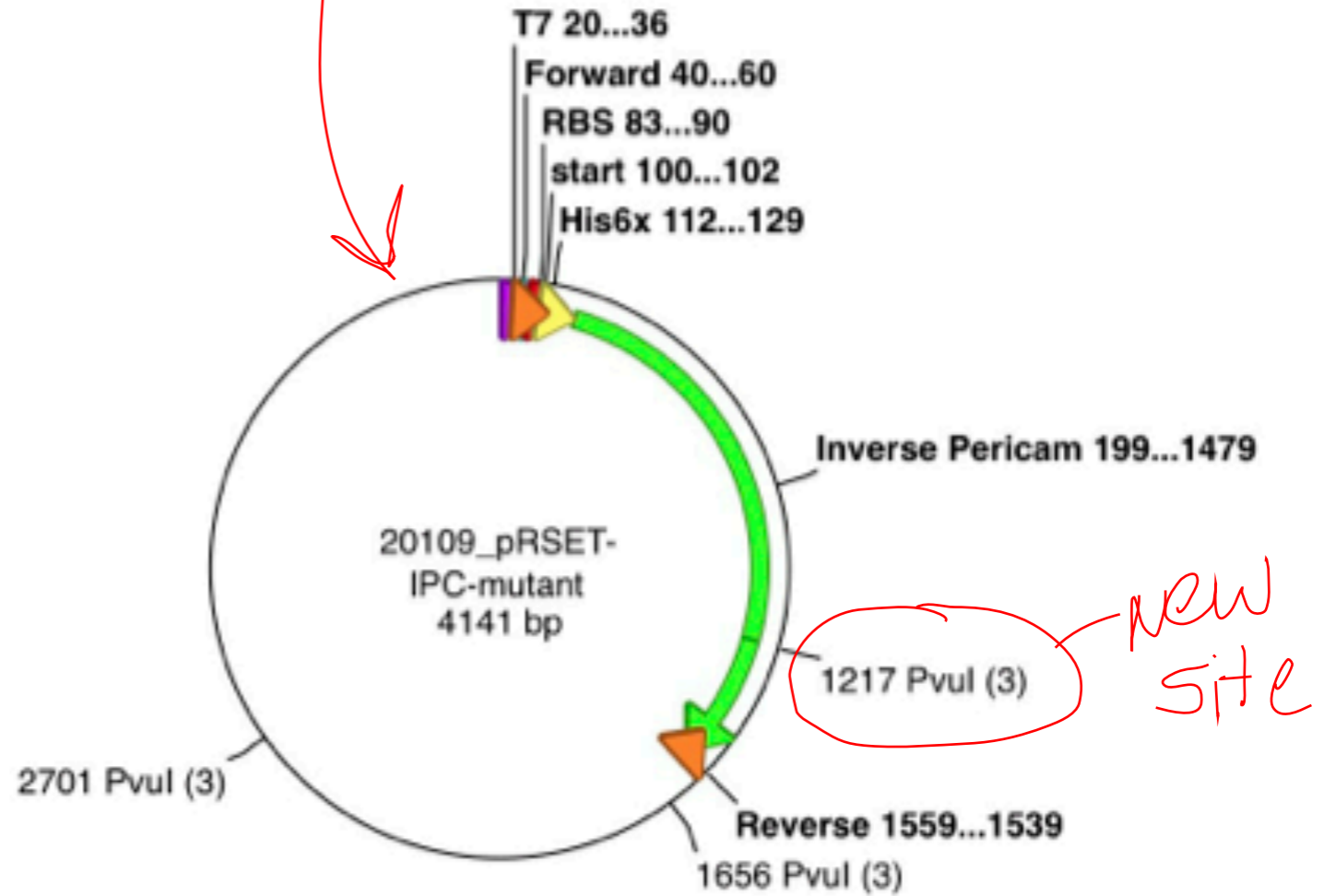


4
3
1

3 subtle shift

← same!

← might be faint



FNT → DO THIS TODAY!

why? compare K_D values of Hill coeff

Lab Math: Descriptive Statistics

Distribution: *replicates*

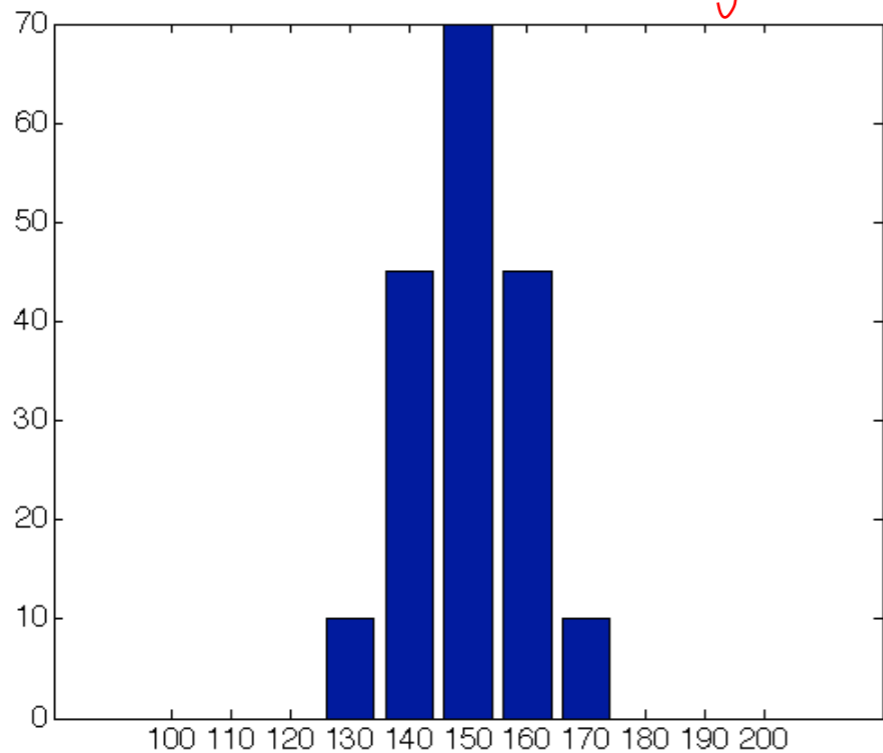
- Gaussian/Normal
- poisson
- bimodal

Mean (\bar{X}): $\frac{\sum x_n}{n}$ $\bar{X} = 150$

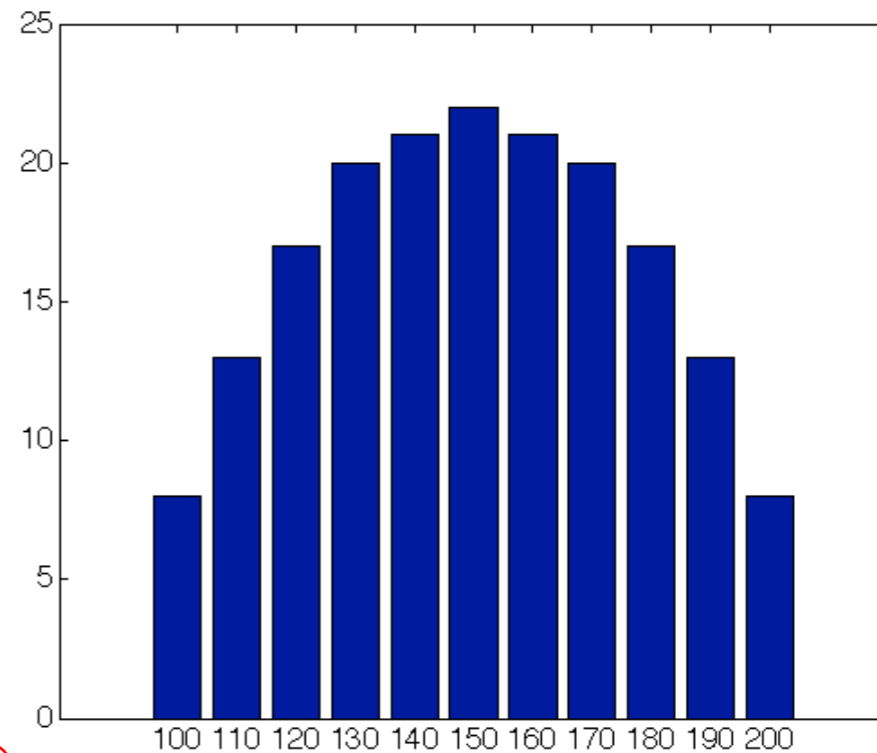
Sample number (n): 200

of subjects

Histogram



fraction of subjects



weight (lbs)

BE professors

Lab Math: Descriptive Statistics

Distribution: Normal/Gaussian

Mean (\bar{X}): 150

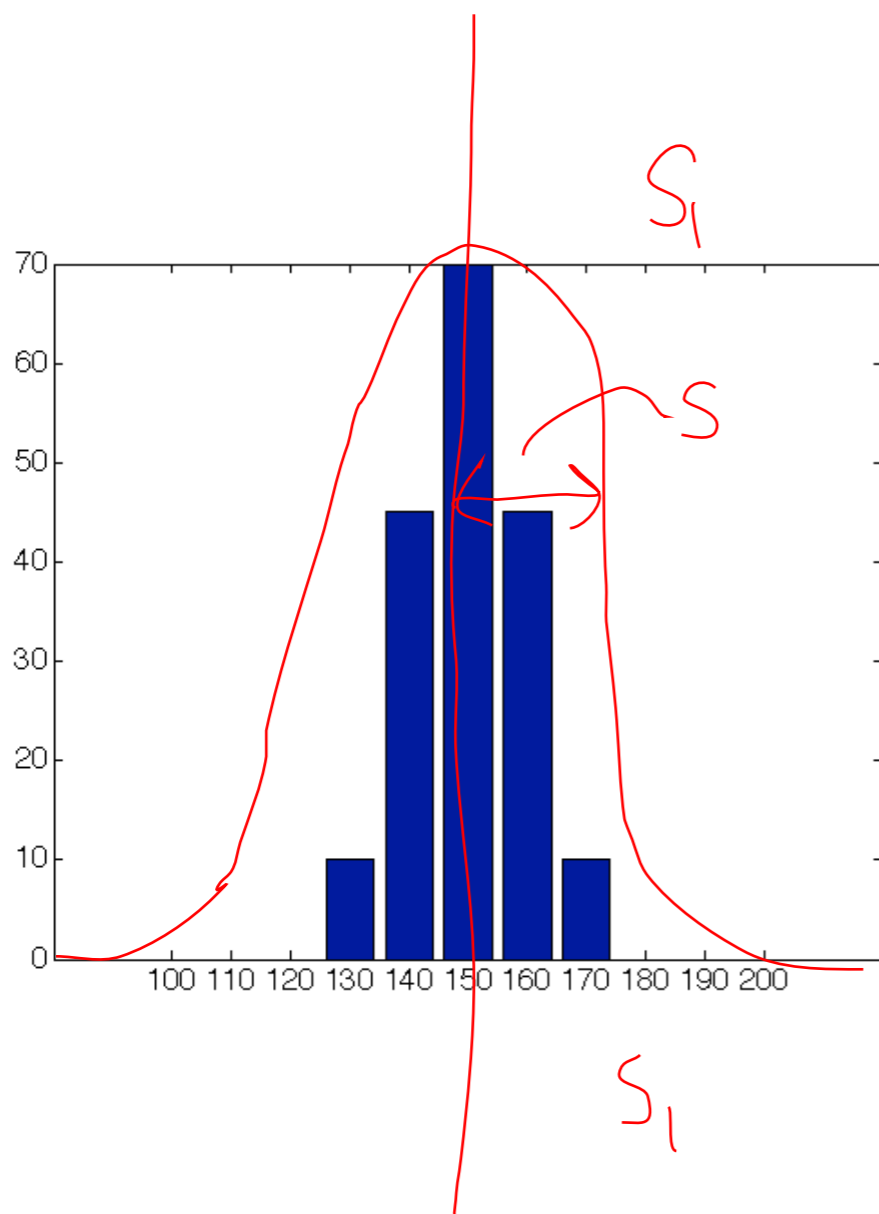
Dispersion / Standard Deviation (s):

*wideth of distribution;
variability*

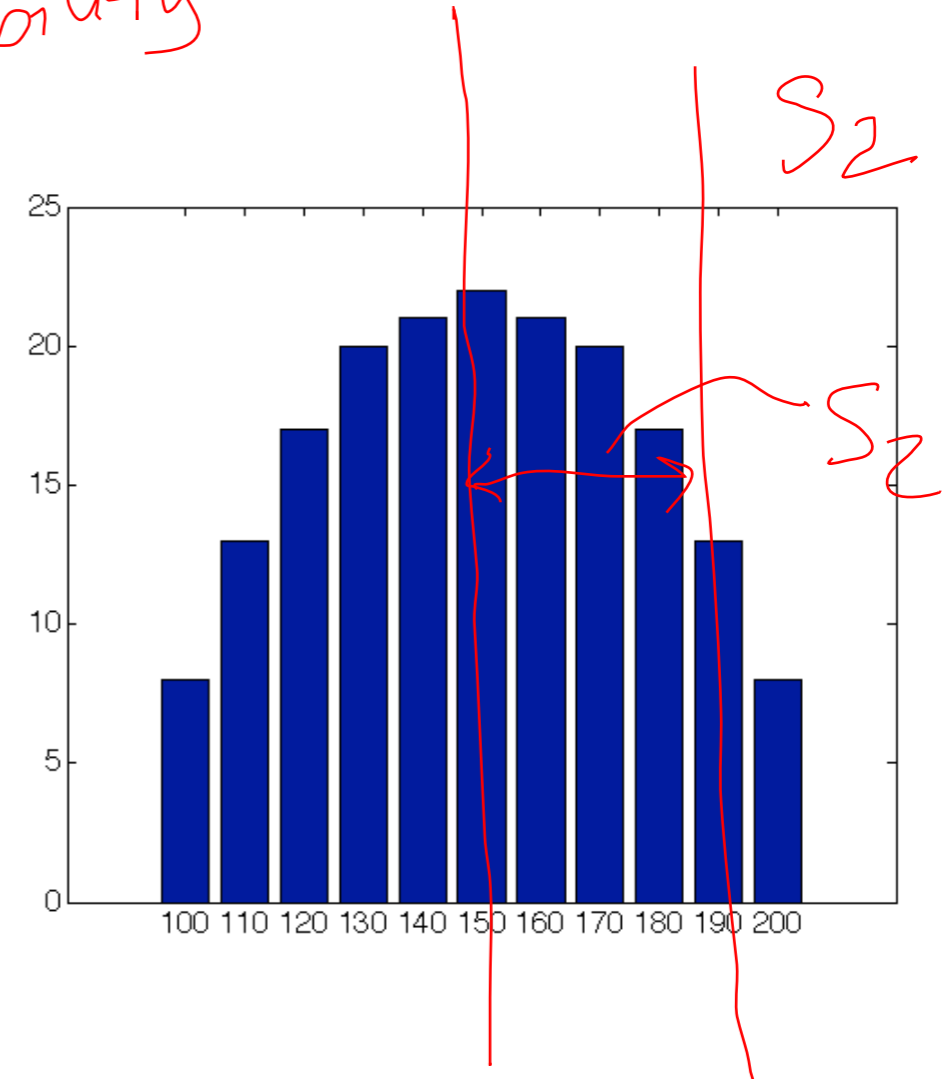
Is captures

68%

of the data



$S_2 > S_1$



Lab Math: Descriptive Statistics

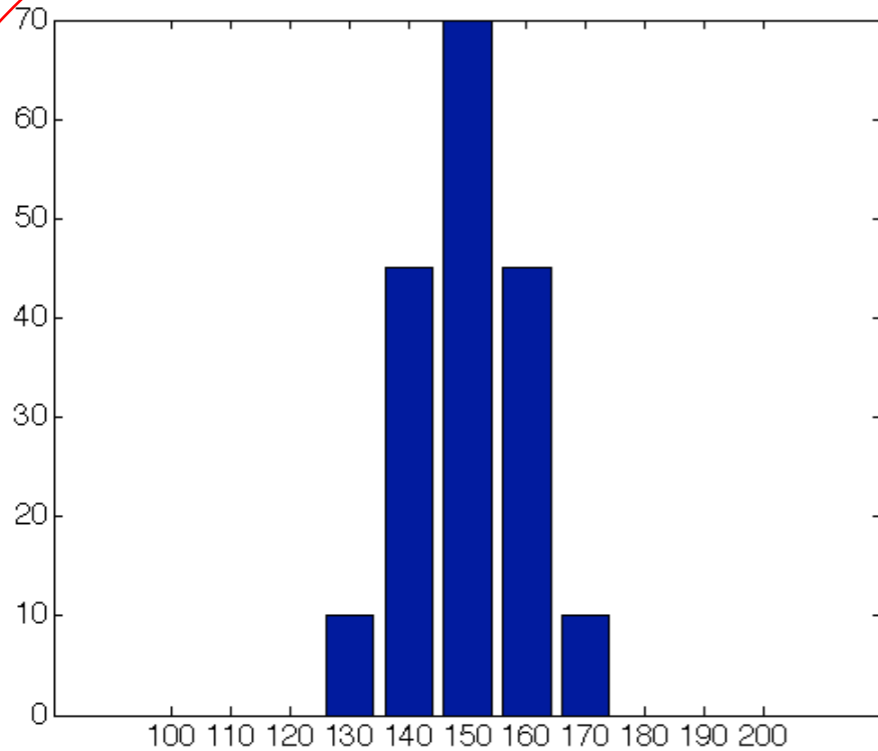
Dispersion / Standard Deviation (s):

$$\bar{X} = 150$$

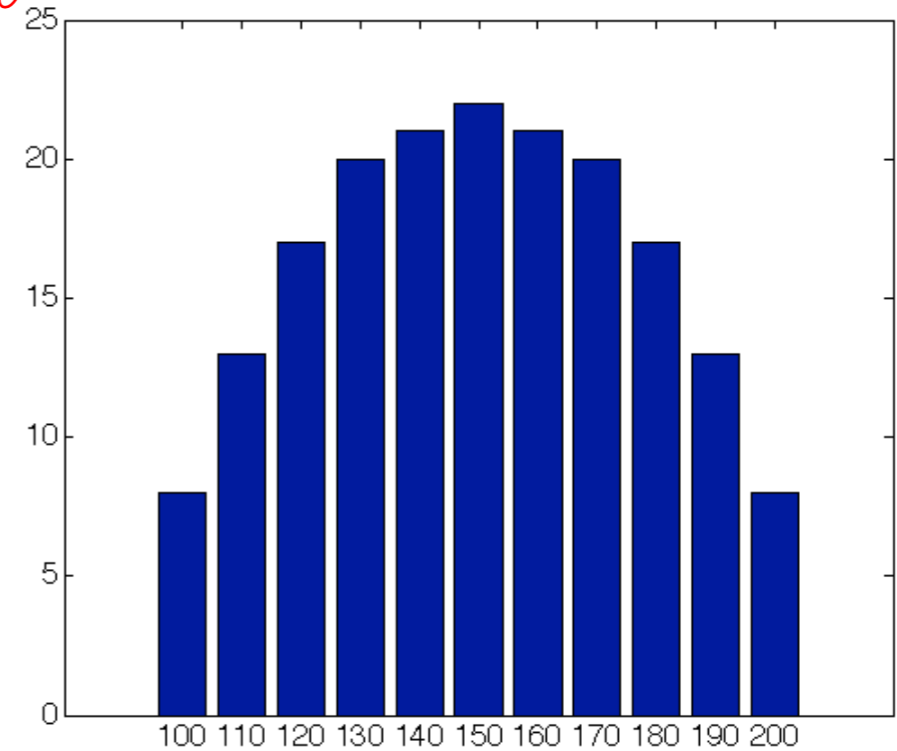
$$n = 200$$

$$\sum \text{error}(X_n - \bar{X}) = 0$$

DOF
constrains
this equation



$$s_1 = 10$$



$$s_2 = 27$$

Degrees of Freedom (DOF): $n - 1$

1) $\bar{X} = 150$ ← something has to be fixed to reach this

2)

Lab Math: Descriptive Statistics

What is our 95% Confidence Interval?

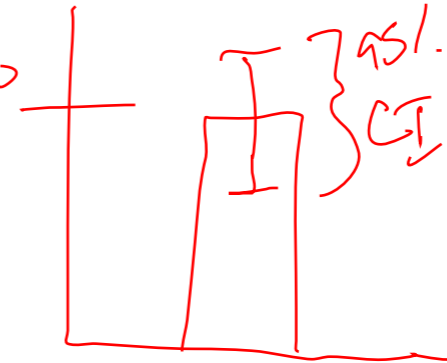
$$\bar{X} = 150$$

$$n = 200$$

$$\text{DOF} = 199$$

$$\mu = \bar{x} \pm \frac{t s}{\sqrt{n}}$$

$$\mu = 150 \pm 3.8$$



What is the t statistic?

$$f(\text{DOF}, \% \text{CI})$$

$$t_{\text{calc}} > t_{\text{table}} \Rightarrow \text{Statistical sig}$$

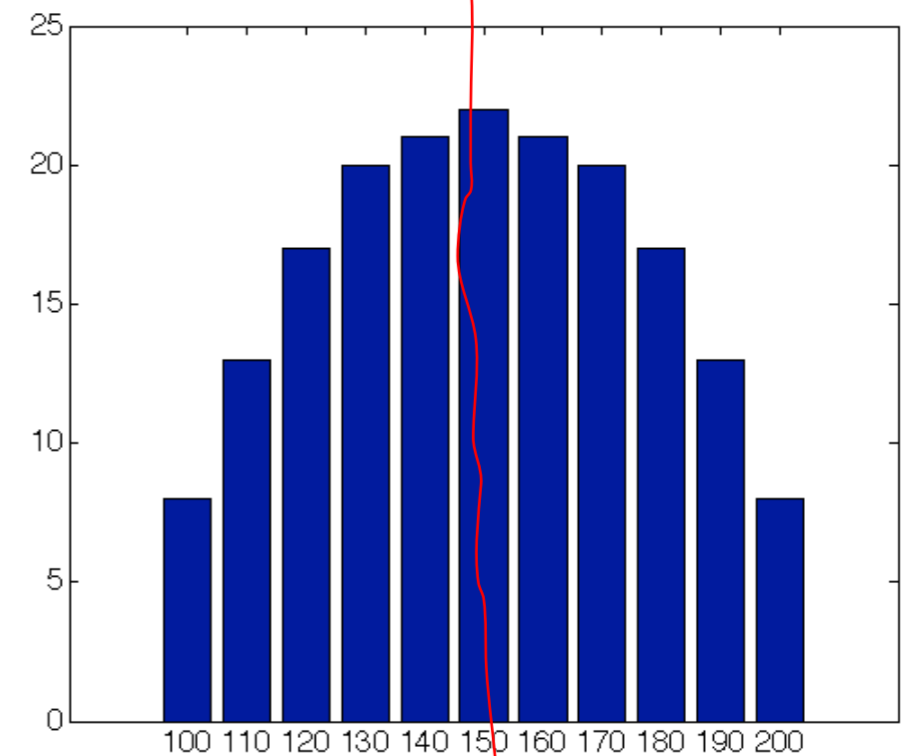
How do we calculate it?

In 20.109: Excel function TINV

90% CI?

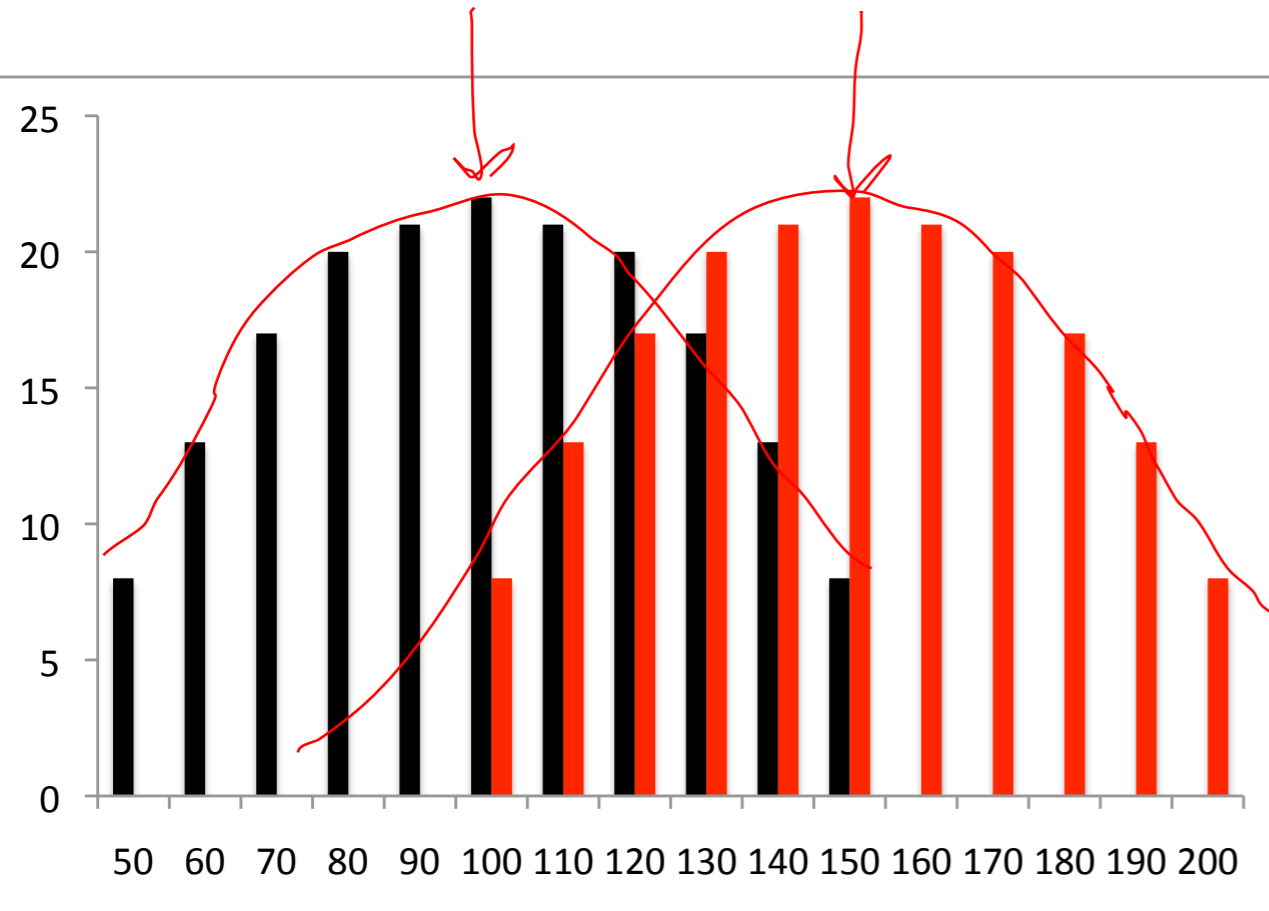
$$> 3.8 \quad < 3.8 = 3.8$$

precision accuracy



$$s_2 = 27$$

Are these two samples different?



Compare two sample means with a t-test.

Every statistical test:

Has *assumptions*

Asks *specific question*

Requires *human interpretation*

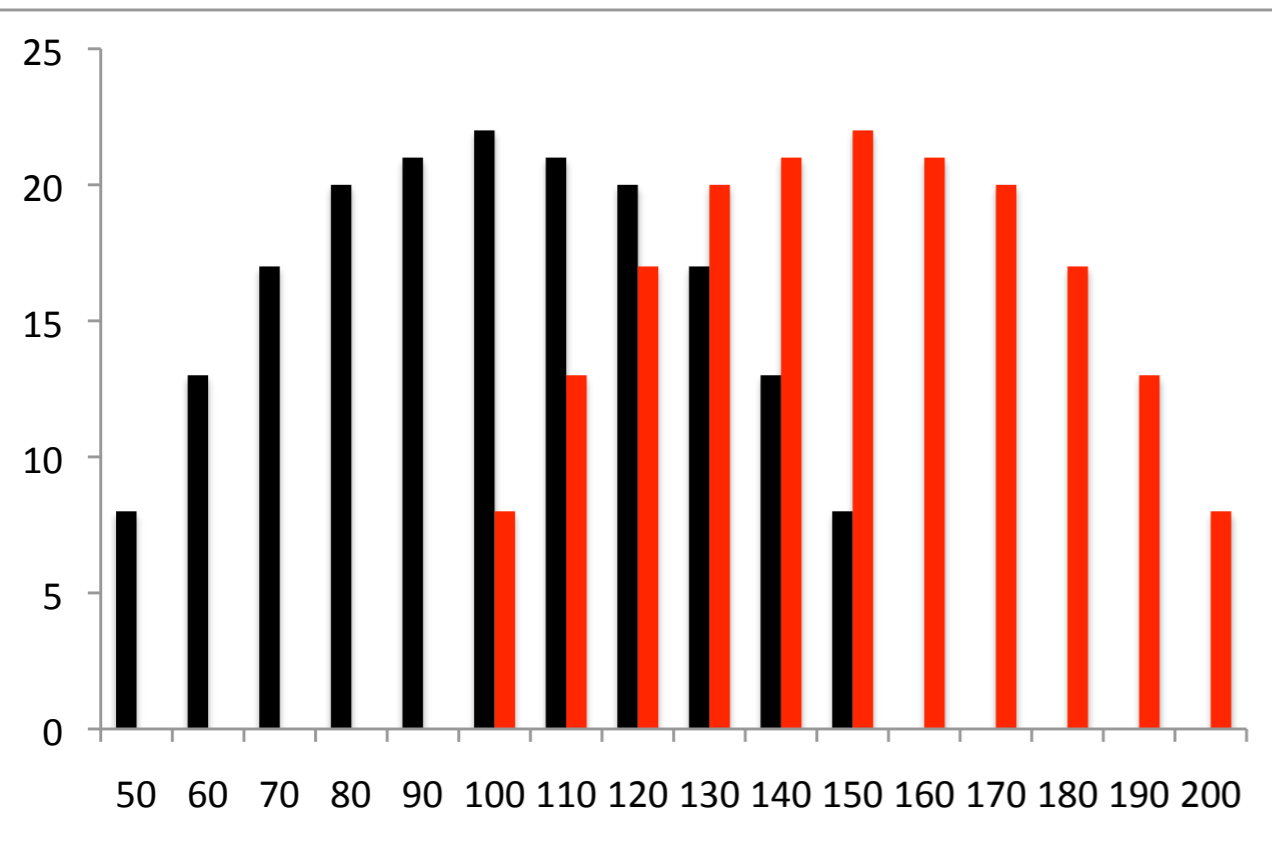
GI GO

Basics of the t-test:

A) Normally distributed data (non-normal Rank-sum test)

B) ^{equal} variance (Excel = type Z)

Are these two samples different?



$s_1 = 27$
 $s_2 = 27$

$$t_{calc} = \frac{\bar{x}_1 - \bar{x}_2}{\underbrace{s^*}_{\text{pooled}}} \sqrt{\frac{n_1 n_2}{n_1 + n_2}}$$

Here $s =$ pooled standard deviation

$$s^* = \sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2}}$$

DOF = $n_1 + n_2 - 2$

If $t_{calc} > t_{table}$ there is a significant difference between the means at this CI.

$t_{table} = 1.96$ (f(DOF, 95% CI) ← 95%

$p = 0.05$ ← 5% time our ped. is wrong

$t_{calc} = 18$ $p = 10^{-9}$

Today in Lab:

★ With time left before gels are done:

● Work on FNT -- this will significantly speed up Friday!

● Complete the statistics practice:

● Men: 73, 70, 67, 68, 67

● Women: 72, 68, 67, 64, 64, 65, 65, 63, 66, 65, 62

★ Transform bacteria: prechill tubes! 30 min incubation

★ Remember to wear NITRILE gloves at gel bench!!