20.109
Laboratory Fundamentals in Biological Engineering

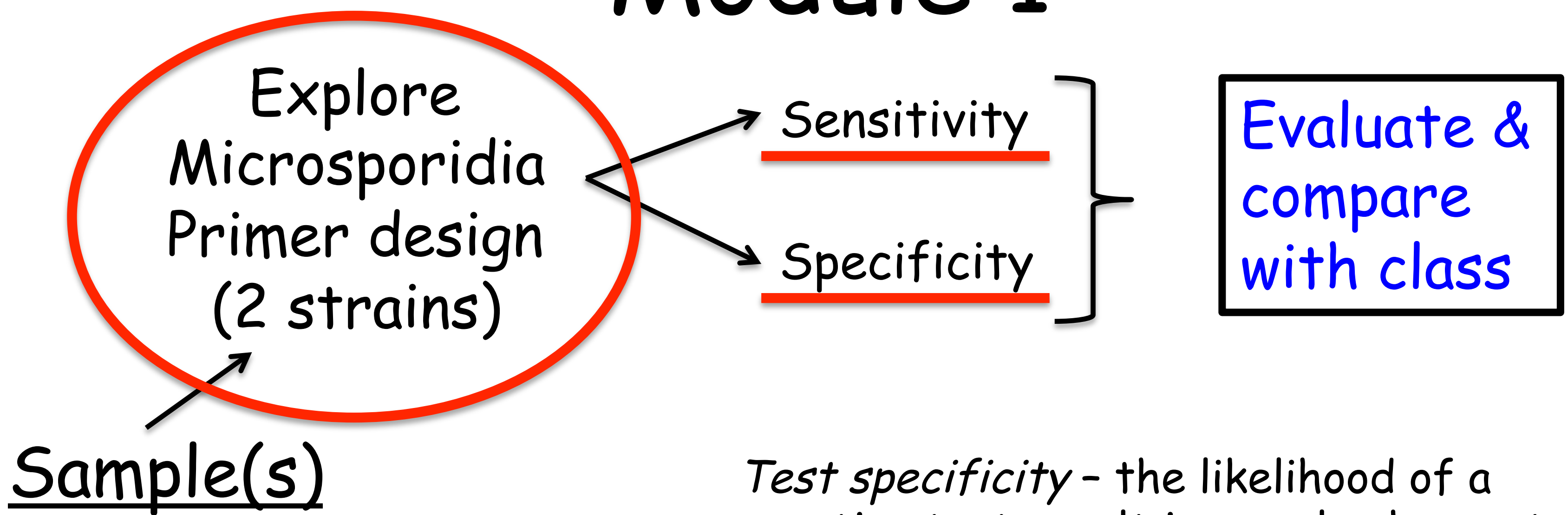
Module 1
Nucleic Acid Engineering
Lecture 6

Office hours: by appt.

Development and Evaluation of a diagnostic test

- Sensitivity
- Specificity

Module 1



Test specificity – the likelihood of a negative test result in samples known to be free of the microbe (pT-/D-).
aka - "true negative rate"

Test sensitivity – the likelihood of a positive test result in samples known to contain the pathogen (pT+/D+).
aka – "true-positive rate" or "operational sensitivity"

Calculating sensitivity and specificity

True status

Test	True status +	True status −
+	a: 10	b: 2
−	c: 5	d: 83

a – true postitive
d – true negative
c – false negative
b – false positive

Sensitivity = a/(a+c) 10/15   d/(b+d) 83/85 = Specificity

Savage Chickens by Doug Savage

Test \ True status	+	−
+	a 10	b 2
−	c 5	d 83

Positive Predictive Value = a/(a+b) 10/12

Negative Predictive Value = d/(c+d) 83/88

Sensitivity = 10/15

83/85 = Specificity

Prevalence and predictive values

True status

Test	+	−
+	a 20 / 10	b 2
−	c 10 / 5	d 68 / 83

Positive Predictive Value = 20/22 ↑

10/12

Negative Predictive Value = 68/78 ↓

83/88

Sensitivity = 67%   98% = Specificity

Improving Predictive values?

True status

Test	+	−
+	a 20	b 2
−	c 10	d 68

Positive Predictive Value = 20/22

Negative Predictive Value = 68/78

Sensitivity = 67%

98% = Specificity

Can sensitivity and specificity of tests differ indirectly?

- An example:
  - Disease burden (eg – heartworm)
  - Breed variation
  - High and low prevalence areas

Liklihood ratios – diagnostic utility of a test

Test	True disease +	True disease –
+	a 20	b 2
–	c 10	d 68

Liklihood Ratio for a Positive Test= $\frac{a/a+c}{1-(d/b+d)}$

$33.5 = \frac{20/30}{1-(68/70)}$

Liklihood Ratio for a Negative Test= $\frac{1-(a/a+c)}{d/b+d}$

$0.33 = \frac{1-(20/30)}{68/70}$

Sensitivity = 67%

98% = Specificity

Test Accuracy

Test	True disease +	True disease −
+	a 20	b 2
−	c 10	d 68

Accuracy =
88/100 = 88%

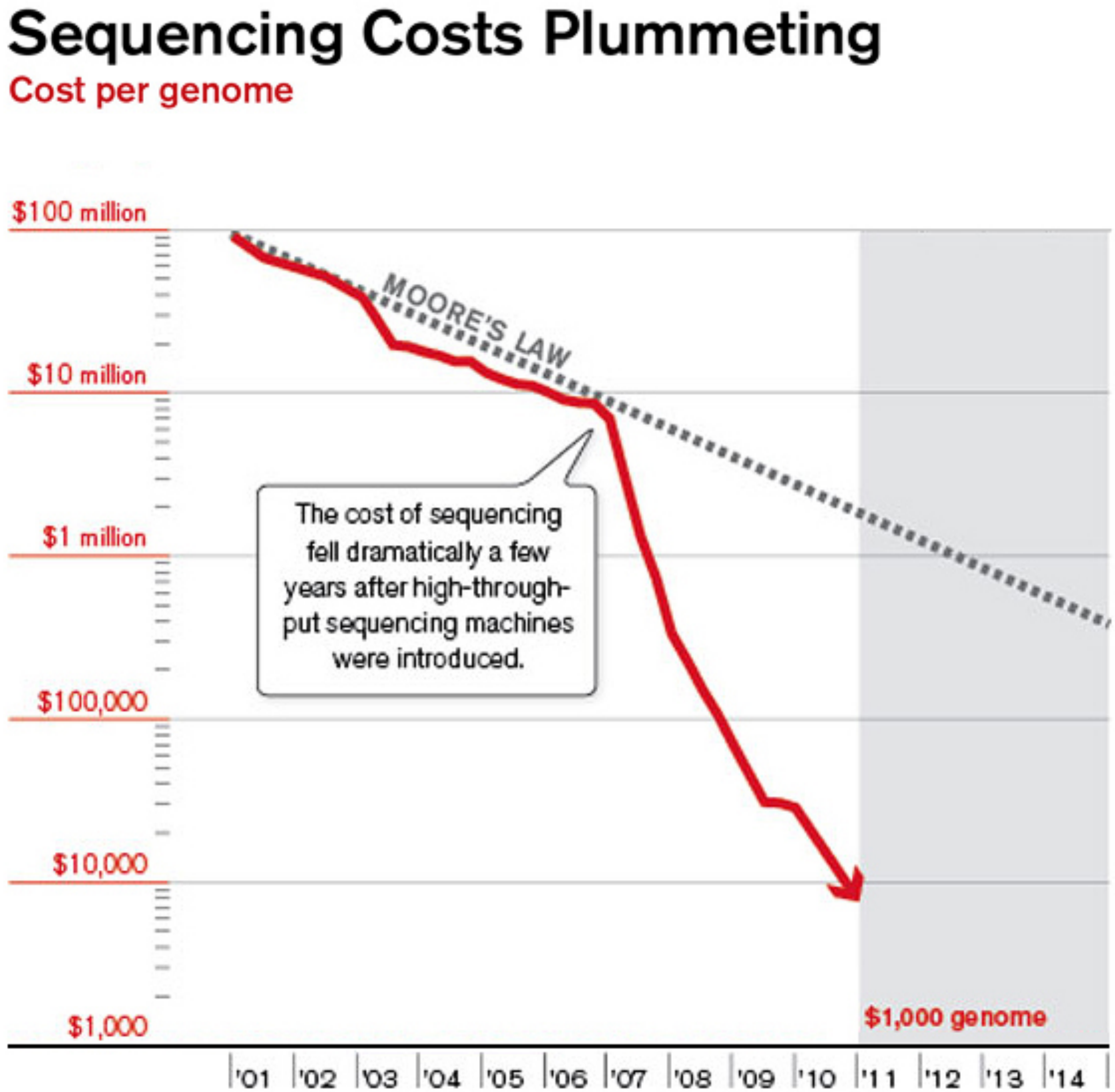
Some sources of bias to consider in evaluating test performance

- Improper standards of validity
- The spectrum of test subjects

Comparing sequencing platforms in microbiome analysis

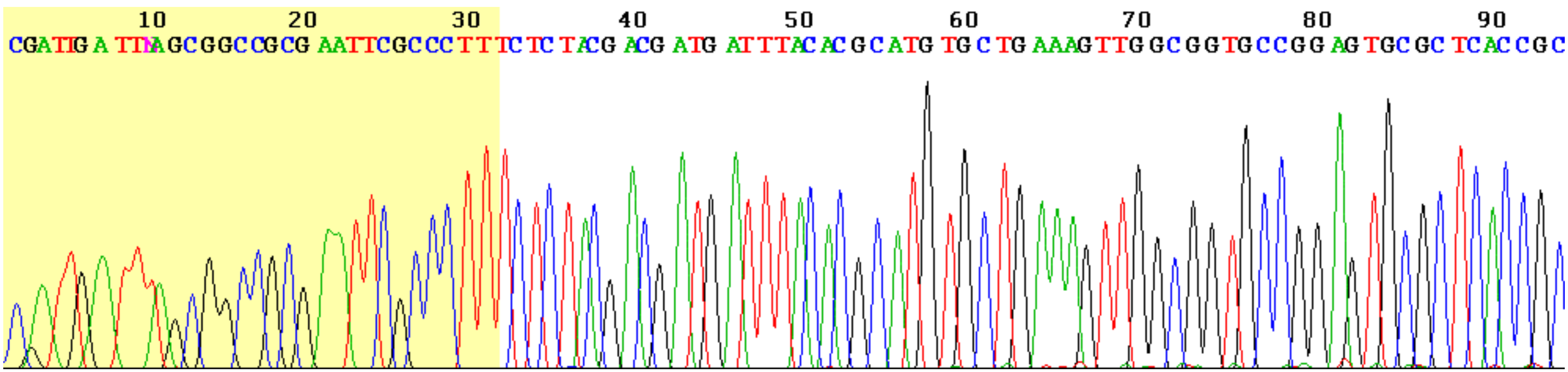
Platform	Method	Reads	16S	Metagenome	Notes
Sanger	Dideoxy terminator	750 bp	2-3 reads to cover	Good for database comparisons	Accurate, costly, slow
Pyroseq.	Light emission	400 bp			Good for 16S but not meta
Illumina	Flourescent step-by-step	100-150		More coverage makes up for short reads	High coverage, low cost
3^{rd} generation	Electronic signal	10-100 kb		Great for assembly	Unknown error, usability

Bases to Bytes (Technology Review April 2012)
Cheap sequencing technology is flooding the world with genomic data.
Can we handle the deluge?

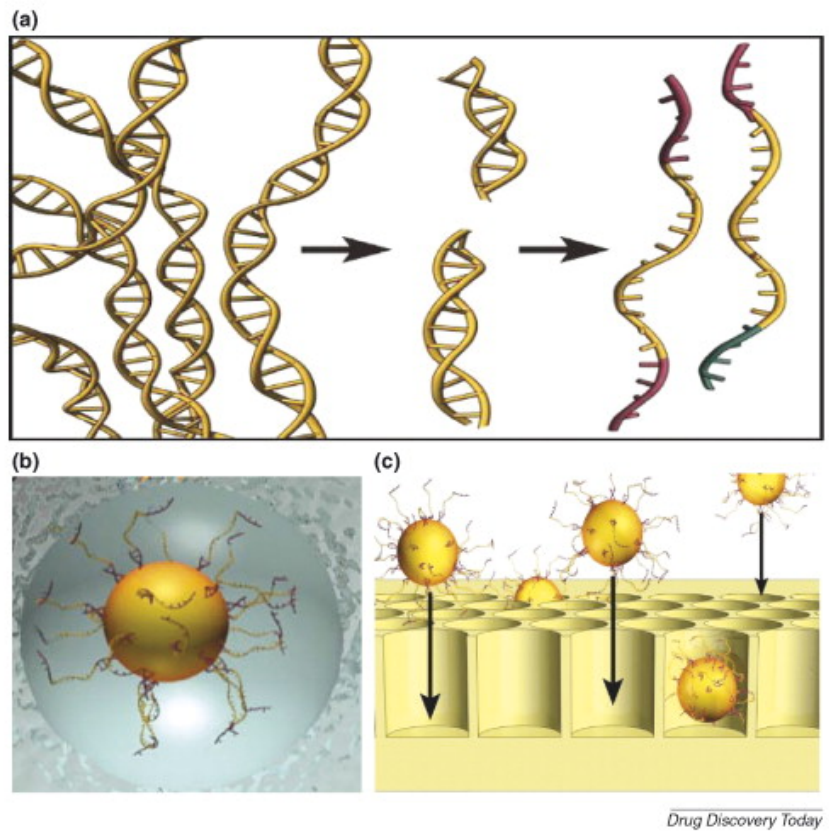


Sanger sequencing

Sanger Sequencing

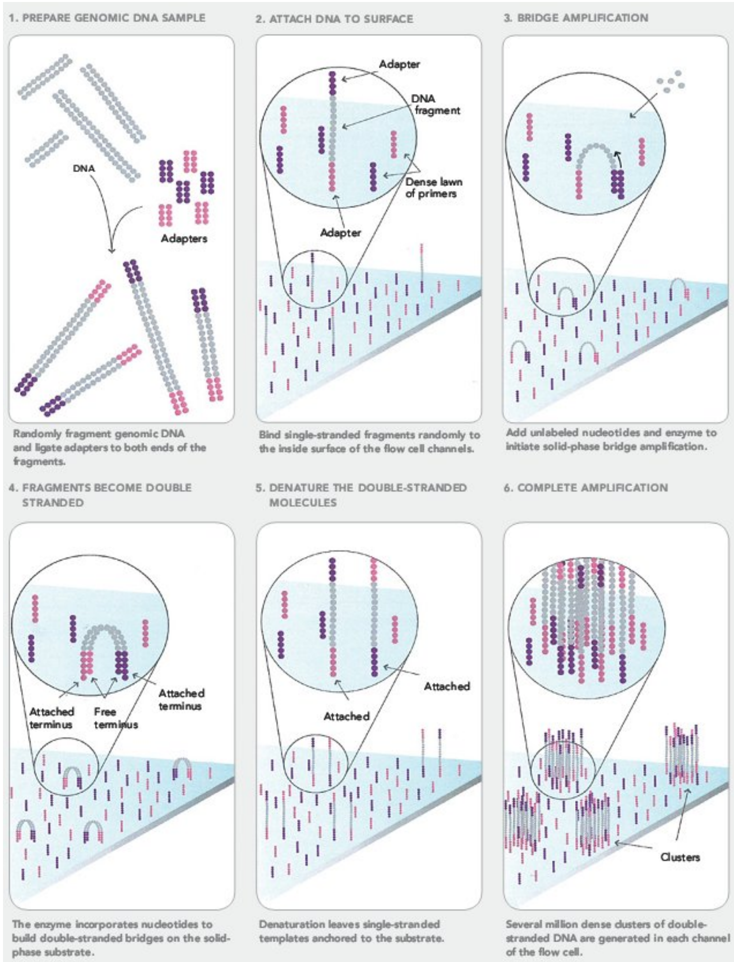


Pyrosequencing



Pyrosequencing

Illumina sequencing



Illumina Sequencing

Nanopore Technology

Oxford Nanopore
Technology