Bio(tech) Interlude: PCR and DNA Sequencing

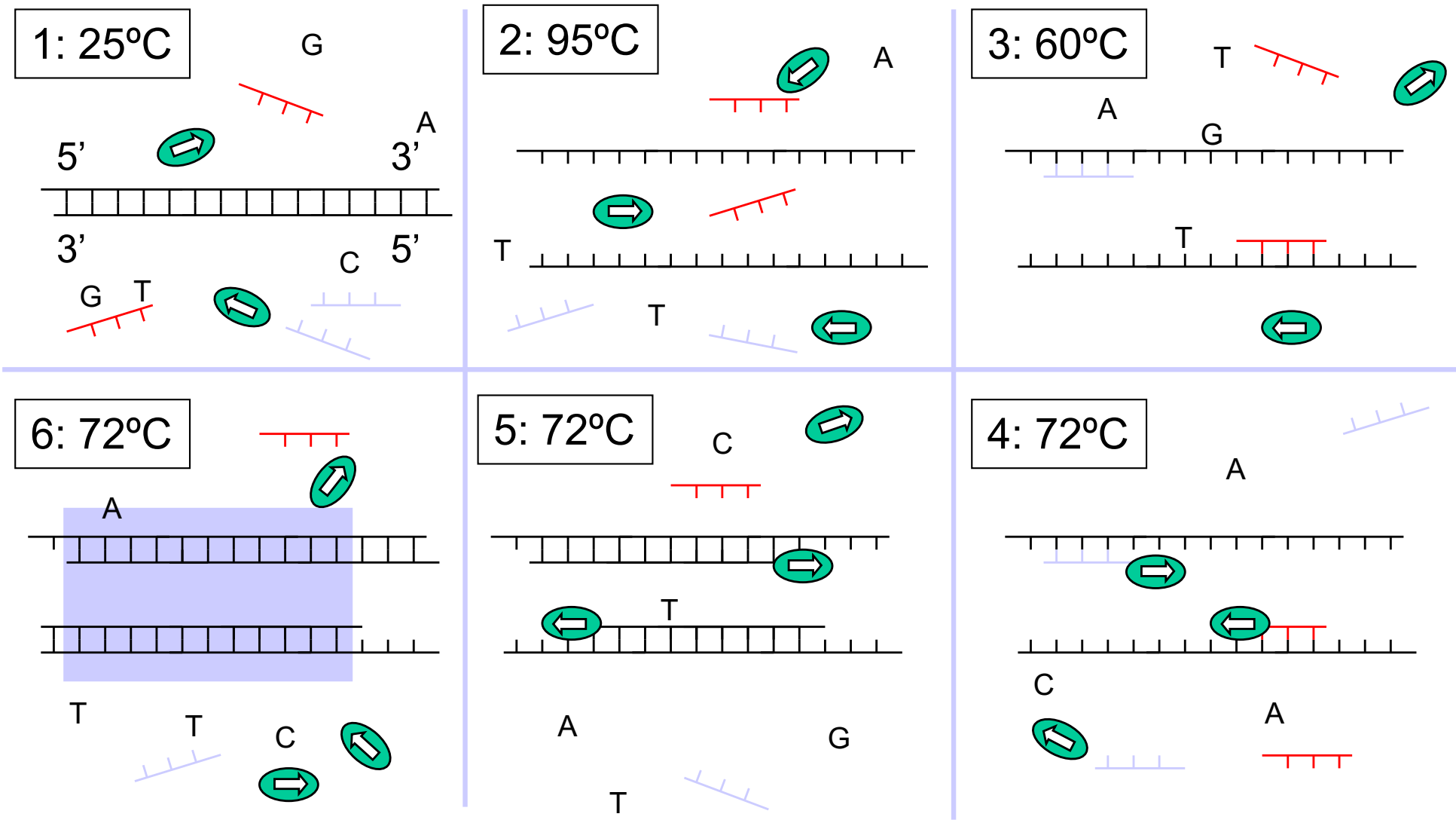
3 Nobel Prizes:

PCR: Kary Mullis, 1993

Electrophoresis: A.W.K. Tiselius, 1948

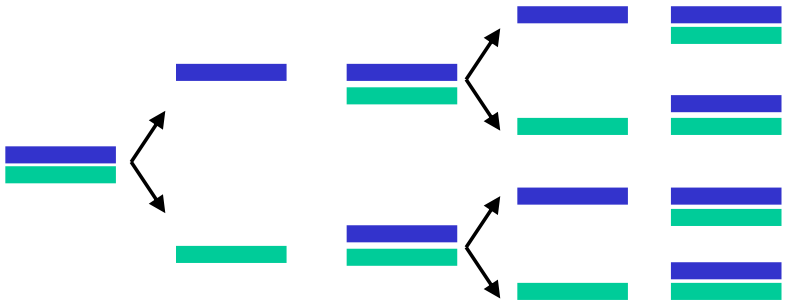
DNA Sequencing: Frederick Sanger, 1980

PCR


1: 25ºC
G
A
5'
3'
3'
5'
C
G
T
2: 95ºC
A
T
T
3: 60ºC
T
A
G
T
6: 72ºC
A
T
T
C
5: 72ºC
C
T
A
G
T
4: 72ºC
A
C
A

Hot spring, near Great Fountain Geyser, Yellowstone National Park

PCR



Ingredients:

- many copies of deoxy nucleotide triphosphates
- many copies of two primer sequences (~20 nt each)
  - readily synthesized
- many copies of Taq polymerase (*Thermus aquaticus*),
  - readily available commercialy
- as little as 1 strand of template DNA
- a programmable “thermal cycler”

Amplification: million to billion fold

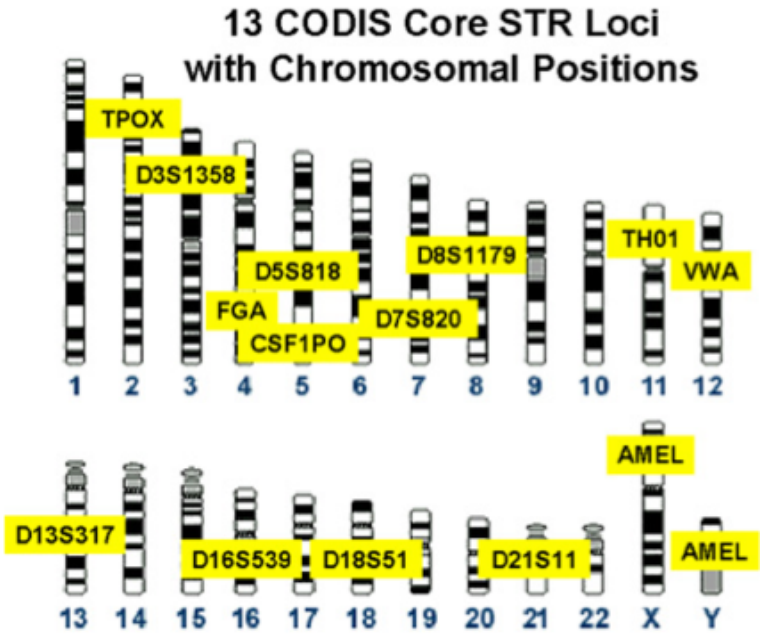
Range: up to 2k bp routinely; 50k with other enzymes & care

Why PCR?

PCR is important for all the reasons that filters and amplifiers are important in electronics, e.g., sample size is reduced from grams of tissue to a few cells, can pull out small signal amidst “noisy” background

Very widely used; forensics, archeology, cloning, sequencing, …

DNA Forensics



E.g. FBI "CODIS" (combined DNA indexing system) data base

As of 1/2013, over 10,142,600 offender profiles

Picked 13 "short tandem repeats", i.e., variable-length regions of human genome flanked by (essentially) invariant sequences (primer targets), several alleles common at each locus, of which you have 2

Amplify each from, e.g., small spot of dried blood

Measure product lengths (next slides)

http://www.fbi.gov/about-us/lab/biometric-analysis/codis

http://www.dna.gov/solving-crimes/cold-cases/howdatabasesaid/codis/

Gel Electrophoresis

DNA/RNA backbone is negatively charged (they're acids)

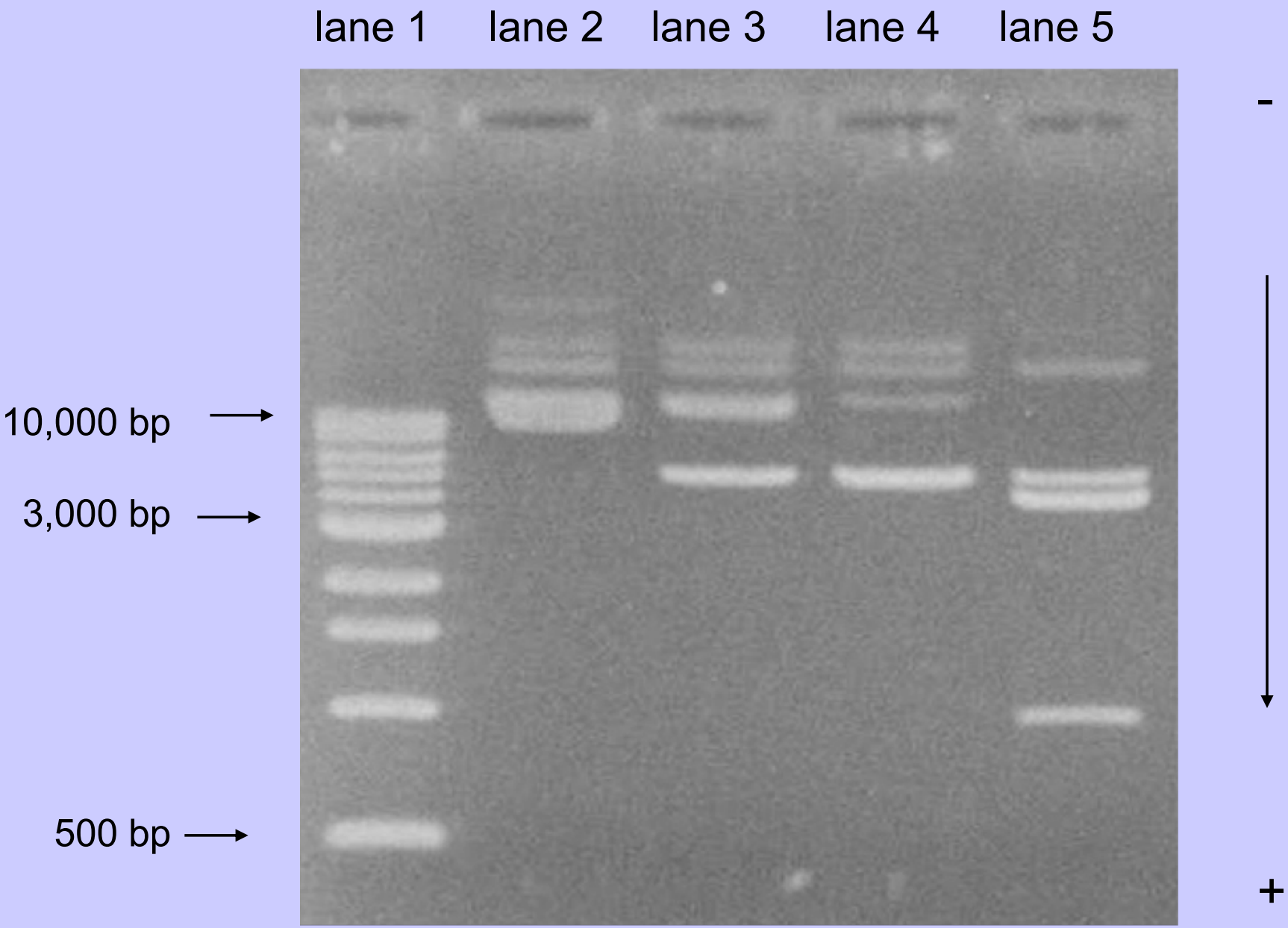
Molecules moves slowly in gels under an electric field

- agarose gels for large molecules
- polyacrylamide gels for smaller ones

Smaller molecules move faster

So, you can *separate DNAs & RNAs by size*

Nobel Chem prize, 1948 Arne Wilhelm Kaurin Tiselius

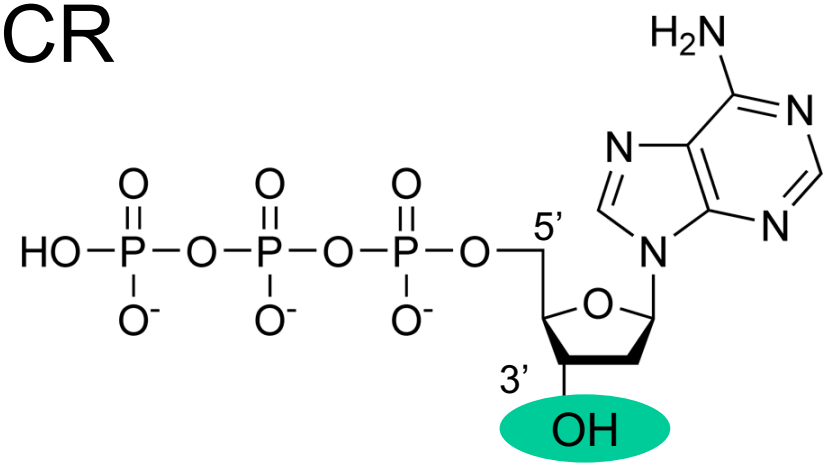

lane 1
lane 2
lane 3
lane 4
lane 5
10,000 bp
3,000 bp
500 bp
-
+

DNA Sequencing – Sanger Method

Like one-cycle, one-primer PCR

Suppose 0.1% of A's:

- are *di*-deoxy adenosine's; backbone can't extend
- carry a green florescent dye



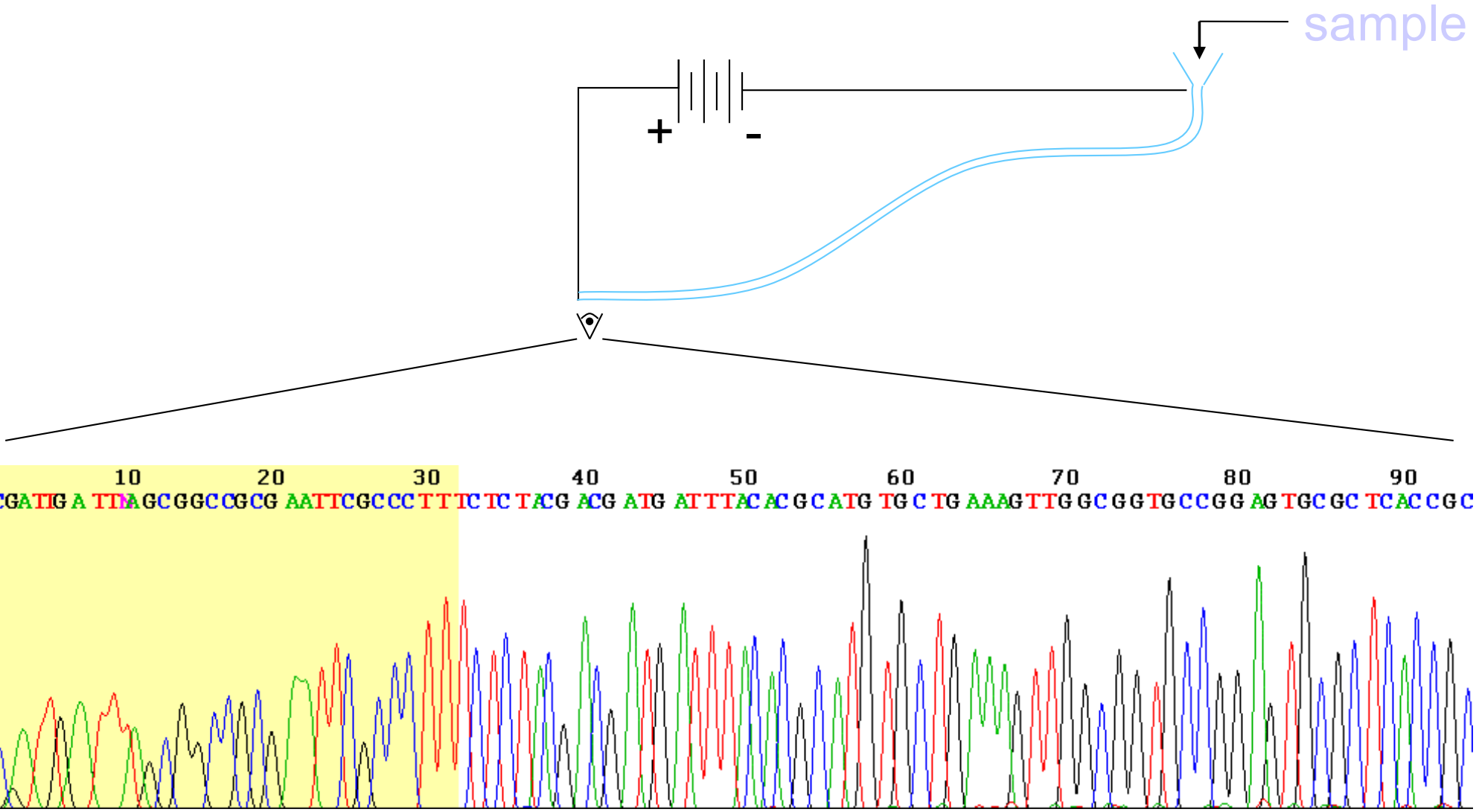
Separate by capillary gel electrophoresis

If frags of length 42, 49, 50, 55 … glow green, those positions are A's

Ditto C's (blue), G's (yellow), T's (red)

DNA Sequencing

Sanger with capillary electrophoresis



Sequencing A Genome

Highly automated

Typical Sanger "read" about 600 nt

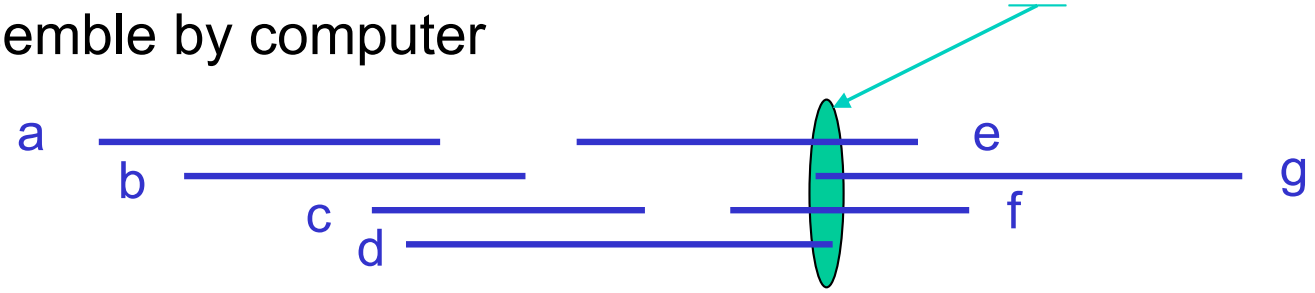
"Whole Genome Shotgun" approach:

randomly fragment (many copies of) genome

sequence many, enough to cover each base 10x or more times

reassemble by computer

E.g., human genome project:
≈ 30Gbases and
≈ $3 \times 10^9 / 600 \times 10$
= 5×10^7 reads



Complications: repeated region, missed regions, sequencing errors, chimeric DNA fragments, …

But overall accuracy ~10^{-4}, if careful

“Next Generation” Sequencing

Many technical improvements to Sanger approach over many years, culminating in highly automated machines used for the HGP

Since then, many innovative new ideas/products:

- Helicos: single molecule flourescence tethered to flow cell
- Illumina: colony PCR; reversible dye terminator
- Ion Torrent: semiconductor detection of ions released by polymerase
- Roche 454: emulsion PCR; pyro sequencing
- Oxford Nanopore
- Pacific Biosciences: single tethered polymerases in “zero mode waveguide” nano-wells, circularized DNA, “real time”
- ABI SOLiD: emulsion PCR, sequence by ligation, “color-space”
- Complete Genomics: rolling circle replication/DNA nanoballs

Technology is changing rapidly!

“Next Generation” Sequencing

~1 billion microscopic PCR “colonies” on 1x2” slide

“Read” ~50-150bp of sequence from (1 or 2) ends of each

Ends fluorescently labeled, blocked, chemically cycled

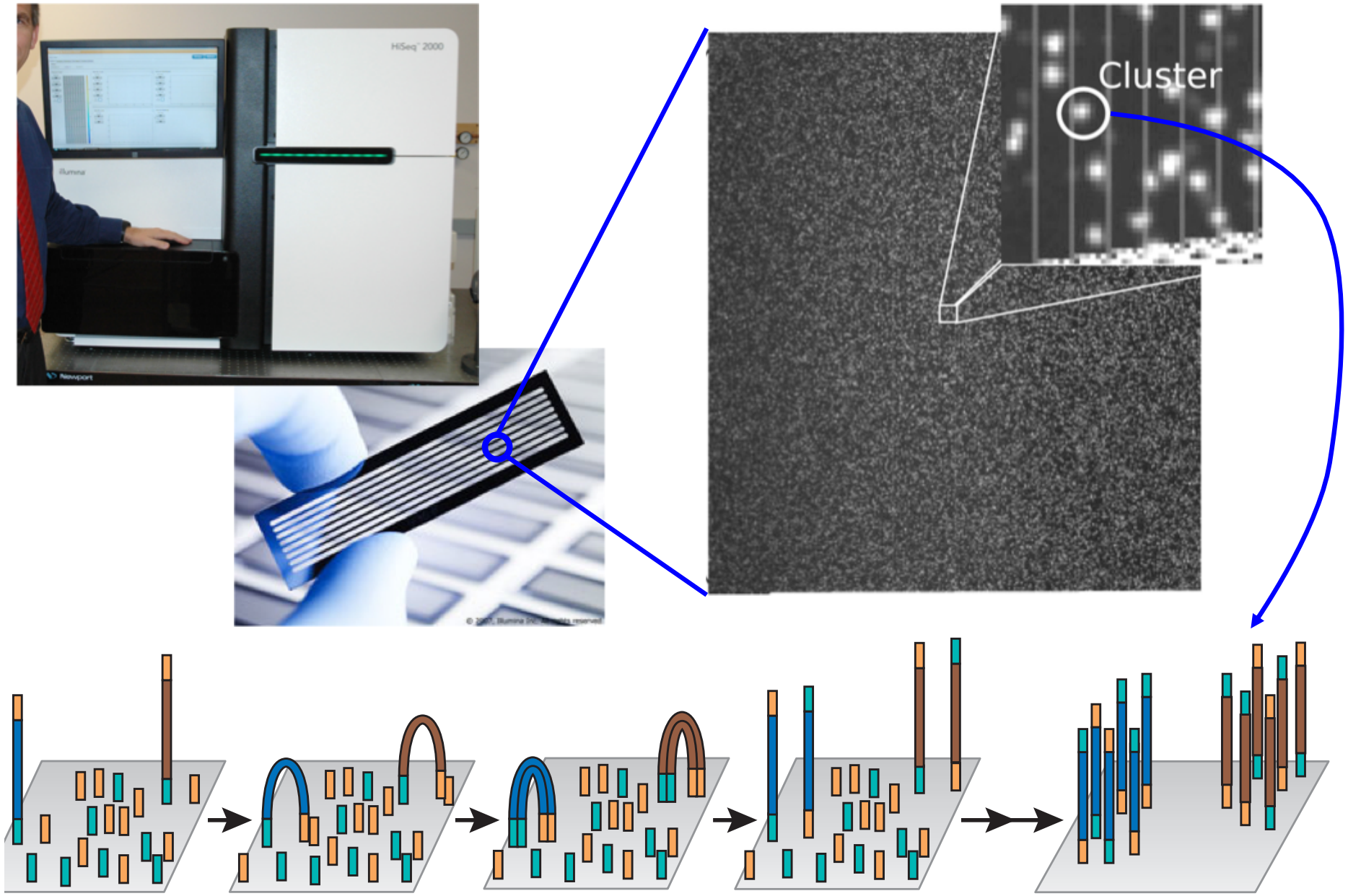
Automated: takes a few days; ~ 100 G bases/day

Costs a few thousand dollars

Generates terabytes of data (mostly images)

I,e., ~ 30x human genome/day (you need 25x-50x to assemble)

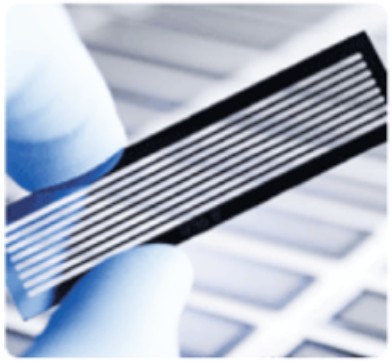
Other approaches: long reads, single molecules,…
Technology is changing rapidly!



http://www.technologyreview.com/sites/default/files/legacy/pgenome_x220.jpghttp://bioinformatics.oxfordjournals.org/content/25/17/2194/F1.large.jpg
http://bioinformatics.oxfordjournals.org/content/25/17/2194/F1.large.jpg
Fig from: Shendure and Ji 2008. “Next-Generation DNA Sequencing..” *Nature Biotechnol* 26 (10) (October): 1135–1145. doi:10.1038/nbt1486.

Modern DNA Sequencing

A table-top box the size of your oven (but costs a bit more … ;-) can generate ~100 billion BP of DNA seq/day; i.e.
= 2008 genbank,
= 30x your genome



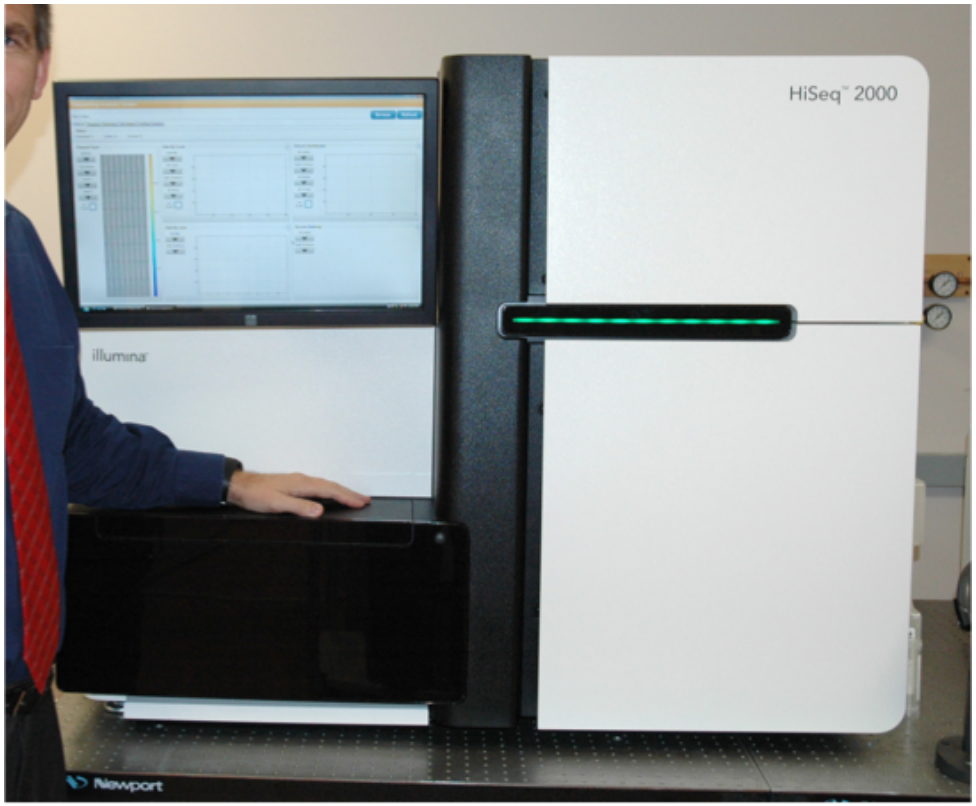


Figure 3: Illumina Sequencing Technology Outpaces Moore's Law for the Price of Whole Human Genome Sequencing

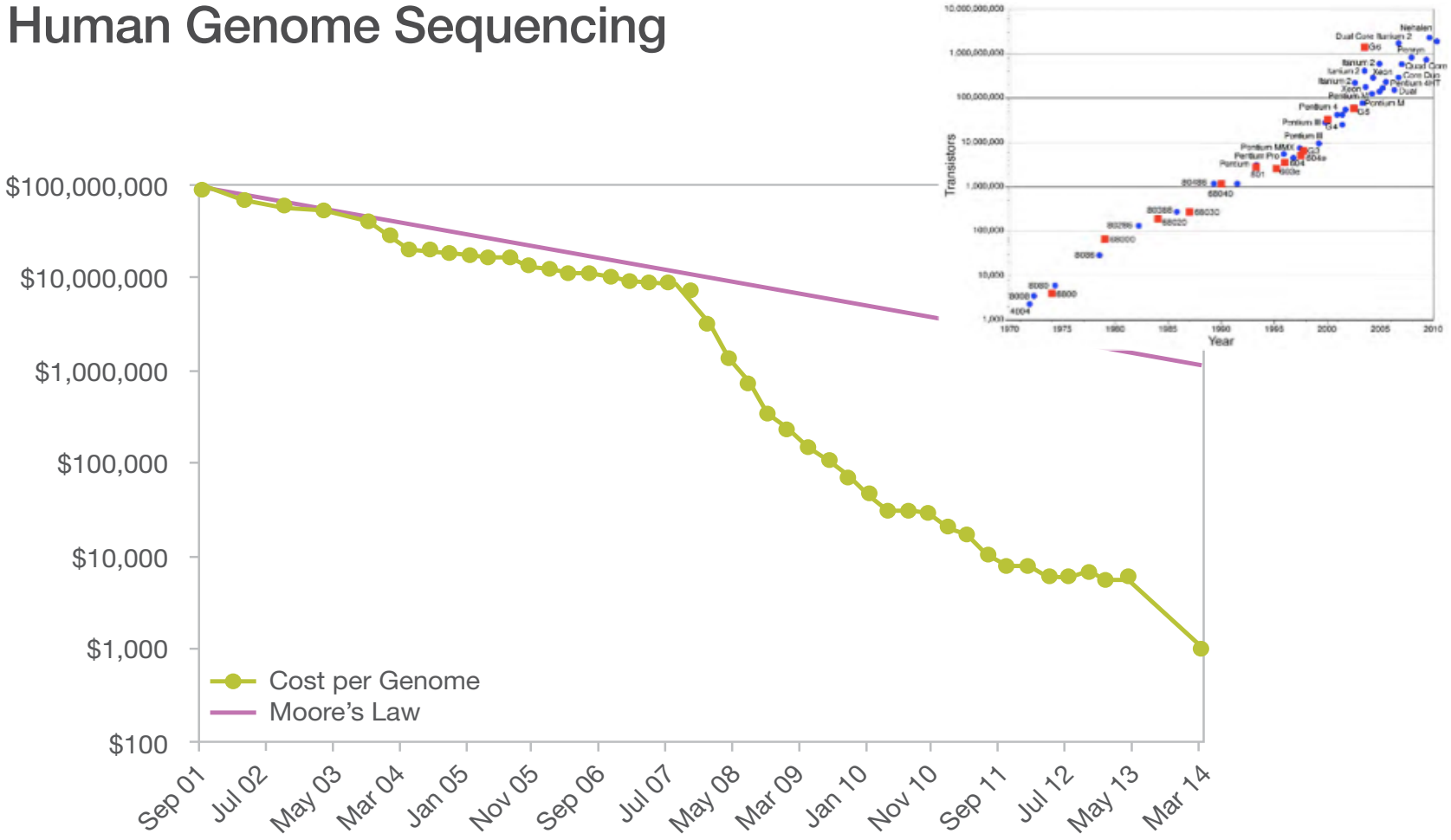


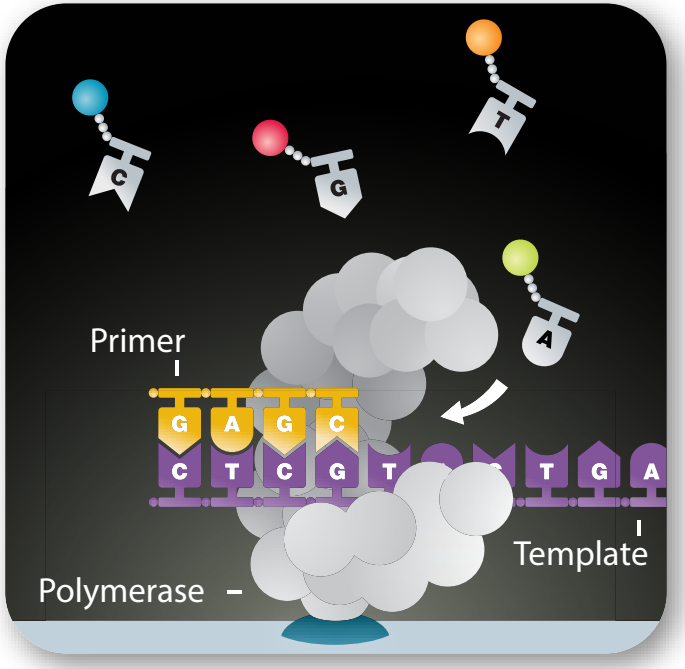
Table 1: HiSeq X Ten Preliminary Performance Parameters*

	Dual Flow Cell	Single Flow Cell
Output/Run	1.6–1.8 Tb	800–900 Gb
Reads Passing Filter†	≤ 6 billion	≤ 3 billion
Supported Read Length	2 × 150	
Run Time	< 3 days	
Quality	≥ 75% of bases above Q30 at 2 × 150 bp	

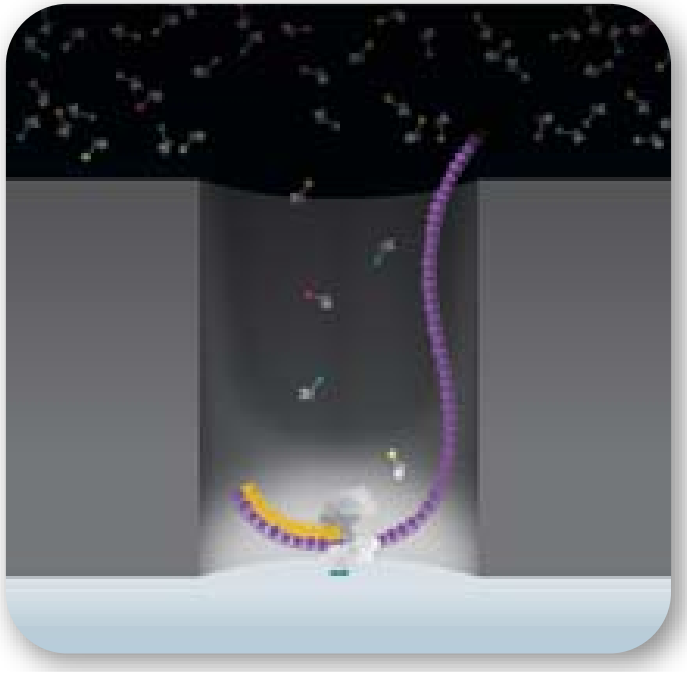
*Specifications based on Illumina PhiX control library at supported cluster densities (between 1,255–1,412 K clusters/mm^2). Supported library preparation kit includes TruSeq Nano DNA HT kit with 350 bp target insert size and HiSeq X HD reagents. HiSeq X was designed and optimized for human whole-genome sequencing; other applications and species are not supported.

†Single-end reads.

Pacific Biosciences



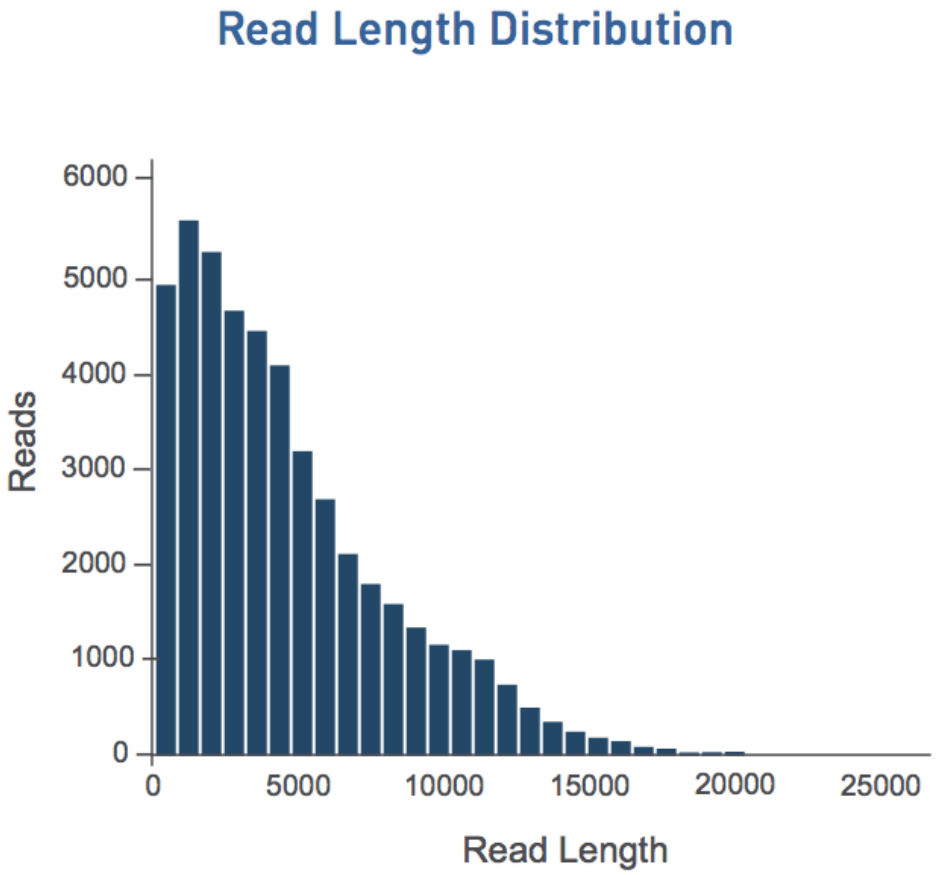
Phospholinked nucleotides



Zero-Mode Waveguides

http://files.pacb.com/pdf/PacBio_RS_II_Brochure.pdf http://www.globenewswire.com/NewsRoom/Attachment/18068

Pacific Biosciences



Advantages:
- single molecules
- long reads
- direct CH_3 detection

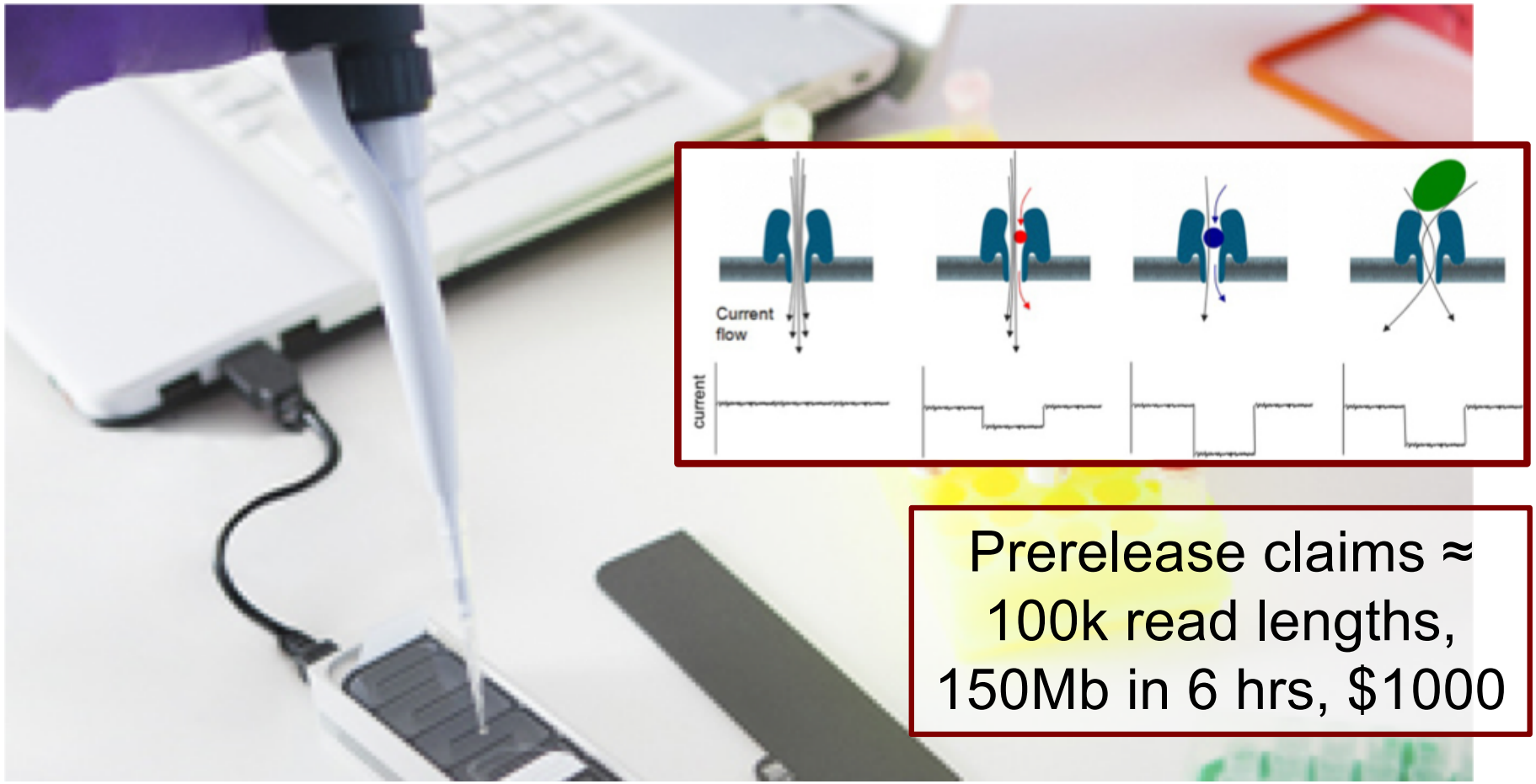
Disadvantages:
- throughput
- error rate; (circularize?)

Read Length:	
Average:	4,606 bp
95th Percentile:	11,792 bp
Maximum:	23,297 bp
Throughput per SMRT® Cell:	216 Mb
	47,197 reads

Based on data from 11 kb plasmid library using a 120 minute movie

http://www.pacificbiosciences.com/img/assets/smrt_sequencing_advantage_readlength_lg.png

Oxford Nanopore



Prerelease claims ≈
100k read lengths,
150Mb in 6 hrs, $1000

http://www.nanoporetech.com/uploads/Technology_New/MinION/MinION_117.jpg

http://www.nanoporetech.com/uploads/Technology_New/Introduction_To_Nanopore_Sensing/Nanopore_sensing_101_0_rs.jpg

Personal Genomes

2001: ~$2.7 billion (Human Genome Project)

2003: ~$300 million

2007: ~$1 million

2008: ~$60 thousand

2009: ~$4400

2014: ~$1000 (?)

bioinformatics not included…

Summary

- PCR allows simple *in vitro* amplification of minute quantities of DNA (having pre-specified boundaries)
- Sanger sequencing uses
  - a PCR-like setup with modified chemistry to generate varying length prefixes of a DNA template with the last nucleotide of each color-coded
  - gel electrophoresis to separate DNA by size, giving sequence
- Sequencing random overlapping fragments allows genome sequencing (and many other applications)
- “Next Gen” sequencing: many innovations
  - throughput up, cost down (lots!)