DNA Sequencing

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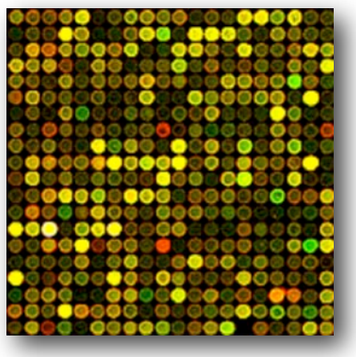
Technology for studying nucleic acids

Sanger DNA sequencing

1977-1990s



DNA Microarrays

Since mid-1990s

2nd-generation DNA sequencing

Since ~2007

3rd-generation & single-molecule DNA sequencing

Since ~2010

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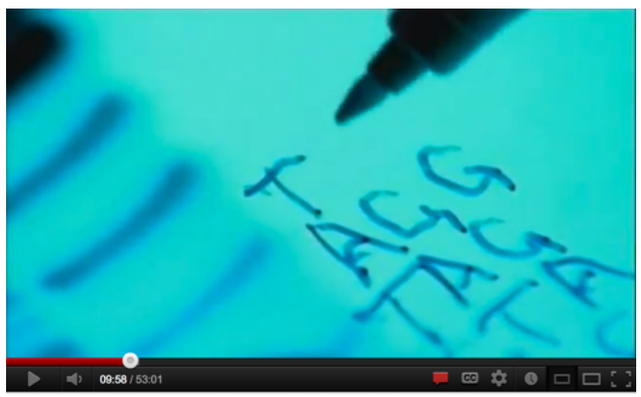
Sanger (“first generation”) DNA sequencing

Sanger sequencing
1977-1990s

Fred Sanger in episode 3 of PBS documentary “DNA”



Not-so-high-throughput Sanger sequencing

First practical method invented by Fred Sanger in 1977. Initially used to sequence shorter genomes, e.g. viral genomes 10,000s of bases long.

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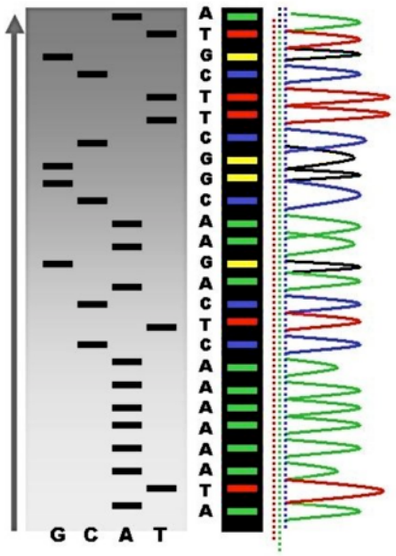
DNA sequencing: Human Genome Project



Improvements both in technology and in algorithms allowed it to eventually be used for human genome project.

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Sequencing

No sequencing technology yet invented can read much more than 10,000 nucleotides at a time with reasonable cost, throughput, accuracy

Instead, there's a vigorous race to see whose sequencer can read "short" fragments of DNA (around 100s of nucleotides) with best cost, throughput, accuracy

Decoding DNA With Semiconductors

By NICHOLAS WADE
Published: July 20, 2011

Company Unveils DNA Sequencing Device Meant to Be Portable, Disposable and Cheap

By ANDREW POLLACK
Published: February 17, 2012

Cost of Gene Sequencing Falls, Raising Hopes for Medical Advances

By JOHN MARKOFF
Published: March 7, 2012

Source: nytimes.com

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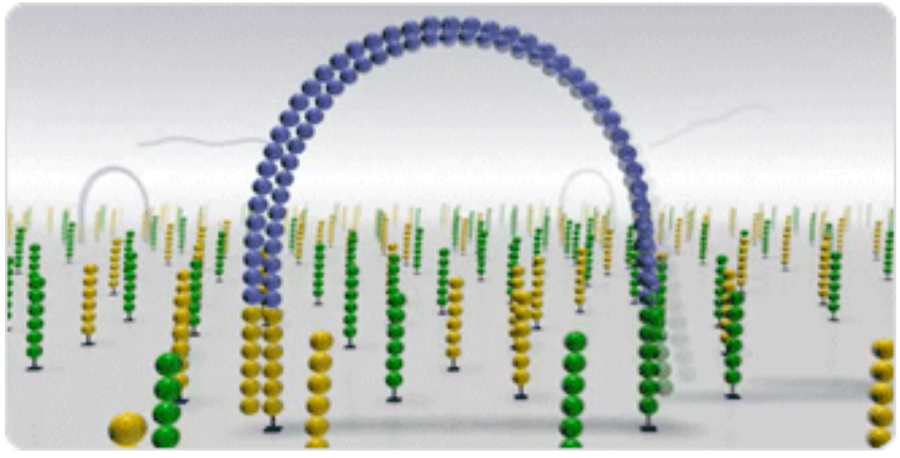
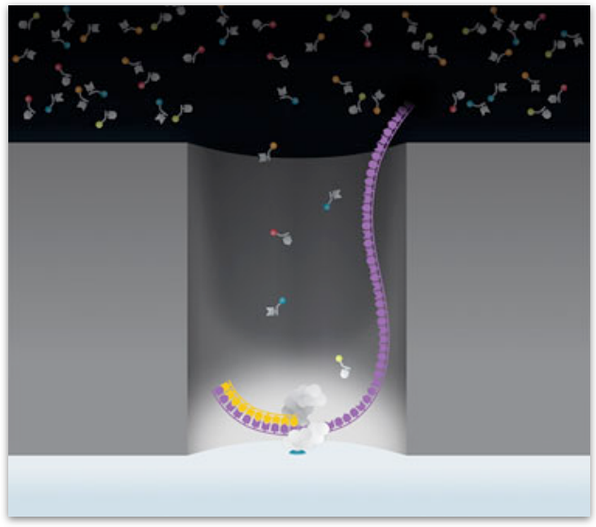
Sequencing

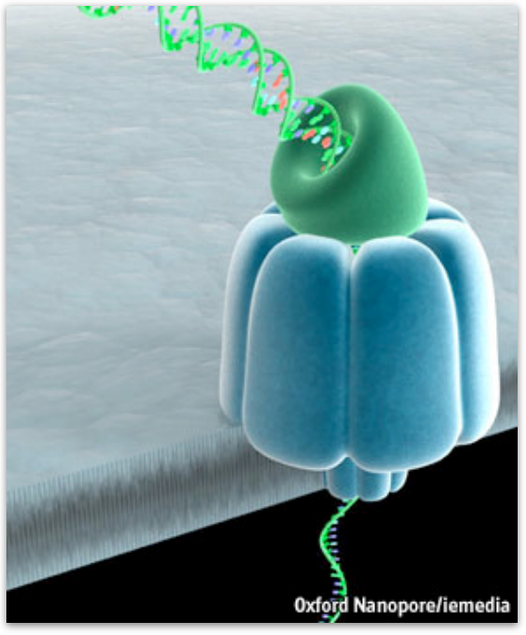
Since 2005, many DNA sequencing instruments have been described and released. They are based on a few different principles



Synthesis / ligation



SMRT cell



Nanopore

Sequencing by synthesis (“massively parallel sequencing”) provides greatest throughput, and is the most prevalent today

Pictures: http://www.illumina.com/systems/miseq/technology.ilmn, http://www.genengnews.com/gen-articles/third-generation-sequencing-debuts/3257/

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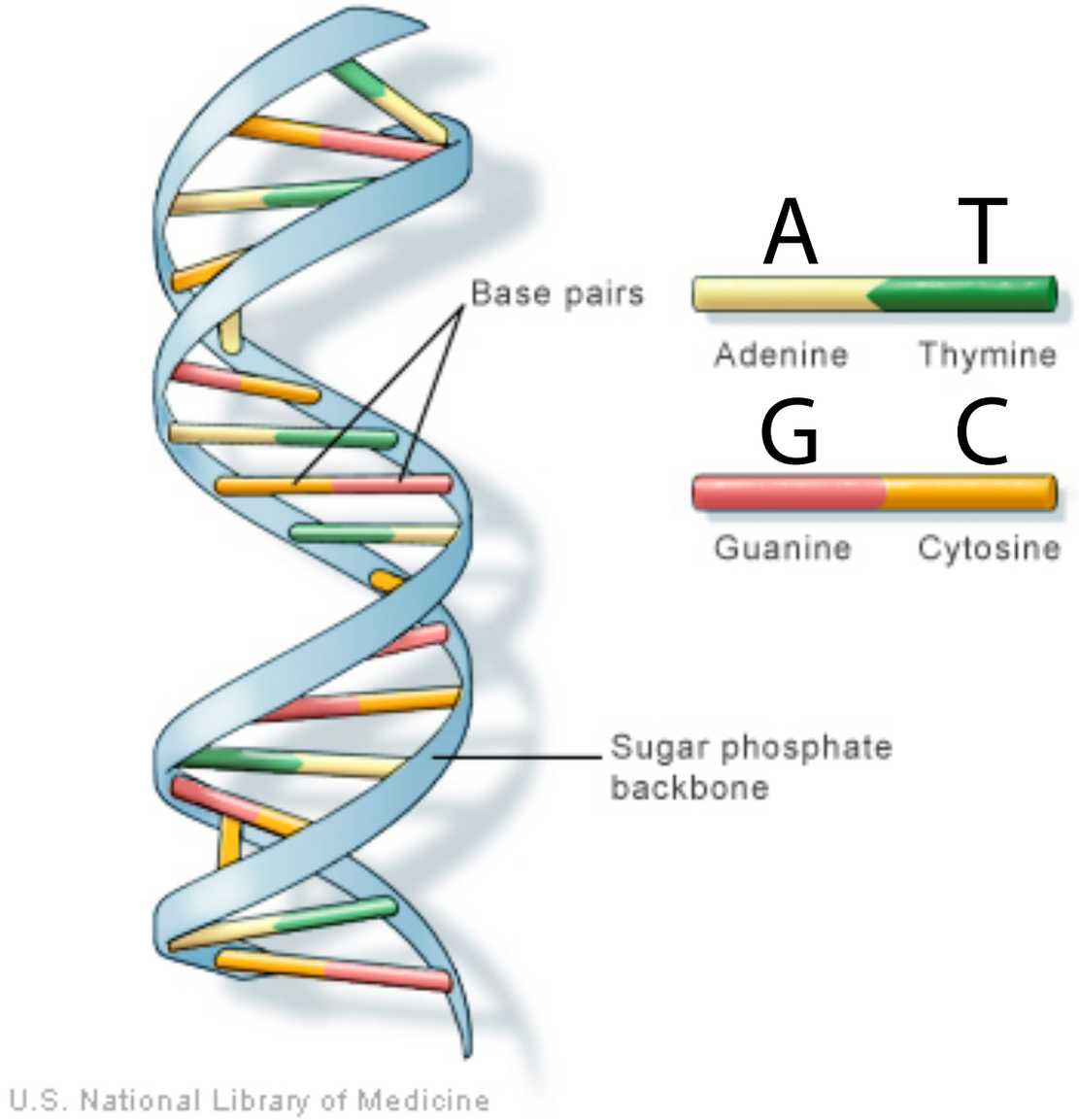
DNA sequencing: double helix



Picture: http://ghr.nlm.nih.gov/handbook/basics/dna

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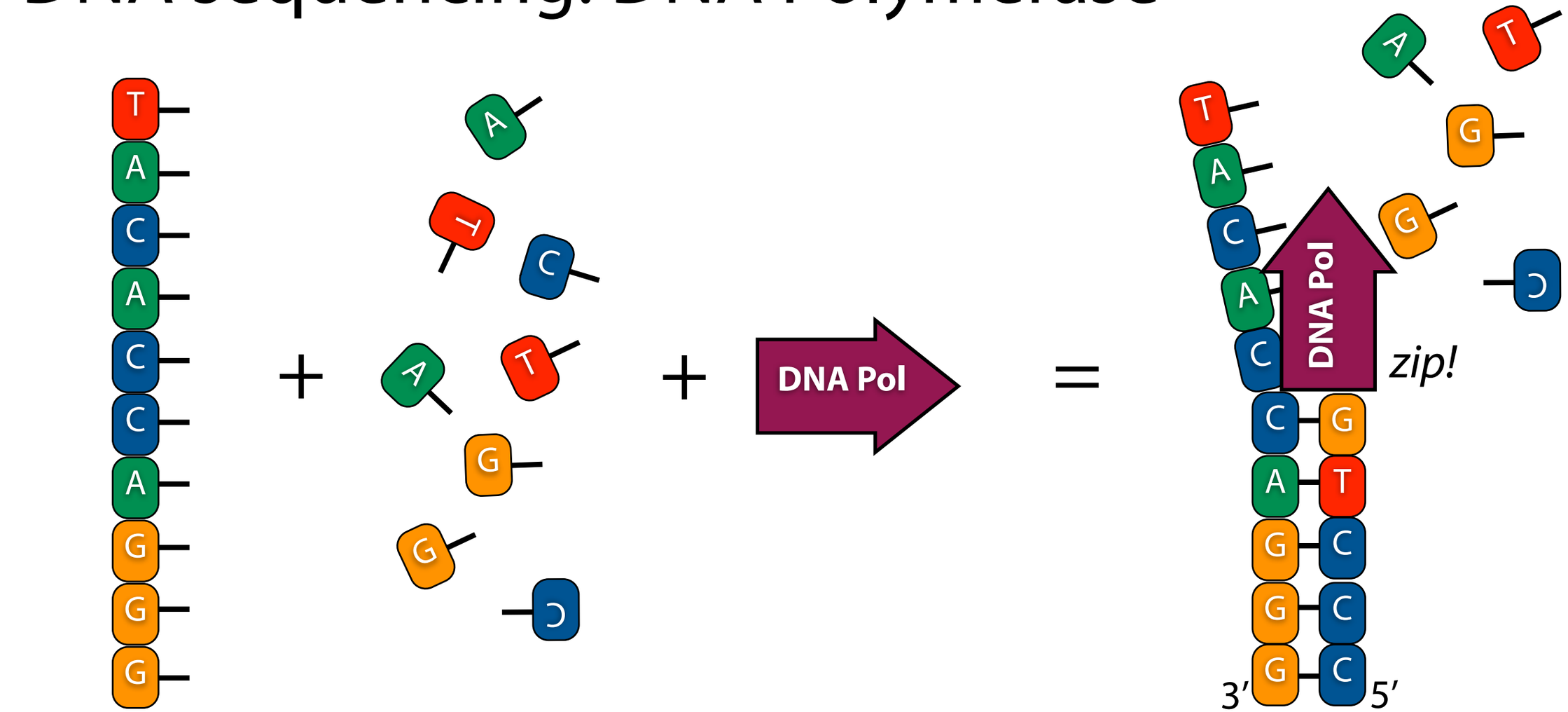
DNA sequencing: DNA Polymerase



Single-stranded DNA *template* | Free nucleotides | DNA polymerase | Strand synthesis

DNA polymerase moves along the template in one direction, integrating complementary nucleotides as it goes

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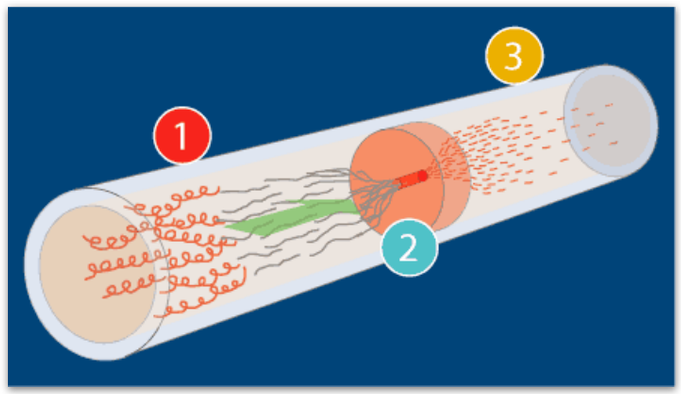
Sequencing by synthesis

1. Take DNA sample, which includes many copies of the genome, and chop it into single-stranded fragments (“templates”)

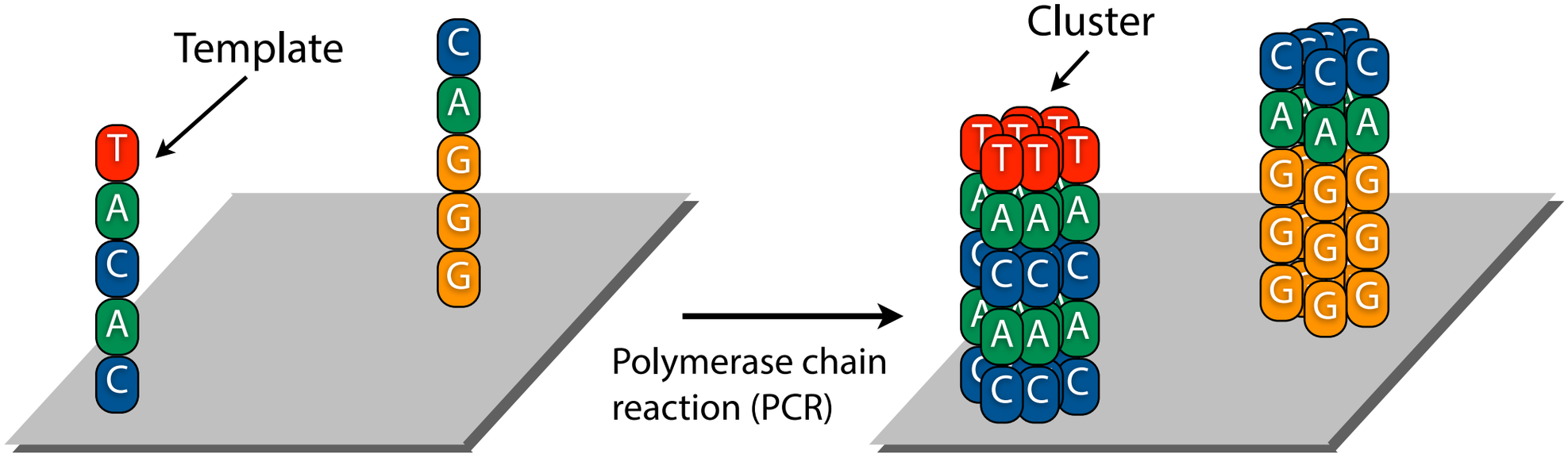
   E.g. with ultrasound waves, water-jet shearing (pictured), divalent cations



Picture: http://www.jgi.doe.gov/sequencing/education/how/how_1.html

2. Attach templates to a surface

3. Make copies so that each template becomes a “cluster” of clones



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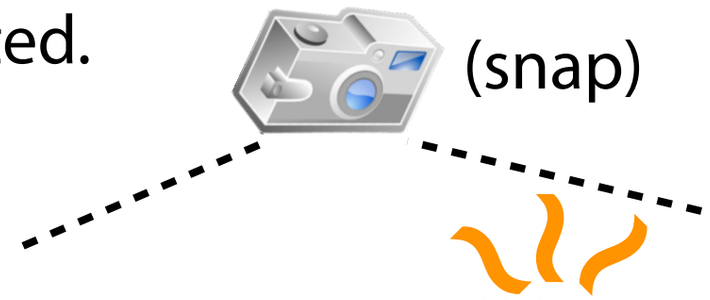
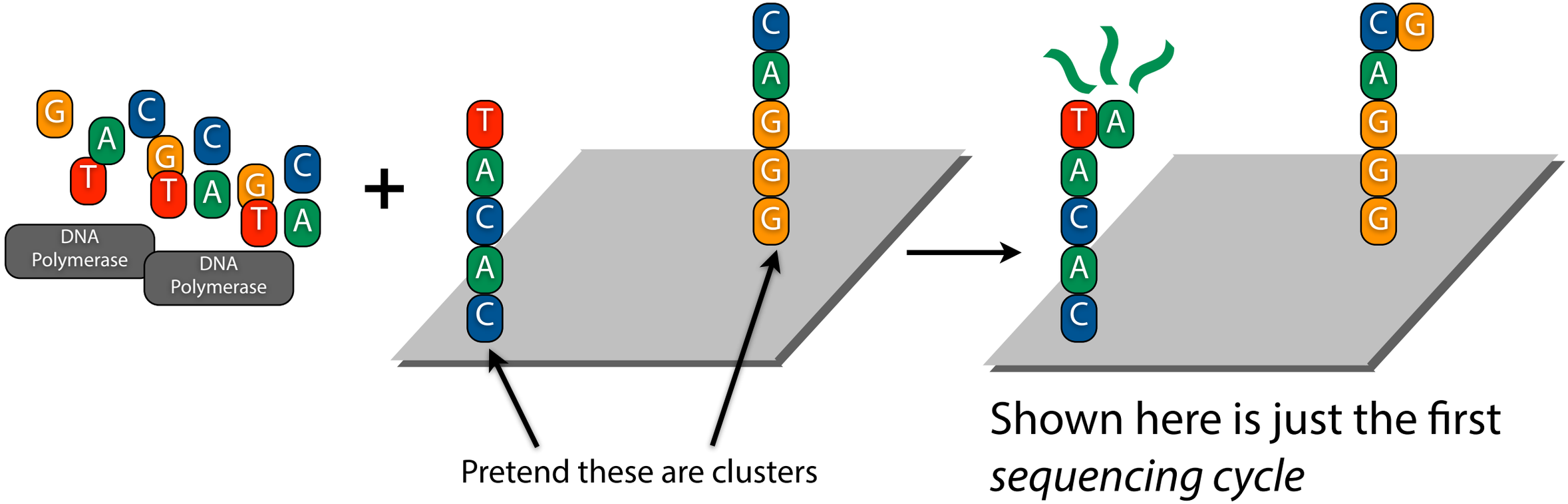
Sequencing by synthesis

4. Repeatedly inject mixture of *color-labeled* nucleotides (A, C, G and T) and DNA polymerase. When a complementary nucleotide is added to a cluster, the corresponding color of light is emitted. Capture images of this as it happens.





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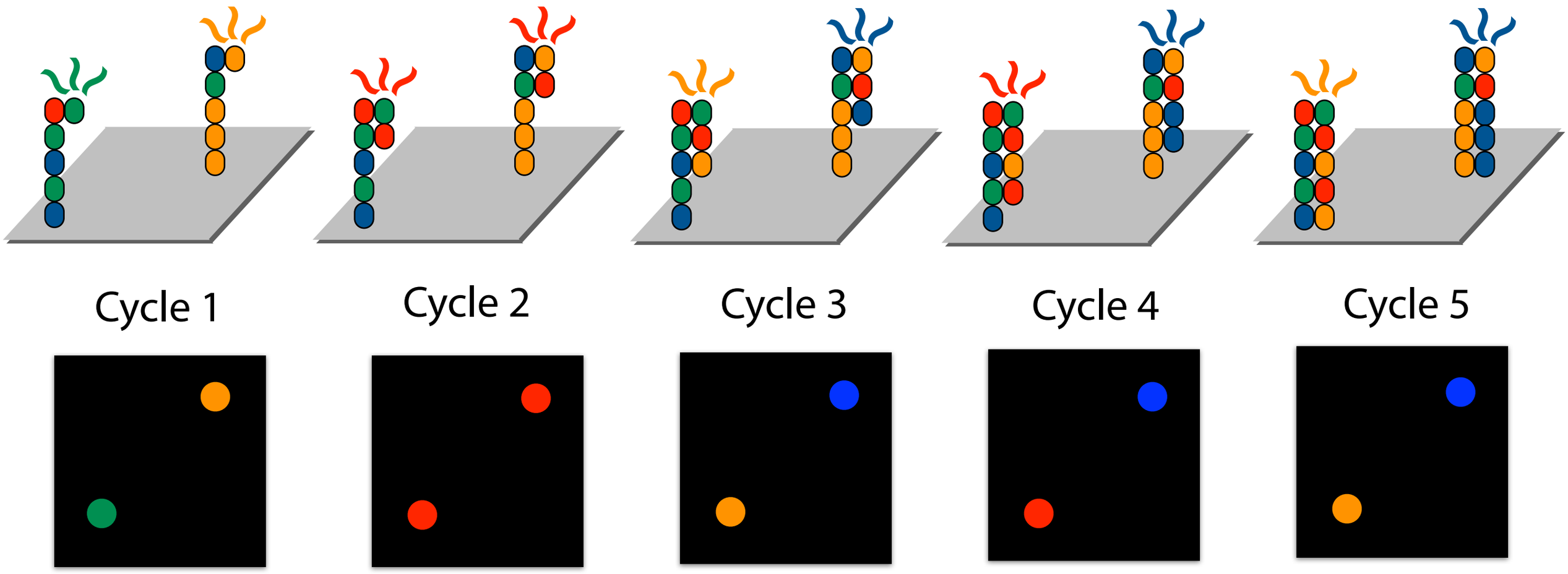
Sequencing by synthesis

5. Line up images and, for each cluster, turn the series of light signals into corresponding series of nucleotides



Sequencing by synthesis

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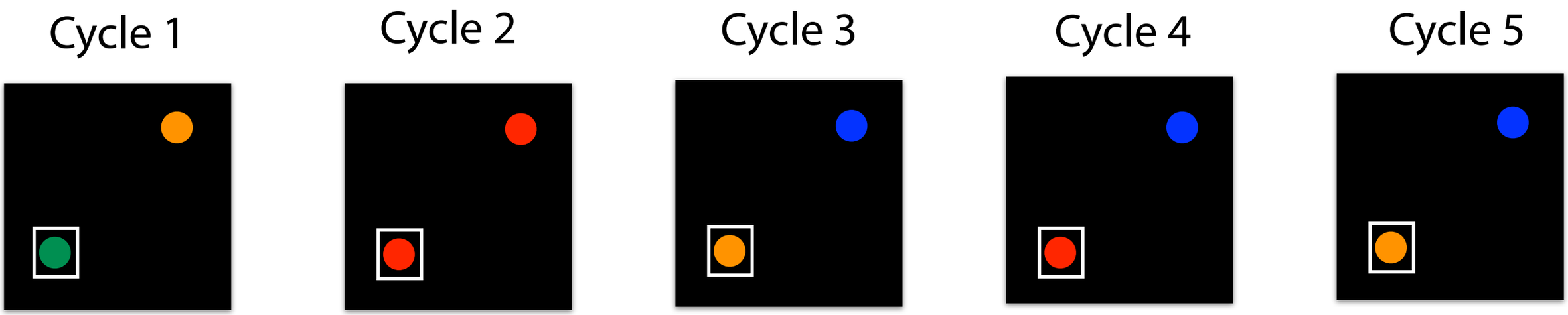
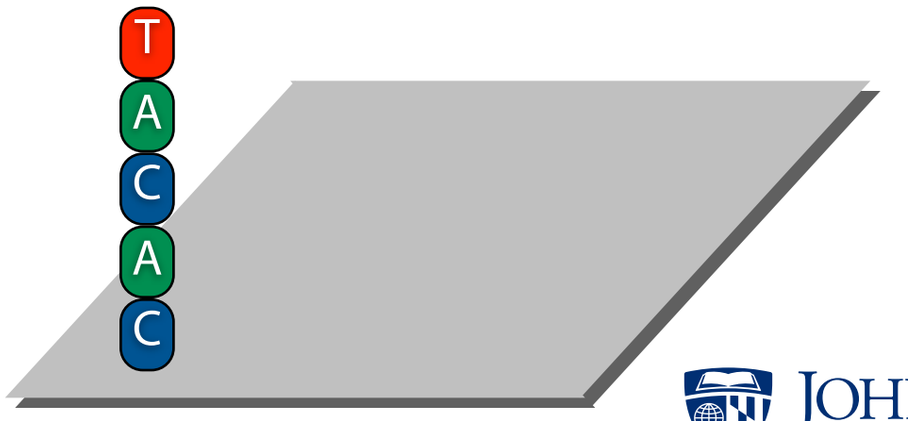


"Base caller" software looks at this cluster across all images and "calls" the complementary nucleotides: TACAC, corresponding to the template sequence



TACAC is a "sequence read," or "read." Actual reads are usually 100 or more nucleotides long.

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Sequencing by synthesis

A modern sequencing-by-synthesis instrument such as the HiSeq sequences *billions* of clusters simultanously

A single "run" takes about 10 days to generate about 600 billion nucleotides of data

Cost of the reagents is $5-10K per run; multiplexing (sequencing many samples per run) further reduces cost per genome

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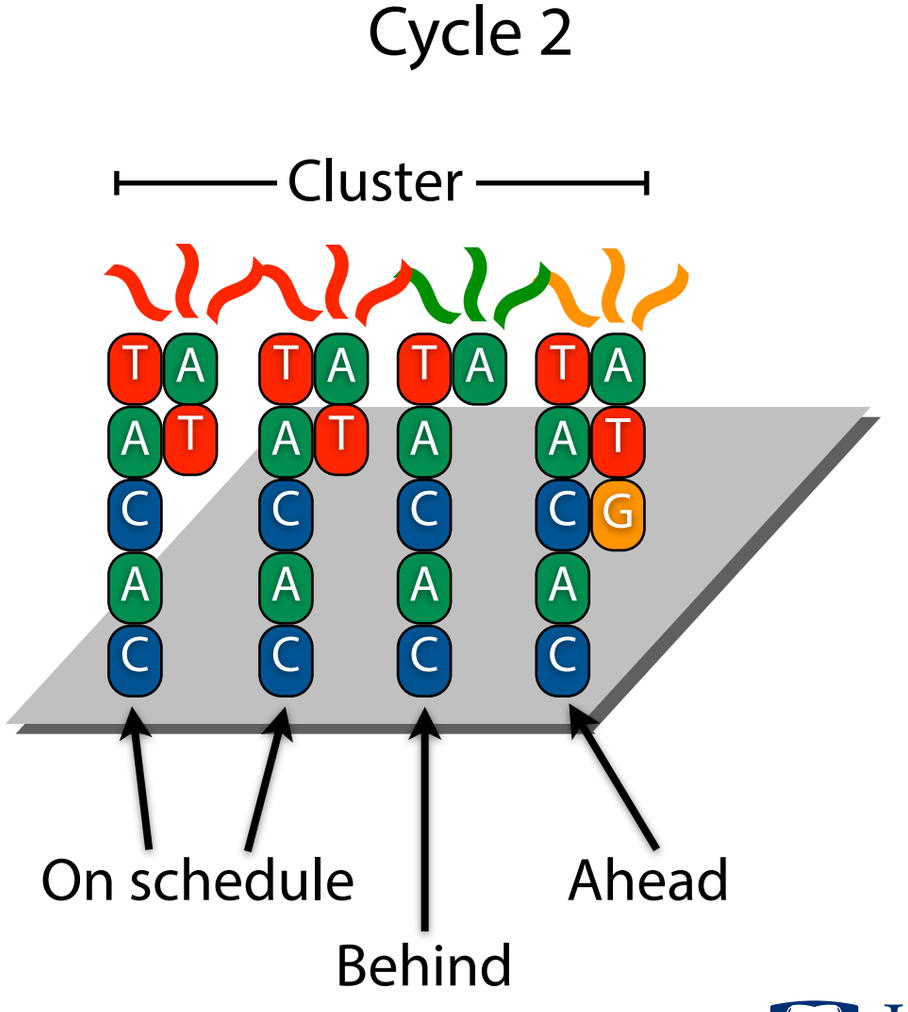
Sequencing by synthesis: errors

Errors creep in when some templates get "out of sync," by missing an incorporation or by incorporating 2 or more nucleotides at once



Base caller must deal with this uncertainty. Actual base callers report a *quality score* (confidence level) along with each nucleotide.

Errors are more common in later sequencing cycles, as proportionally more templates fall out of sync

Sequencing: read format

Below is a FASTQ file. A chunk of 4 lines describes a read. For each read, the 4 lines are (1) read name, (2) nucleotide sequence, (3) (placeholder), (4) quality value sequence.

Read 1	Name
	Nucleotides
	(placeholder)
	Quality values
Read 2	Name
	Nucleotides
	(placeholder)
	Quality values
Read 3	Name
	Nucleotides
	(placeholder)
	Quality values
Read 4	Name
	Nucleotides
	(placeholder)
	Quality values
Read 5	Name
	Nucleotides
	(placeholder)
	Quality values

reads — Example — bash — 104×25

```
$ head -20 SRA_HISEQ2000_FC1.shuffle.2M.1.fastq
@509.6.64.20524.149722
AGCTCTGGTGACCCATGGGCAGCTGCTAGGGAGCCTTCTCTCCACCCTGAAAATAGCTTCTGGCTGNTGGGTGAACTATGGAGAGAAAGCGTTTTATTAT
+
HHHHHHHGHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHIIHHIHFHHFHHHIHGEHHIIFIHBC#@:@9,--541436D9?;E###############
@509.4.62.19231.2763
GTTGATAAGCAAGCATCTCATTTTGTGCATATACCTGGTCTTTCGTATTCTGGCGTGAAGTCGCCGNCTGAATGCCAGCAATCTCTTTTTGAGTCTCATT
+
HHHHHHHHHHHHHHEHHHHHHHHHHHHHHHHHHHHHHHDHHHHHHGHGHHHHHHHHHH=EF?DHE4#555=;===GGHEGGEGHG@C@<7<3@?F<A9@<
@509.6.47.3027.76579
CCTTTTCGACTAGAGACTGCCAAGTGCCAAAATATCCACTTGCAGATACTACAACAAGAGTGTTTCNAAACTGCTCAATCAAAAGAAATGTTCAACTCTT
+
HHHHHHHHHHHHHHHHHHHIHHHHHHHHHHHHHHHHHHHHGHHHHHHHHGHHHHHHHHHHEH?HH4#554DDADDHHHHHH@GHHFGBBFFHFHFHEHHH
@509.2.7.2951.186312
AAAGATACAACATACCACAATCTTTGAGACACACCTAAGACAATAAGGCAGTGTTAAGAGGAAAATTAATAGCACTAAATGCCCACATCAAAAAGTTAGA
+
HHHHHHHHHHHHHHHHHHHGHDHHHHHFHEHHHGHHGHHHHHHHHHGHHHHHHEHEF<?<@=BBFFFGCFFE?<;@AFG=GA;@D@D?FDFFB=B;F=>AA@
@509.6.25.8102.140546
GGACACATTCAAACCATTGCATCCATCCTCTGCATTCAGAAAGATAGTCCAACAGAAAGATCTGGANTCAAGAGACCCAGCTGATTACCAATTCCAGTTT
+
HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHIHHHHHHHIHHHIHHHIHG#FFDCDD@@GGGHHFIHEGIFIEIIIIGIIGFGF
$
```

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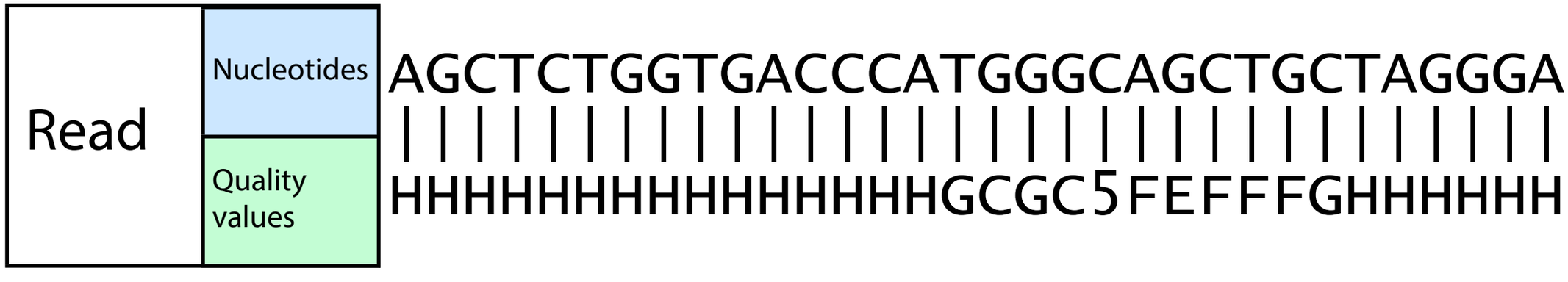
Sequencing: qualities

Nucleotides and quality values line up 1-to-1:



A quality value is an ASCII encoding of a number, Q

where $Q = -10 \log_{10} p$

where p is sequencer's estimate of the probability that the nucleotide at that position was called *incorrectly*

$Q = 10$: error probability is 1 in 10
$Q = 20$: error probability is 1 in 100
$Q = 30$: error probability is 1 in 1,000

Sometimes called "Phred scale"

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See also: http://en.wikipedia.org/wiki/Fastq

Sequencing: qualities

Typical ASCII encoding is “Phred+33”:

take integer Q, add 33, convert to character

```
def phred33ToQ(qual):
  """ Turn Phred+33 ASCII-encoded quality into Phred-scaled integer """
  return ord(qual)-33
```

(converts character to integer)

```
def QtoPhred33(Q):
  """ Turn Phred-scaled integer into Phred+33 ASCII-encoded quality """
  return chr(Q + 33)
```

(converts integer to character)

```
def QtoP(Q):
  """ Turn Phred-scaled integer into error probability """
  return 10.0 ** (-0.1 * Q)
```

(exponentiation)

```
def PtoQ(p):
  """ Turn error probability into Phred-scaled integer """
  import math
  return -10.0 * math.log10(p)
```