Introduction to second-generation sequencing

CMSC858B Spring 2012
Many slides courtesy of Ben Langmead

Second-Generation Sequencing

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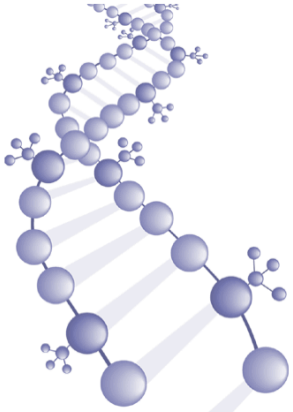
Human Epigenome Project

HEP
Human
Epigenome
Project



Methylation

epigenomics | The Wellcome Trust Sanger Institute | CNG

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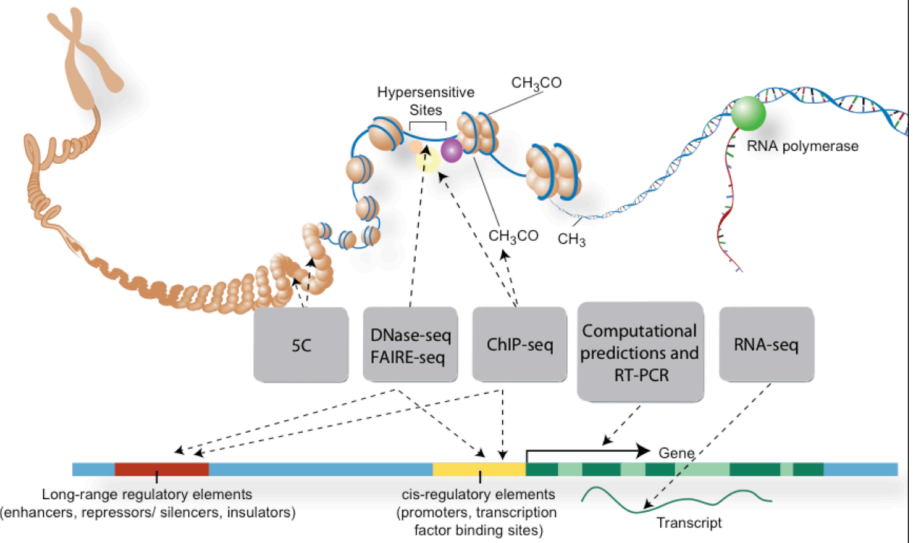
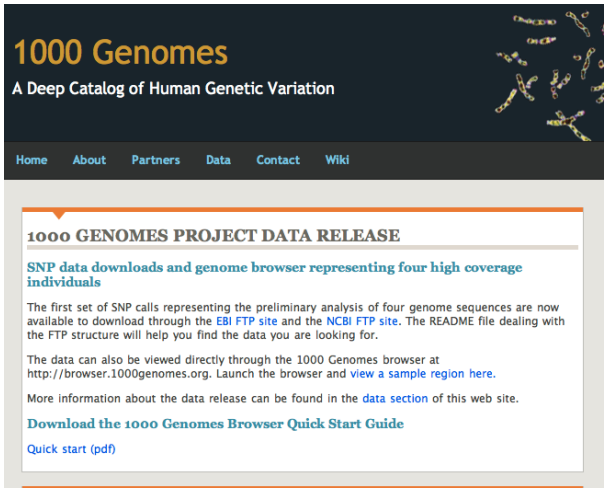
ENCODE project



http://www.genome.gov/10005107

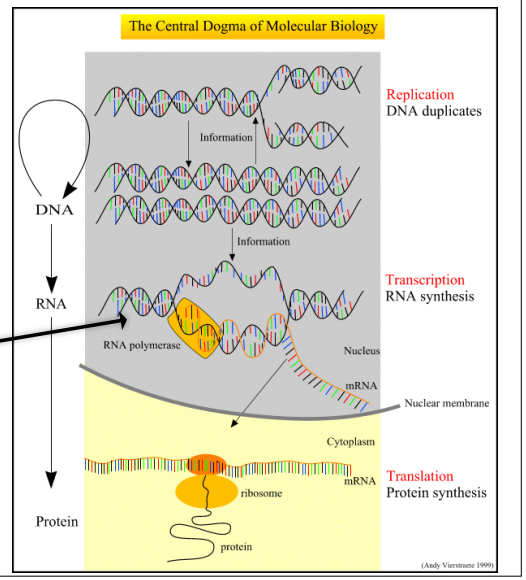
1000 Genomes Project



What Do They Measure?

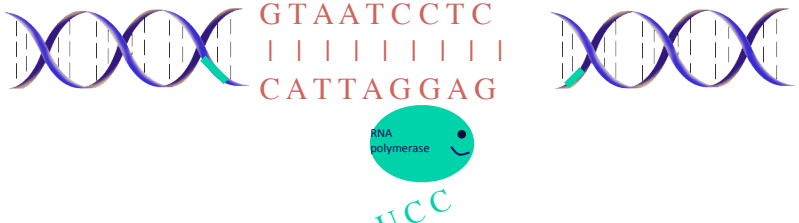
Microarrays

Gene Expression Arrays
Exon Arrays



Transcription

DNA

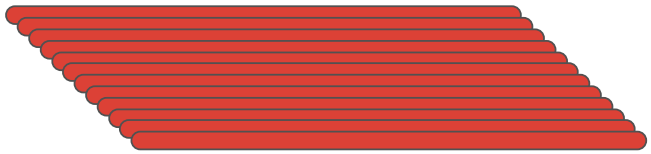


From DNA to mRNA

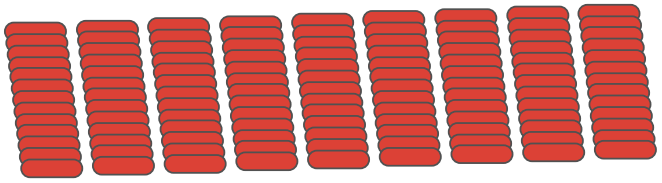
Reverse transcription

Clone cDNA strands, complementary to the mRNA

Sec-gen Sequencing

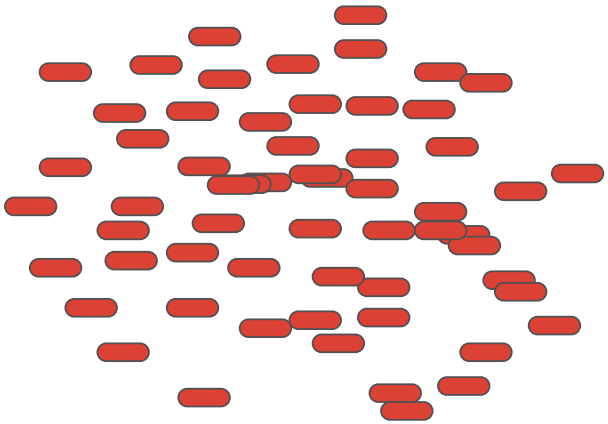


Sec-gen Sequencing



Fragmentation is random,
i.e., not equal-sized (but hard to draw)

Sec-gen Sequencing



Second-Generation Sequencing

- "Ultra high throughput" DNA sequencing
  - 3 gigabases / day vs.
  - 3 gigabases / 13 years (human genome project, more or less)

Platforms

illumina

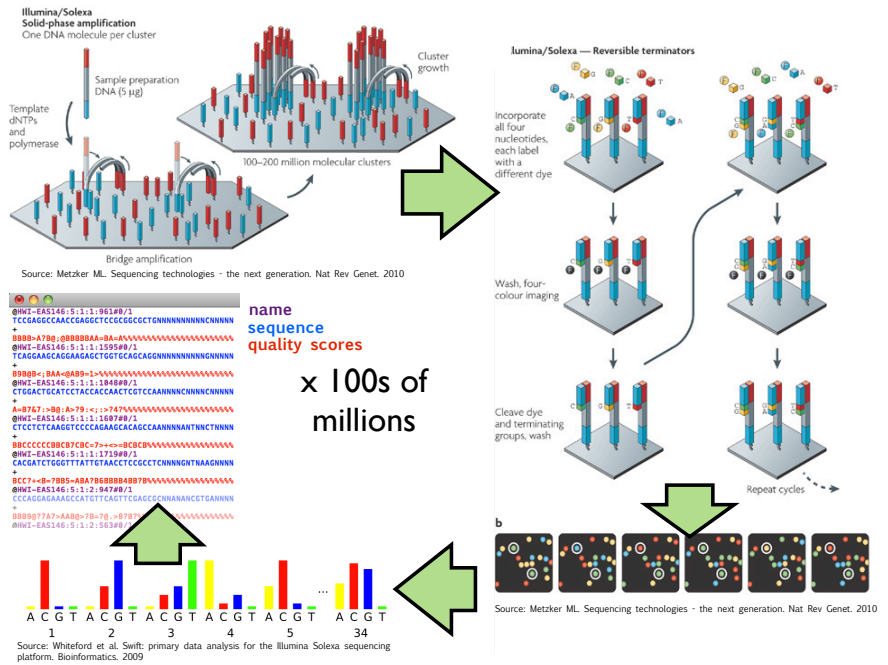
life technologies | Semiconductor Sequencing for Life™ ion torrent

454 SEQUENCING | Roche

NANOPORE

Applied Biosystems

- Millions of short DNA fragments (~100 bp) sequenced in parallel



Sequencing throughput

GA II
1.6 billion bp per day
(2008)

GA IIx
5 billion bp per day
(2009)

HiSeq 2000
25 billion bp per day
(2010)

Images: www.illumina.com/systems
Numbers: www.politigenomics.com/next-generation-sequencing-informatics
Dates: Illumina press releases

Sequencing throughput

GA II
1.6 billion bp per day
(2008)

GA IIx
5 billion bp per day
(2009)

HiSeq 2500
60 billion bp per day
(2012)

Images: www.illumina.com/systems
Numbers: www.politigenomics.com/next-generation-sequencing-informatics
Dates: Illumina press releases

Sequencing throughput

End of 2009 Mid 2010 Late 2011/Early 2012

SOLiD 3+ System
Up to 50 Gb

New SOLiD™ Systems		
Specifications	SOLiD™ 4 System	SOLiD™ 4*hq* System*
Throughput/Run**	Up to 100 Gb	Up to 300 Gb
System Accuracy	99.94%	99.99%
Cost/Genome***	As low as $6,000	As low as $3,000
Read Length	• Fragment: 50 bp • Mate-pair: 2 x 50 bp • Paired-end: 50 x 25 bp	• Fragment: 75 bp • Mate-pair: 2 x 75 bp • Paired-end: 75 x 35 bp
Multiplexing	• 48 RNA barcodes • 96 DNA barcodes	• 96 RNA barcodes • 96 DNA barcodes
Run Time	• 3 days for 35 bp • 11 days for 50 x 25 bp • 12 days for 2 x 50 bp	• 3 days for 35 bp • 12 days for 75 x 35 bp • 14 days for 2 x 75 bp

* These systems are under development and the specifications are subject to change.
** Expected throughput.
*** Prices reflect US list at optimal running efficiencies. Regional pricing may vary.

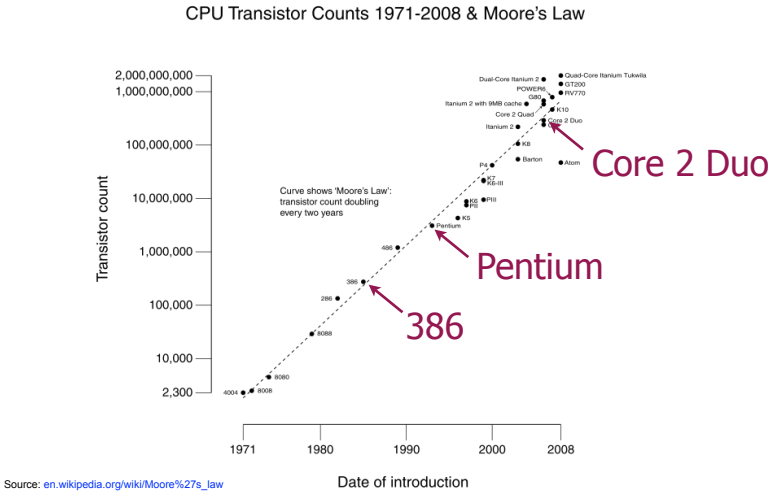
Source: www3.appliedbiosystems.com/cms/groups/mcb_marketing/documents/generaldocuments/cms_061241.pdf

Computational throughput

Moore's Law:

The number of transistors that can be placed inexpensively on an integrated circuit doubles approximately every two years.

Computational throughput

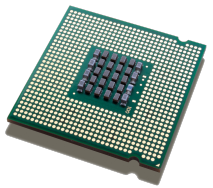


Source: en.wikipedia.org/wiki/Moore%27s_law

Throughput growth gap

4-5x per year

>



2x per 2 years

ionTorrent

Ion Semiconductor Sequencing

Speed, Scalability, Simplicity

Ion semiconductor benchtop sequencers perform real-time measurements of hydrogen ions produced during DNA replication. Ion semiconductor chips employ a massively parallel array of semiconductor sensors, to directly translate genetic information (DNA) to digital information (DNA sequence). This groundbreaking technology enables rapid and scalable sequencing across a range of applications.

Technology

Ion PGM™ Sequencer

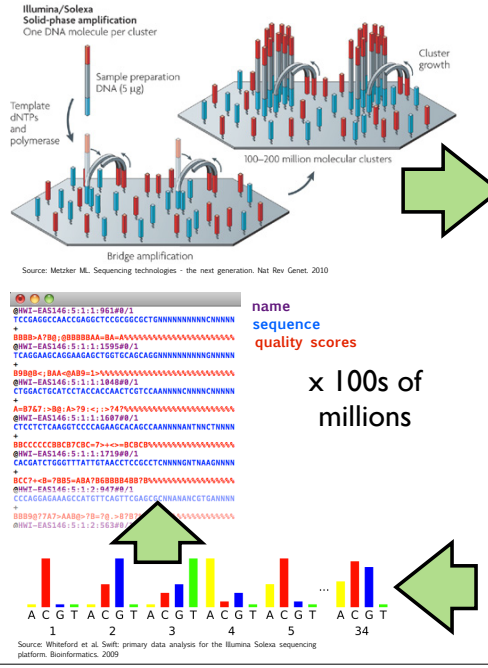
The fastest and most affordable benchtop sequencer

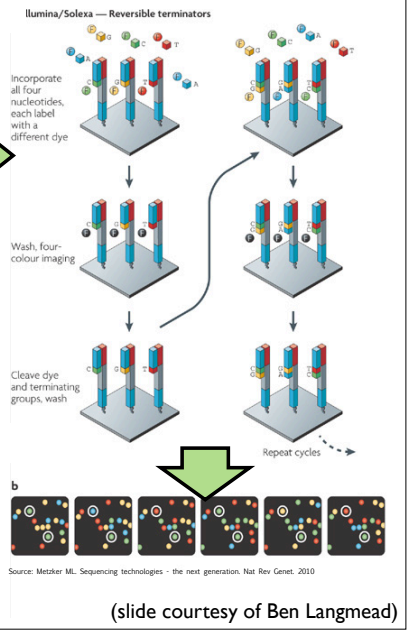
Ion Proton™ Sequencer

Genome and exome sequencing on your benchtop

Oxford Nanopore

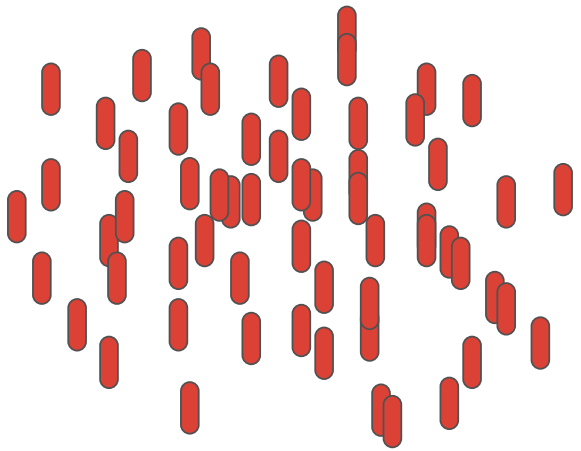
- Nanopore technology
- ultralong reads (48kb genome sequenced as one read)





(slide courtesy of Ben Langmead)

Sec-gen Sequencing



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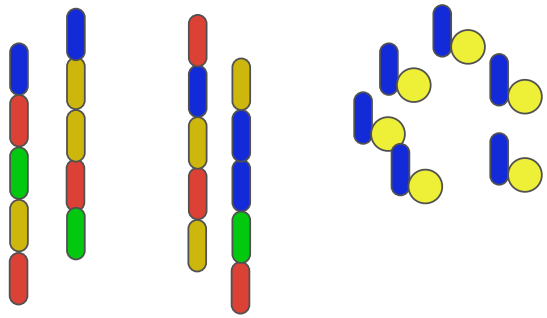
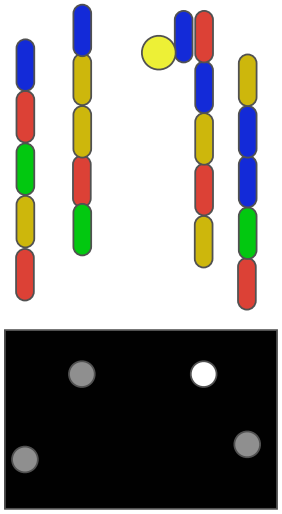
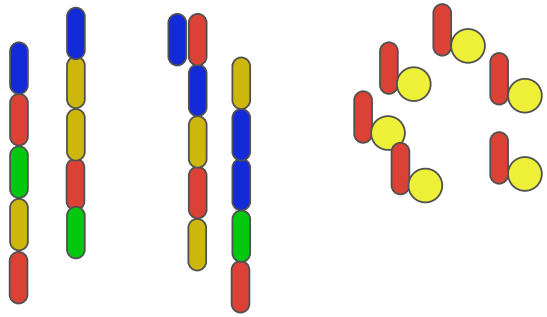
Sec-gen Sequencing



Sec-gen Sequencing



Sec-gen Sequencing



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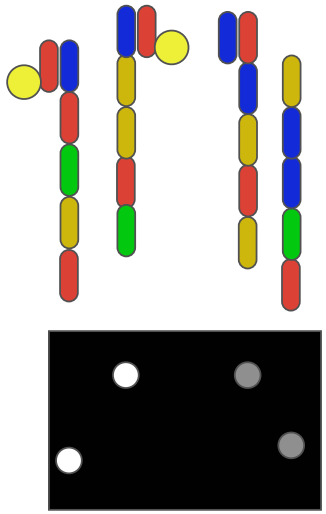
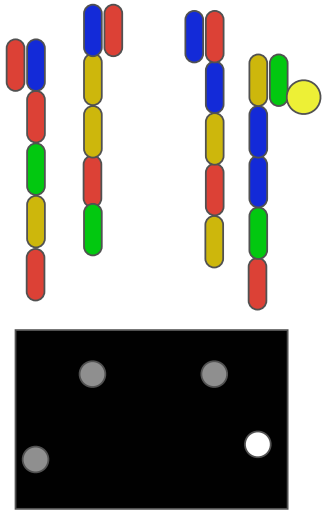


Sec-gen Sequencing



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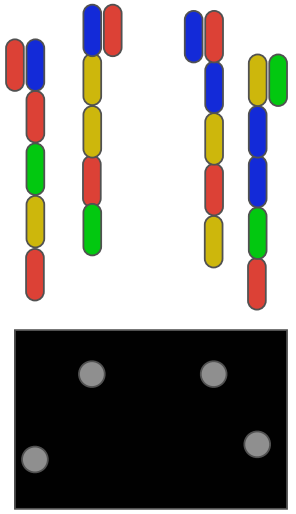
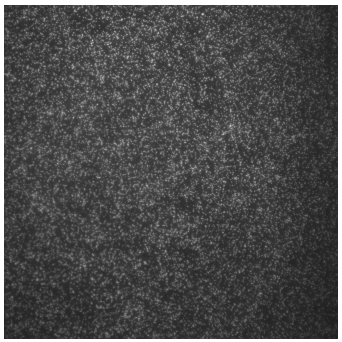
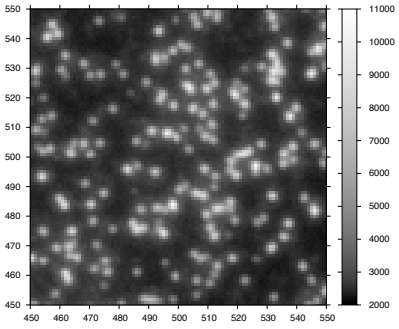


Image Analysis



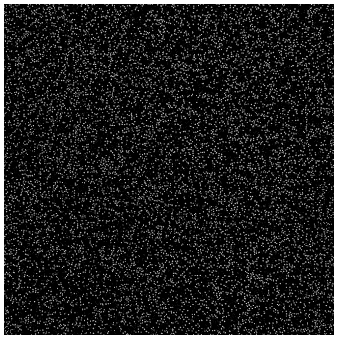


An input image and zoomed in section

Image Analysis

- 4 images per cycle
- ~100 tiles
- Analysis:
  - Filtering
  - Background subtraction
  - Thresholding
- Each image analysis independent (so can parallelize)

Image Analysis



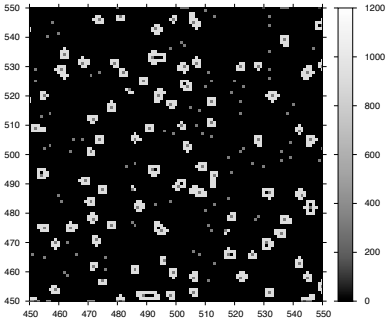
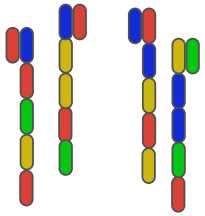
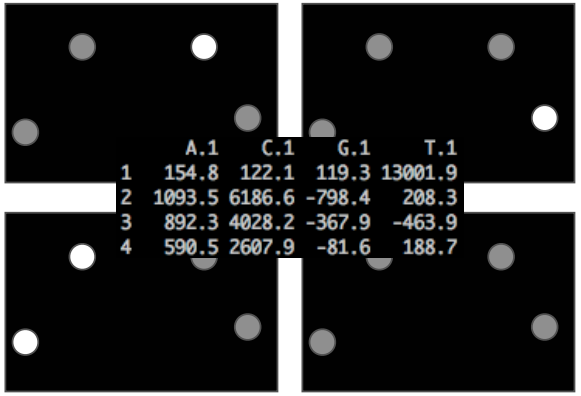


Image after processing. *This is old, cluster density is much higher now*

Sec-gen Sequencing



First Cycle



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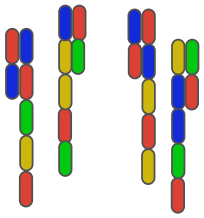
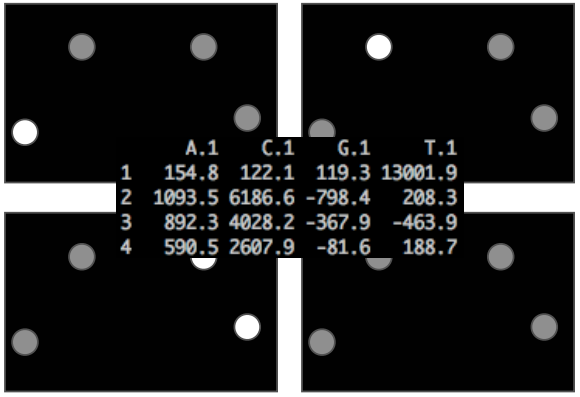
Sec-gen Sequencing



Second Cycle



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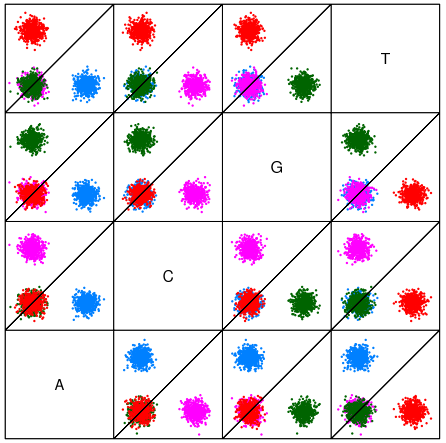
A Thought Experiment



Four-channel fluorescence intensity, cycle 1

Color coded by call made: A, C, G, T

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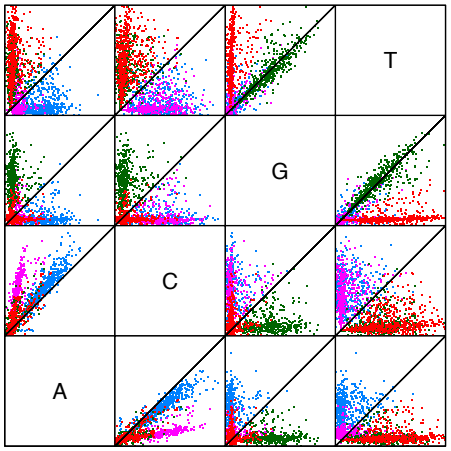
Fluorescence Intensity



Four–channel fluorescence intensity, cycle 1

Color coded by call made: A, C, G, T

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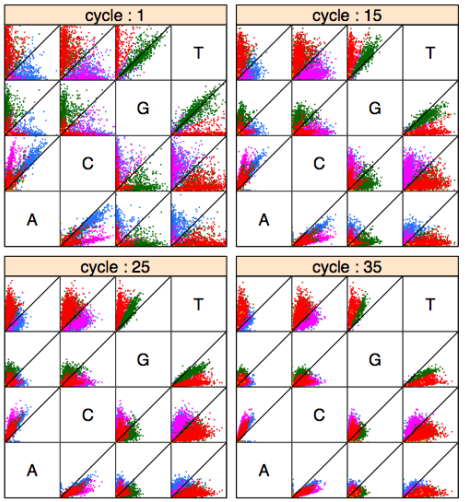
Fluorescence Intensity

MORE ON THIS LATER IN THE COURSE!



Four–channel fluorescence intensity

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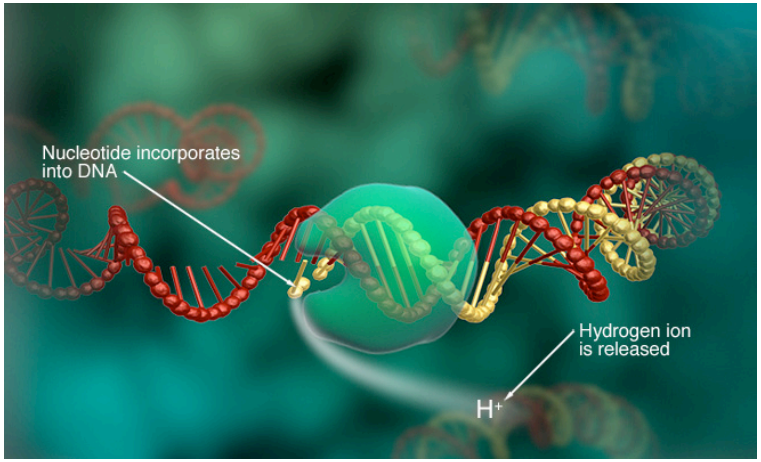
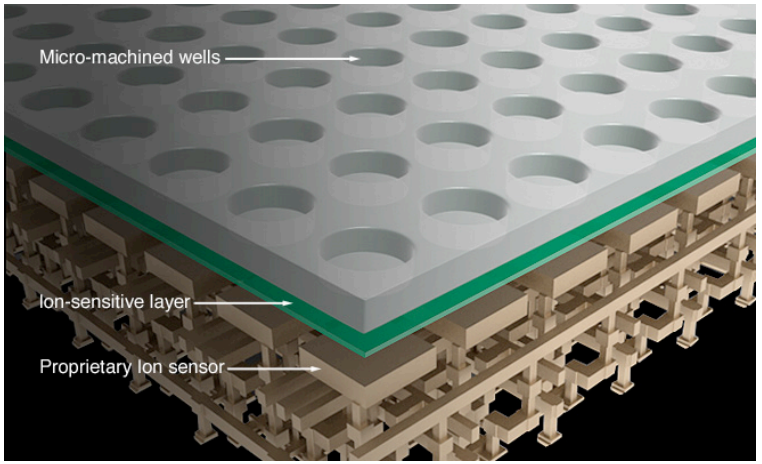
Ion Torrent



Ion Torrent



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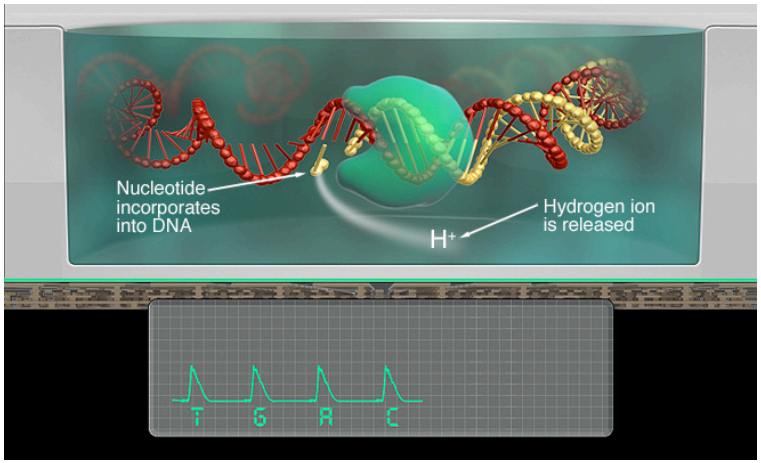

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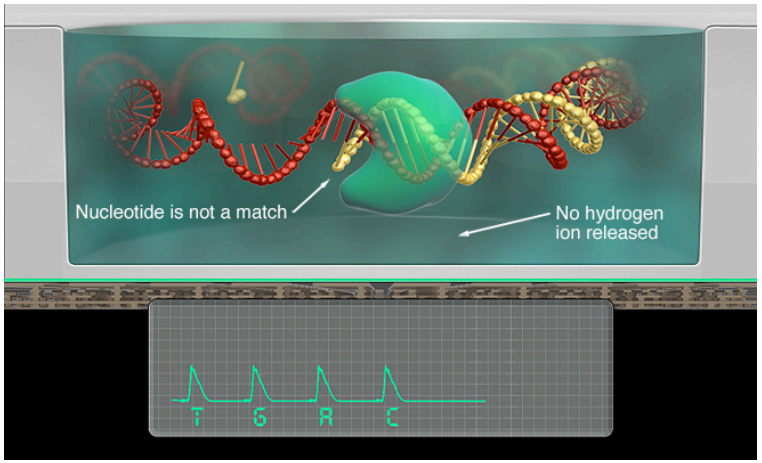

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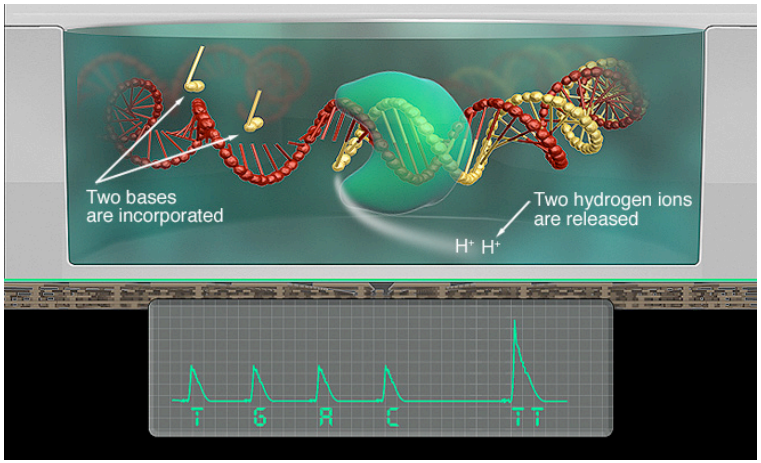
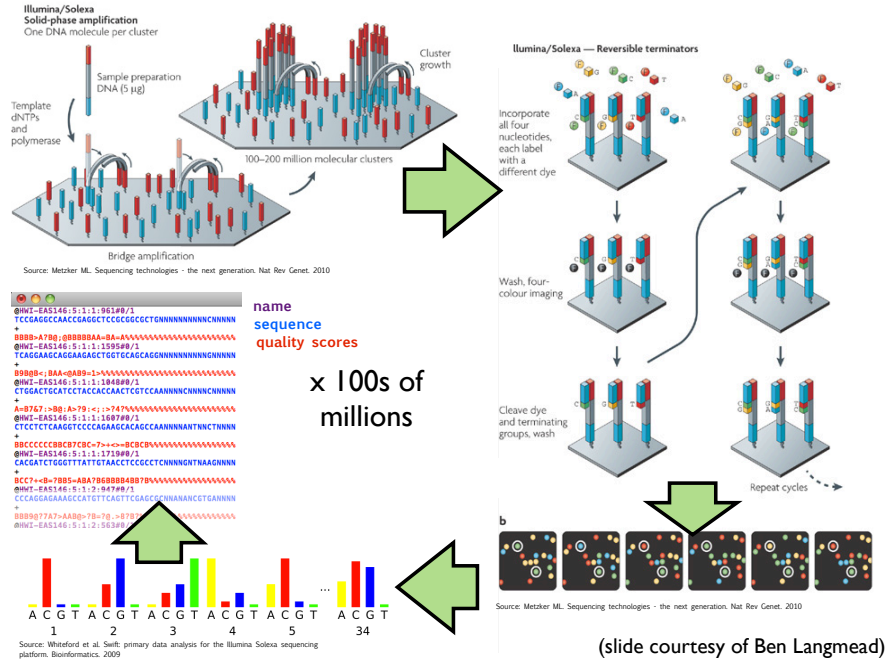
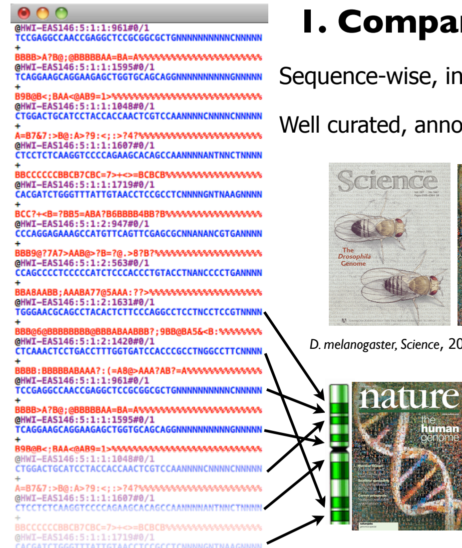




(slide courtesy of Ben Langmead)

From reads to evidence

From reads to evidence



1. Comparative

Sequence-wise, individuals of a species are nearly identical

Well curated, annotated "reference" genomes exist

D. melanogaster, *Science*, 2000 — *H. sapiens*, *Nature*, 2000 and *Science*, 2000 — *M. musculus*, *Nature*, 2002

Idea: "Map" reads to their point of origin with respect to a reference, then study differences

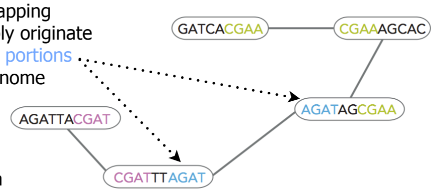
From reads to evidence

2. *de novo*

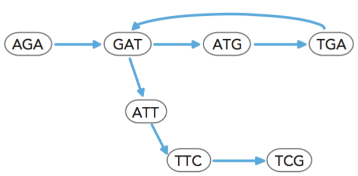
Assume nothing! - let reads tell us everything

Reads with overlapping sequence probably originate from overlapping portions of the subject genome

Encode overlap relationships as a graph



Source: De Novo Assembly Using Illumina Reads. Illumina. 2010



The full genome sequence is a "tour" of the graph

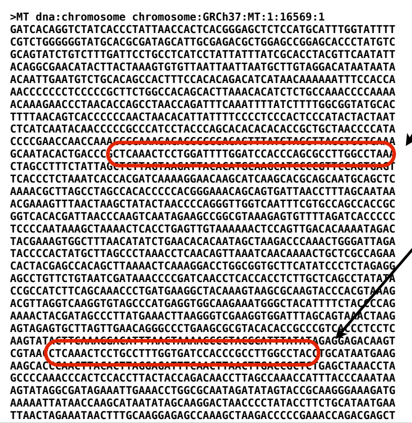
Source: De Novo Assembly Using Illumina Reads. Illumina. 2010
http://www.illumina.com/Documents/products/technotes/technote_denovo_assembly.pdf

Mapping

Take a read:

```
CTCAAACTCCTGACCTTTGGTGATCCACCCGCCTNGGCCTTC
```

And a reference sequence:



How do we determine the read's point of origin with respect to the reference?

Answer: sequence similarity

Hypothesis 1:

Read

```
CTCAAAGACCTGACCTTTGGTGATCCACCC-----GCCTNGGCCTTC
||||||  ||||   ||||  |||||||||     |||| |||||
CTCAAACTCCTGGATTTTG--GATCCACCCAGCTGGCCTTGGCCTAA
```

Reference

Hypothesis 2:

Read

```
CTCAAACTCCTGACCTTTGGTGATCCACCCGCCTNGGCCTTC
||||||||||||| |||||||||||||||||||||| ||||| |
CTCAAACTCCTG-CCTTTGGTGATCCACCCGCCTTGGCCTAC
```

Reference

Which hypothesis is better?

Say hypothesis 2 is correct. Why are there still mismatches and gaps?

Mapping

Read

CTCAAACTCCTGACCTTTGGTGATCCACCCGCCTNGGCCTTC

Reference

>MT dna:chromosome chromosome:GRCh37:MT:1:16569:1

GATCACAGGTCTATCACCCTATTAACCACTCACGGGAGCTCTCCATGCATTTGGTATTTT
CGTCTGGGGGGTATGCACGCGATAGCATTGCGAGACGCTGGAGCCGGAGCACCCTATGTC
GCAGTATCTGTCTTTGATTCCTGCCTCATCCTATTATTTATCGCACCTACGTTCAATATT
ACAGGCGAACATACTTACTAAAGTGTGTTAATTAATTAATGCTTGTAGGACATAATAATA
ACAATTGAATGTCTGCACAGCCACTTTCCACACAGACATCATAACAAAAAATTTCCACCA
AACCCCCCCTCCCCCGCTTCTGGCCACAGCACTTAAACACATCTCTGCCAAACCCCAAAA
ACAAAGAACCCTAACACCAGCCTAACCAGATTTCAAATTTTATCTTTTGGCGGTATGCAC
TTTTAACAGTCACCCCCCAACTAACACATTATTTTCCCCTCCCACTCCCATACTACTAAT
CTCATCAATACAACCCCCGCCCATCCTACCCAGCACACACACACCGCTGCTAACCCCATA
CCCCGAACCAACCAAACCCCAAAGACACCCCCCACAGTTTATGTAGCTTACCTCCTCAAA
GCAATACACTGACCCGCTCAAACTCCTGGATTTTGTGATCCACCCAGCGCCTTGGCCTAA
CTAGCCTTTCTATTAGCTCTTAGTAAGATTACACATGCAAGCATCCCCGTTCCAGTGAGT
TCACCCTCTAAATCACCACGATCAAAAGGAACAAGCATCAAGCACGCAGCAATGCAGCTC
AAAACGCTTAGCCTAGCCACACCCCCACGGGAAACAGCAGTGATTAACCTTTAGCAATAA
ACGAAAGTTTAACTAAGCTATACTAACCCCAGGGTTGGTCAATTTCGTGCCAGCCACCGC
GGTCACACGATTAACCCAAGTCAATAGAAGCCGGCGTAAAGAGTGTTTTAGATCACCCCC
TCCCCAATAAAGCTAAAACTCACCTGAGTTGTAAAAAACTCCAGTTGACACAAAATAGAC
TACGAAAGTGGCTTTAACATATCTGAACACACAATAGCTAAGACCCAAACTGGGATTAGA
TACCCCACTATGCTTAGCCTAAACCTCAACAGTTAAATCAACAAAACTGCTCGCCAGAA
CACTACGAGCCACAGCTTAAAACTCAAAGGACCTGGCGGTGCTTCATATCCCTCTAGAGG
AGCCTGTTCTGTAATCGATAAACCCCGATCAACCTCACCACCTCTTGCTCAGCCTATATA
CCGCCATCTTCAGCAAACCCTGATGAAGGCTACAAAGTAAGCGCAAGTACCCACGTAAAG
ACGTTAGGTCAAGGTGTAGCCCATGAGGTGGCAAGAAATGGGCTACATTTTCTACCCCAG
AAAACTACGATAGCCCTTATGAAACTTAAGGGTCGAAGGTGGATTTAGCAGTAAACTAAG
AGTAGAGTGCTTAGTTGAACAGGGCCCTGAAGCGCGTACACACCGCCCGTCACCCTCCTC
AAGTATACTTCAAAGGACATTTAACTAAAACCCCTACGCATTTATATAGAGGAGACAAGT
CGTAACCTCAAACTCCTGGCCTTTGGTGATCCACCCGCCTTGGCCTACCTGCATAATGAA
AAGCACCCAACTTACACTTAGGAGATTTCAACTTAACTTGACCGCTCTGAGCTAAACCTA
GCCCCAAACCCACTCCACCTTACTACCAGACAACCTTAGCCAAACCATTTACCCAAATAA
AGTATAGGCGATAGAAATTGAAACCTGGCGCAATAGATATAGTACCGCAAGGGAAAGATG
AAAAATTATAACCAAGCATAATATAGCAAGGACTAACCCCTATACCTTCTGCATAATGAA
TTAACTAGAAATAACTTTGCAAGGAGAGCCAAAGCTAAGACCCCCGAAACCAGACGAGCT
ACCTAAGAACAGCTAAAAGAGCACACCCGTCTATGTAGCAAAATAGTGGGAAGATTTATA
GGTAGAGGCGACAAACCTACCGAGCCTGGTGATAGCTGGTTGTCCAAGATAGAATCTTAG

This is an **alignment**:

Read

CTCAAAGACCTGACCTTTGGTGATCCACCC------GCCTNGGCCTTC
CTCAAACTCCTGGATTTTG--GATCCACCCAGCTGGCCTTGGCCTAA

Reference

Software programs that compare reads to references and find alignments are **aligners**.

Alignment is computationally difficult because references (e.g. human) are **very long** (more than 1M times longer than what's shown to the left) and sequencers produce data very rapidly, e.g. up to **25 billion bases per day** in 2010.

Sequencing throughput increases by ~5x per year, whereas computers get faster at a rate closer to ~2x every 2 years.

Mapping

Take a read:

CTCAAACTCCTGACCTTTGGTGATCCA

And a reference sequence:

>MT dna:chromosome chromosome:GRCh37:MT:1:16569:1

GATCACAGGTCTATCACCCTATTAACCACTCACGGGAGCTCTCCATGCATTTGGTATTTT
CGTCTGGGGGGTATGCACGCGATAGCATTGCGAGACGCTGGAGCCGGAGCACCCTATGTC
GCAGTATCTGTCTTTGATTCCTGCCTCATCCTATTATTTATCGCACCTACGTTCAATATT
ACAGGCGAACATACTTACTAAAGTGTGTTAATTAATTAATGCTTGTAGGACATAATAATA
ACAATTGAATGTCTGCACAGCCACTTTCCACACAGACATCATAACAAAAAATTTCCACCA
AACCCCCCCTCCCCCGCTTCTGGCCACAGCACTTAAACACATCTCTGCCAAACCCCAAAA
ACAAAGAACCCTAACACCAGCCTAACCAGATTTCAAATTTTATCTTTTGGCGGTATGCAC
TTTTAACAGTCACCCCCCAACTAACACATTATTTTCCCCTCCCACTCCCATACTACTAAT
CTCATCAATACAACCCCCGCCCATCCTACCCAGCACACACACACCGCTGCTAACCCCATA
CCCCGAACCAACCAAACCCCAAAGACACCCCCCACAGTTTATGTAGCTTACCTCCTCAAA
GCAATACACTGACCCGCTCAAACTCCTGGATTTTGTGATCCACCCAGCGCCTTGGCCTAA
CTAGCCTTTCTATTAGCTCTTAGTAAGATTACACATGCAAGCATCCCCGTTCCAGTGAGT
TCACCCTCTAAATCACCACGATCAAAAGGAACAAGCATCAAGCACGCAGCAATGCAGCTC
AAAACGCTTAGCCTAGCCACACCCCCACGGGAAACAGCAGTGATTAACCTTTAGCAATAA
ACGAAAGTTTAACTAAGCTATACTAACCCCAGGGTTGGTCAATTTCGTGCCAGCCACCGC
GGTCACACGATTAACCCAAGTCAATAGAAGCCGGCGTAAAGAGTGTTTTAGATCACCCCC
TCCCCAATAAAGCTAAAACTCACCTGAGTTGTAAAAAACTCCAGTTGACACAAAATAGAC
TACGAAAGTGGCTTTAACATATCTGAACACACAATAGCTAAGACCCAAACTGGGATTAGA
TACCCCACTATGCTTAGCCTAAACCTCAACAGTTAAATCAACAAAACTGCTCGCCAGAA
CACTACGAGCCACAGCTTAAAACTCAAAGGACCTGGCGGTGCTTCATATCCCTCTAGAGG
AGCCTGTTCTGTAATCGATAAACCCCGATCAACCTCACCACCTCTTGCTCAGCCTATATA
CCGCCATCTTCAGCAAACCCTGATGAAGGCTACAAAGTAAGCGCAAGTACCCACGTAAAG
ACGTTAGGTCAAGGTGTAGCCCATGAGGTGGCAAGAAATGGGCTACATTTTCTACCCCAG
AAAACTACGATAGCCCTTATGAAACTTAAGGGTCGAAGGTGGATTTAGCAGTAAACTAAG
AGTAGAGTGCTTAGTTGAACAGGGCCCTGAAGCGCGTACACACCGCCCGTCACCCTCCTC
AAGTATACTTCAAAGGACATTTAACTAAAACCCCTACGCATTTATATAGAGGAGACAAGT
CGTAACCTCAAACTCCTGGCCTTTGGTGATCCACCCGCCTTGGCCTACCTGCATAATGAA
AAGCACCCAACTTACACTTAGGAGATTTCAACTTAACTTGACCGCTCTGAGCTAAACCTA
GCCCCAAACCCACTCCACCTTACTACCAGACAACCTTAGCCAAACCATTTACCCAAATAA
AGTATAGGCGATAGAAATTGAAACCTGGCGCAATAGATATAGTACCGCAAGGGAAAGATG
AAAAATTATAACCAAGCATAATATAGCAAGGACTAACCCCTATACCTTCTGCATAATGAA
TTAACTAGAAATAACTTTGCAAGGAGAGCCAAAGCTAAGACCCCCGAAACCAGACGAGCT

Which hypothesis is better?

Hypothesis 1:

Read

CTCAAACTCCTGACCTTTGGTGATCCA
CTCAAACTCCTGCCCTTTGGTGATCCA

Reference

Hypothesis 2:

Read

CTCAAACTCCTGACCTTTGGTGATCCA
CTCAAACTCCTGACCTTTCGTGATCCA

Reference

Is there any way to break the tie?

Mapping

Recall that reads come with per-cycle quality values (in red)

In FASTQ format (left), qualities are encoded as ASCII characters like B, = or %, but really they're integers [0, 40]

A quality value Q is a function of the probability P that the sequencing machine called the wrong base:

$$Q = -10 \cdot \log_{10}(P)$$

Q = 10: 1 in 10 chance that base was miscalled
Q = 20: 1 in 100 chance
Q = 30: 1 in 1000 chance

Higher is "better."

Qs are estimated by the sequencer's software and aren't necessarily accurate

Mapping

Take a read:

CTCAAACTCCTGACCTTTGGTGATCCA

And a reference sequence:

>MT dna:chromosome chromosome:GRCh37:MT:1:16569:1

GATCACAGGTCTATCACCCTATTAACCACTCACGGGAGCTCTCCATGCATTTGGTATTTT
CGTCTGGGGGGTATGCACGCGATAGCATTGCGAGACGCTGGAGCCGGAGCACCCTATGTC
GCAGTATCTGTCTTTGATTCCTGCCTCATCCTATTATTTATCGCACCTACGTTCAATATT
ACAGGCGAACATACTTACTAAAGTGTGTTAATTAATTAATGCTTGTAGGACATAATAATA
ACAATTGAATGTCTGCACAGCCACTTTCCACACAGACATCATAACAAAAAATTTCCACCA
AACCCCCCCTCCCCCGCTTCTGGCCACAGCACTTAAACACATCTCTGCCAAACCCCAAAA
ACAAAGAACCCTAACACCAGCCTAACCAGATTTCAAATTTTATCTTTTGGCGGTATGCAC
TTTTAACAGTCACCCCCCAACTAACACATTATTTTCCCCTCCCACTCCCATACTACTAAT
CTCATCAATACAACCCCCGCCCATCCTACCCAGCACACACACACCGCTGCTAACCCCATA
CCCCGAACCAACCAAACCCCAAAGACACCCCCCACAGTTTATGTAGCTTACCTCCTCAAA
GCAATACACTGACCCGCTCAAACTCCTGGATTTTGTGATCCACCCAGCGCCTTGGCCTAA
CTAGCCTTTCTATTAGCTCTTAGTAAGATTACACATGCAAGCATCCCCGTTCCAGTGAGT
TCACCCTCTAAATCACCACGATCAAAAGGAACAAGCATCAAGCACGCAGCAATGCAGCTC
AAAACGCTTAGCCTAGCCACACCCCCACGGGAAACAGCAGTGATTAACCTTTAGCAATAA
ACGAAAGTTTAACTAAGCTATACTAACCCCAGGGTTGGTCAATTTCGTGCCAGCCACCGC
GGTCACACGATTAACCCAAGTCAATAGAAGCCGGCGTAAAGAGTGTTTTAGATCACCCCC
TCCCCAATAAAGCTAAAACTCACCTGAGTTGTAAAAAACTCCAGTTGACACAAAATAGAC
TACGAAAGTGGCTTTAACATATCTGAACACACAATAGCTAAGACCCAAACTGGGATTAGA
TACCCCACTATGCTTAGCCTAAACCTCAACAGTTAAATCAACAAAACTGCTCGCCAGAA
CACTACGAGCCACAGCTTAAAACTCAAAGGACCTGGCGGTGCTTCATATCCCTCTAGAGG
AGCCTGTTCTGTAATCGATAAACCCCGATCAACCTCACCACCTCTTGCTCAGCCTATATA
CCGCCATCTTCAGCAAACCCTGATGAAGGCTACAAAGTAAGCGCAAGTACCCACGTAAAG
ACGTTAGGTCAAGGTGTAGCCCATGAGGTGGCAAGAAATGGGCTACATTTTCTACCCCAG
AAAACTACGATAGCCCTTATGAAACTTAAGGGTCGAAGGTGGATTTAGCAGTAAACTAAG
AGTAGAGTGCTTAGTTGAACAGGGCCCTGAAGCGCGTACACACCGCCCGTCACCCTCCTC
AAGTATACTTCAAAGGACATTTAACTAAAACCCCTACGCATTTATATAGAGGAGACAAGT
CGTAACCTCAAACTCCTGGCCTTTGGTGATCCACCCGCCTTGGCCTACCTGCATAATGAA
AAGCACCCAACTTACACTTAGGAGATTTCAACTTAACTTGACCGCTCTGAGCTAAACCTA
GCCCCAAACCCACTCCACCTTACTACCAGACAACCTTAGCCAAACCATTTACCCAAATAA
AGTATAGGCGATAGAAATTGAAACCTGGCGCAATAGATATAGTACCGCAAGGGAAAGATG
AAAAATTATAACCAAGCATAATATAGCAAGGACTAACCCCTATACCTTCTGCATAATGAA
TTAACTAGAAATAACTTTGCAAGGAGAGCCAAAGCTAAGACCCCCGAAACCAGACGAGCT

Which hypothesis is better?

Hypothesis 1:

Q=30

Read

CTCAAACTCCTGACCTTTGGTGATCCA
CTCAAACTCCTGCCCTTTGGTGATCCA

Reference

Hypothesis 2:

Q=10

Read

CTCAAACTCCTGACCTTTGGTGATCCA
CTCAAACTCCTGACCTTTCGTGATCCA

Reference

Mapping

Take a read:

```
CTCAAACTCCTGACCTTTGGTGATCCA
```

And a reference sequence:

```
>MT dna:chromosome chromosome:GRCh37:MT:1:16569:1
GATCACAGGTCTATCACCCTATTAACCACTCACGGGAGCTCTCCATGCATTTGGTATTTT
CGTCTGGGGGGTATGCACGCGATAGCATTGCGAGACGCTGGAGCCGGAGCACCCTATGTC
GCAGTATCTGTCTTTGATTCCTGCCTCATCCTATTATTTATCGCACCTACGTTCAATATT
ACAGGCGAACATACTTACTAAAGTGTGTTAATTAATTAATGCTTGTAGGACATAATAATA
ACAATTGAATGTCTGCACAGCCACTTTCCACACAGACATCATAACAAAAAATTTCCACCA
AACCCCCCCTCCCCCGCTTCTGGCCACAGCACTTAAACACATCTCTGCCAAACCCCAAAA
ACAAAGAACCCTAACACCAGCCTAACCAGATTTCAAATTTTATCTTTTGGCGGTATGCAC
TTTTAACAGTCACCCCCCAACTAACACATTATTTTCCCCTCCCACTCCCATACTACTAAT
CTCATCAATACAACCCCCGCCCATCCTACCCAGCACACACACACCGCTGCTAACCCCATA
CCCCGAACCAACCAAACCCCAAAGACACCCCCCACAGTTTATGTAGCTTACCTCCTCAAA
GCAATACACTGACCCGCTCAAACTCCTGGATTTTGTGATCCACCCAGCGCCTTGGCCTAA
CTAGCCTTTCTATTAGCTCTTAGTAAGATTACACATGCAAGCATCCCCGTTCCAGTGAGT
TCACCCTCTAAATCACCACGATCAAAAGGAACAAGCATCAAGCACGCAGCAATGCAGCTC
AAAACGCTTAGCCTAGCCACACCCCCACGGGAAACAGCAGTGATTAACCTTTAGCAATAA
ACGAAAGTTTAACTAAGCTATACTAACCCCAGGGTTGGTCAATTTCGTGCCAGCCACCGC
GGTCACACGATTAACCCAAGTCAATAGAAGCCGGCGTAAAGAGTGTTTTAGATCACCCCC
TCCCCAATAAAGCTAAAACTCACCTGAGTTGTAAAAAACTCCAGTTGACACAAAATAGAC
TACGAAAGTGGCTTTAACATATCTGAACACACAATAGCTAAGACCCAAACTGGGATTAGA
TACCCCACTATGCTTAGCCCTAAACCTCAACAGTTAAATCAACAAAACTGCTCGCCAGAA
CACTACGAGCCACAGCTTAAAACTCAAAGGACCTGGCGGTGCTTCATATCCCTCTAGAGG
AGCCTGTTCTGTAATCGATAAACCCCGATCAACCTCACCACCTCTTGCTCAGCCTATATA
CCGCCATCTTCAGCAAACCCTGATGAAGGCTACAAAGTAAGCGCAAGTACCCACGTAAAG
ACGTTAGGTCAAGGTGTAGCCCATGAGGTGGCAAGAAATGGGCTACATTTTCTACCCCAG
AAAACTACGATAGCCCTTATGAAACTTAAGGGTCGAAGGTGGATTTAGCAGTAAACTAAG
AGTAGAGTGCTTAGTTGAACAGGGCCCTGAAGCGCGTACACACCGCCCGTCACCCTCCTC
AAGTATACTTCAAAGGACATTTAACTAAAACCCCTACGCATTTATATAGAGGAGACAAGT
CGTAACCTCAAACTCCTGGCCTTTGGTGATCCACCCGCCTTGGCCTACCTGCATAATGAA
AAGCACCCAACTTACACTTAGGAGATTTCAACTTAACTTGACCGCTCTGAGCTAAACCTA
GCCCCAAACCCACTCCACCTTACTACCAGACAACCTTAGCCAAACCATTTACCCAAATAA
AGTATAGGCGATAGAAATTGAAACCTGGCGCAATAGATATAGTACCGCAAGGGAAAGATG
AAAAATTATAACCAAGCATAATATAGCAAGGACTAACCCCTATACCTTCTGCATAATGAA
TTAACTAGAAATAACTTTGCAAGGAGAGCCAAAGCTAAGACCCCCGAAACCAGACGAGCT
```

Which hypothesis is better?

Hypothesis 1:

Read

```
CTCAAACTCCTGACCTTTGGTGATCCA
||||||||||| ||||||||||||||
CTCAAACTCCTG-CCTTTGGTGATCCA
```

Reference

Hypothesis 2:

Read

```
CTCAAACTCCTGACCTTTGGTGATCCA
|||||||||||||||||| ||||||||
CTCAAACTCCTGACCTTTCGTGATCCA
```

Reference

Is there any way to break the tie?

Hint: In Illumina sequencing, sequencing errors almost never manifest as gaps

Mapping

Aligners can employ **penalties** to account for the relative probability of seeing different dissimilarities

Estimates vary, but small gaps ("indels") occur in humans at 1 in ~10-100K positions.

$$\begin{aligned} Pen_{\text{gap}} &\equiv -10\log_{10}(P_{\text{gap}}) \\ &= -10\log_{10}(0.00005) \\ &\approx 45 \end{aligned}$$

SNPs occur in humans at 1 in ~1K positions, but depending on Q, sequencing error may be more likely

$$\begin{aligned} Pen_{\text{mm}} &\equiv \arg\min(-10\log_{10}(P_{\text{miscall}}), -10\log_{10}(P_{\text{SNP}})) \\ &= \arg\min(Q, -10\log_{10}(0.001)) \\ &= \arg\min(Q, 30) \end{aligned}$$

Read

```
CTCAAACTCCTGACCTTTGGTGATCCA
||||||||||| ||||||||||||||
CTCAAACTCCTG-CCTTTGGTGATCCA
```

Reference

Penalty = 45

Q=10

Read

```
CTCAAACTCCTGACCTTTGGTGATCCA
||||| |||||||||||||| ||||||
CTCAA-CTCCTGACCTTTGGCGATCCA
```

Reference

Penalty = 55

Q=40

Read

```
CTCAAACTCCTGACCTTTGGTGATCCA
|||||||||||||||||||||| ||||
CTCAAACTCCTGACCTTTGGTGCTCCA
```

Reference

Penalty = 30

Resources

- Bowtie: ultra-fast mapping of short reads to reference genome

Bowtie
An ultrafast memory-efficient short read aligner

Bowtie is an ultrafast, memory-efficient short read aligner. It aligns short DNA sequences (reads) to the human genome at a million 35-bp reads per hour. Bowtie indexes the genome with a Burrows-Wheeler index to keep its memory footprint small: t GB for the human genome (2.9 GB for paired-end).

- http://bowtie-bio.sourceforge.net

RNA-seq differential expression