Statistics applied to second-generation sequencing data

Second-Generation Sequencing

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News

The death of microarrays?

High-throughput gene sequencing seems to be stealing a march on microarrays. Heidi Ledford looks at a genome technology facing intense competition.

Heidi Ledford

Faster, cheaper DNA sequencing technology is revolutionizing the burgeoning field of personal genomics. But it is having another, more subtle effect.

Tools

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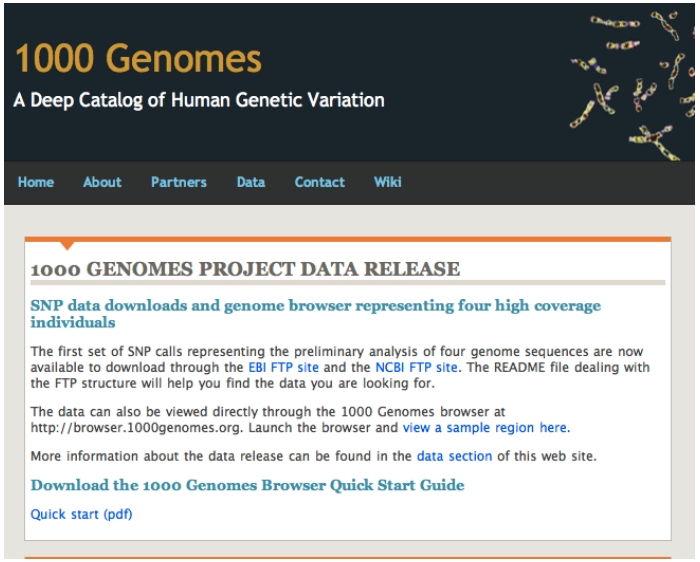
Second-Generation Sequencing

- "Ultra high throughput" DNA sequencing
  - 3 gigabases / week vs.
  - 3 gigabases / 13 years (more or less)

1000 Genomes Project



Genotyping

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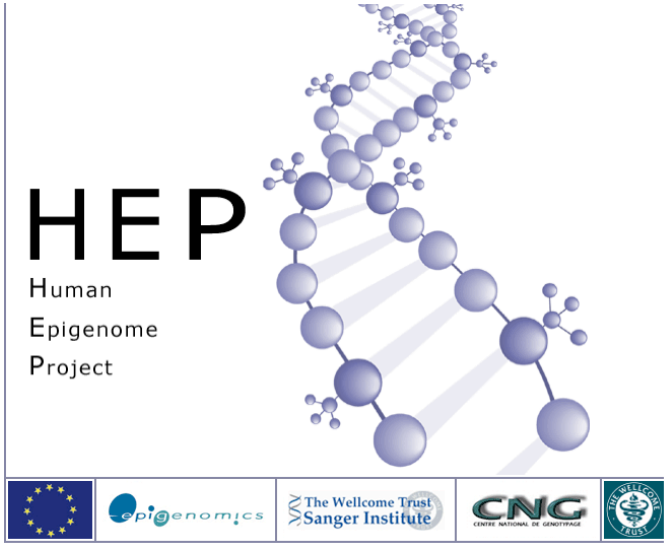
Human Epigenome Project



Methylation

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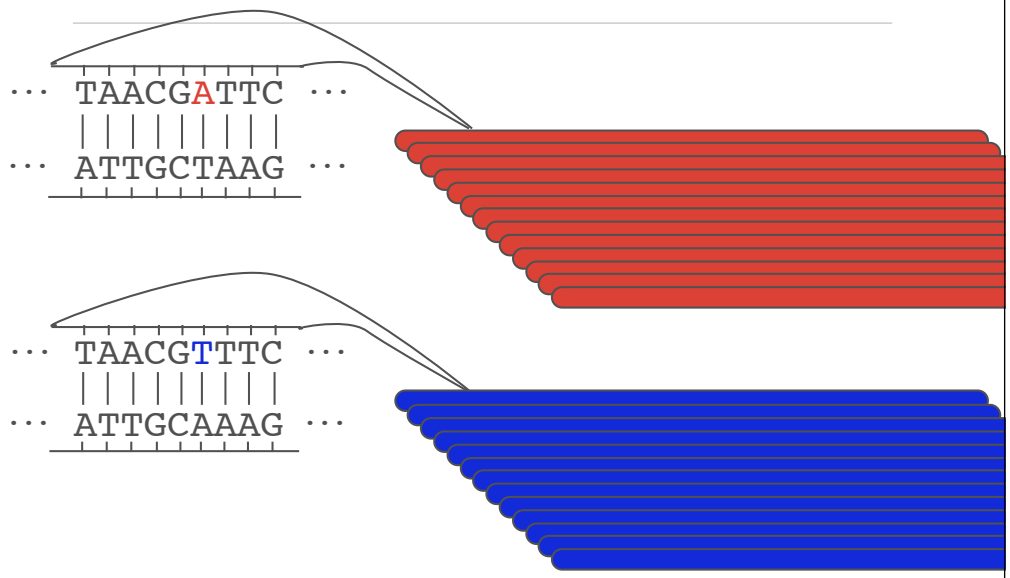
Platforms

- Millions of short DNA fragments (~36-70 bp) sequenced in parallel

Sec-gen Sequencing



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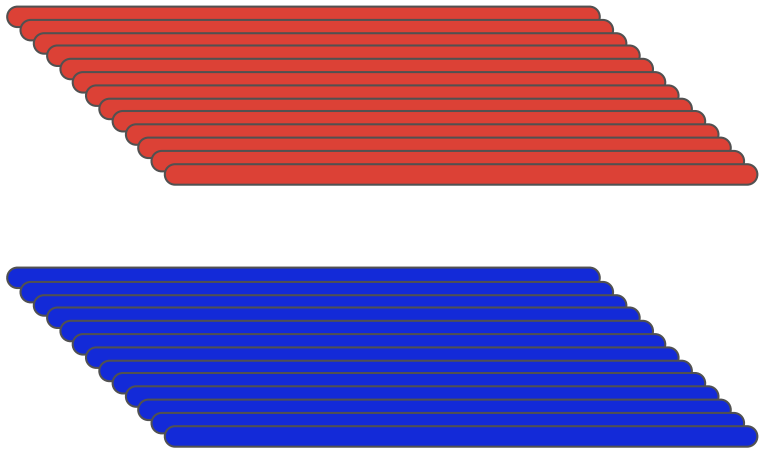
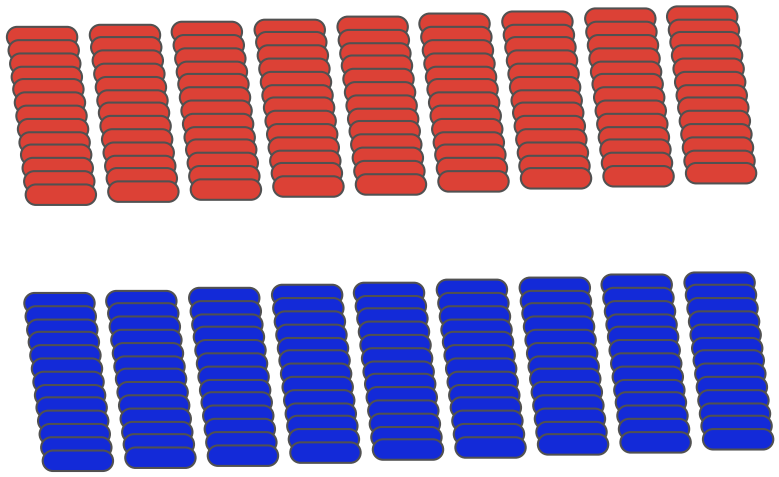
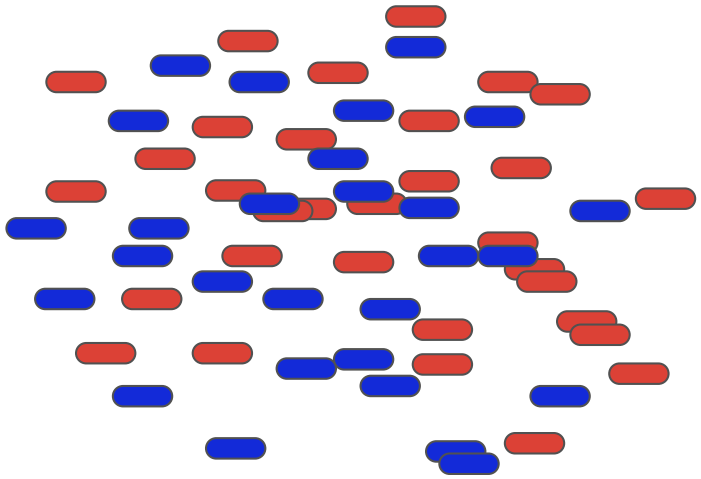
Sec-gen Sequencing



Sec-gen Sequencing



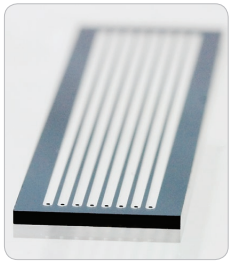
Sec-gen Sequencing



A Set of Short Reads

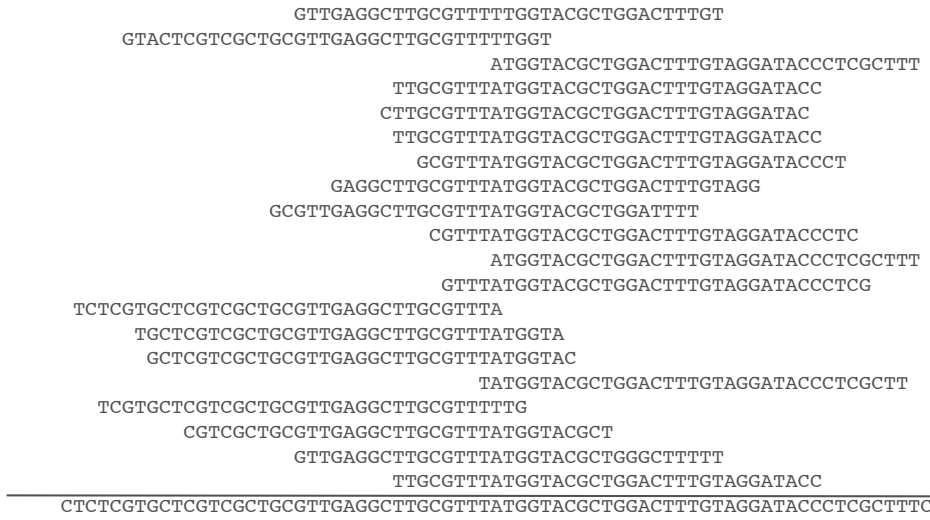
```
GTTGAGGCTTGCGTTTTTGGTACGCTGGACTTTGT
GTACTCGTCGCTGCGTTGAGGCTTGCGTTTTTGGT
ATGGTACGCTGGACTTTGTAGGATACCCTCGCTTT
TTGCGTTTATGGTACGCTGGACTTTGTAGGATACC
CTTGCGTTTATGGTACGCTGGACTTTGTAGGATAC
TTGCGTTTATGGTACGCTGGACTTTGTAGGATACC
GCGTTTATGGTACGCTGGACTTTGTAGGATACCCT
GAGGCTTGCGTTTATGGTACGCTGGACTTTGTAGG
GCGTTGAGGCTTGCGTTTATGGTACGCTGGATTTT
CGTTTATGGTACGCTGGACTTTGTAGGATACCCTC
ATGGTACGCTGGACTTTGTAGGATACCCTCGCTTT
GTTTATGGTACGCTGGACTTTGTAGGATACCCTCG
TCTCGTGCTCGTCGCTGCGTTGAGGCTTGCGTTTA
TGCTCGTCGCTGCGTTGAGGCTTGCGTTTATGGTA
GCTCGTCGCTGCGTTGAGGCTTGCGTTTATGGTAC
TATGGTACGCTGGACTTTGTAGGATACCCTCGCTT
TCGTGCTCGTCGCTGCGTTGAGGCTTGCGTTTTTG
CGTCGCTGCGTTGAGGCTTGCGTTTATGGTACGCT
GTTGAGGCTTGCGTTTATGGTACGCTGGGCTTTTT
TTGCGTTTATGGTACGCTGGACTTTGTAGGATACC
```

Illumina/Solexa



- Eight lanes
- 100 tiles/lane
- ~200K fragments per tile
- ~160M short reads (~50-70 bp) per run

Matching



Use Blast?

- Matching 10,000,000 32 bp reads:

BLAST takes more than 6 months

BLAT takes 2 months

MAQ takes 1 day and half

Bowtie takes 17 minutes

MATCHING

```
                        GTTGAGGCTTGCGTTTTTGGTACGCTGGACTTTGT
  GTACTCGTCGCTGCGTTGAGGCTTGCGTTTTTGGT
                                              ATGGTACGCTGGACTTTGTAGGATACCCTCGCTTT
                                 TTGCGTTTATGGTACGCTGGACTTTGTAGGATACC
                                CTTGCGTTTATGGTACGCTGGACTTTGTAGGATAC
```

Bowtie

An ultrafast memory-efficient short read aligner

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Bowtie is an ultrafast, memory-efficient short read aligner. It aligns short DNA sequences (reads) to the human genome at a rate of over 25 million 35-bp reads per hour. Bowtie indexes the genome with a Burrows-Wheeler index to keep its memory footprint small: typically about 2.2 GB for the human genome (2.9 GB for paired-end).

OSI certified

```
          TGCTCGTCGCTGCGTTGAGGCTTGCGTTTATGGTA
           GCTCGTCGCTGCGTTGAGGCTTGCGTTTATGGTAC
                                              TATGGTACGCTGGACTTTGTAGGATACCCTCGCTT
      TCGTGCTCGTCGCTGCGTTGAGGCTTGCGTTTTTG
             CGTCGCTGCGTTGAGGCTTGCGTTTATGGTACGCT
                        GTTGAGGCTTGCGTTTATGGTACGCTGGGCTTTTT
                                 TTGCGTTTATGGTACGCTGGACTTTGTAGGATACC
  CTCTCGTGCTCGTCGCTGCGTTGAGGCTTGCGTTTATGGTACGCTGGACTTTGTAGGATACCCTCGCTTTC
```


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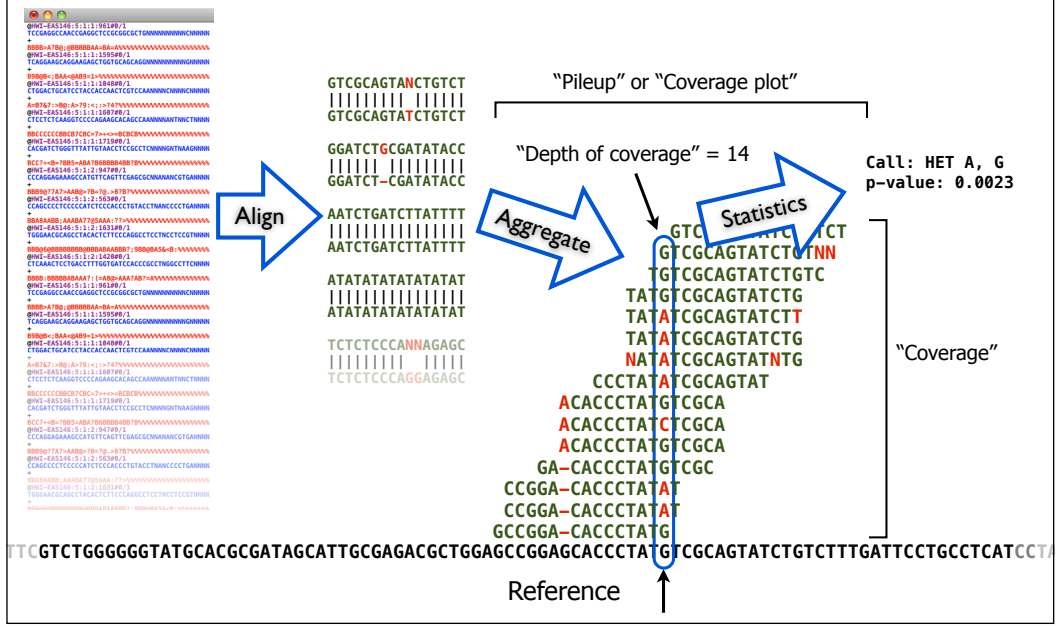
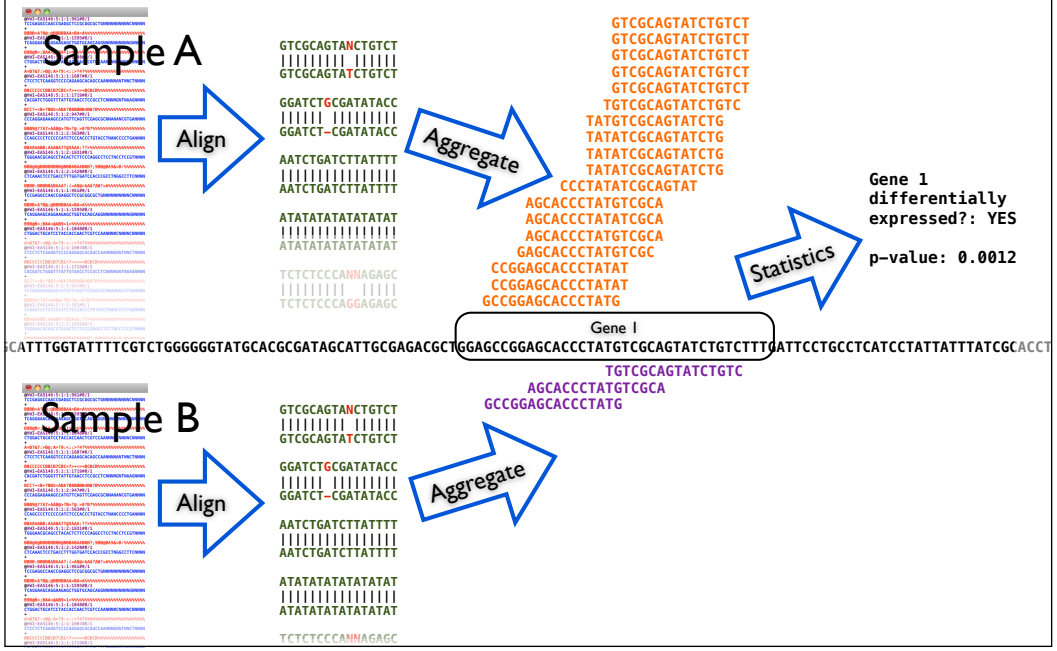
Workflow examples

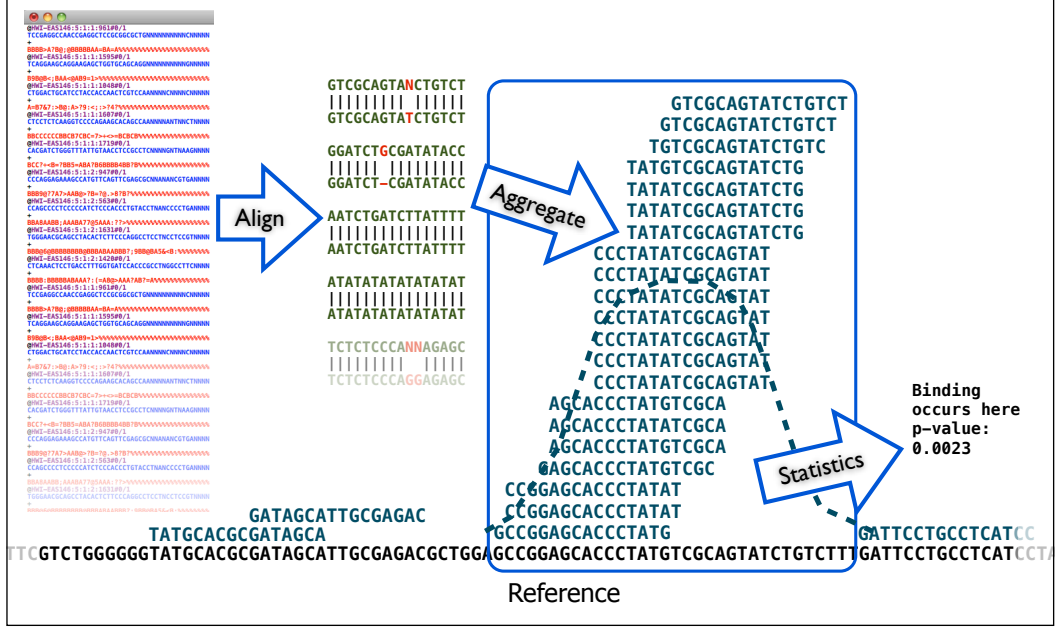
Variant detection



RNA-seq differential expression

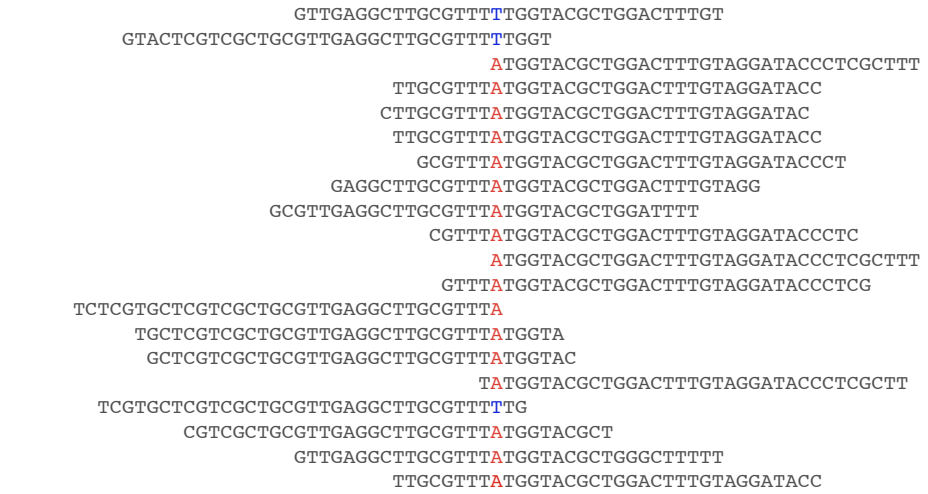


ChIP-seq

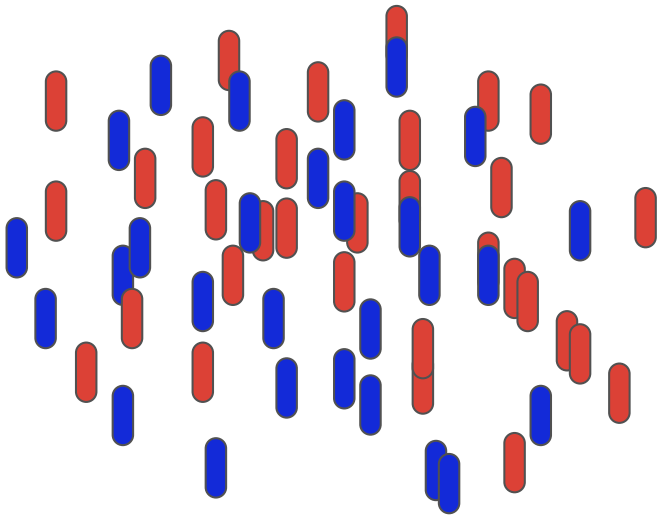

Align
Aggregate
Statistics
Binding occurs here
p-value: 0.0023
Reference

The End

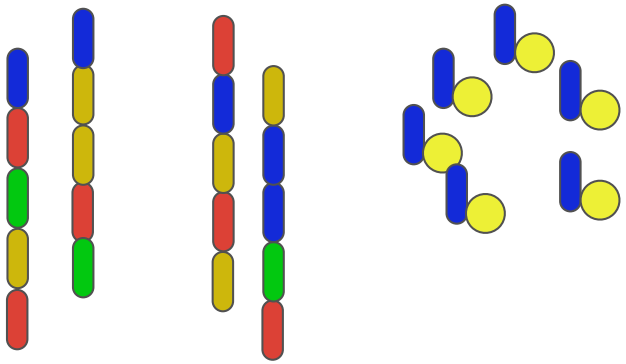
SNPs



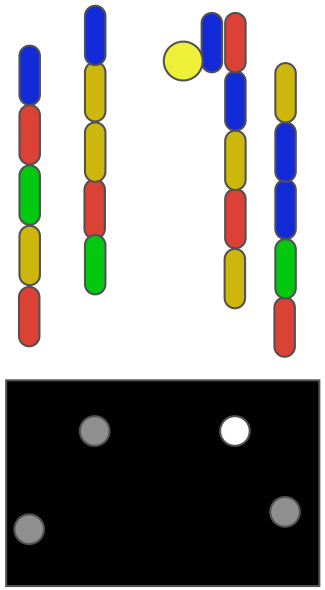
Sec-gen Sequencing



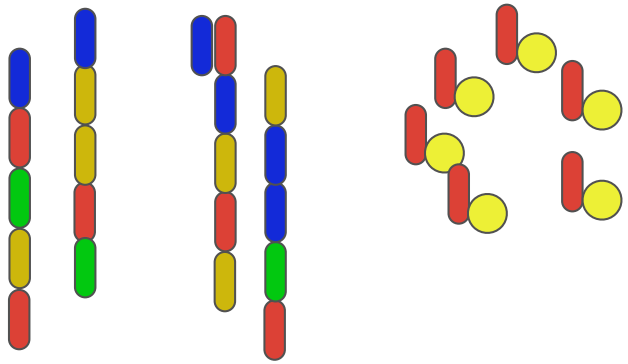
Sec-gen Sequencing



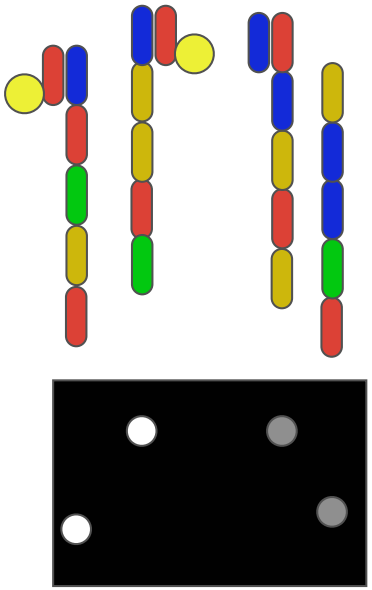
Sec-gen Sequencing



Sec-gen Sequencing



Sec-gen Sequencing



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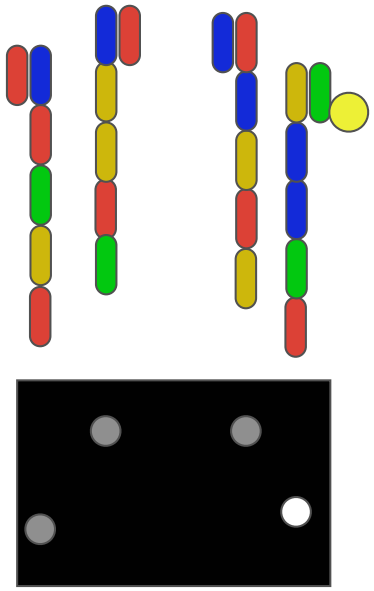

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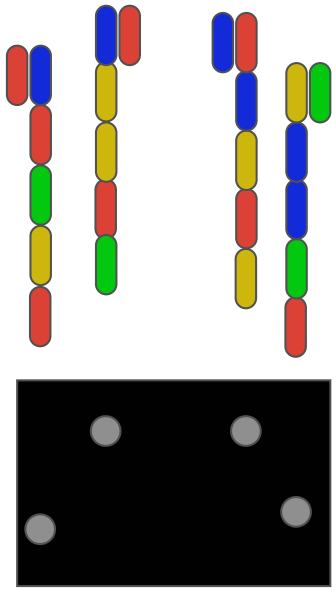

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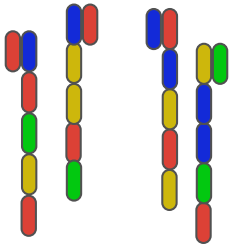
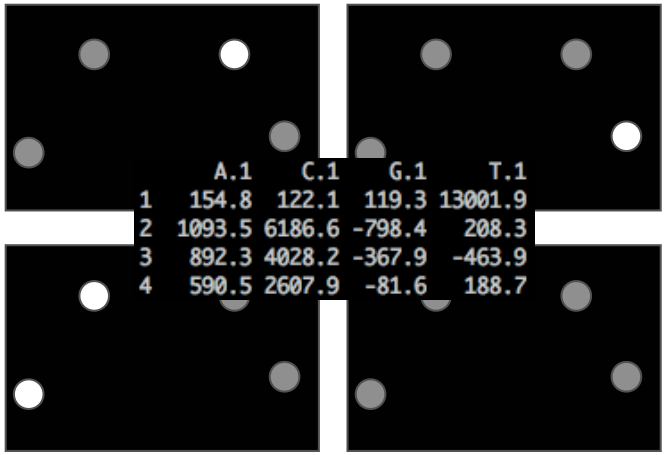


First Cycle



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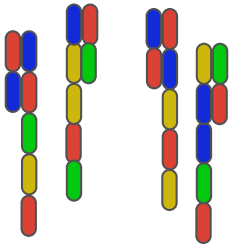
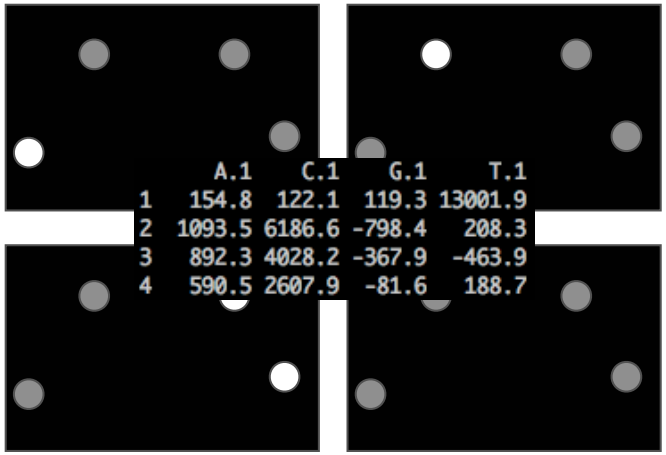
Sec-gen Sequencing



Second Cycle



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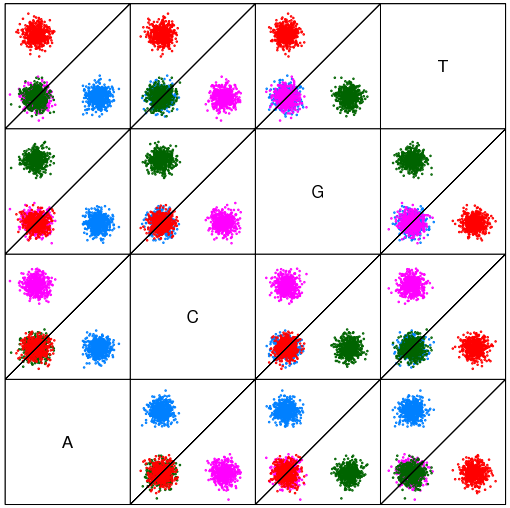
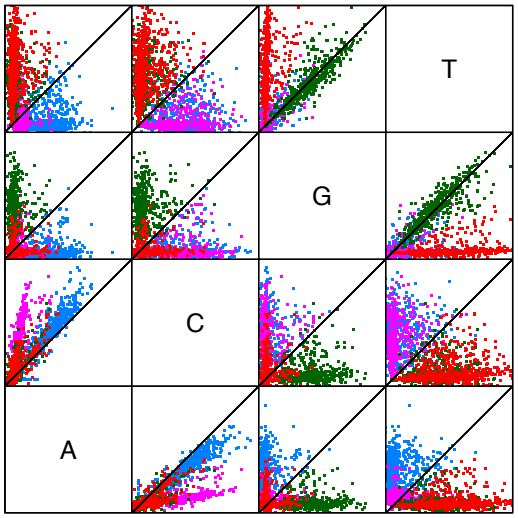
A Thought Experiment



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

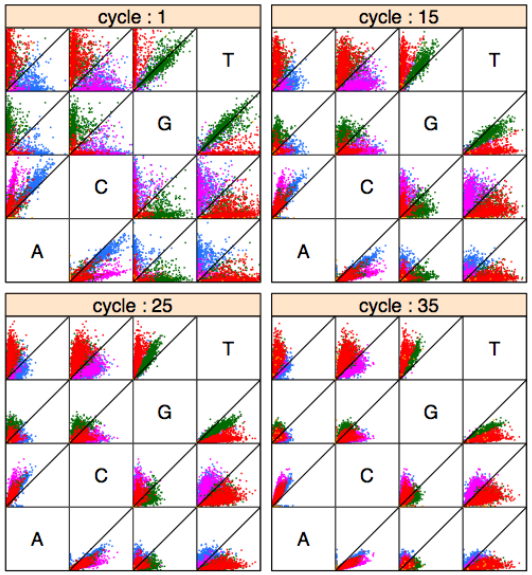
Fluorescence Intensity



Four–channel fluorescence intensity, cycle 1

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

Fluorescence Intensity



Four−channel fluorescence intensity

Challenges

- Base-calling is the result of a complicated procedure on noisy measurements
  - Base-calls are not made with the same certainty
- Statistical: How to model this uncertainty?
- Computational: Can we manage this model at sec-gen data scale?

Intensity Model

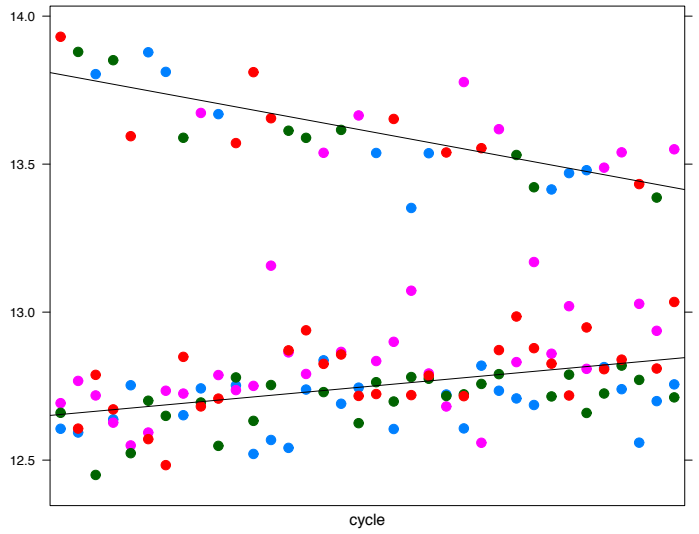
- log intensity read *i*, cycle *j*, channel *c*

$$u_{ijc} = \underline{\Delta_{ijc}}(\mu_{cj\alpha} + x_j^T \alpha_i + \epsilon_{ijc}^{\alpha}) + \underline{(1 - \Delta_{ijc})}(\mu_{cj\beta} + x_j^T \beta_i + \epsilon_{ijc}^{\beta})$$

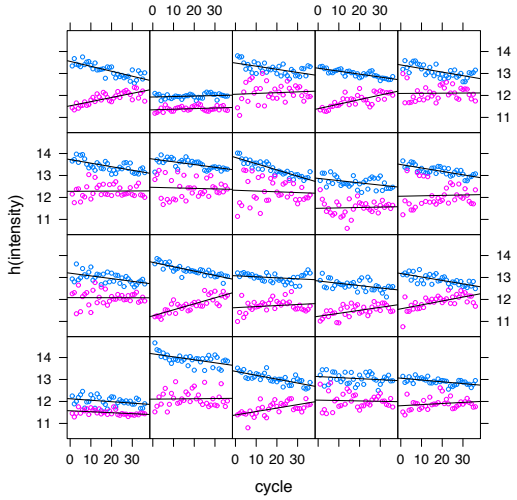
- indicators of nucleotide identity, read *i*, pos. *j*

$$\Delta_{ijc} = \begin{cases} 1 & \text{if } c \text{ is the nucleotide in read } i \text{ position } j \\ 0 & \text{otherwise} \end{cases}$$

Intensity Model (read-cycle effect)



Read & Cycle Effects



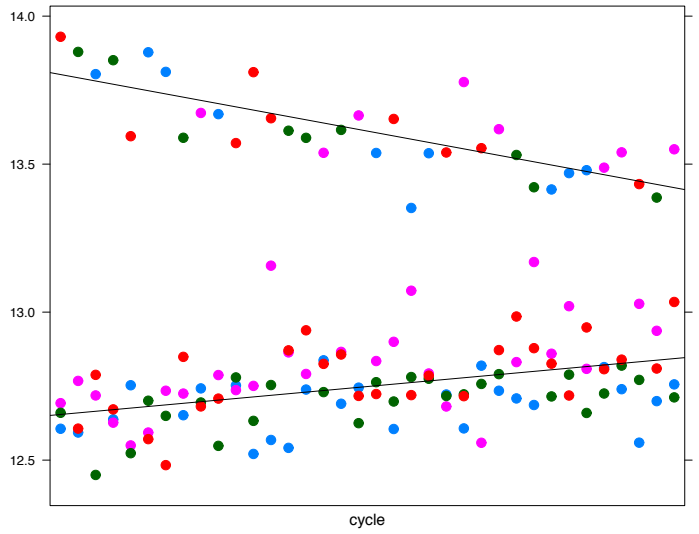
Intensity Model

- log intensity read *i*, cycle *j*, channel *c*

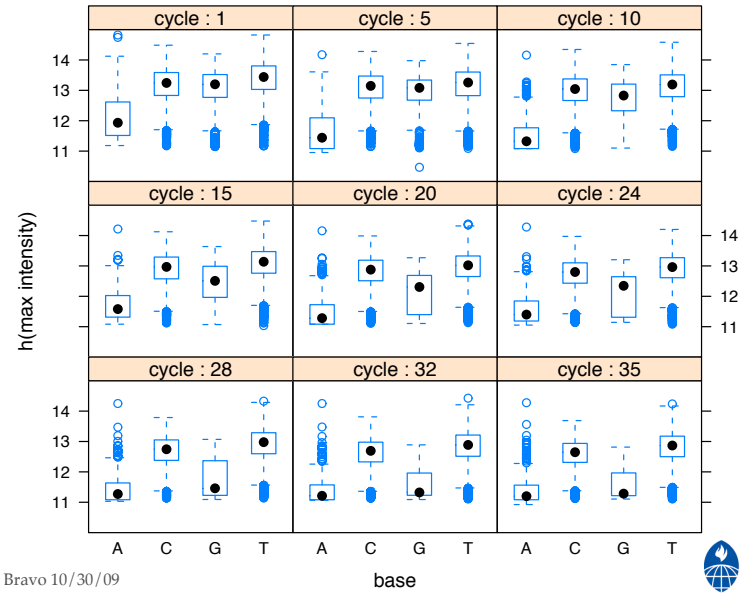
$$u_{ijc} = \Delta_{ijc}(\mu_{cj\alpha} + \underline{x_j^T \alpha_i} + \epsilon_{ijc}^{\alpha}) + (1 - \Delta_{ijc})(\mu_{cj\beta} + \underline{x_j^T \beta_i} + \epsilon_{ijc}^{\beta})$$

- read-specific linear models

Intensity Model (read-cycle effect)



Intensity Model (Base-Cycle Effect)



Intensity Model

- log intensity read *i*, cycle *j*, channel *c*

$$u_{ijc} = \Delta_{ijc}(\underline{\mu_{cj\alpha}} + x_j^T \alpha_i + \epsilon_{ijc}^{\alpha}) + (1 - \Delta_{ijc})(\underline{\mu_{cj\beta}} + x_j^T \beta_i + \epsilon_{ijc}^{\beta})$$

- base-cycle effects

Intensity Model

- log intensity read *i*, cycle *j*, channel *c*

$$u_{ijc} = \Delta_{ijc}(\mu_{cj\alpha} + x_j^T \alpha_i + \underline{\epsilon_{ijc}^{\alpha}}) + (1 - \Delta_{ijc})(\mu_{cj\beta} + x_j^T \beta_i + \underline{\epsilon_{ijc}^{\beta}})$$

- random noise

$$\epsilon_{ijc}^{\alpha} \sim N(0, \sigma_{\alpha i}^2) \qquad \epsilon_{ijc}^{\beta} \sim N(0, \sigma_{\beta i}^2)$$

Intensity Model

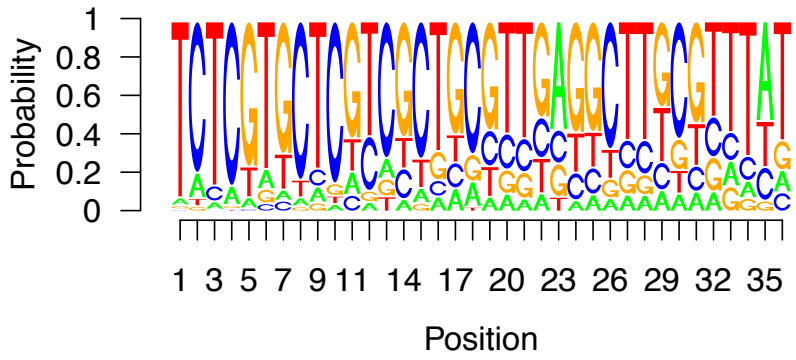
- log intensity read *i*, cycle *j*, channel *c*

$$u_{ijc} = \Delta_{ijc}(\mu_{cj\alpha} + x_j^T \alpha_i + \epsilon_{ijc}^{\alpha}) + (1 - \Delta_{ijc})(\mu_{cj\beta} + x_j^T \beta_i + \epsilon_{ijc}^{\beta})$$

- estimate parameters with EM algorithm
- which also estimates

$$z_{ijc} := \mathrm{E}\{\Delta_{ijc} = 1 | u_{ij}\} = P(\Delta_{ijc} = 1 | u_{ij})$$

Base Identity Probability Profiles



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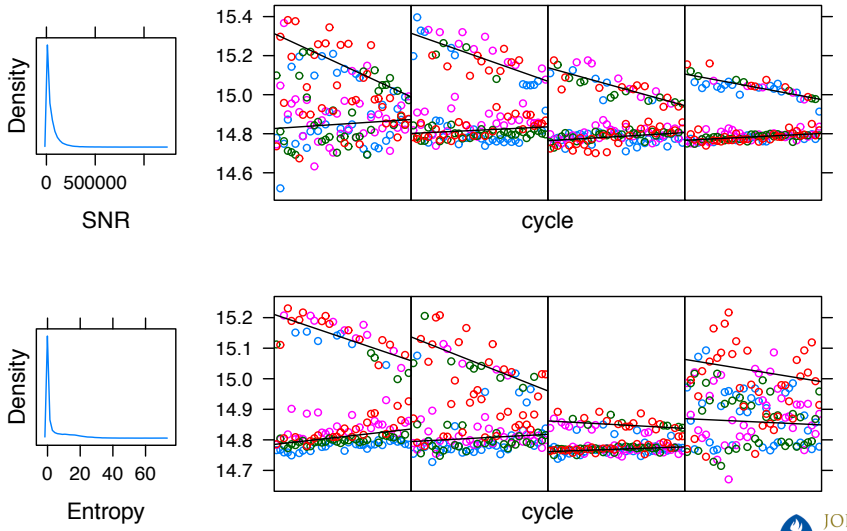
Quality Metrics

1. Entropy: Certainty according to probability profiles in each read position

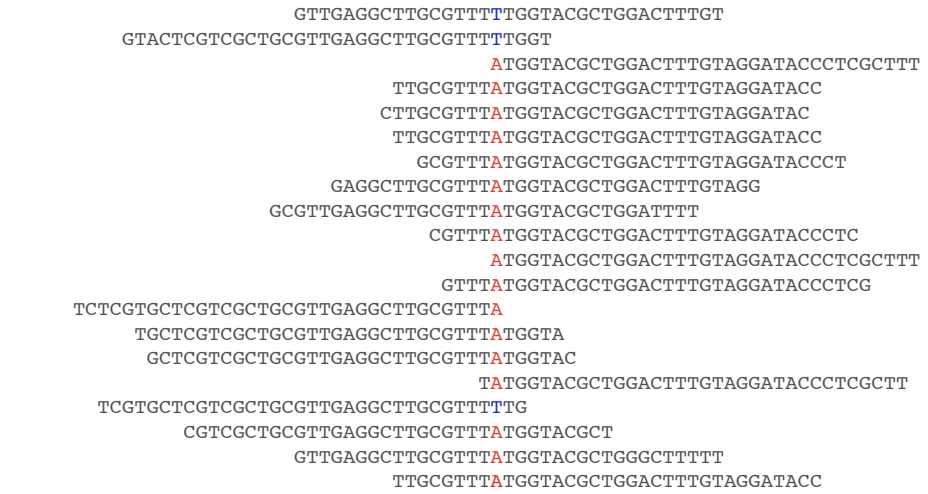
$$H_{ij} = -\sum_{c} z_{ijc} \log_2 z_{ijc}$$

$$H_i = -\sum_{cj} z_{ijc} \log_2 z_{ijc}$$

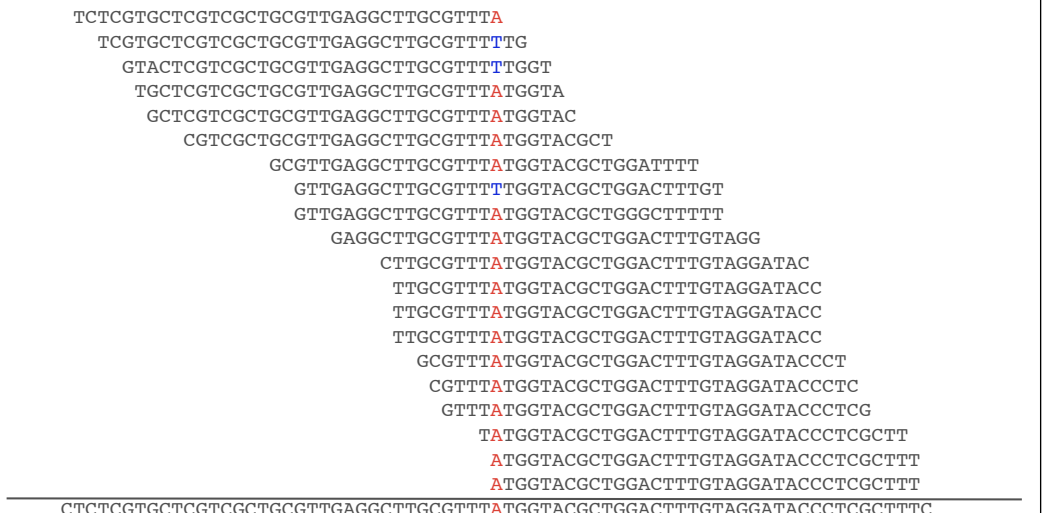
Quality Metrics



SNPs



SNPs



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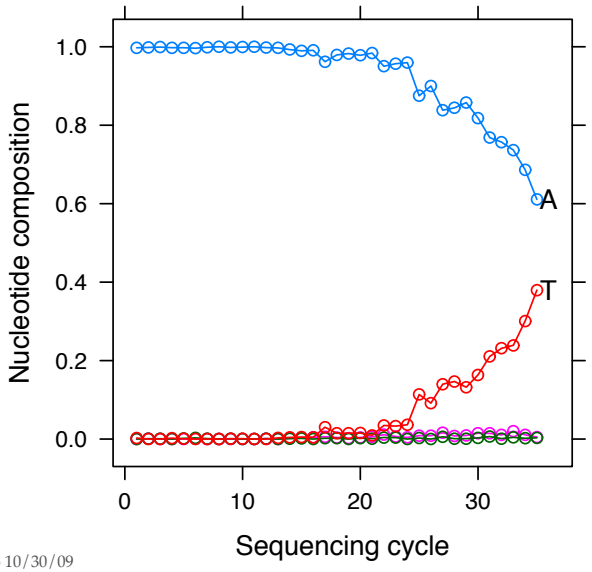
SNPs



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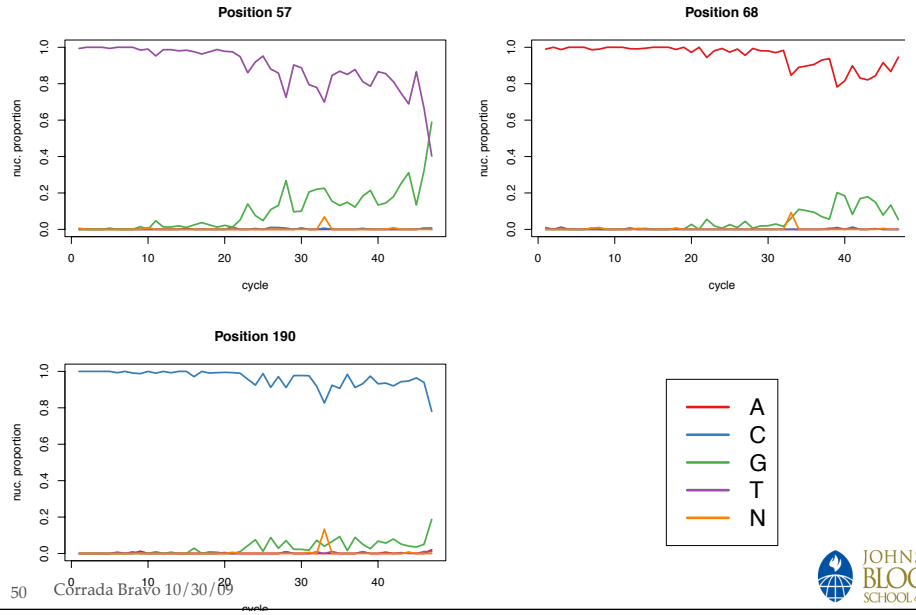
SNPs



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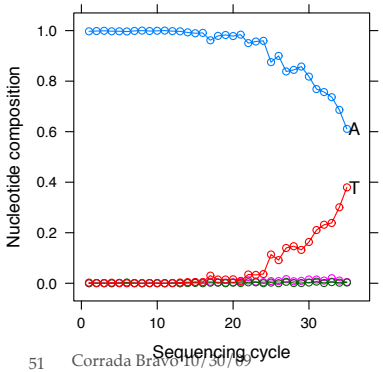
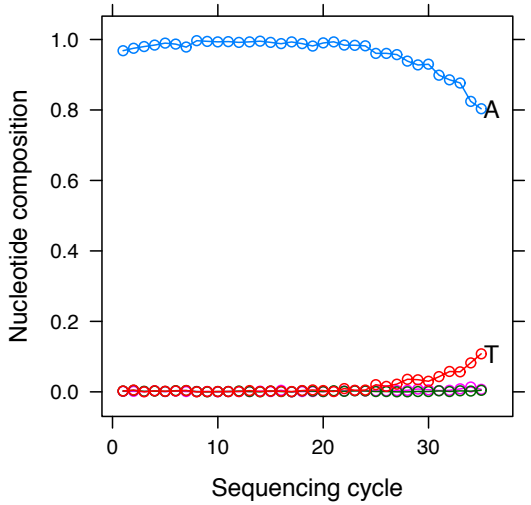
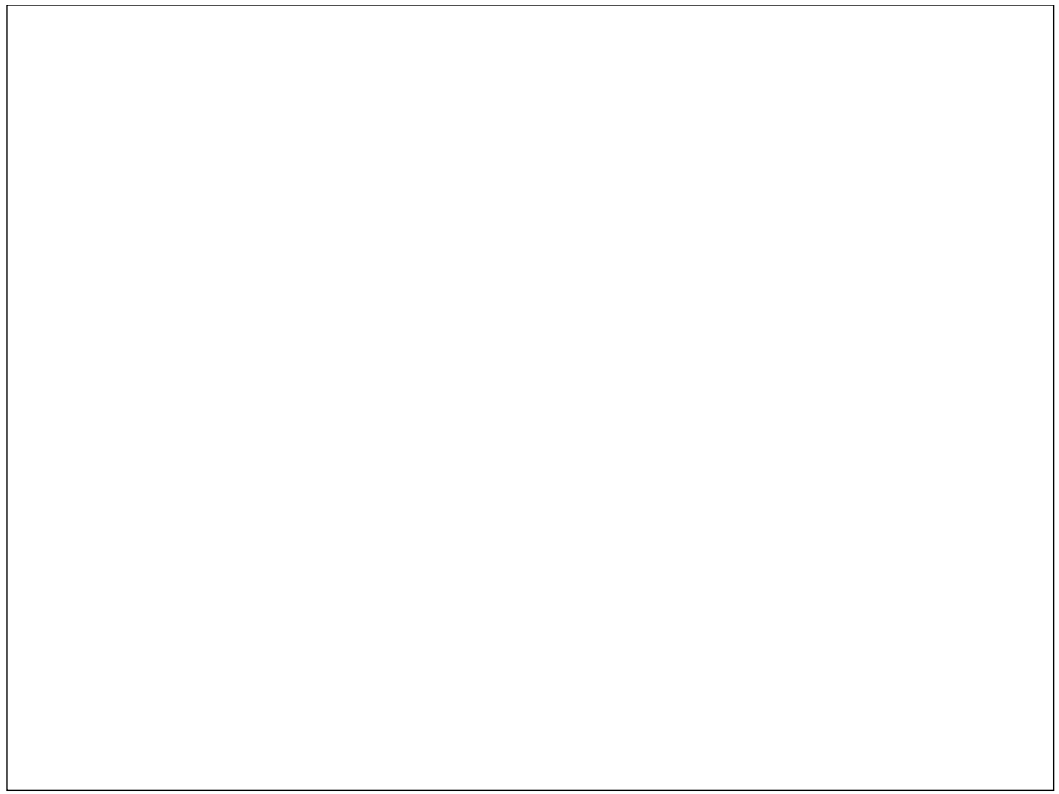
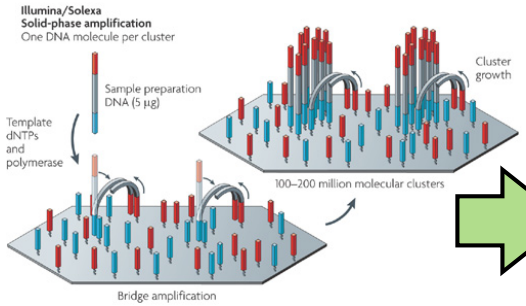
SNPs





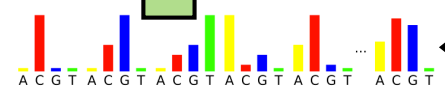




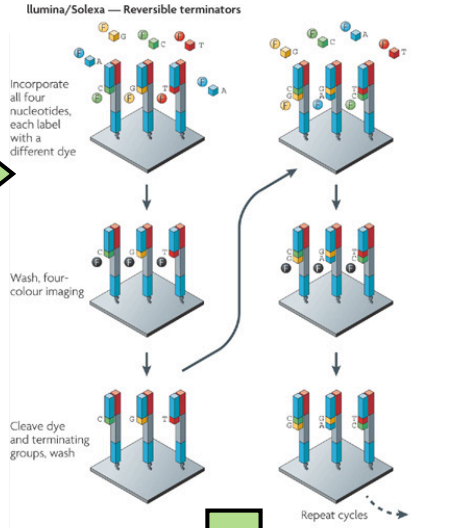
Source: Metzker ML. Sequencing technologies - the next generation. Nat Rev Genet. 2010

name
sequence
quality scores

x 100s of millions



Source: Whiteford et al. Swift: primary data analysis for the Illumina Solexa sequencing platform. Bioinformatics. 2009



Source: Metzker ML. Sequencing technologies - the next generation. Nat Rev Genet. 2010

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

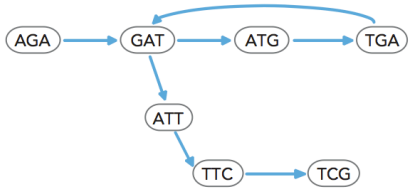
2. *de novo*

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:

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

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
TACCCCACTATGCTTAGCCCTAAACCTCAACAGTTAAATCAACAAAACTGCTCGCCAGAA
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
TACCCCACTATGCTTAGCCCTAAACCTCAACAGTTAAATCAACAAAACTGCTCGCCAGAA
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:

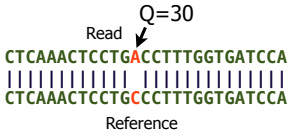
CTCAAACTCCTGACCTTTGGTGATCCA

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
TACCCCACTATGCTTAGCCCTAAACCTCAACAGTTAAATCAACAAAACTGCTCGCCAGAA
CACTACGAGCCACAGCTTAAAACTCAAAGGACCTGGCGGTGCTTCATATCCCTCTAGAGG
AGCCTGTTCTGTAATCGATAAACCCCGATCAACCTCACCACCTCTTGCTCAGCCTATATA
CCGCCATCTTCAGCAAACCCTGATGAAGGCTACAAAGTAAGCGCAAGTACCCACGTAAAG
ACGTTAGGTCAAGGTGTAGCCCATGAGGTGGCAAGAAATGGGCTACATTTTCTACCCCAG
AAAACTACGATAGCCCTTATGAAACTTAAGGGTCGAAGGTGGATTTAGCAGTAAACTAAG
AGTAGAGTGCTTAGTTGAACAGGGCCCTGAAGCGCGTACACACCGCCCGTCACCCTCCTC
AAGTATACTTCAAAGGACATTTAACTAAAACCCCTACGCATTTATATAGAGGAGACAAGT
CGTAACCTCAAACTCCTGGCCTTTGGTGATCCACCCGCCTTGGCCTACCTGCATAATGAA
AAGCACCCAACTTACACTTAGGAGATTTCAACTTAACTTGACCGCTCTGAGCTAAACCTA
GCCCCAAACCCACTCCACCTTACTACCAGACAACCTTAGCCAAACCATTTACCCAAATAA
AGTATAGGCGATAGAAATTGAAACCTGGCGCAATAGATATAGTACCGCAAGGGAAAGATG
AAAAATTATAACCAAGCATAATATAGCAAGGACTAACCCCTATACCTTCTGCATAATGAA
TTAACTAGAAATAACTTTGCAAGGAGAGCCAAAGCTAAGACCCCCGAAACCAGACGAGCT
```

Which hypothesis is better?

Hypothesis 1:



Hypothesis 2:

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

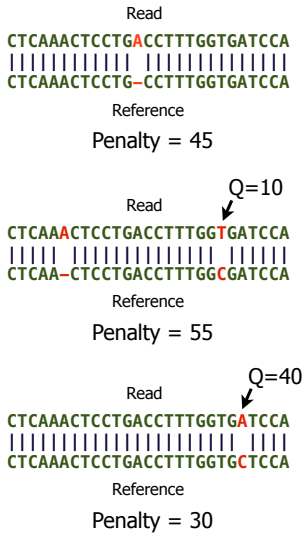
Aligners can employ **penalties** to account for the relative probability of seeing different dissimilarities

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

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




```
\begin{align*}
Pen _\textrm{gap}& \equiv -10 \log _{10} (P_\textrm{gap}) \\
&= -10 \log _{10} (0.00005) \\
&\approx 45
\end{align*}

\begin{align*}
Pen _\textrm{mm}& \equiv \arg\min(-10 \log _{10} (P_\textrm{miscall}), -10 \log _
{10} (P_\textrm{SNP})) \\
&= \arg\min(Q, -10 \log _{10} (0.001)) \\
&= \arg\min(Q, 30) \\
\end{align*}
```