Introduction to Sequencing

BCB 504: Applied Bioinformatics

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Outline

History

Roche 454 Pyrosequencing

Illumina Solexa

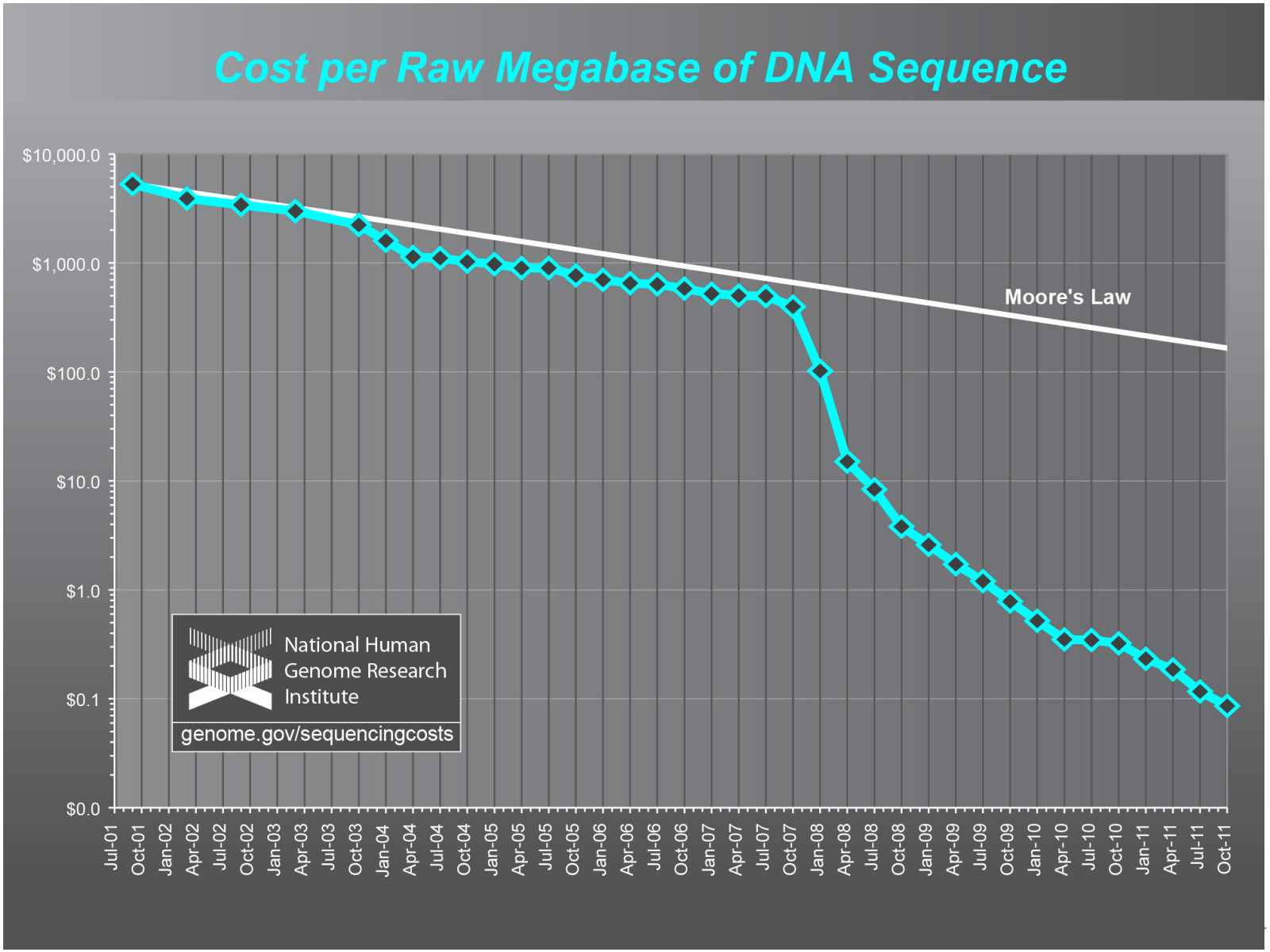
Sequence Data

Library Preparation

Other sequencing technologies

Evolution of DNA Sequencing

Jan - 2012: $0.09 per Megabase, $7,950 per Genome (30x coverage)



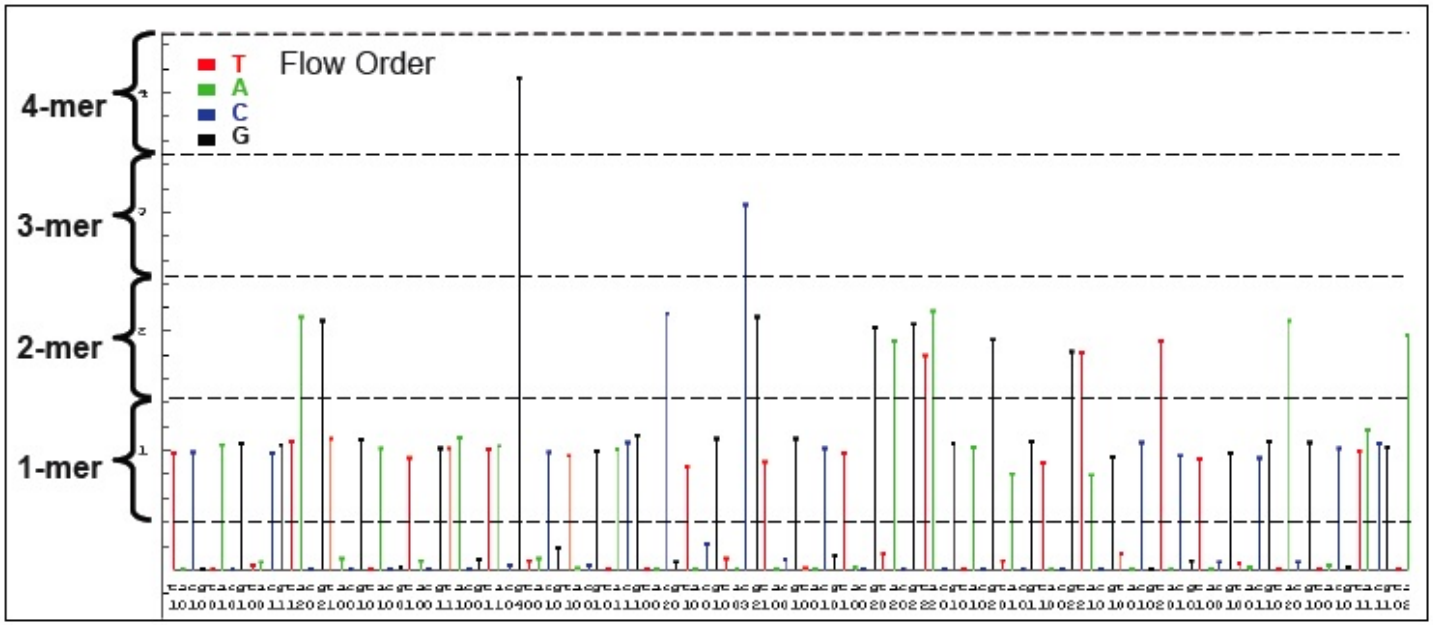
Roche 454 Workflow

- Library Construction
- QA - Library Quantification (Titration)
- emulsion PCR (emPCR)
- Picotiter Plate Loading
- Sequencing
- Image extraction
- Flowgram extraction

Roche 454 Workflow Video

454 Video

Roche 454 Flowgrams



Illumina Workflow

- Library Construction
- Cluster Generation
- Sequencing
- image extraction

Illumina Workflow Video

Illumina Video

Illumina Read Naming Conventions

@EAS139:136:FC706VJ:2:2104:15343:197393 1:Y:18:ATCACG

EAS139	the unique instrument name
136	the run id
FC706VJ	the flowcell id
2	flowcell lane
2104	tile number within the flowcell lane
15343	'x'-coordinate of the cluster within the tile
197393	'y'-coordinate of the cluster within the tile
1	the member of a pair, 1 or 2 (paired-end or mate-pair reads only)
Y	Y if the read fails filter (read is bad), N otherwise
18	0 when none of the control bits are on, otherwise it is an even number
ATCACG	index sequence

454 Read Naming Convections

>EBO6PME01EGNVK

Timestamp EB06PM

Randomized E

Plate Region 01

X,Y coord EGNVK

The timestamp, hash character and X,Y location use a base-36 encoding (where values 0-25 are the letters 'A'-'Z' and the values 26-35 are the digits '0'-'9'). An accession thus consists only of letters and digits, and is case-insensitive.

fasta,qual and fastq files

- fasta files

  >sequence1
  ACCCATGATTTGCGA
- qual files

  >sequence1
  40 40 39 39 40 39 40 40 40 40 20 20 36 39 39
- fastq files

  @sequence1
  ACCCATGATTTGCGA
  +
  IIHHIHIIII55EHH

phred scores

$$Q = -10 log_{10} P$$

Phred Quality Score	Probability of incorrect base call	Base call accuracy
10	1 in 10	90%
20	1 in 100	99%
30	1 in 1000	99.9%
40	1 in 10000	99.99%

phred score conversion

$Q_{sanger} = -10 log_{10} P$ - based on probability (aka phred)
$Q_{solexa} = -10 log_{10} \frac{P}{1-P}$ - based on odds

S - Sanger	Phred+33,	raw reads typically (0, 40)
X - Solexa	Solexa+64,	raw reads typically (-5, 40)
I - Illumina 1.3+	Phred+64,	raw reads typically (0, 40)
J - Illumina 1.5+	Phred+64,	raw reads typically (3, 40)
L - Illumina 1.8+	Phred+33,	raw reads typically (0, 41)

Library Preparation types

- Shotgun - randomly fragmented DNA (100bp - 1kb)
- RNA - Random nanomers or 3' bias
- Amplicons
- Paired end / Mate pair

Ion Torrent Workflow Video

Ion Torrent Video

Pacific Biosciences Workflow Video

Pacific Biosciences Video