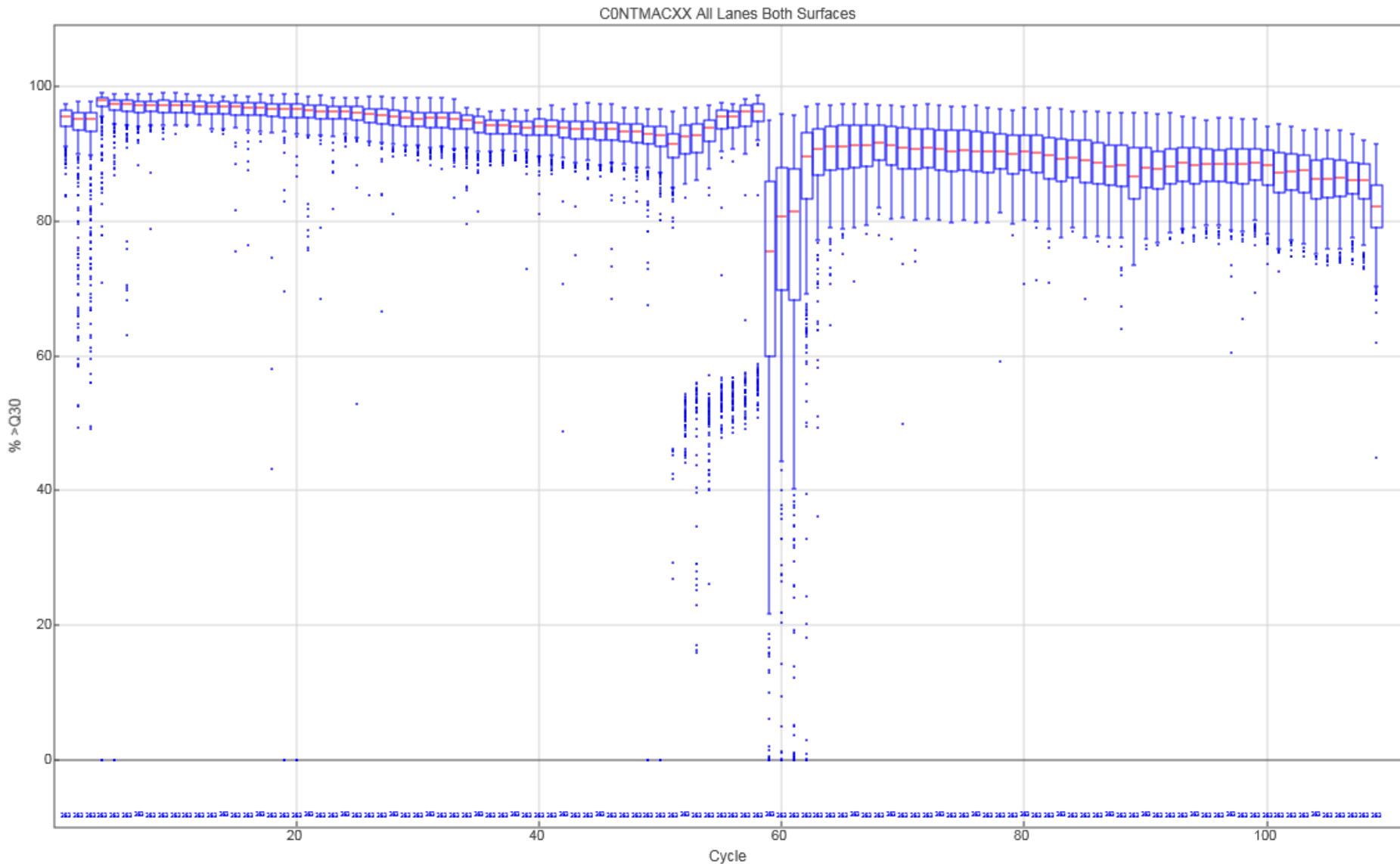# Percent Q30 Scores per cycle for all lanes and both surfaces
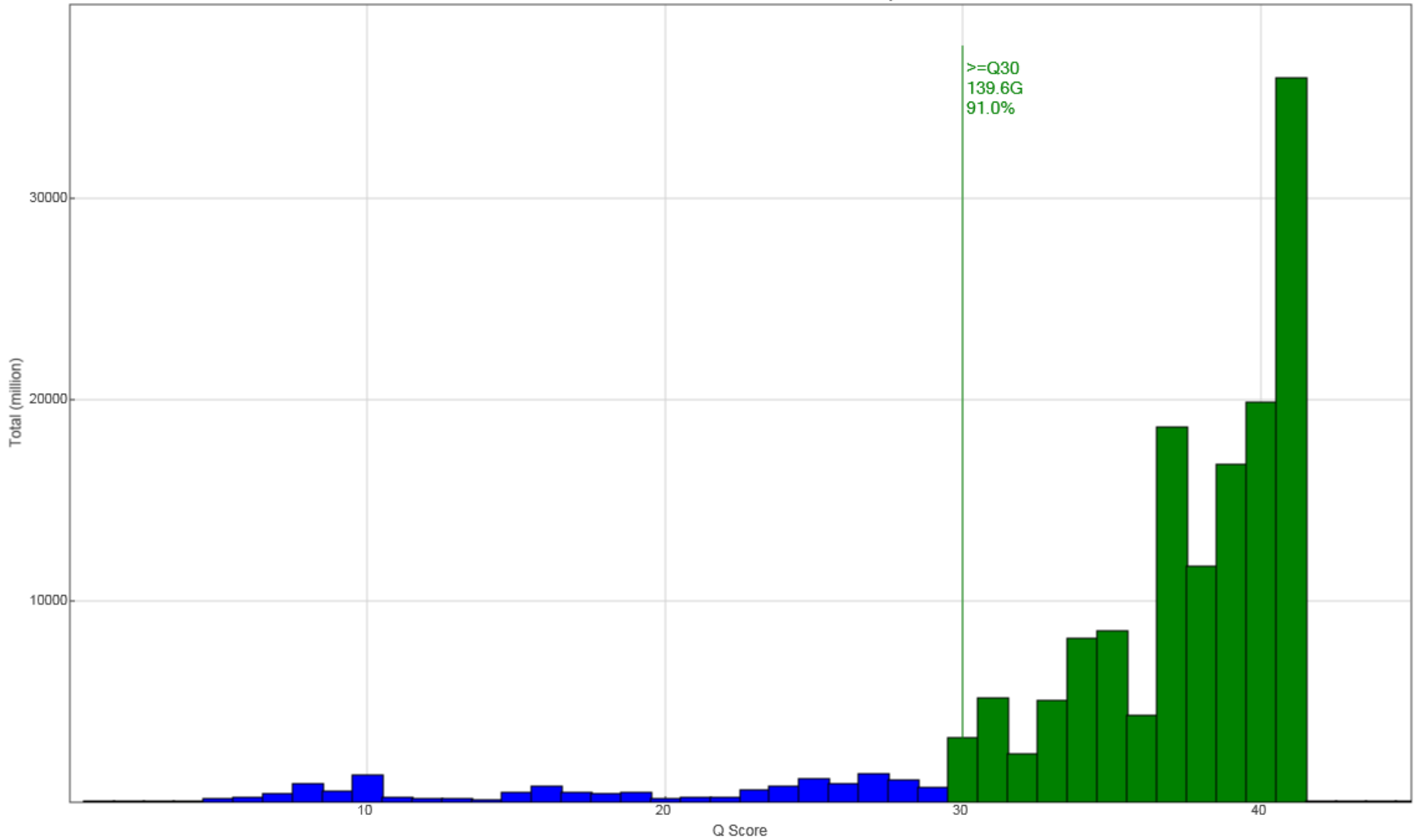

C0NTMACXX All Lanes Both Surfaces

Cluster Density
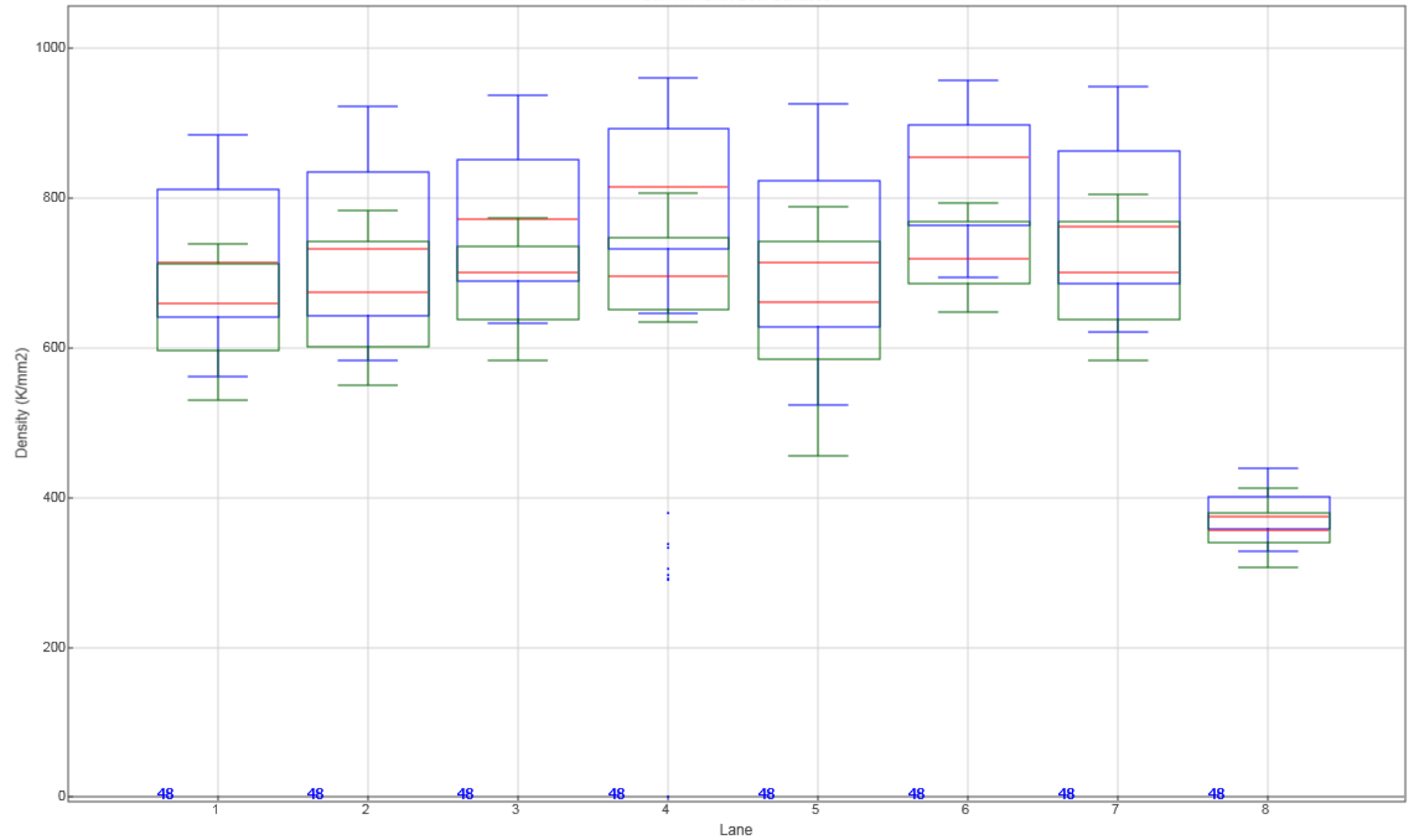
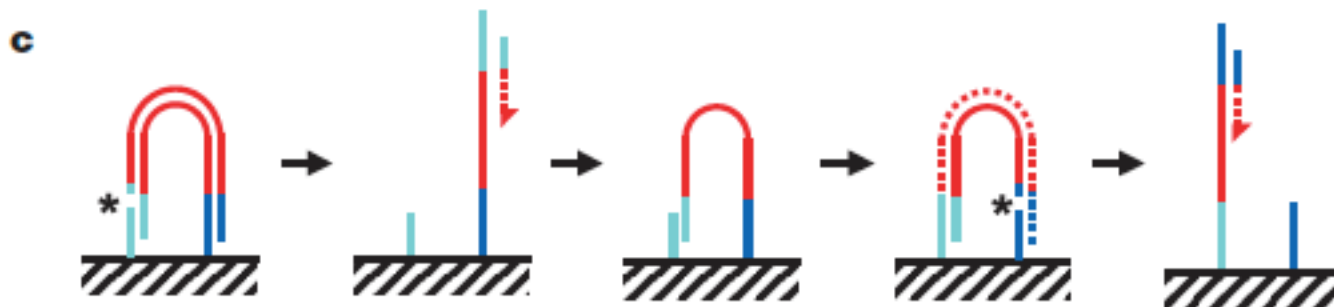# Q Scores

C0NTMACXX All Lanes Both Surf. All Reads All Cycles

c. Paired End Sequencing

d. Mate Paired End Sequenicng

c.

DNA to Data	Sample Prep
~7 days 36x2 bp reads inc. sample prep	3 hours hands-on

Adapters containing attachment sequences (A1 & A2) and sequencing primer sites (SP1 & SP2) are ligated onto DNA fragments (e.g., genomic DNA). The resulting library of single molecules is attached to a flow cell. Each end of every template is read sequentially.

d.

Mate pair library preparation is designed to generate short fragments consisting of two segments that originally had a separation of several kilobases in the genome. Fragments of sample genomic DNA is end-biotinylated to tag the eventual mate pair segments. Self-circularization and refragmentation of these large fragments generates a population of small fragments, some of which contain both mate pair segments with no intervening sequence. These mate pair fragments are enriched using their biotin tag. Mate pairs are sequenced using a similar two-adaptor strategy as described for paired-end sequencing.

DNA to Data	Sample Prep
~8 days 36x2 bp reads inc. sample prep	4¼ hours hands-on

ABI SOLiD

Di base Probes

3′ TTnnnzzz 5′

3′ GTnnnzzz 5′

3′ CTnnnzzz 5′

3′ ATnnnzzz 5′

Cleavage Site

TEMPLATE
2nd Base

	A	C	G	T
A				
C				
G				
T				

1st Base

SOLiD™ Substrate

1 μm bead

5′ P1 Adapter Template Sequence 3′

Glass Slide

35bp/template
9 Billion bp/run

1. Ligate

2. Image

3. Cap

4. Cleave

5. Repeat ligation cycle for n cycles

6. Primer Reset

7. Repeat Steps 1-6 for 5 Primer rounds

PRIMER ROUND 1
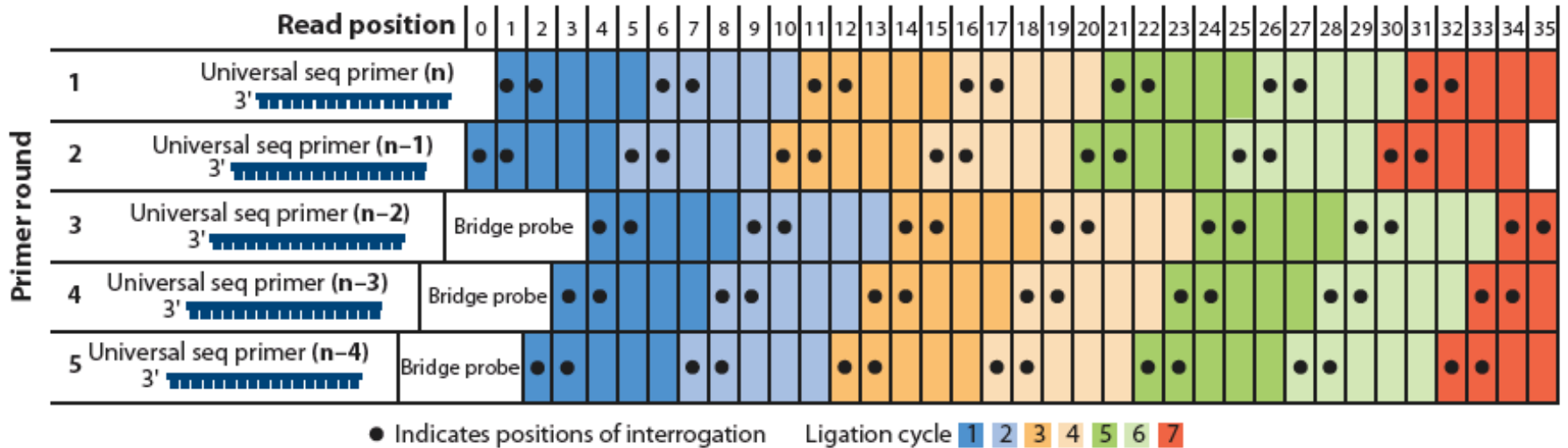
POH

+ Ligase

Universal seq primer (n) AT

3′ TA

1 μm bead P1 Adapter Template Sequence 3′

Excite Fluorescence

TA

Phosphatase

PO₄

3′

Cleavage Agent

HO

AT P

TA

3′

Ligation cycle 1 2 3 4 5 6 7 ... **(n cycles)**

3′

Universal seq primer (n-1)

3′ 2. Primer reset

1. Melt off extended sequence

1 μm bead 3′

1 base shift

PRIMER ROUND 2

-1

Universal seq primer (n-1)
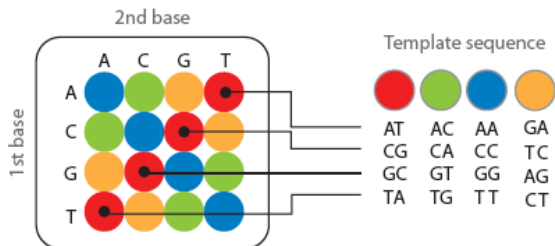
3′ AA CA CG TC AA TA CC

1 μm bead T GT GC AG TT AT GG 3′

SOLiD

8. Repeat Reset with , n–2, n–3, n–4 primers

| Read position | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 |

Primer round

1 — Universal seq primer (n)
2 — Universal seq primer (n–1)
3 — Universal seq primer (n–2) Bridge probe
4 — Universal seq primer (n–3) Bridge probe
5 — Universal seq primer (n–4) Bridge probe

● Indicates positions of interrogation   Ligation cycle 1 2 3 4 5 6 7

Decoding

Color space sequence

TA	AC	AA	GA
GC	CA	CC	TC
CG	GT	GG	AG
AT	TG	TT	CT

Base zero → AT TG

Possible dinucleotides

AT TG GG GA   Decoded sequence

A T G G A   Base space sequence

Possible dinucleotides encoded by each color

2nd base

1st base: A C G T

	A	C	G	T
A				
C				
G				
T				

Template sequence

AT	AC	AA	GA
CG	CA	CC	TC
GC	GT	GG	AG
TA	TG	TT	CT

Double interrogation

With 2 base encoding each base is defined twice
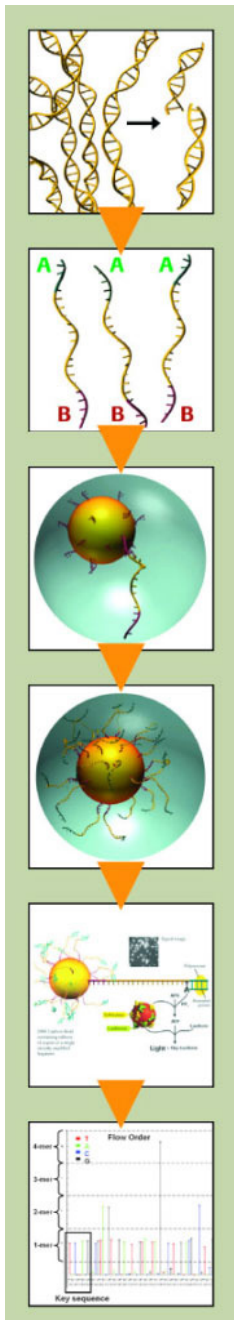
A ←→ T ←→ G ←→ G ←→ A
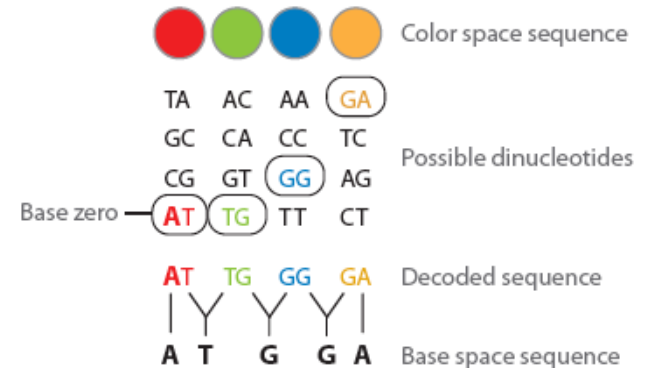
454 LifeSciences
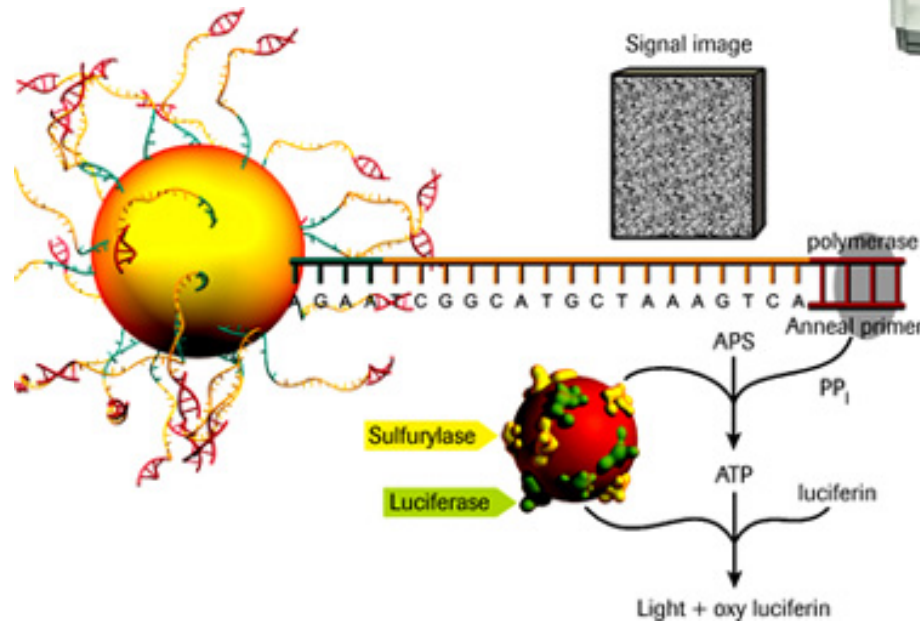
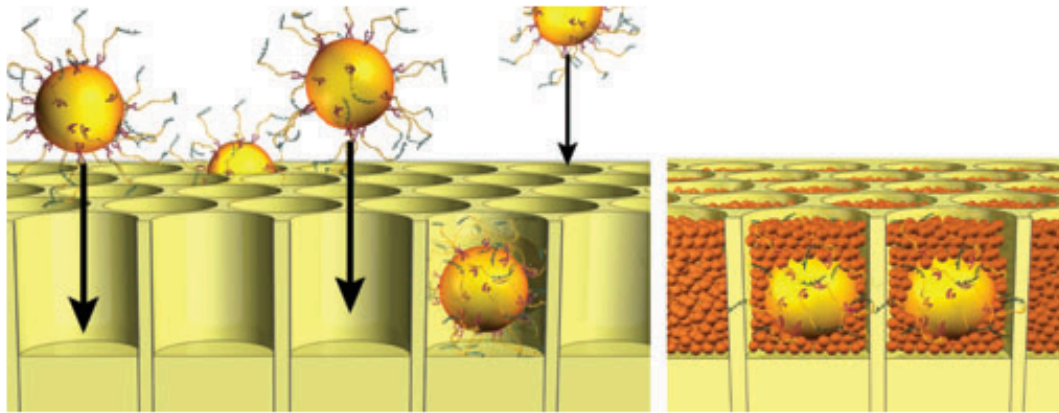

With Titanium Chemistry can generate up to 1000bp/template
700 Million bases/run

454

Sequencing

7.5 hours

- Well diameter: average of 44 μm
- 400,000 reads obtained in parallel
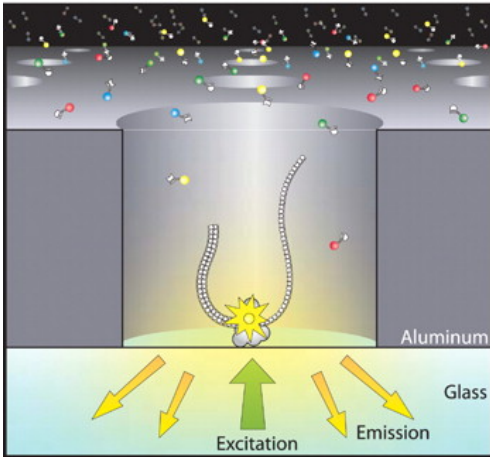- A single cloned amplified sstDNA bead is deposited per well

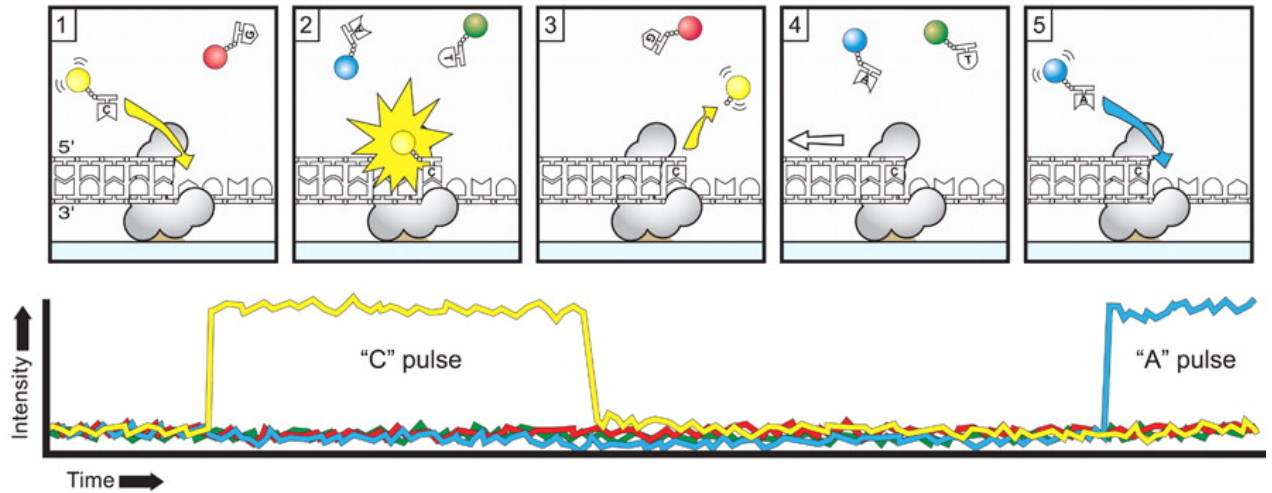Amplified sstDNA library beads → Quality filtered bases
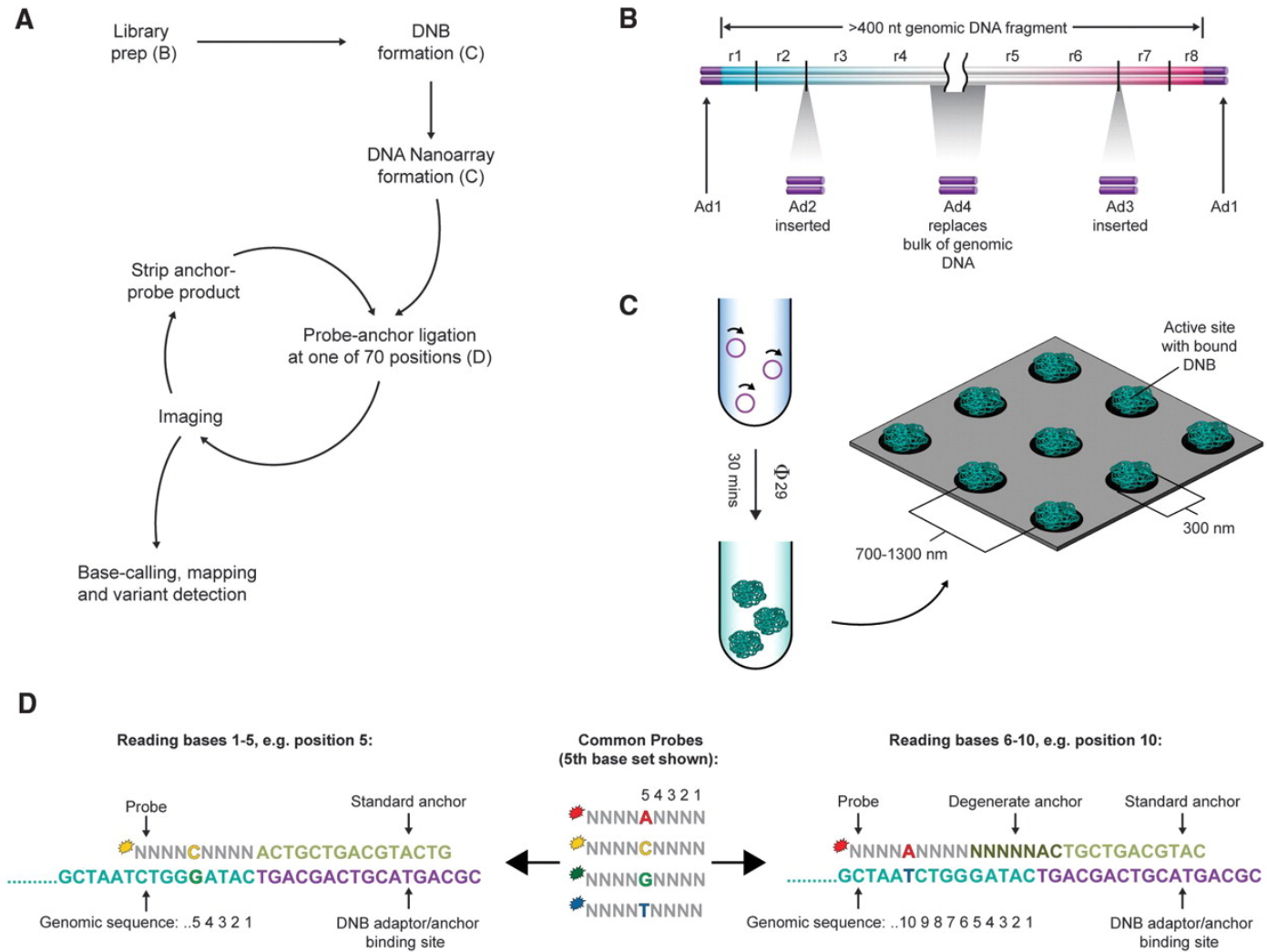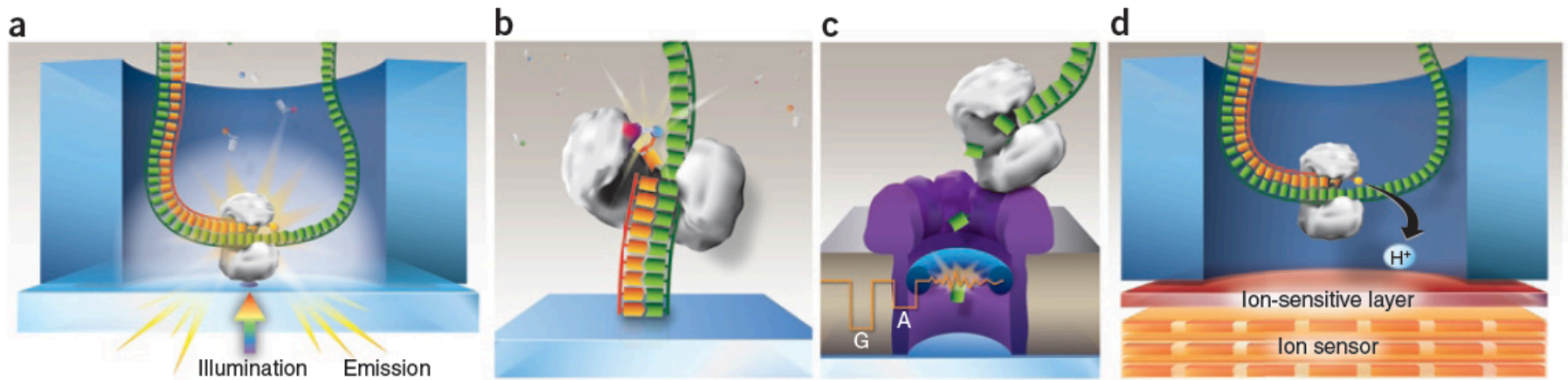
Pacific Biosciences Technology

Complete Genomics Technology

R Drmanac et al. Science 2010;327:78-81

Next Next-Gen Sequencing (3G Seq)

Figure 1 Third-generation sequencing platforms. (**a**) Pacific Biosciences SMRT (single-molecule real-time) DNA sequencing method. The platform uses a DNA polymerase anchored to the bottom surface of a ZMW (pictured in cross section). Differentially labeled nucleotides enter the ZMW via diffusion and occupy the 'detection volume' (white translucent halo area) or microseconds. During an incorporation event, the labeled nucleotide is 'held' within the detection volume by the polymerase for tens of milliseconds. As each nucleotide is incorporated, the label, located on the terminal phosphate, is cleaved off and diffuses out of the ZMW. (**b**) Life Technologies FRET sequencing platform uses base fluorescent labeling technology, a DNA polymerase modified with a quantum dot and DNA template molecules immobilized onto a solid surface. During an incorporation event, energy is transferred from the quantum dot to an acceptor fluorescent moiety on each labeled base. Light emission can only emanate from labeled nucleotides as they are being incorporated. (**c**) The Oxford nanopore sequencing platform uses an exonuclease coupled to a modified α-hemolysin nanopore (purple, pictured in cross section) positioned within a lipid bilayer. As sequentially cleaved bases are directed through the nanopore, they are transiently bound by a cyclodextrin moiety (blue), disturbing current through the nanopore in a manner characteristic for each base. (**d**) The Ion Torrent sequencing platform uses a semiconductor-based high-density array of microwell reaction chambers positioned above an ion-sensitive layer and an ion sensor. Single nucleotides are added sequentially, and incorporation is recorded by measuring hydrogen ions released as a by-product of nucleotide chain elongation.

Sequencing DNA

Human Whole Genome Sequencing

- Initial Ref Sequence $300 million and took about a decade. (Draft reported in 2001)

- Craig Venter's Genome for ~$10 million. (Pub Oct 2007).

- Yoruban from Nigeria in 8 weeks for $250,000. Approx. 30X coverage. (Pub Nov. 2008)

- Han Chinese in 8 weeks for ~$500,000 at approx. 36X coverage.

- Korean Individual (Pub July 2009).

- Female patient with AML. Sequenced normal and tumor from same patient. 98 full runs on GAI for tumor DNA and 34 full runs for normal skin cell DNA. ~1.5 years to compete both genomes.

- As of January 2012 a human genome can be sequenced for about $5,000 at an average read depth of 30X in 10 days

DNA Sequencing with Next-Generation Technologies

A Draft Sequence of the Neandertal Genome

Richard E. Green,[1*†‡] Johannes Krause,[1†§] Adrian W. Briggs,[1†§] Tomislav Maricic,[1†§] Udo Stenzel,[1†§] Martin Kircher,[1†§] Nick Patterson,[2†§] Heng Li,[2†] Weiwei Zhai,[3†‖] Markus Hsi-Yang Fritz,[4†] Nancy F. Hansen,[5†] Eric Y. Durand,[3†] Anna-Sapfo Malaspinas,[3†]

Sequencing the nuclear genome of the extinct woolly mammoth

Webb Miller[1], Daniela I. Michael D. Packard[1], Fan Kerstin Lindblad-Toh[5], Eri Sharon Sheridan[7], Tom P

Genetic history of an archaic hominin group from Denisova Cave in Siberia

David Reich[1,2*], Richard E. Green[3,4*], Martin Kircher[3*], Johannes Krause[3,5*], Nick Patterson[2*], Eric Y. Durand[6*], Bence Viola[3,7*], Adrian W. Briggs[1,3], U Can Alkan[10], Qiaome Michael Richards[7,13], Montgomery Slatkin[6]

Insights into hominid evolution from the gorilla genome sequence

Aylwyn Scally[1], Julien Y. Dutheil[2]†, LaDeana W. Hillier[3], Gregory E. Jordan[4], Ian Goodhead[1]†, Javier Herrero[4], Asger Hobolth[2], Tuuli Lappalainen[5], Thomas Mailund[2], Tomas Marques-Bonet[3,6,7], Shane McCarthy[1], Stephen H. Montgomery[8], Petra C. Schwalie[4], Y. Amy Tang[1], Michelle C. Ward[9,10], Yali Xue[1], Bryndis Yngvadottir[1]†, Can Alkan[3,11], Lars N. Andersen[2], Qasim Ayub[1], Edward V. Ball[12], Kathryn Beal[4], Brenda J. Bradley[8,13], Yuan Chen[1], Chris M. Clee[1], Stephen Fitzgerald[4], Tina A. Graves[14], Yong Gu[1], Paul Heath[1], Andreas Heger[15], Emre Karakoc[3], Anja Kolb-Kokocinski[1], Gavin K. Laird[1], Gerton Lunter[16], Stephen Meader[15], Matthew Mort[12], James C. Mullikin[17], Kasper Munch[2], Timothy D. O'Connor[8], Andrew D. Phillips[12], Javier Prado-Martinez[6], Anthony S. Rogers[1]†, Saba Sajjadian[3], Dominic Schmidt[9,10], Katy Shaw[12], Jared T. Simpson[1], Peter D. Stenson[12], Daniel J. Turner[1]†, Linda Vigilant[18], Albert J. Vilella[4], Weldon Whitener[1], Baoli Zhu[19]†, David N. Cooper[12], Pieter de Jong[19], Emmanouil T. Dermitzakis[5], Evan E. Eichler[3,11], Paul Flicek[4], Nick Goldman[4], Nicholas I. Mundy[8], Zemin Ning[1], Duncan T. Odom[1,9,10], Chris P. Ponting[15], Michael A. Quail[1], Oliver A. Ryder[20], Stephen M. Searle[1], Wesley C. Warren[14], Richard K. Wilson[14], Mikkel H. Schierup[2], Jane Rogers[1]†, Chris Tyler-Smith[1] & Richard Durbin[1]

Applications

- Whole Genome Sequencing

- Exome Sequencing

- Targeted Genomic Sequencing

- Chromatin-IP-Sequencing

- DNAse I Hypersensitivity Sequencing

- Methyl-Seq (RRBS, MeDIP, etc)

- Microbiome Sequencing

- Metagenomics