High-throughput sequencing

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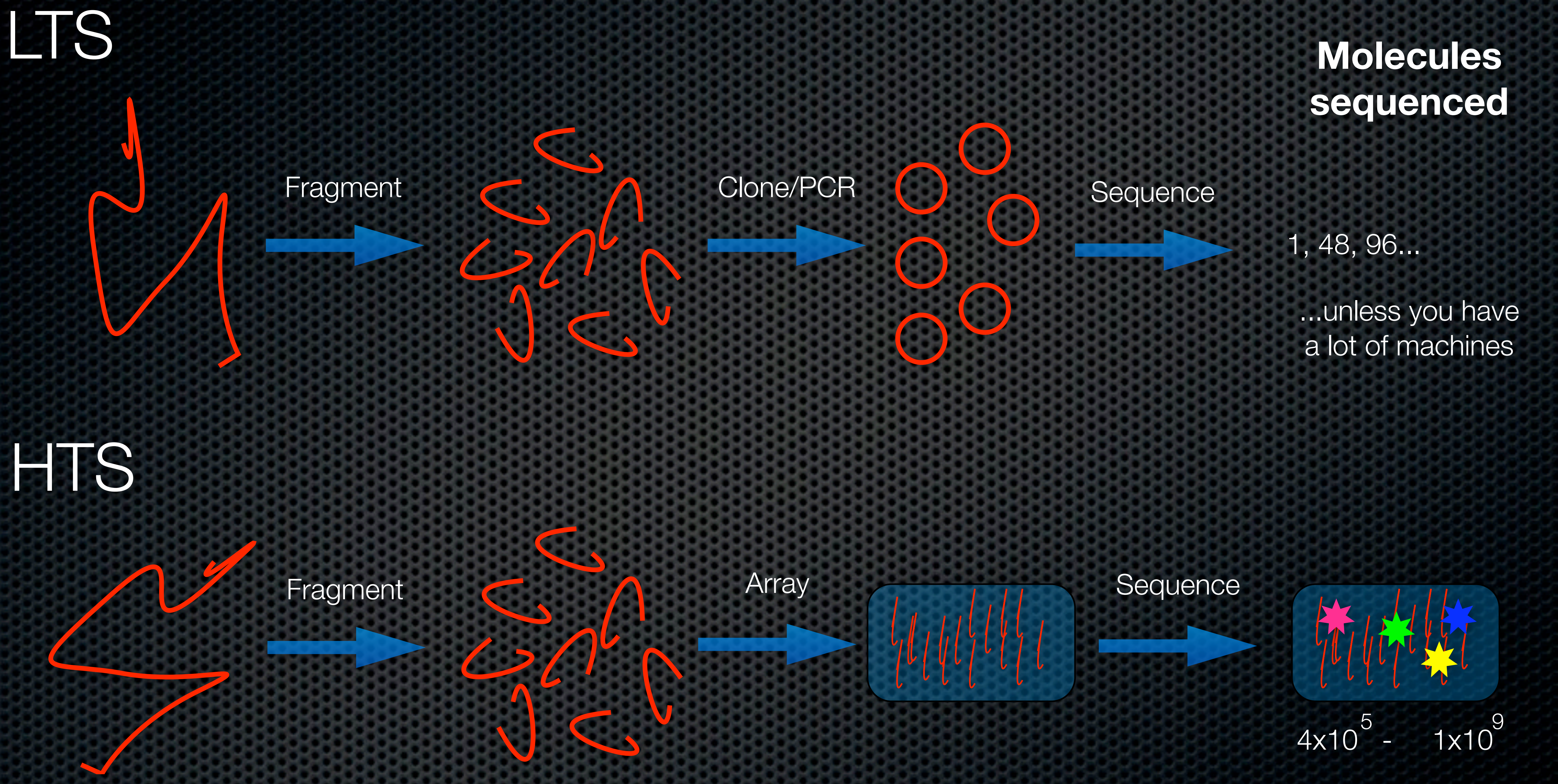
Overview

- Technology
- Data and analysis
- Applications

Technology

Sequencing past, present and future

Sequencing: old and next



LTS

Molecules sequenced

Fragment

Clone/PCR

Sequence

1, 48, 96...

...unless you have a lot of machines

HTS

Fragment

Array

Sequence

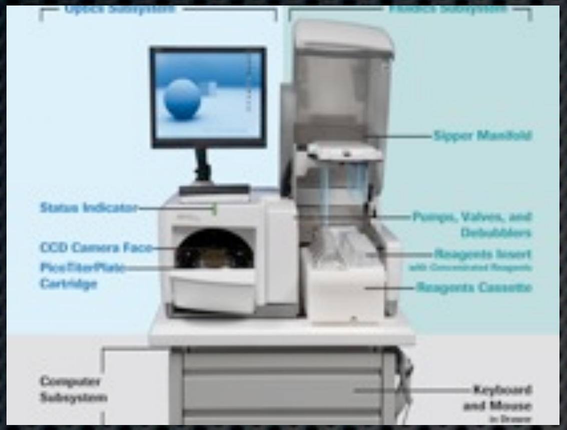
$4x10^5$ - $1x10^9$

...on one machine

Massively parallel

HTS systems available

454



Roche

Solexa

Illumina

SOLiD

ABI

HeliScope

Helicos

Others in 2010

High-throughput sequencing core facility

Department of Medical Genetics, Oslo University Hospital Ullevål

Illumina Genome Analyzer II

- Cluster station
- Paired-end module
- iPAR
- Genome Analyzer Pipeline

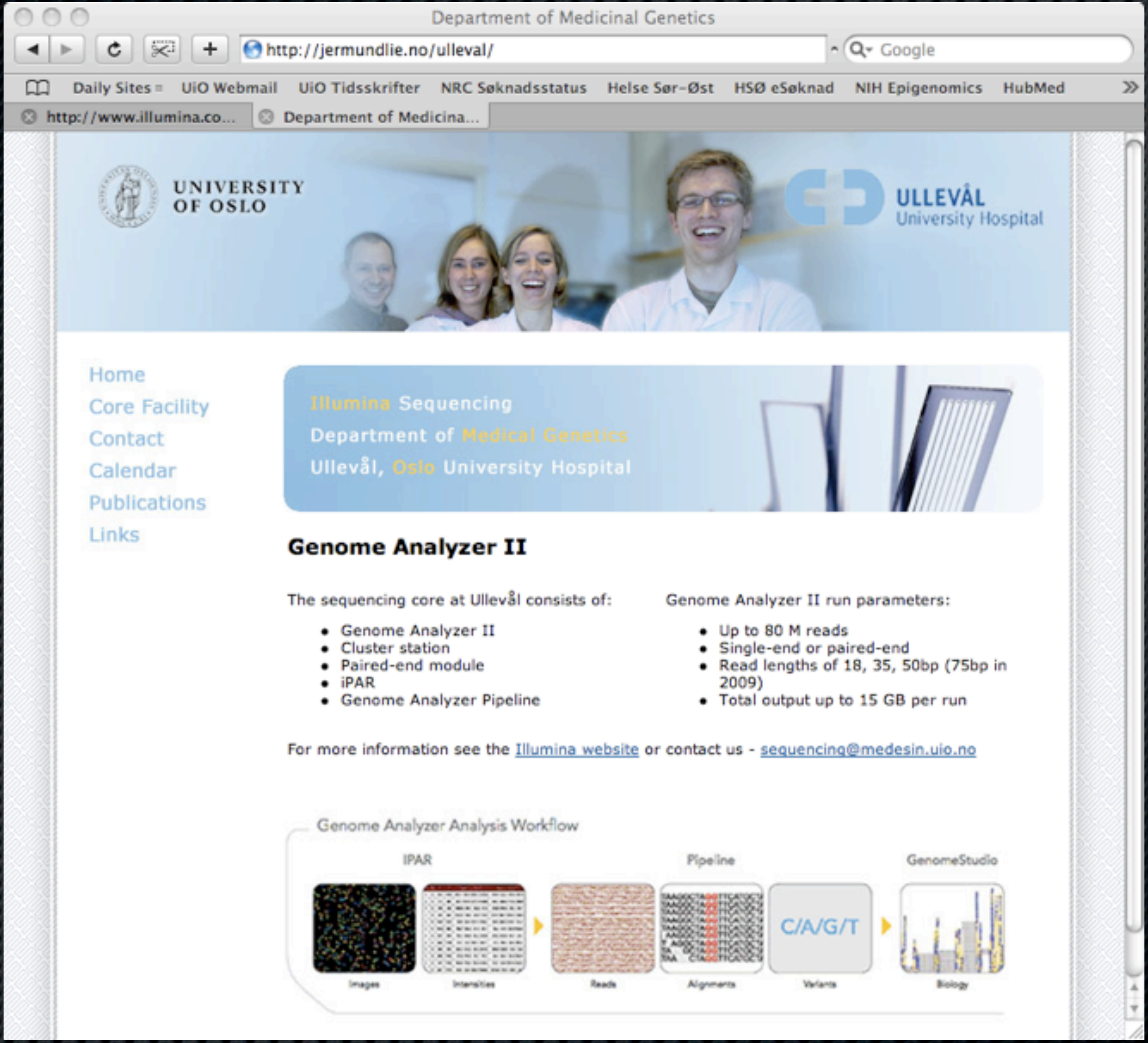
- Up to 160 M reads
- Single-end or paired-end
- Read lengths of 18, 35, 50, 75
- Total output up to 15 GB per run

UNIVERSITETET I OSLO

ULLEVÅL universitetssykehus

HELSE SØR-ØST

www.med.uio.no/ulleval/medgen/sequencing


Department of Medicinal Genetics
http://jermundlie.no/ulleval/
Google
Daily Sites
UiO Webmail
UiO Tidsskrifter
NRC Søknadsstatus
Helse Sør-Øst
HSØ eSøknad
NIH Epigenomics
HubMed
http://www.illumina.co...
Department of Medicina...
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Illumina Sequencing
Department of Medical Genetics
Ullevål, Oslo University Hospital

Genome Analyzer II

The sequencing core at Ullevål consists of:

- Genome Analyzer II
- Cluster station
- Paired-end module
- iPAR
- Genome Analyzer Pipeline

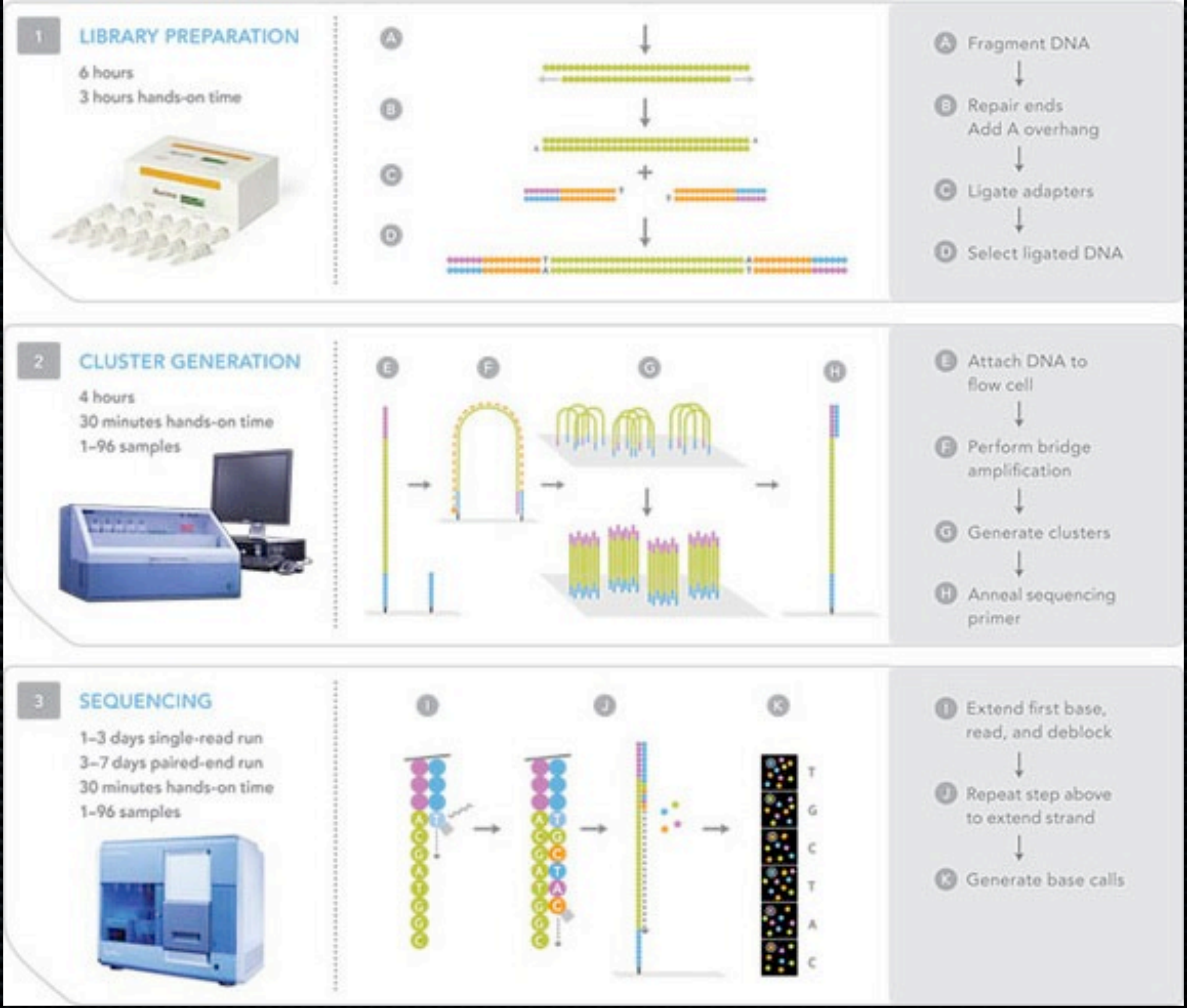
Genome Analyzer II run parameters:

- Up to 80 M reads
- Single-end or paired-end
- Read lengths of 18, 35, 50bp (75bp in 2009)
- Total output up to 15 GB per run

For more information see the Illumina website or contact us - sequencing@medesin.uio.no

sequencing@medisin.uio.no

Illumina sequencing technology



Illumina Genome Analyzer IIx and beyond

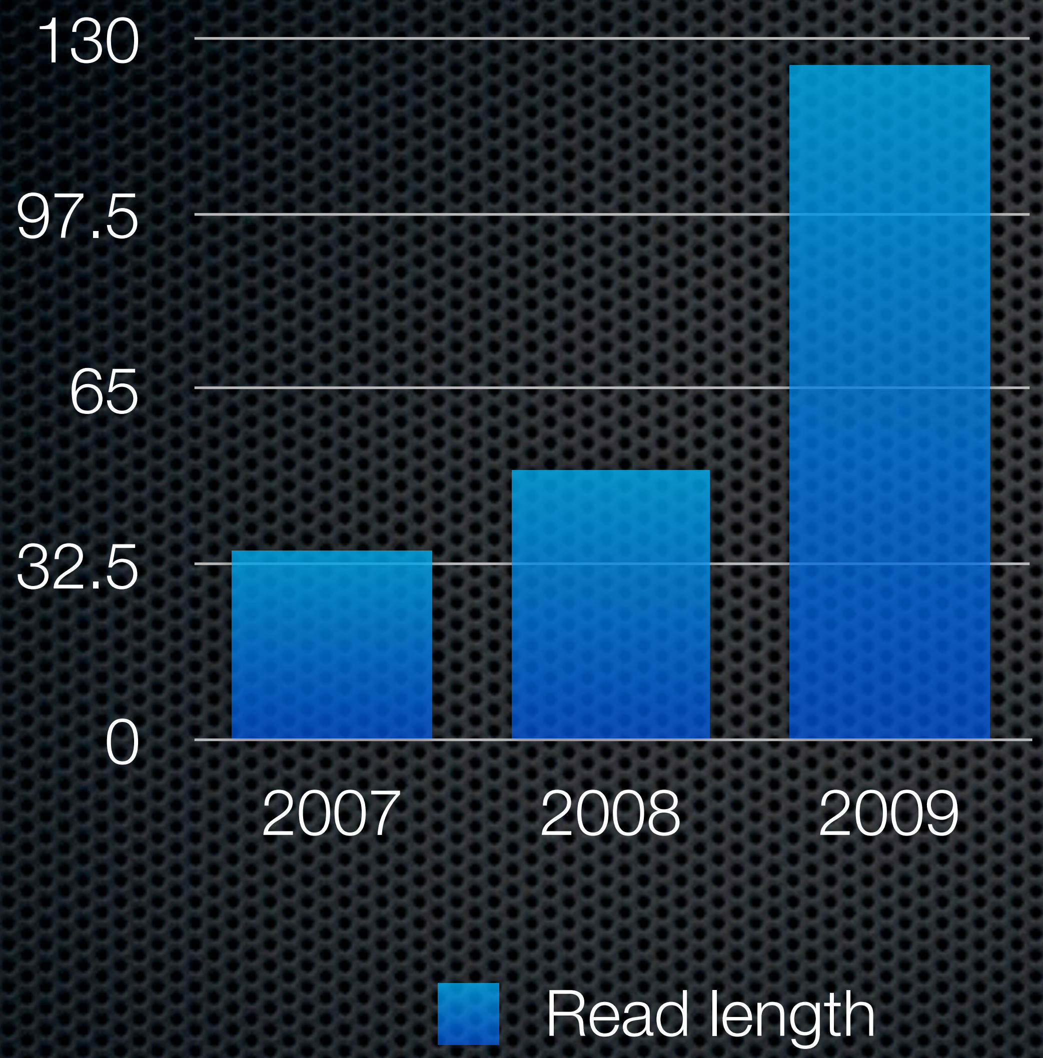
- Hardware upgrade
  - Increased reagent volume
  - Improved scanning
- Software
  - Improved cluster detection algorithms

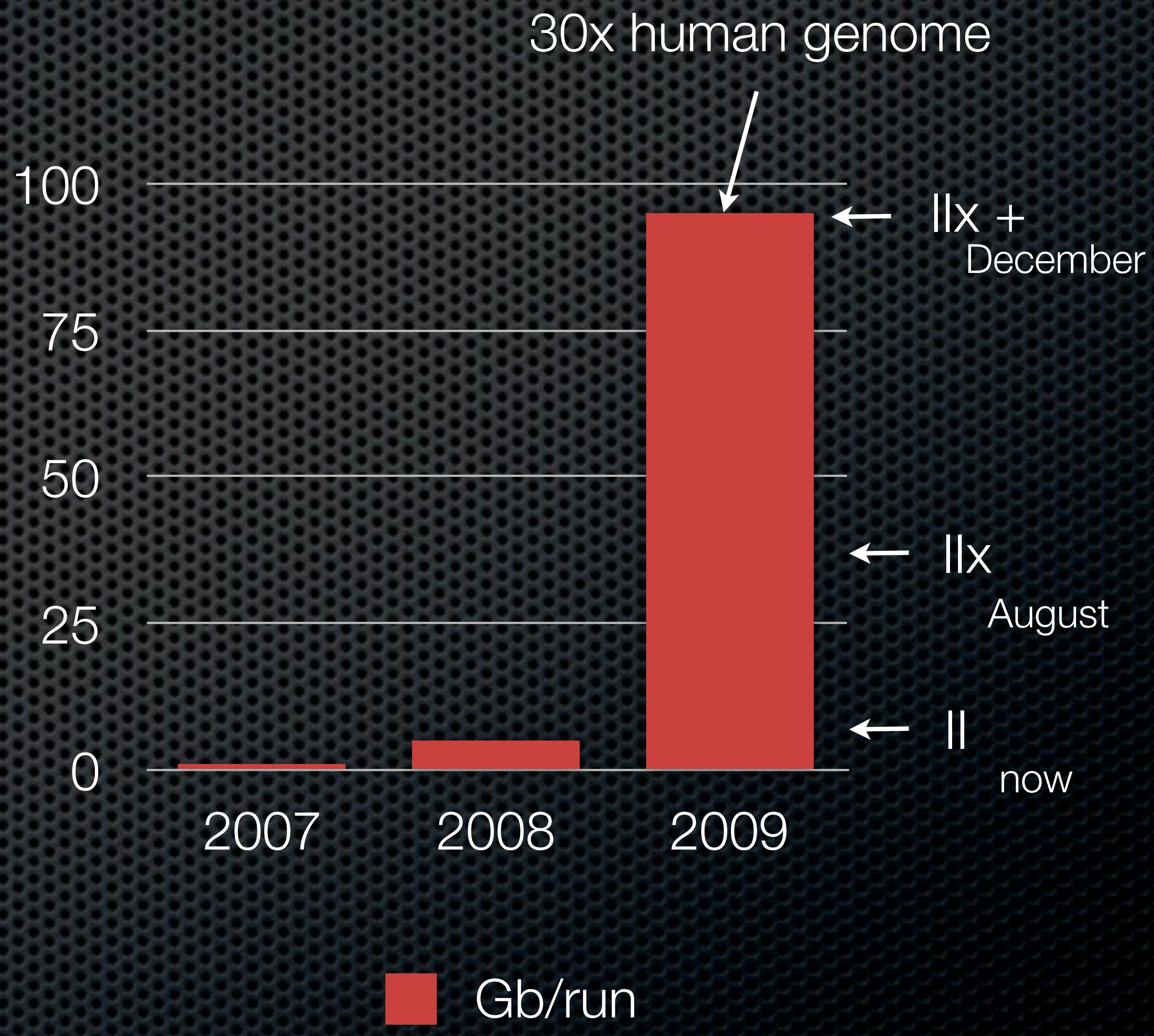
Installation
August 2009

- Flow cell
  - Ordered arrays
  - Submicron features

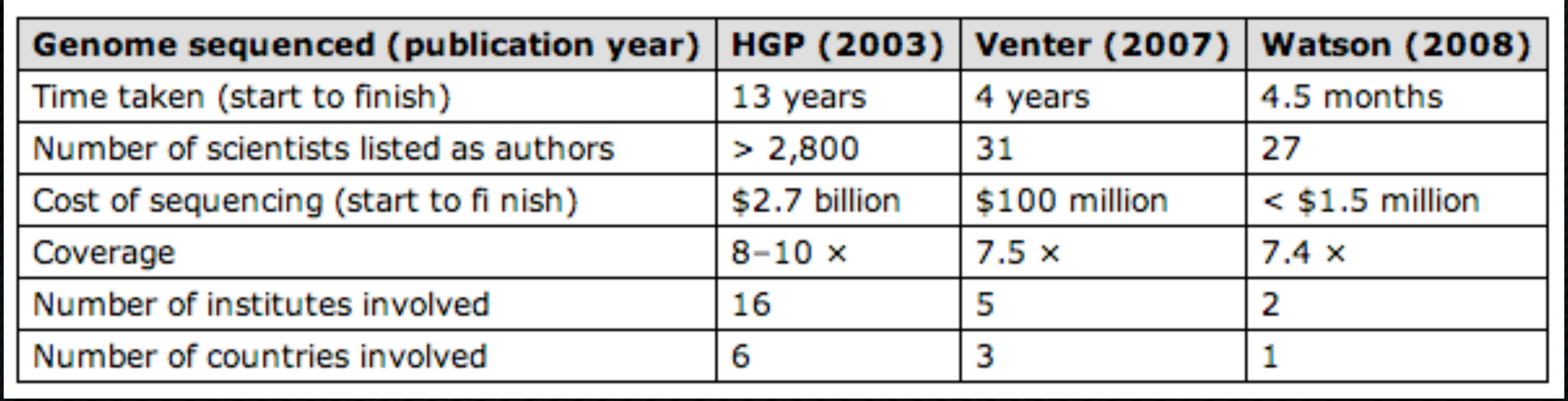
End 2009

Illumina throughput





Sequencing throughput in practice



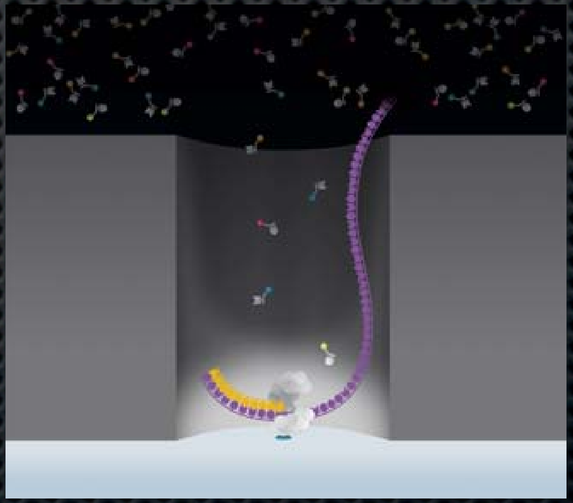
Genome sequenced (publication year)	HGP (2003)	Venter (2007)	Watson (2008)
Time taken (start to finish)	13 years	4 years	4.5 months
Number of scientists listed as authors	> 2,800	31	27
Cost of sequencing (start to finish)	$2.7 billion	$100 million	< $1.5 million
Coverage	8–10 ×	7.5 ×	7.4 ×
Number of institutes involved	16	5	2
Number of countries involved	6	3	1

ABI/SOLiD - Yoruban (12x) - $60 000

Illumina (2009) - Hsa 30x - $30 000 (?)

3rd generation technologies

- Intelligent Biosystems
- Visigen
- Oxford Nanopore
- Reveo
- ZS Genetics
- Complete Genomics (sell whole human genomes in 2009 for $5,000?)
- Pacific Biosciences



Single molecule sequencing
(no amplification bias)

Data and analysis

Illumina sequence data

- Random DNA library of short fragments ~300 bp
- ~100-200 million DNA sequences
- 18, 36, 50, 75, 125 bp long
- Single-end reads
- Paired-end reads

- Run time: 1-10 days
- Data volume: 300 GB.....8 TB

Data issues

- Up to 4 TB/week

- Data storage and backup
- Network speed
- Security (human data)

Analysis hardware

System	Specifications
Pipeline server	
Processor	HP Proliant dl580 g5 rack server (4 quad-core 2.93GHz 64-bit Intel Xeon)
Memory	32 GB
Storage	21 TB (HP 60 MSA)
Operating system	Linux
iPAR	
Processor	HP DL 380 (2 × 5460 3.16 GHz)
Memory	16 GB
Storage	3.2 TB (HP SmartArray P800)
Operating system	Linux/XP
Mac Pro (x2)	
Processor	2 quad-core 2.66 GHz 64-bit Intel Nehalem
Memory	16 GB
Storage	4 TB
Operating system	OS X

NorStore,Titan.....

Analysis pipeline

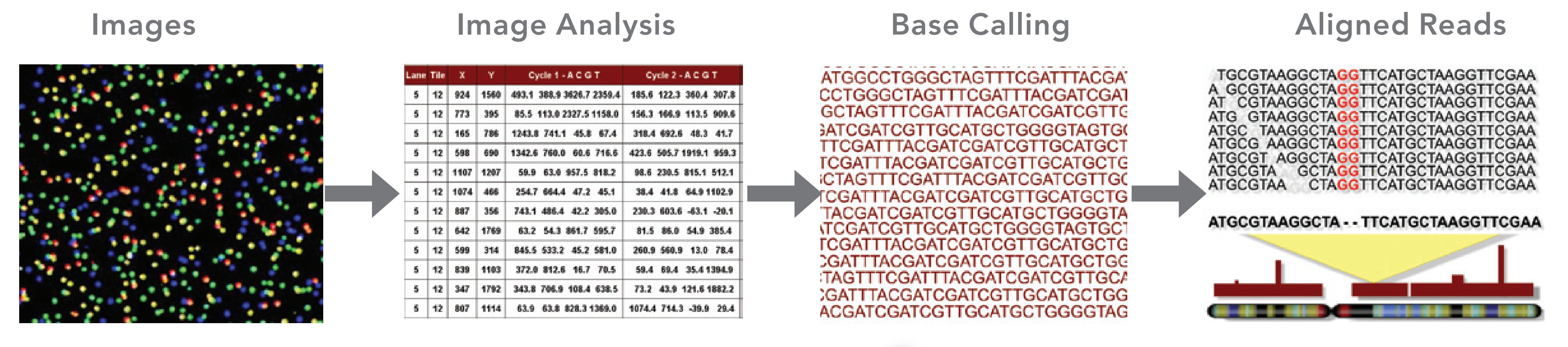
Illumina Pipeline 1.4

SCS

Firecrest

Bustard

GERALD



FASTQ format

```
@EAS54_6_R1_2_1_413_324
CCCTTCTTGTCTTCAGCGTTTCTCC
+
;;3;;;;;;;;;;;;7;;;;;;;88
@EAS54_6_R1_2_1_540_792
TTGGCAGGCCAAGGCCGATGGATCA
+
;;;;;;;;;;;7;;;;;-;;;3;83
@EAS54_6_R1_2_1_443_348
GTTGCTTCTGGCGTGGGTGGGGGGG
+EAS54_6_R1_2_1_443_348
;;;;;;;;;;;9;7;;.7;39333
```

Other software/analyses

Integrated solutions
* CLCbio Genomics Workbench - *de novo* and reference assembly of Sanger, Roche FLX, Illumina, Helicos, and SOLiD data. Commercial next-gen-seq software that extends the CLCbio Main Workbench software. Includes SNP detection, CHiP-seq, browser and other features. Commercial. Windows, Mac OS X and Linux.
* Galaxy - Galaxy = interactive and reproducible genomics. A job webportal.
* Genomatix - Integrated Solutions for Next Generation Sequencing data analysis.
* JMP Genomics - Next gen visualization and statistics tool from SAS. They are working with NCGR to refine this tool and produce others.
* NextGENe - *de novo* and reference assembly of Illumina, SOLiD and Roche FLX data. Uses a novel Condensation Assembly Tool approach where reads are joined via "anchors" into mini-contigs before assembly. Includes SNP detection, CHiP-seq, browser and other features. Commercial. Win or MacOS.
* SeqMan Genome Analyser - Software for Next Generation sequence assembly of Illumina, Roche FLX and Sanger data integrating with Lasergene Sequence Analysis software for additional analysis and visualization capabilities. Can use a hybrid templated/de novo approach. Commercial. Win or Mac OS X.
* SHORE - SHORE, for Short Read, is a mapping and analysis pipeline for short DNA sequences produced on a Illumina Genome Analyzer. A suite created by the 1001 Genomes project. Source for POSIX.
* SlimSearch - Fledgling commercial product.

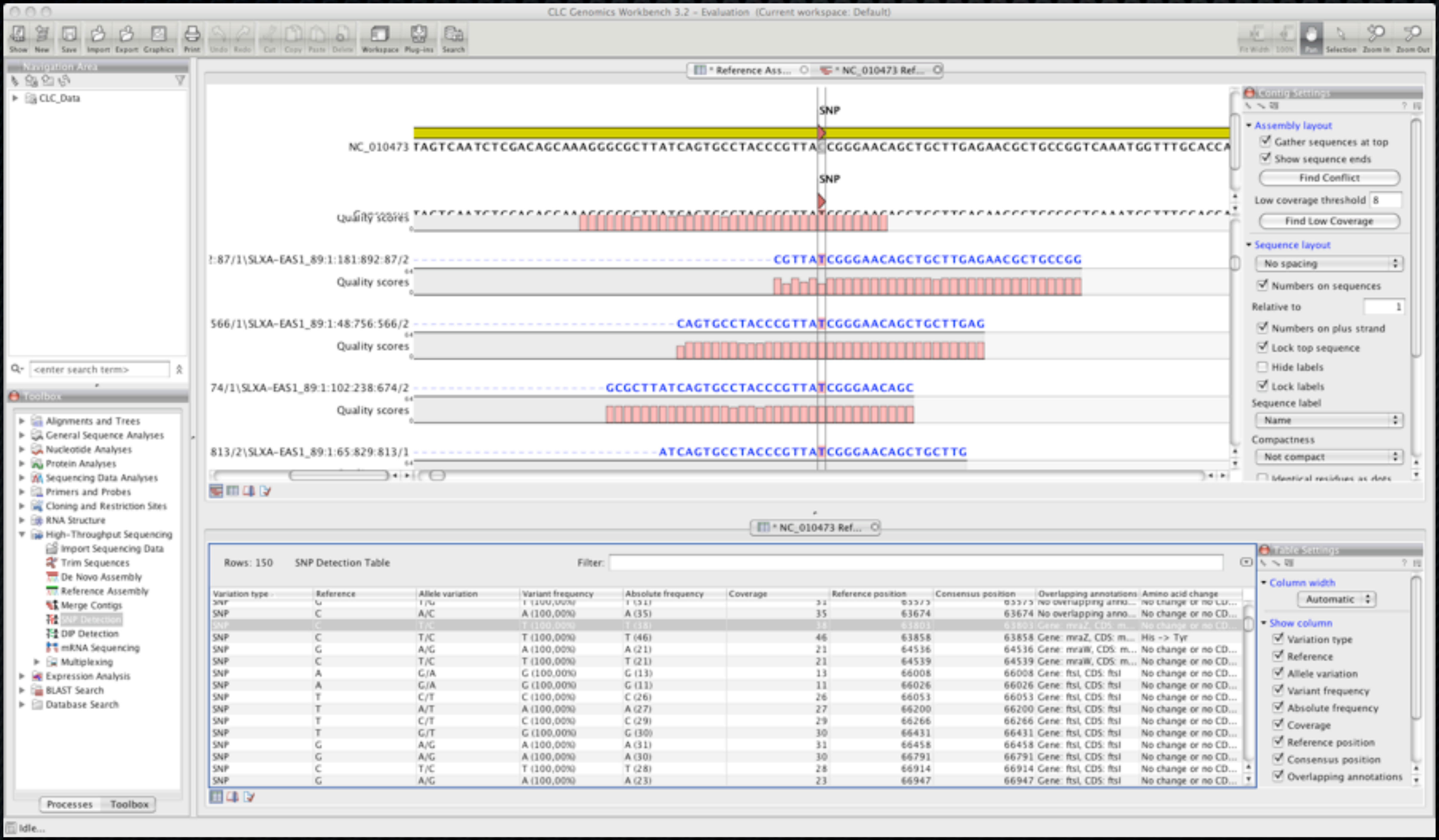
Align/Assemble to a reference
* BFAST - Blat-like Fast Accurate Search Tool. Written by Nils Homer, Stanley F. Nelson and Barry Merriman at UCLA.
* Bowtie - Ultrafast, memory-efficient short read aligner. It aligns short DNA sequences (reads) to the human genome at a rate of 25 million reads per hour on a typical workstation with 2 gigabytes of memory. Uses a Burrows-Wheeler-Transformed (BWT) index. Link to discussion thread here. Written by Ben Langmead and Cole Trapnell. Linux, Windows, and Mac OS X.
* BWA - Heng Lee's BWT Alignment program - a progression from Maq. BWA is a fast light-weighted tool that aligns short sequences to a sequence database, such as the human reference genome. By default, BWA finds an alignment within edit distance 2 to the query sequence. C++ source.
* ELAND - Efficient Large-Scale Alignment of Nucleotide Databases. Whole genome alignments to a reference genome. Written by Illumina author Anthony J. Cox for the Solexa 1G machine.
* Exonerate - Various forms of pairwise alignment (including Smith-Waterman-Gotoh) of DNA/protein against a reference. Authors are Guy St C Slater and Ewan Birney from EMBL. C for POSIX.
* GenomeMapper - GenomeMapper is a short read mapping tool designed for accurate read alignments. It quickly aligns millions of reads either with ungapped or gapped alignments. A tool created by the 1001 Genomes project. Source for POSIX.
* GMAP - GMAP (Genomic Mapping and Alignment Program) for mRNA and EST Sequences. Developed by Thomas Wu and Colin Watanabe at Genentec. C/Perl for Unix.
* gnumap - The Genomic Next-generation Universal MAPper (gnumap) is a program designed to accurately map sequence data obtained from next-generation sequencing machines (specifically that of Solexa/Illumina) back to a genome of any size. It seeks to align reads from nonunique repeats using statistics. From authors at Brigham Young University. C source/Unix.
* MAQ - Mapping and Assembly with Qualities (renamed from MAPASS2). Particularly designed for Illumina with preliminary functions to handle ABI SOLiD data. Written by Heng Li from the Sanger Centre. Features extensive supporting tools for DIP/SNP detection, etc. C++ source
* MOSAIK - MOSAIK produces gapped alignments using the Smith-Waterman algorithm. Features a number of support tools. Support for Roche FLX, Illumina, SOLiD, and Helicos. Written by Michael Strömberg at Boston College. Win/Linux/MacOSX
* MrFAST and MrsFAST - mrFAST & mrsFAST are designed to map short reads generated with the Illumina platform to reference genome assemblies; in a fast and memory-efficient manner. Robust to INDELs and MrsFAST has a bisulphite mode. Authors are from the University of Washington. C as source.
* MUMmer - MUMmer is a modular system for the rapid whole genome alignment of finished or draft sequence. Released as a package providing an efficient suffix tree library, seed-and-extend alignment, SNP detection, repeat detection, and visualization tools. Version 3.0 was developed by Stefan Kurtz, Adam Phillippy, Arthur L Delcher, Michael Smoot, Martin Shumway, Corina Antonescu and Steven L Salzberg - most of whom are at The Institute for Genomic Research in Maryland, USA. POSIX OS required.
* Novocraft - Tools for reference alignment of paired-end and single-end Illumina reads. Uses a Needleman-Wunsch algorithm. Can support Bis-Seq. Commercial. Available free for evaluation, educational use and for use on open not-for-profit projects. Requires Linux or Mac OSX.
* PASS - It supports Illumina, SOLiD and Roche-FLX data formats and allows the user to modulate very finely the sensitivity of the alignments. Spaced-seed intial filter, then NW dynamic algorithm to a SW(like) local alignment. Authors are from CRIBI in Italy. Win/Linux.
* RMAP - Assembles 20 - 64 bp Illumina reads to a FASTA reference genome. By Andrew D. Smith and Zhenyu Xuan at CSHL. (published in BMC Bioinformatics). POSIX OS required.
* SeqMap - Supports up to 5 or more bp mismatches/INDELs. Highly tunable. Written by Hui Jiang from the Wong lab at Stanford. Builds available for most OS's.
* SHRiMP - Assembles to a reference sequence. Developed with Applied Biosystem's colourspace genomic representation in mind. Authors are Michael Brudno and Stephen Rumble at the University of Toronto. POSIX.
* Slider- An application for the Illumina Sequence Analyzer output that uses the probability files instead of the sequence files as an input for alignment to a reference sequence or a set of reference sequences. Authors are from BCGSC. Paper is here.
* SOAP - SOAP (Short Oligonucleotide Alignment Program). A program for efficient gapped and ungapped alignment of short oligonucleotides onto reference sequences. The updated version uses a BWT. Can call SNPs and INDELs. Author is Ruiqiang Li at the Beijing Genomics Institute. C++, POSIX.
* SSAHA - SSAHA (Sequence Search and Alignment by Hashing Algorithm) is a tool for rapidly finding near exact matches in DNA or protein databases using a hash table. Developed at the Sanger Centre by Zemin Ning, Anthony Cox and James Mullikin. C++ for Linux/Alpha.
* SOCS - Aligns SOLiD data. SOCS is built on an iterative variation of the Rabin-Karp string search algorithm, which uses hashing to reduce the set of possible matches, drastically increasing search speed. Authors are Ondov B, Varadarajan A, Passalacqua KD and Bergman NH.
* SWIFT - The SWIFT suit is a software collection for fast index-based sequence comparison. It contains: SWIFT — fast local alignment search, guaranteeing to find epsilon-matches between two sequences. SWIFT BALSAM — a very fast program to find semiglobal non-gapped alignments based on k-mer seeds. Authors are Kim Rasmussen (SWIFT) and Wolfgang Gerlach (SWIFT BALSAM)
* SXOligoSearch - SXOligoSearch is a commercial platform offered by the Malaysian based Synamatix. Will align Illumina reads against a range of Refseq RNA or NCBI genome builds for a number of organisms. Web Portal. OS independent.
* Vmatch - A versatile software tool for efficiently solving large scale sequence matching tasks. Vmatch subsumes the software tool REPuter, but is much more general, with a very flexible user interface, and improved space and time requirements. Essentially a large string matching toolbox. POSIX.
* Zoom - ZOOM (Zillions Of Oligos Mapped) is designed to map millions of short reads, emerged by next-generation sequencing technology, back to the reference genomes, and carry out post-analysis. ZOOM is developed to be highly accurate, flexible, and user-friendly with speed being a critical priority. Commercial. Supports Illumina and SOLiD data.

***De novo* Align/Assemble**
* ABySS - Assembly By Short Sequences. ABySS is a de novo sequence assembler that is designed for very short reads. The single-processor version is useful for assembling genomes up to 40-50 Mbases in size. The parallel version is implemented using MPI and is capable of assembling larger genomes. By Simpson JT and others at the Canada's Michael Smith Genome Sciences Centre. C++ as source.
* ALLPATHS - ALLPATHS: De novo assembly of whole-genome shotgun microreads. ALLPATHS is a whole genome shotgun assembler that can generate high quality assemblies from short reads. Assemblies are presented in a graph form that retains ambiguities, such as those arising from polymorphism, thereby providing information that has been absent from previous genome assemblies. Broad Institute.
* Edena - Edena (Exact DE Novo Assembler) is an assembler dedicated to process the millions of very short reads produced by the Illumina Genome Analyzer. Edena is based on the traditional overlap layout paradigm. By D. Hernandez, P. François, L. Farinelli, M. Osteras, and J. Schrenzel. Linux/Win.
* EULER-SR - Short read *de novo* assembly. By Mark J. Chaisson and Pavel A. Pevzner from UCSD (published in Genome Research). Uses a de Bruijn graph approach.
* MIRA2 - MIRA (Mimicking Intelligent Read Assembly) is able to perform true hybrid de-novo assemblies using reads gathered through 454 sequencing technology (GS20 or GS FLX). Compatible with 454, Solexa and Sanger data. Linux OS required.
* SEQAN - A Consistency-based Consensus Algorithm for De Novo and Reference-guided Sequence Assembly of Short Reads. By Tobias Rausch and others. C++, Linux/Win.

http://seqanswers.com

Majority Unix

CLC genomics workbench



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NC_010473 TAGTCAATCTCGACAGCAAAGGGCGCTTATCAGTGCCTACCCGTTACCGGGAACAGCTGCTTGAGAACGCTGCCGGTCAAATGGTTTGCACCA
:87/1\SLXA-EAS1_89:1:181:892:87/2   CGTTATCGGGAACAGCTGCTTGAGAACGCTGCCGG
566/1\SLXA-EAS1_89:1:48:756:566/2   CAGTGCCTACCCGTTATCGGGAACAGCTGCTTGAG
74/1\SLXA-EAS1_89:1:102:238:674/2   GCGCTTATCAGTGCCTACCCGTTATCGGGAACAGC
813/2\SLXA-EAS1_89:1:65:829:813/1   ATCAGTGCCTACCCGTTATCGGGAACAGCTGCTTG
```

Contig Settings

- Assembly layout
  - ☑ Gather sequences at top
  - ☑ Show sequence ends
  - Find Conflict
  - Low coverage threshold: 8
  - Find Low Coverage
- Sequence layout
  - No spacing
  - ☑ Numbers on sequences
  - Relative to: 1
  - ☑ Numbers on plus strand
  - ☑ Lock top sequence
  - □ Hide labels
  - ☑ Lock labels
  - Sequence label: Name
  - Compactness: Not compact
  - □ Identical residues as dots

Rows: 150 SNP Detection Table Filter:

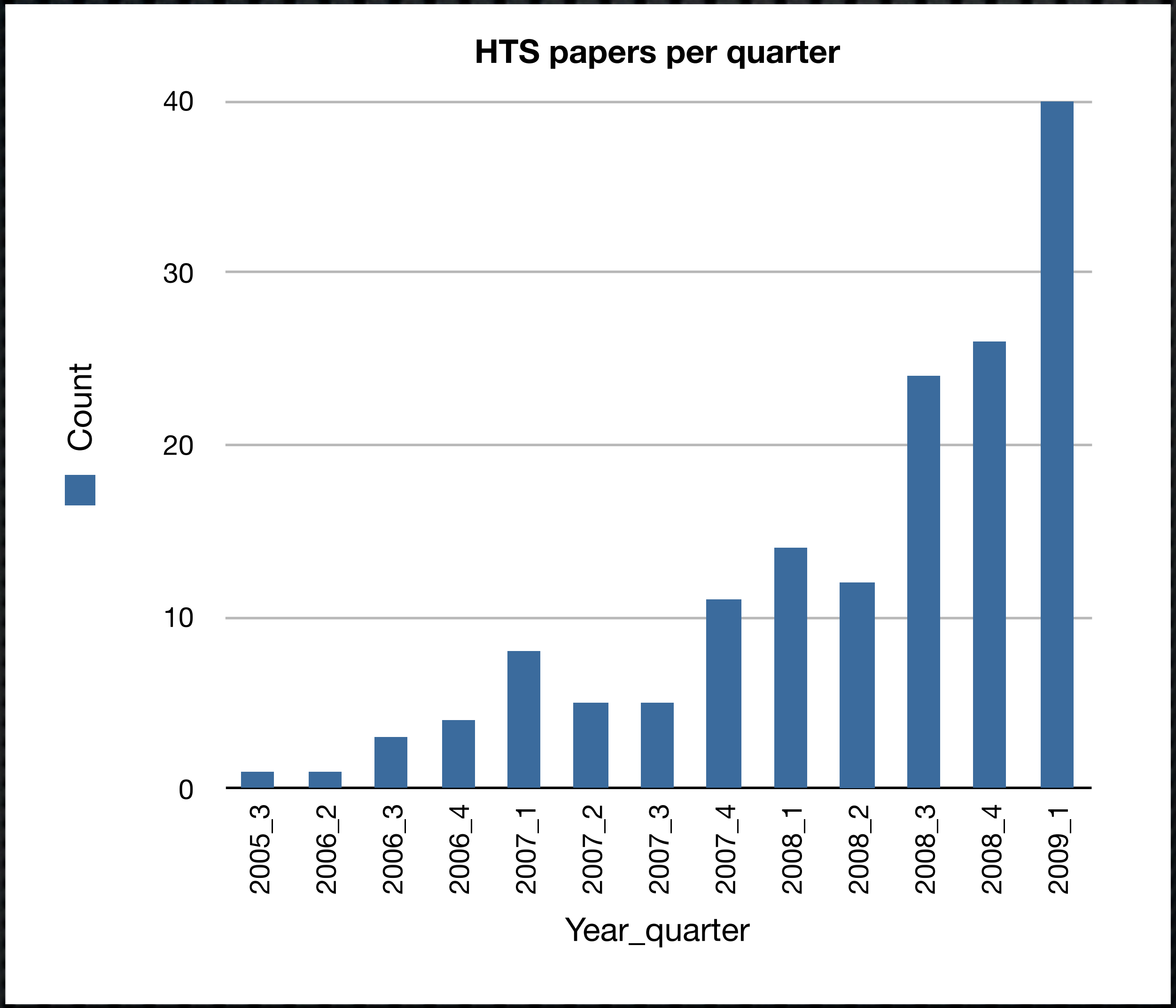
Variation type	Reference	Allele variation	Variant frequency	Absolute frequency	Coverage	Reference position	Consensus position	Overlapping annotations	Amino acid change
SNP	G	T/G	T (100,00%)	T (31)	31	63575	63575	No overlapping anno...	No change or no CD...
SNP	C	A/C	A (100,00%)	A (35)	35	63674	63674	No overlapping anno...	No change or no CD...
SNP	C	T/C	T (100,00%)	T (38)	38	63803	63803	Gene: mraZ, CDS: m...	No change or no CD...
SNP	C	T/C	T (100,00%)	T (46)	46	63858	63858	Gene: mraZ, CDS: m...	His -> Tyr
SNP	G	A/G	A (100,00%)	A (21)	21	64536	64536	Gene: mraW, CDS: m...	No change or no CD...
SNP	C	T/C	T (100,00%)	T (21)	21	64539	64539	Gene: mraW, CDS: m...	No change or no CD...
SNP	A	G/A	G (100,00%)	G (13)	13	66008	66008	Gene: ftsI, CDS: ftsI	No change or no CD...
SNP	A	G/A	G (100,00%)	G (11)	11	66026	66026	Gene: ftsI, CDS: ftsI	No change or no CD...
SNP	T	C/T	C (100,00%)	C (26)	26	66053	66053	Gene: ftsI, CDS: ftsI	No change or no CD...
SNP	T	A/T	A (100,00%)	A (27)	27	66200	66200	Gene: ftsI, CDS: ftsI	No change or no CD...
SNP	T	C/T	C (100,00%)	C (29)	29	66266	66266	Gene: ftsI, CDS: ftsI	No change or no CD...
SNP	T	G/T	G (100,00%)	G (30)	30	66431	66431	Gene: ftsI, CDS: ftsI	No change or no CD...
SNP	G	A/G	A (100,00%)	A (31)	31	66458	66458	Gene: ftsI, CDS: ftsI	No change or no CD...
SNP	G	A/G	A (100,00%)	A (30)	30	66791	66791	Gene: ftsI, CDS: ftsI	No change or no CD...
SNP	C	T/C	T (100,00%)	T (28)	28	66914	66914	Gene: ftsI, CDS: ftsI	No change or no CD...
SNP	G	A/G	A (100,00%)	A (23)	23	66947	66947	Gene: ftsI, CDS: ftsI	No change or no CD...

Table Settings

- Column width: Automatic
- Show column
  - ☑ Variation type
  - ☑ Reference
  - ☑ Allele variation
  - ☑ Variant frequency
  - ☑ Absolute frequency
  - ☑ Coverage
  - ☑ Reference position
  - ☑ Consensus position
  - ☑ Overlapping annotations

Applications

Research publications



Applications

Application	**Project**
Resequencing	whole genome linkage/association mutation detection
de novo sequencing	metagenomics new species
Expression	transcriptome SAGE miRNA
Epigenetics	DNA methylation ChIP
Variation	SNPs CNVs

Users I

User	Institute	Experiment	Species
Gregor/Kristina	IMG	Reseq/Epigen	Human
Beate Skinningsrud	IMG	RNAseq	Human
Eystein Husebye	UiB	RNAseq	Human
Randi Aamodt	CIGENE	DGE	Bee
Susanne Lorenz	CIGENE	RNAseq	Salmon
Elin Kure	OUSU	miRNA	Human
Matthew Kent/Sigbjørn Lien	CIGENE	Genome seq	Cattle/Cod/Pig
Arne Klungland	UiO	miRNA	Mouse
Gaute Brede	UiTrondheim	miRNA	Human
Gregor/SvenOlaf	IMG	reseq	Human
Ingar Olsen	OUS Riks	metagenomics	Bacteria
Hedda Hovik	UiO	Bact RNA seq	Bacteria
Gregor/Robert	IMG/Ullevål	ChIP/Bisulphite	Human
Kristina/Robert	IMG/Ullevål	Bisulphite	Human

Users II

- Many users
- Many institutes
- Many applications

→ Bioinformatic challenge

Three research areas...

- 1000 genomes
- Resequencing - finding variants (SNPs, CNVs)
- Epigenetics

1000 genomes project

- International consortium
- Sequence 1200 genomes
- Produce a nearly complete catalog of common human genetic variants (defined as frequency 1% or higher; SNPs, CNVs)
  - mutation detection in Mendelian disease
  - accelerate fine-mapping efforts association studies
  - enabling design of next-generation genotyping arrays - improve the power of future genetic association studies
  - improve our ability to “impute” or “predict” untyped genetic variants
- Frequent public data releases

1000genomes.org

Resequencing

- Compare test sequence to a reference sequence
- Identify genetic variation
  - Single-nucleotide polymorphisms (SNPs)
  - Insertions/deletions
  - Copy-number variation (CNVs)

Resequencing: mutation detection

Genomic region known

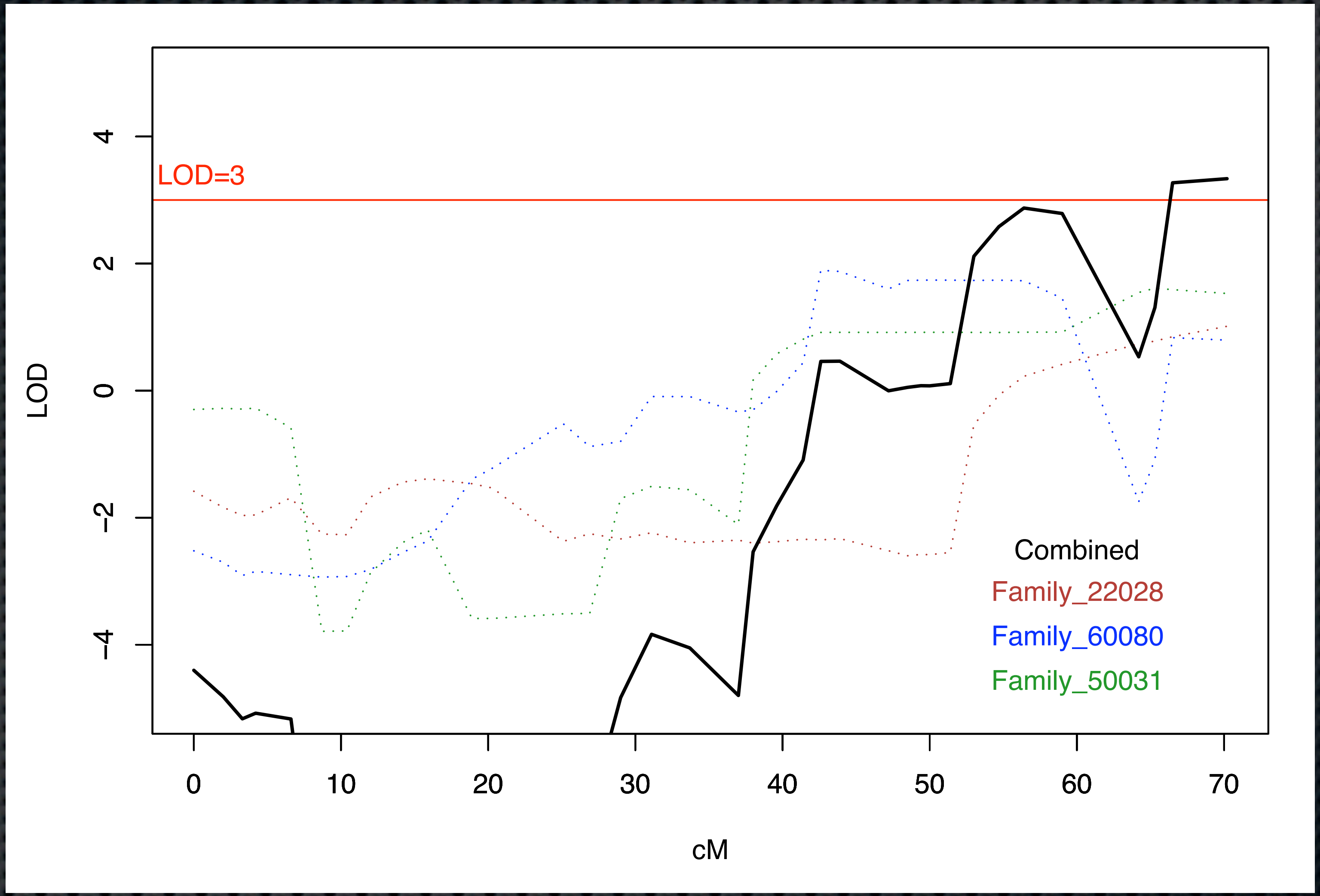
- Linkage peak
- Sequence capture - region of interest

Genomic region unknown

- Rare Mendelian disorders
- Sequence capture - exome
- RNAseq

Region known

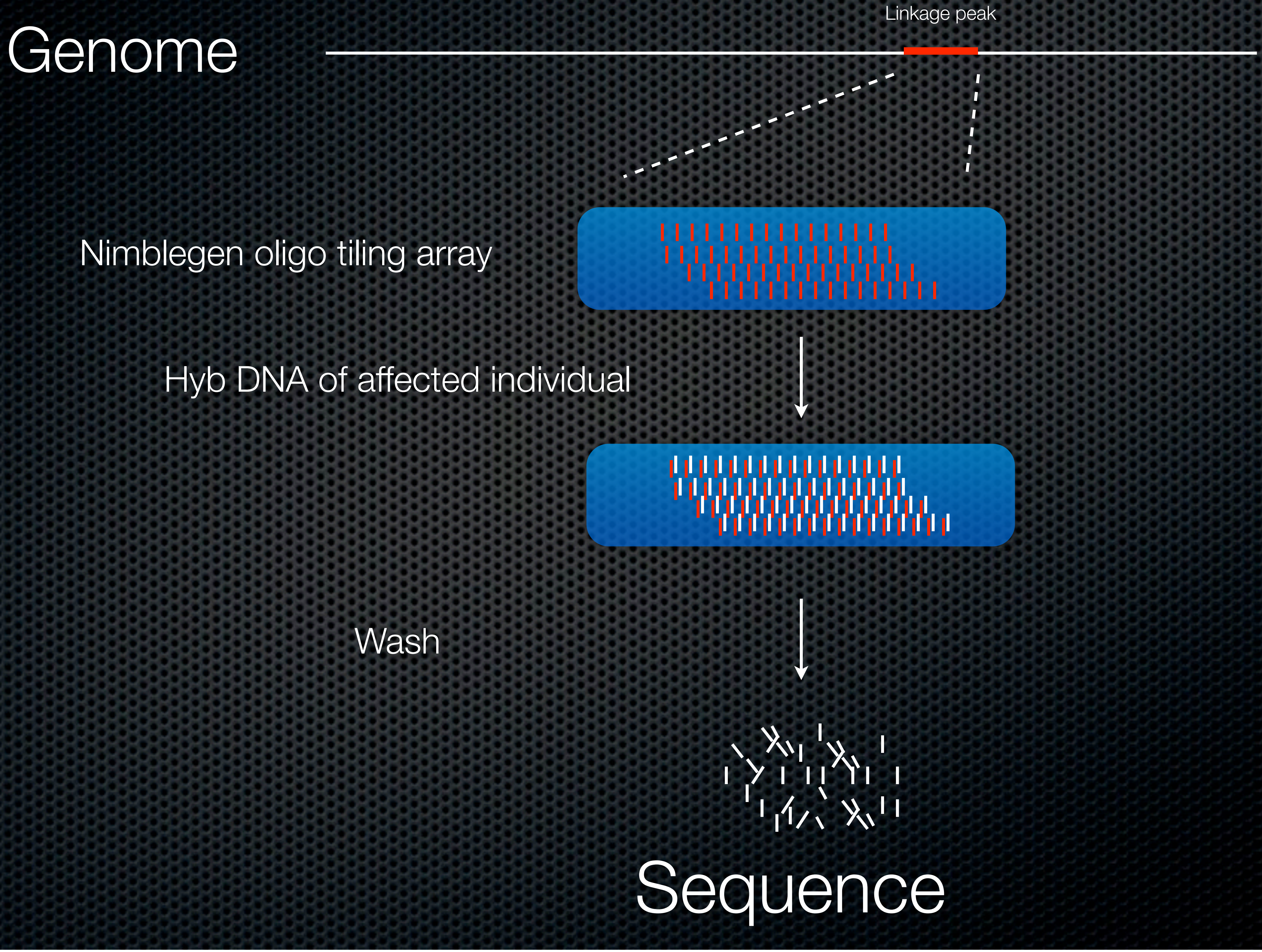
Linkage



1-10 Mb?

How can we capture this region to sequence?

Sequence capture



Genome

Linkage peak

Nimblegen oligo tiling array

Hyb DNA of affected individual

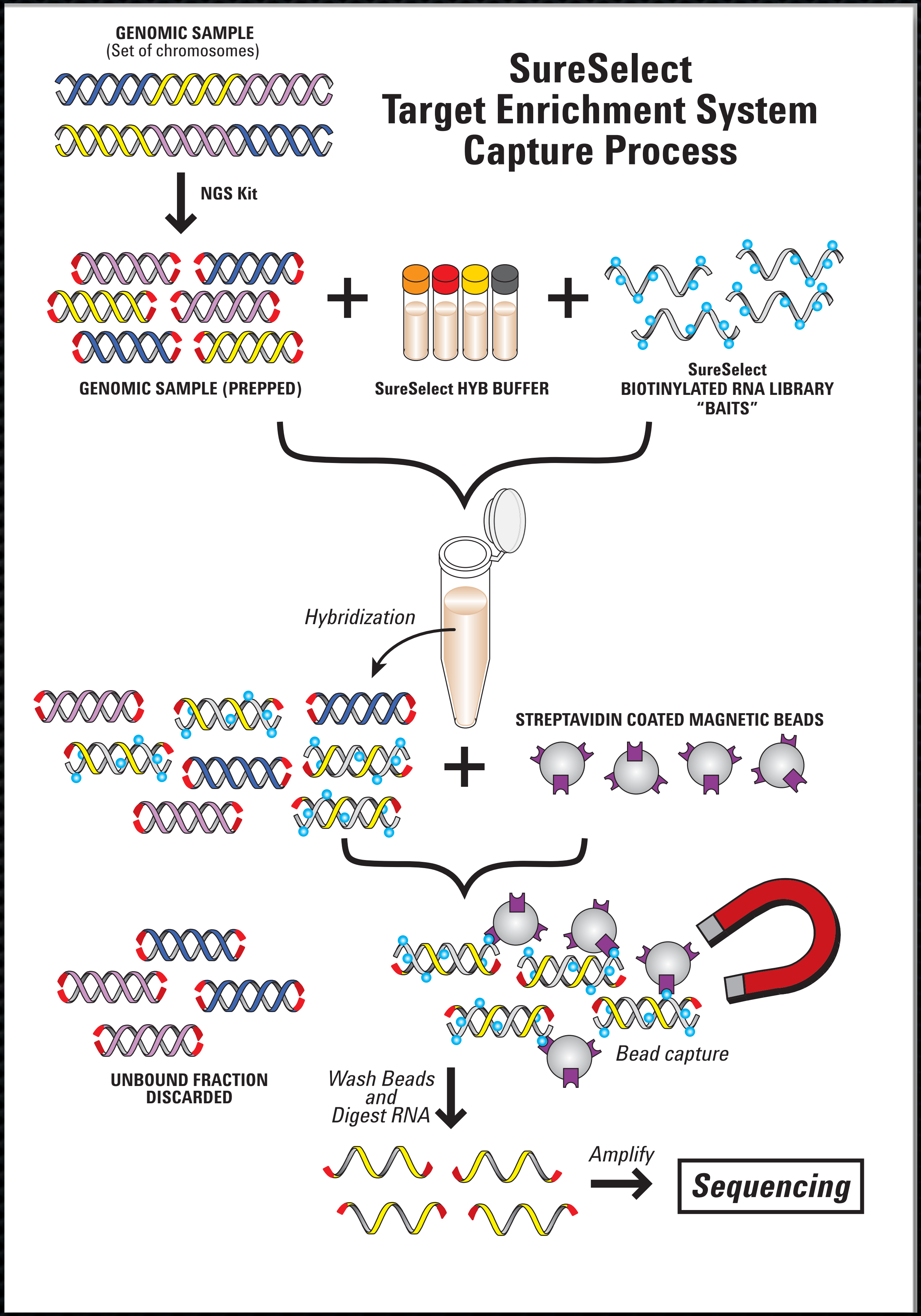
Wash

Sequence

Agilent SureSelect

- RNA oligonucleotides
- >100 bp
- custom design

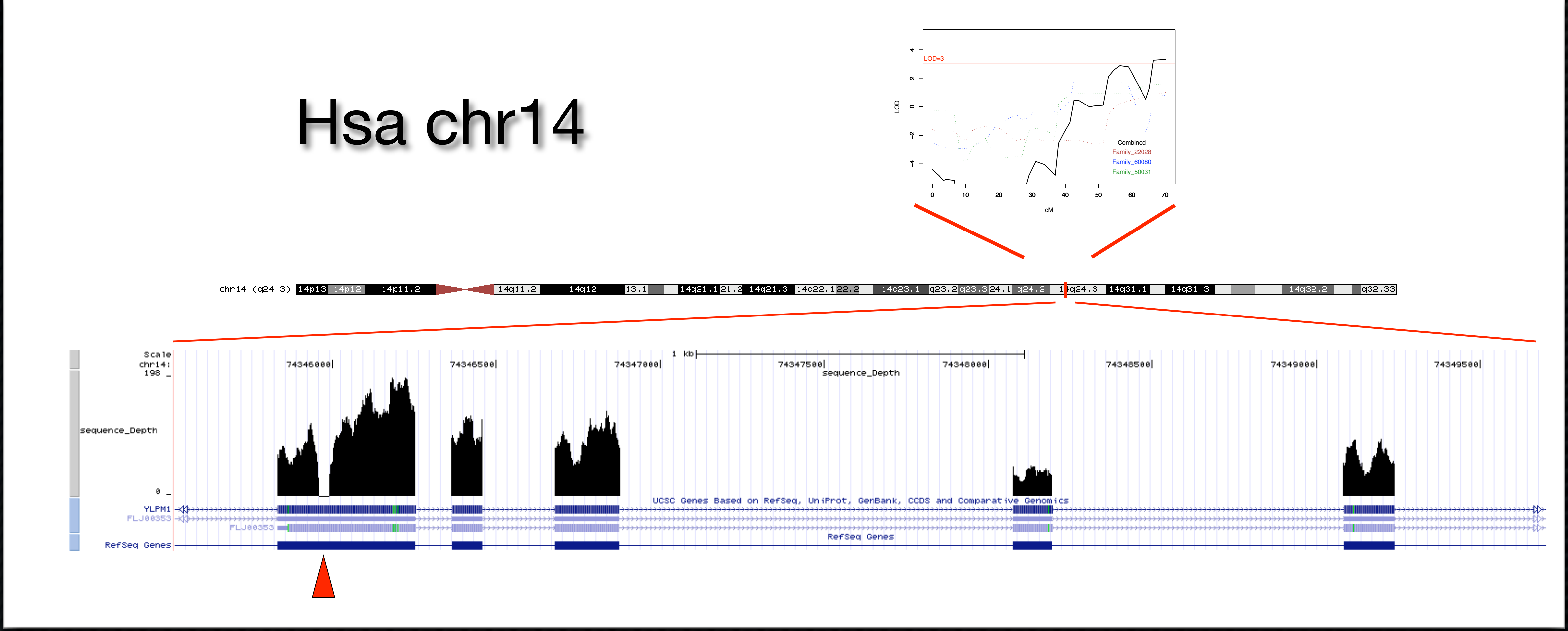
www.agilent.com



Analyzing resequencing data

- Capture DNA and sequence
- Prepare sequence files (Perl...)
- Align to reference (MAQ etc.)
- Format/filter output files (Perl...)
  - .bed, .gtf
  - View on genome browser
  - identify variants

Analyzing resequencing data



Hsa chr14

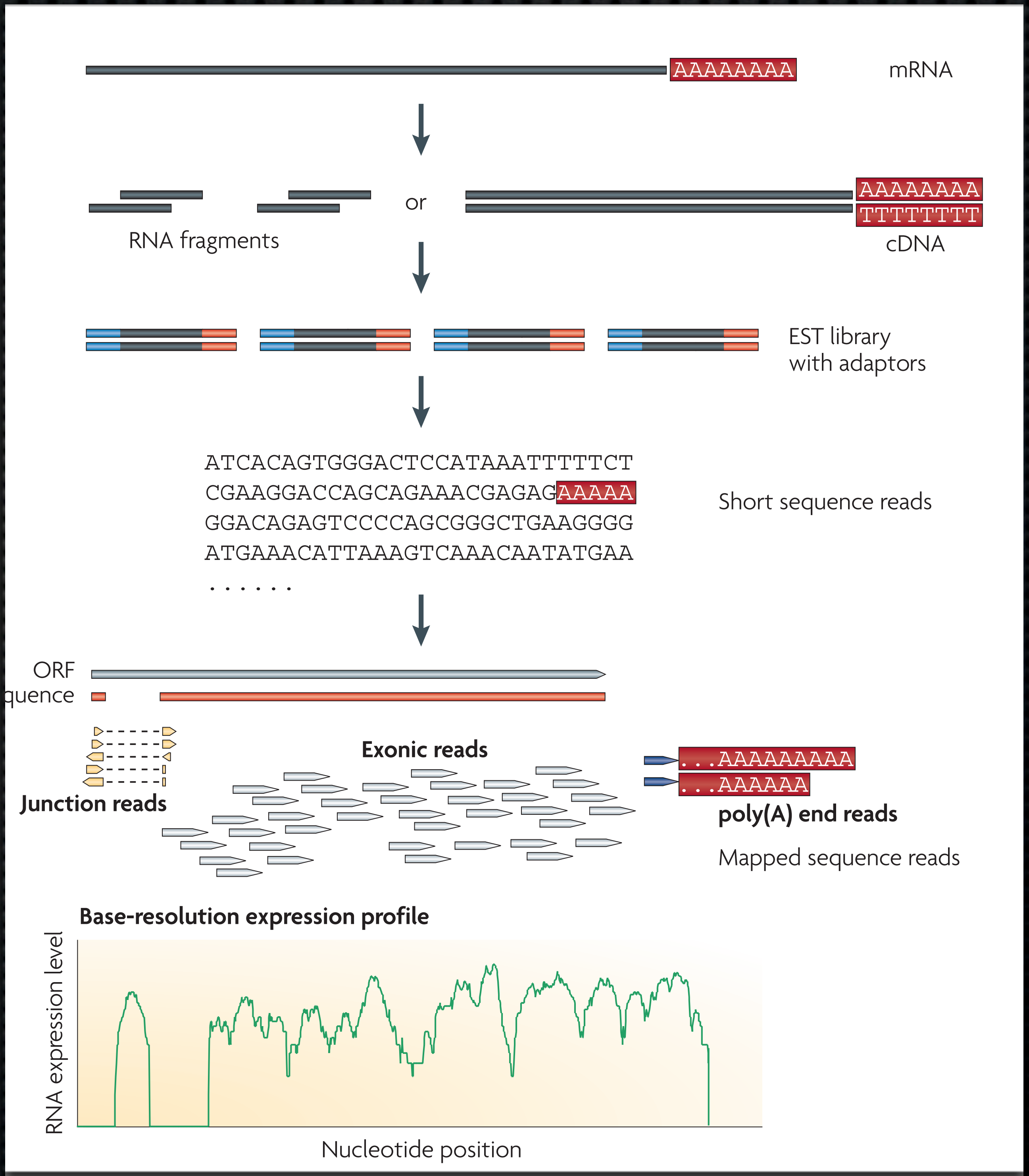
13 bp insertion

Identifying relevant variants is the hard part

Region unknown

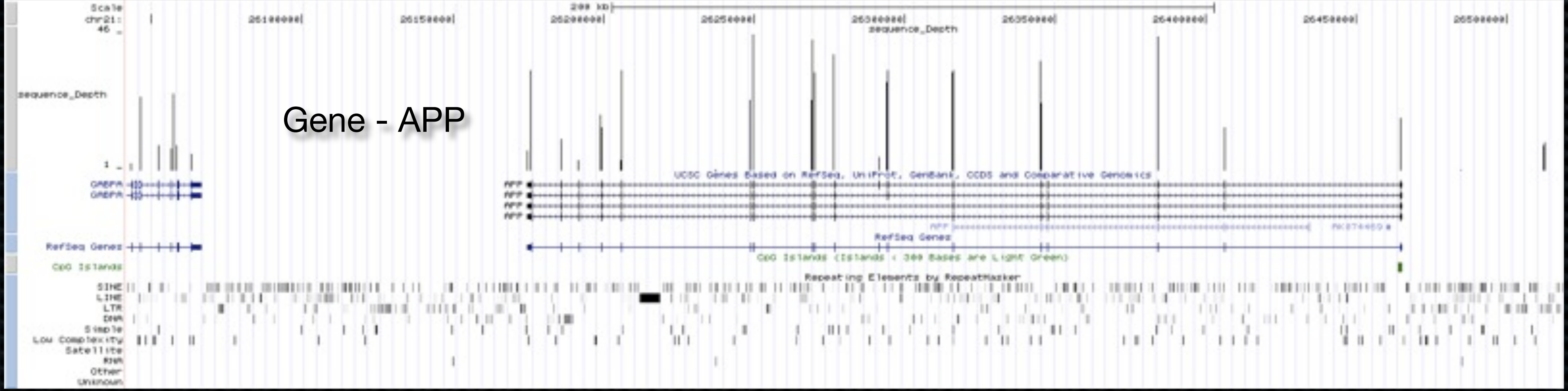
- Sequence capture - exome: sequence all exons

- RNAseq
- Sequence total polyA RNA
- Map reads to reference
- Identify mutations/variants



RNAseq data

Sequence depth



Position along Hsa chr21

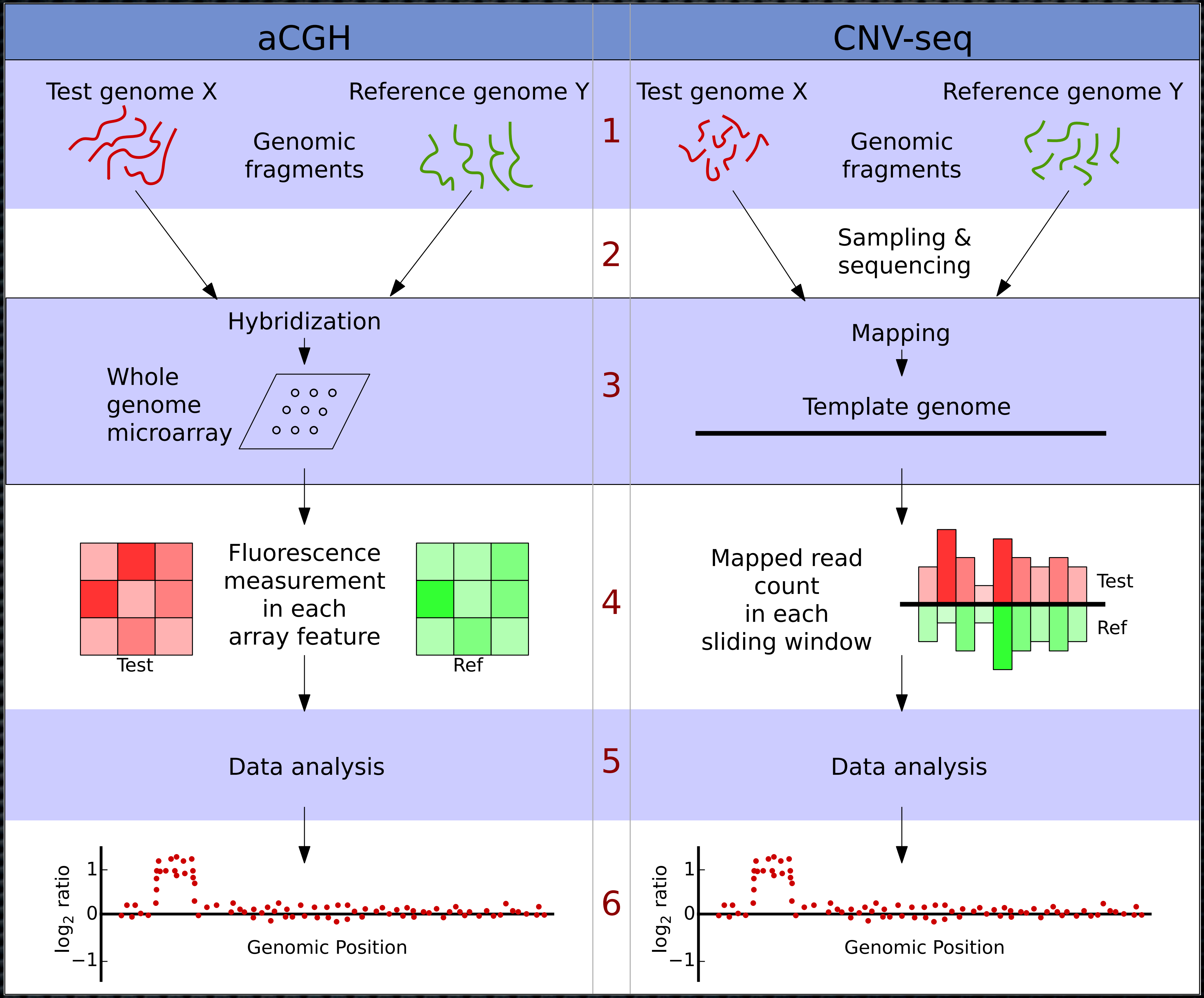
Identifying relevant variants is the hard part

HTS and CNVs

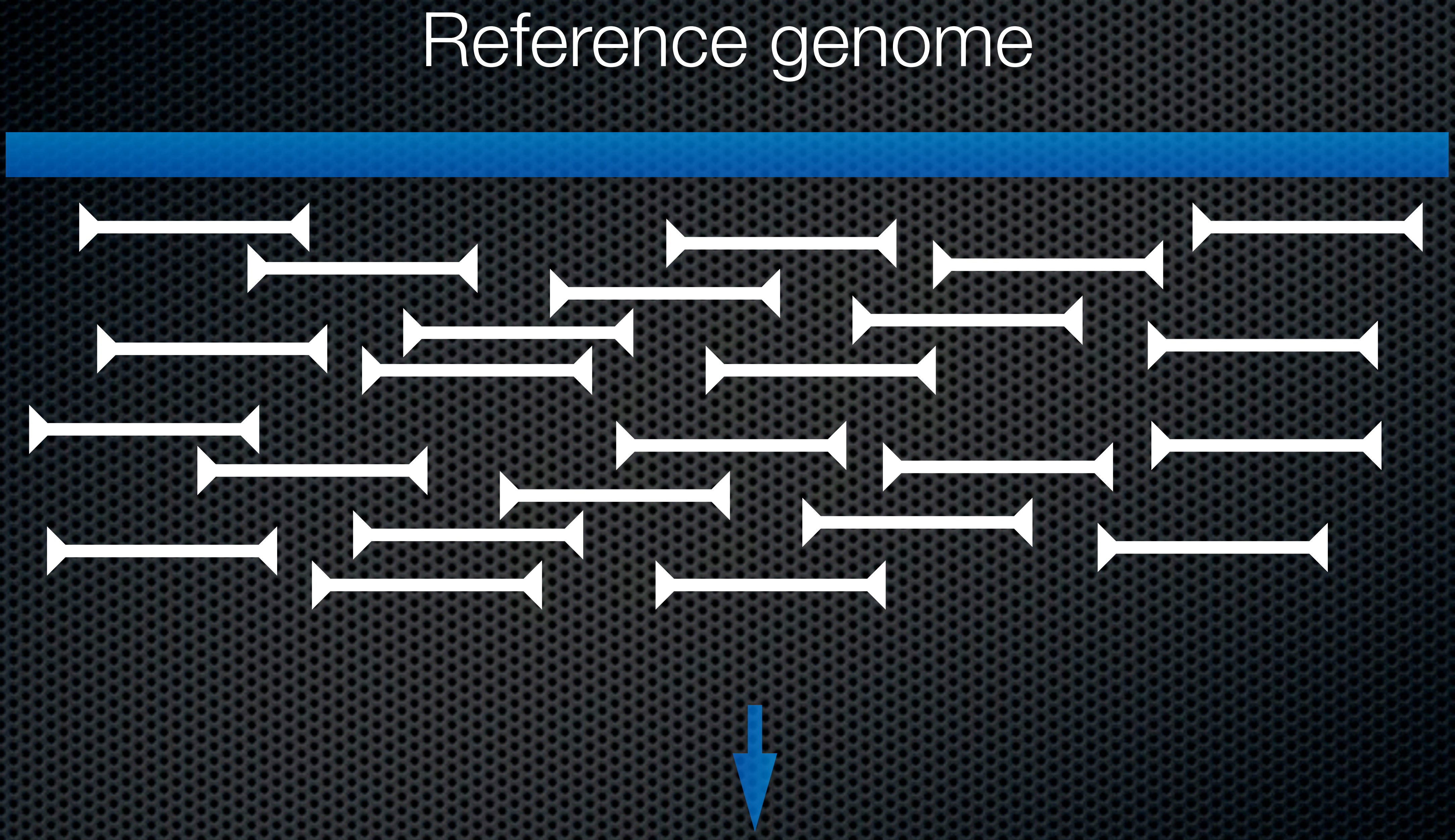
Two strategies to detect CNVs with HTS data

- Read map counting
- Mapping paired-end reads
  - Read map location
  - Read map distance
  - Read map orientation

Read map counting

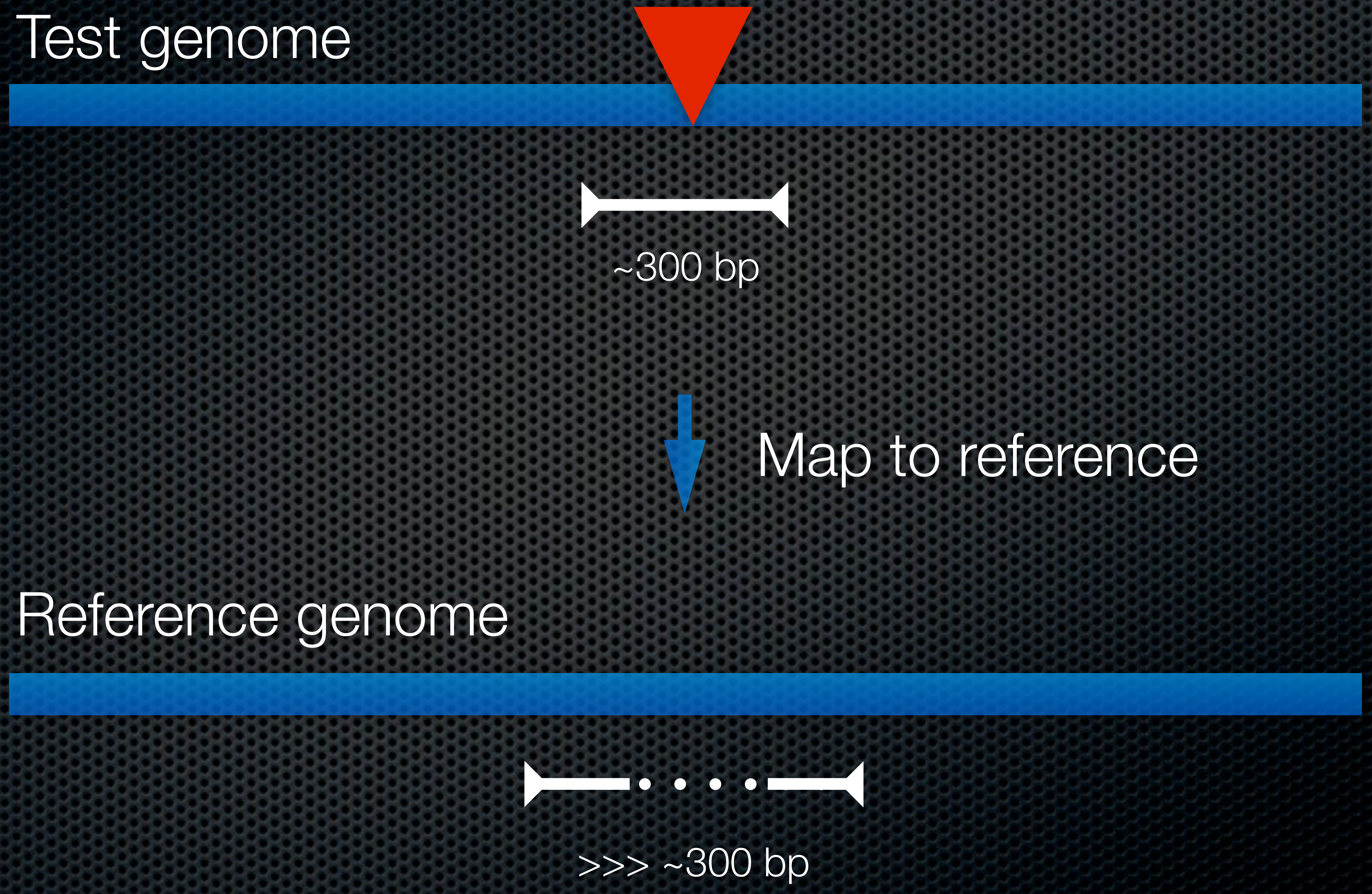


Map paired-end reads

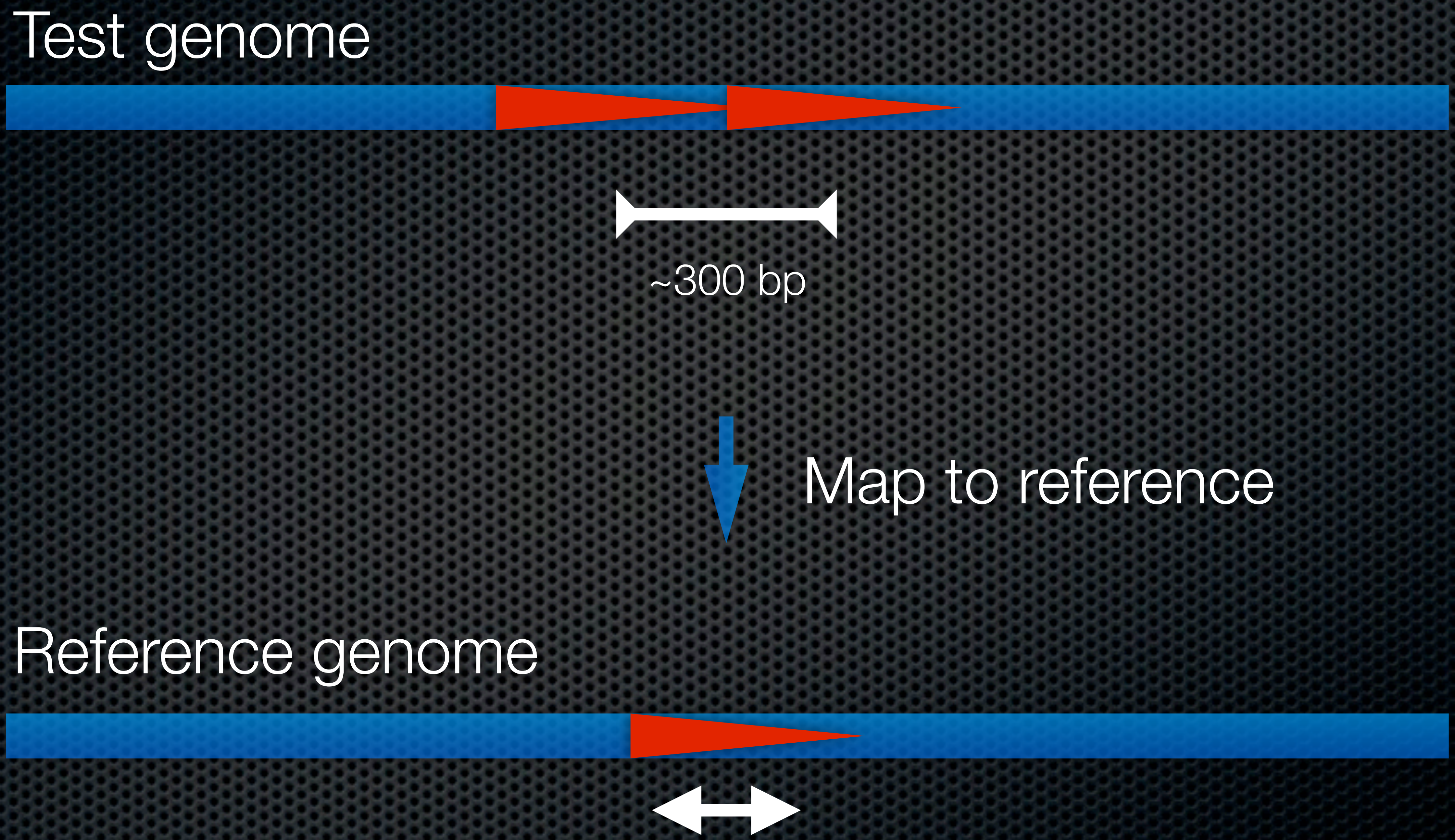


Consensus sequence
identify variants, mutations

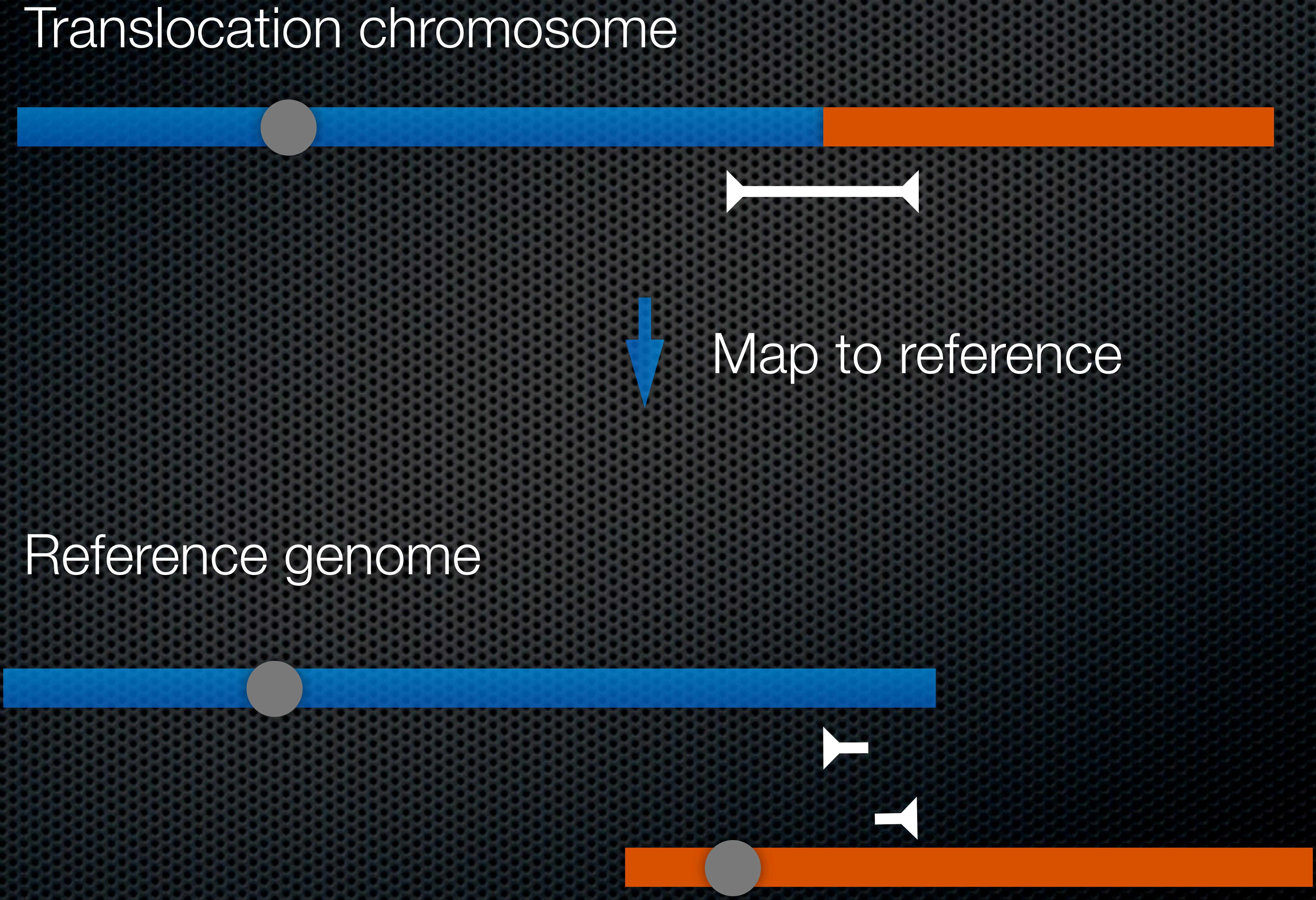
Deletion



Duplication



Balanced translocation

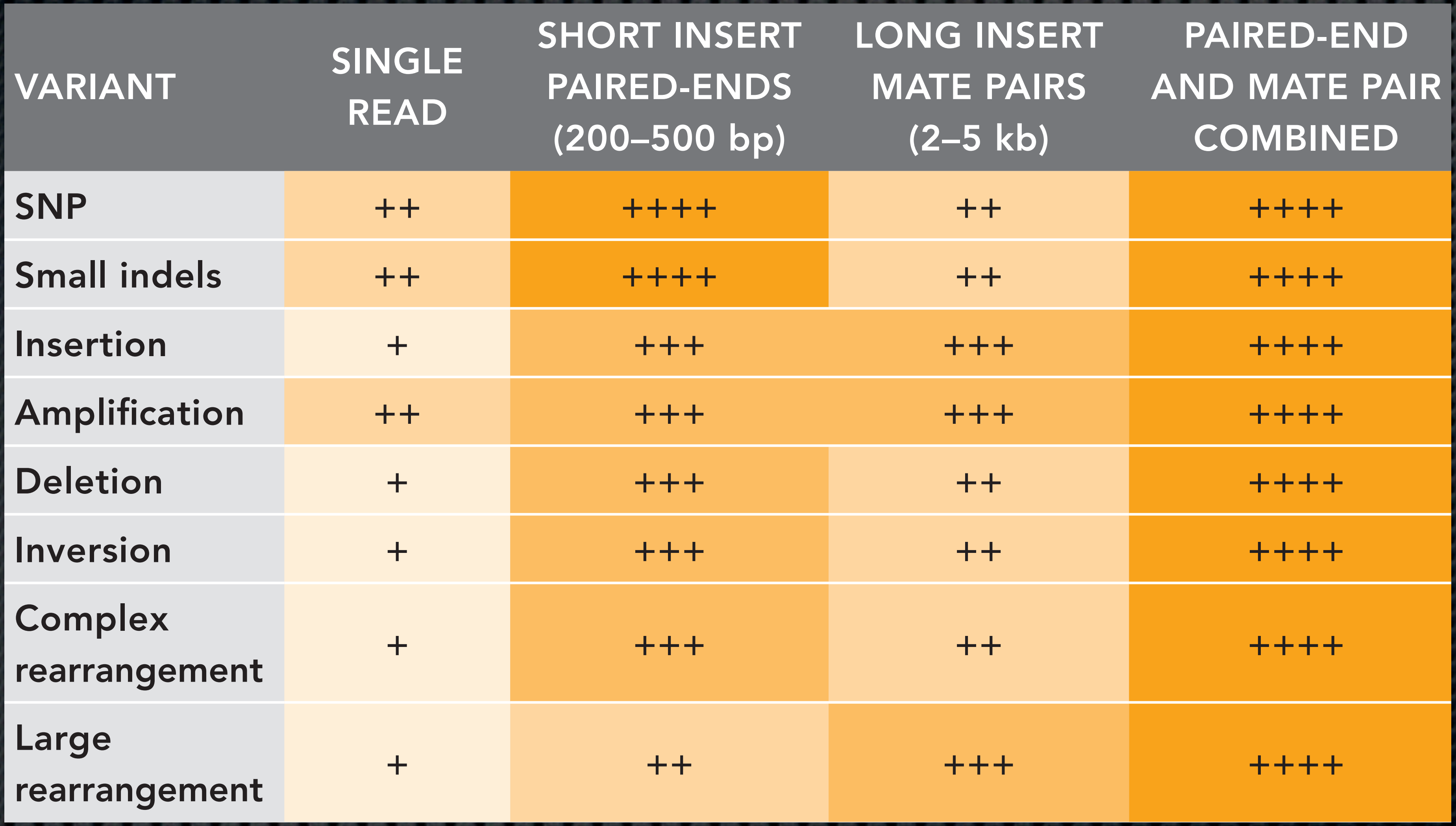


Translocation chromosome

Map to reference

Reference genome

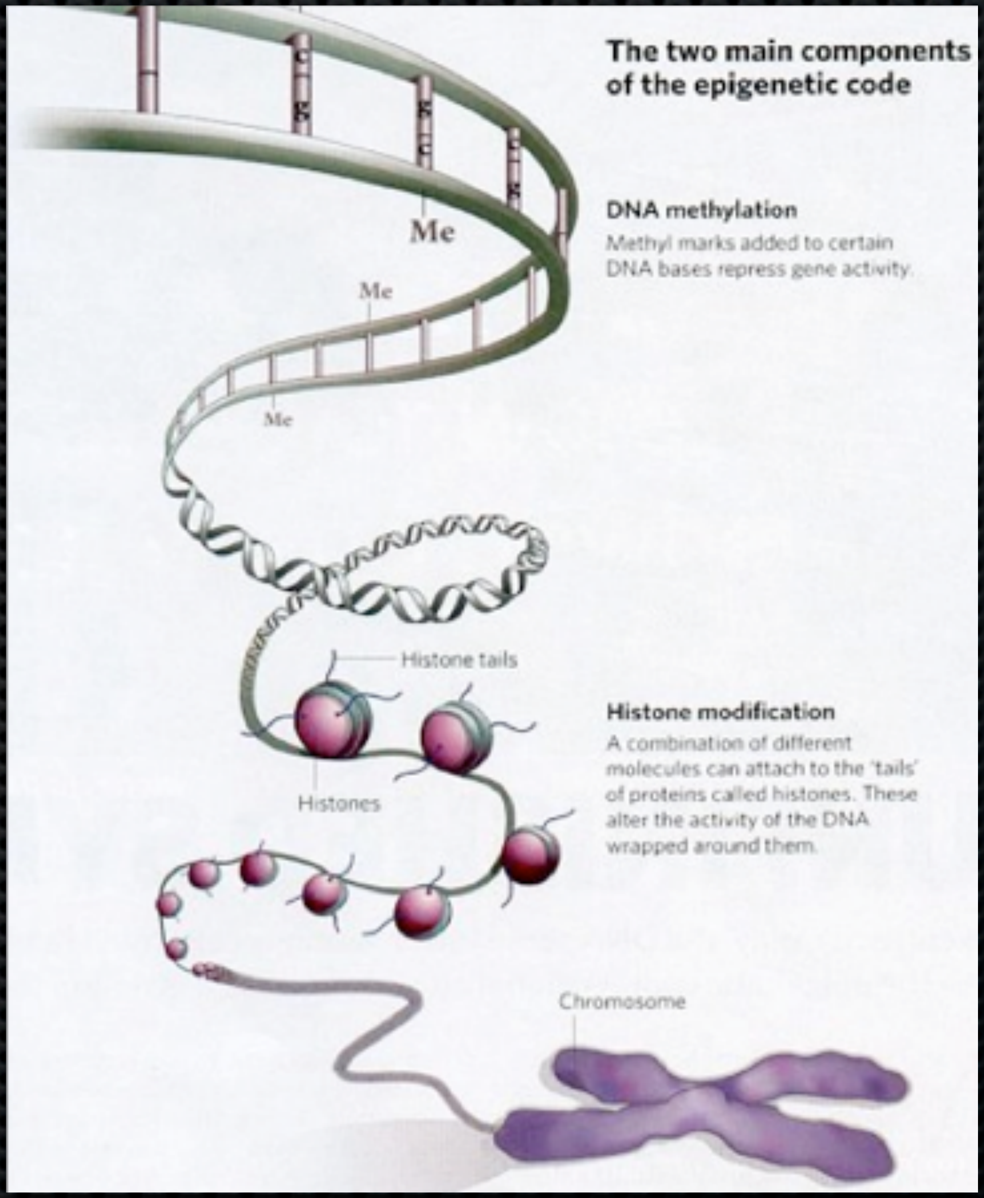
Detecting all variants



VARIANT	SINGLE READ	SHORT INSERT PAIRED-ENDS (200–500 bp)	LONG INSERT MATE PAIRS (2–5 kb)	PAIRED-END AND MATE PAIR COMBINED
SNP	++	++++	++	++++
Small indels	++	++++	++	++++
Insertion	+	+++	+++	++++
Amplification	++	+++	+++	++++
Deletion	+	+++	++	++++
Inversion	+	+++	++	++++
Complex rearrangement	+	+++	++	++++
Large rearrangement	+	++	+++	++++

Epigenetics

- DNA methylation
  - CpG dinucleotides
- Histone modifications
  - acetylation
  - phosphorylation
  - methylation
  - ubiquitination



The two main components of the epigenetic code

DNA methylation
Methyl marks added to certain DNA bases repress gene activity.

Histone modification
A combination of different molecules can attach to the 'tails' of proteins called histones. These alter the activity of the DNA wrapped around them.

→ Control of gene expression

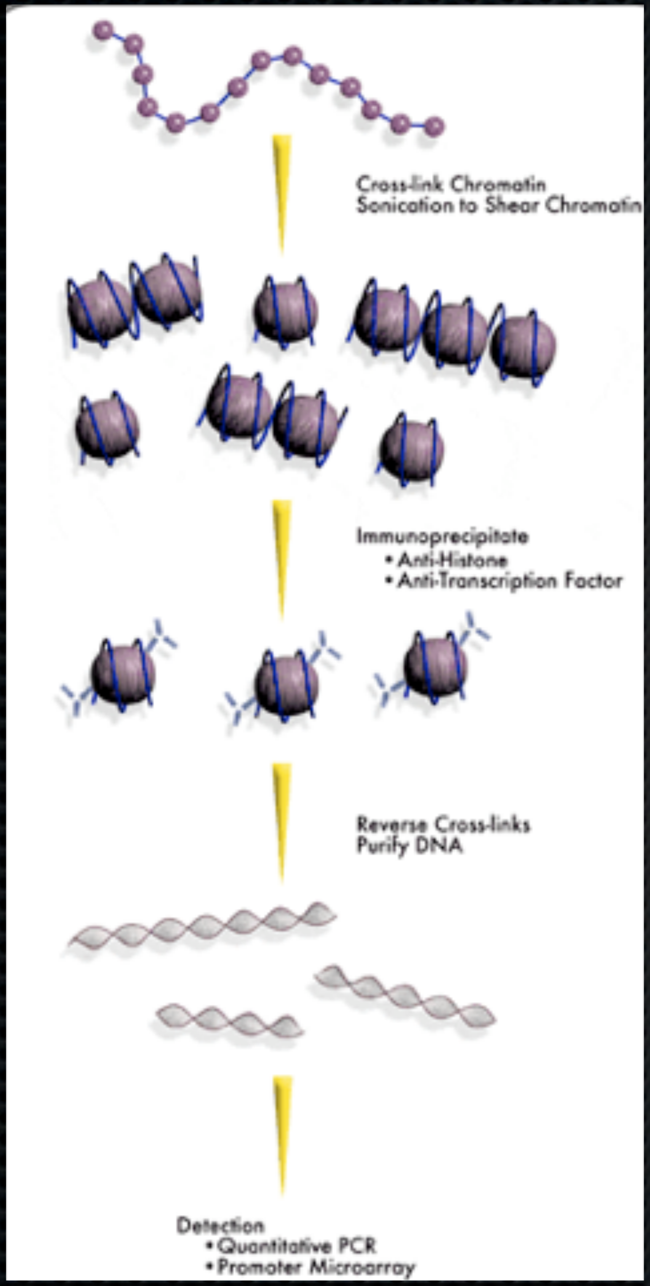
Epigenetics II

- DNA methylation
  - Long-term epigenetic silencing of specific sequences
  - transposons, imprinted genes, pluripotency genes
- Histone modifications
  - Short term, flexible epigenetic control

→ Control of gene expression

HTS and epigenetics

ChIP
chromatin immunoprecipitation



Quantifying DNA methylation

Bisulphite sequencing (BiS)

AGCTGT$\overset{m}{\text{C}}$GATTAGCCG → AGTTGTCGATTAGTTG

methylated

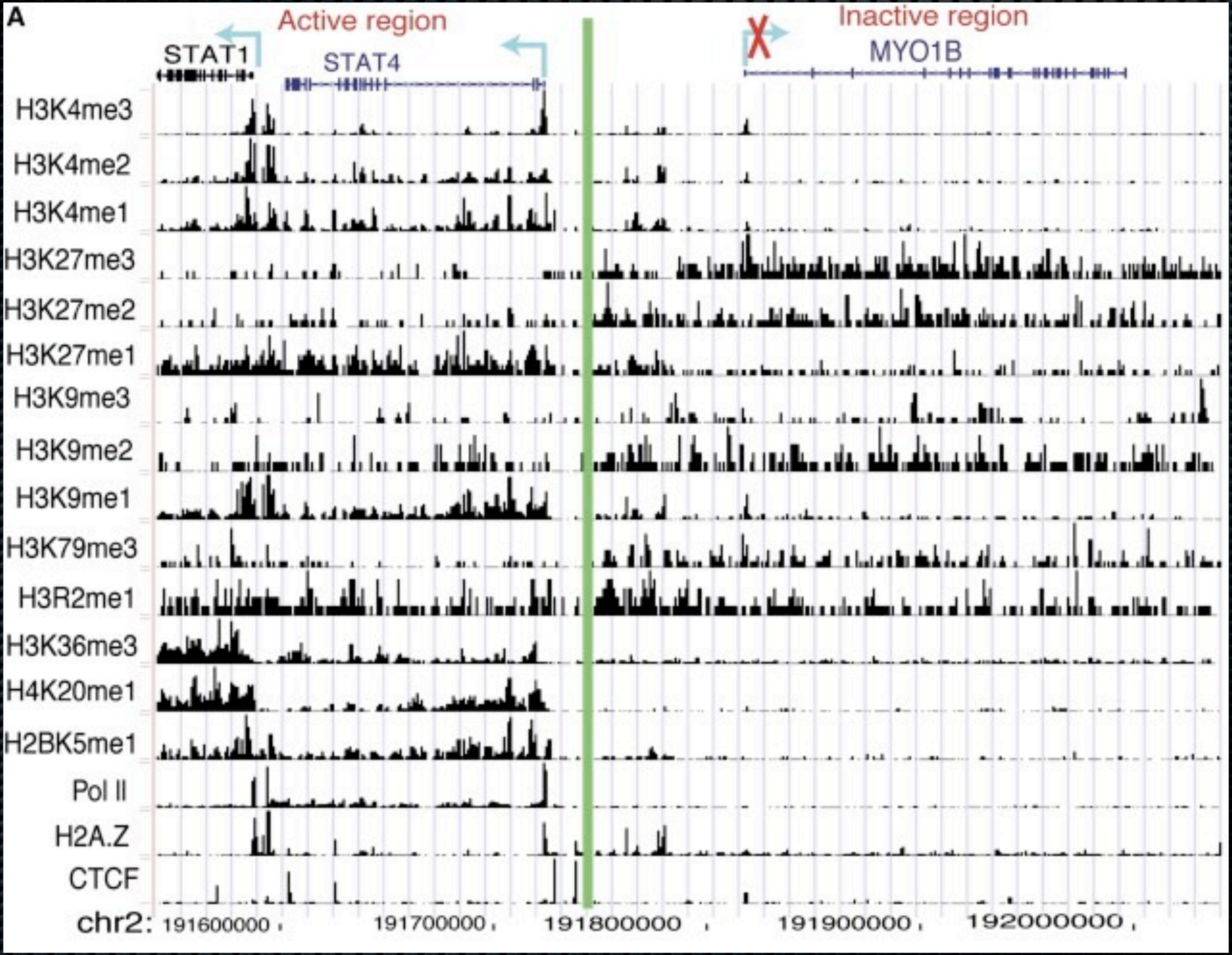
1. bisulphite treat
2. PCR region of interest
3. sequence

AGCTGTCGATTAGCCG → AGTTGTTGATTAGTTG

unmethylated

HTS to identify genome-wide status/variation

ChIP-seq example



Barski et al, Cell 129, 823–837, May 18, 2007

Summary

- High-throughput sequencing
  - Dramatic increase in sequence production
  - Many applications on one platform
  - Field new and moving very quickly

- Bioinformatics challenges/opportunities
  - Data storage
  - Data analysis

Visit?

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