LEIDEN UNIVERSITY MEDICAL CENTER

The Dutch Variant Database.

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Introduction

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Other participants:

- UMCG Groningen.
- AMC Amsterdam.
- EMC Rotterdam.
- VUMC Amsterdam.
- NBIC.

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In *exome sequencing*, we select genomic regions of interest using a *target-enrichment strategy*.

- PCR.
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- In-solution capture.

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- Fragmentation.
- Size selection.
- Linker ligation.
- Capture.

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These regions are then *sequenced*.

- ABI SOLiD 5500.
- Illumina HiSeq 2000.

Introduction

Figure 1: SOLiD 5500XL.

Figure 2: HiSeq 2000.

Paired end, high throughput, cheap.

Introduction

Raw data aquisition.

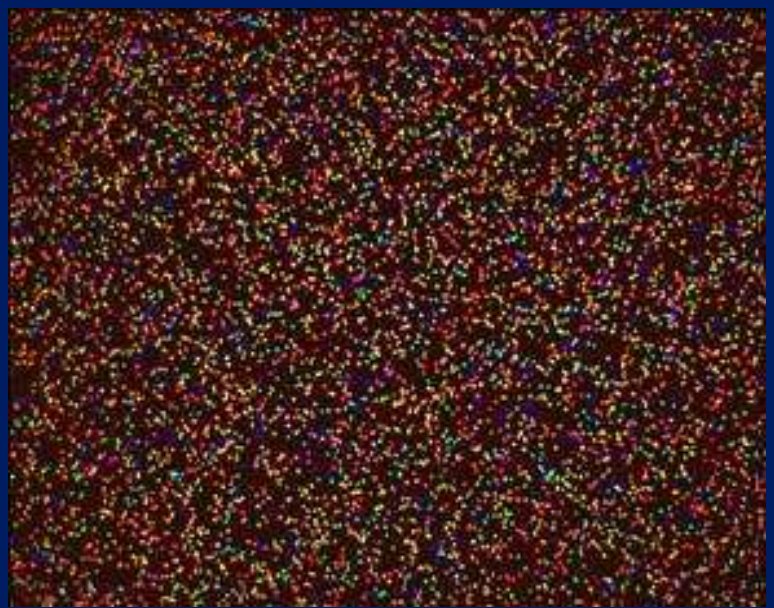

Figure 3: Raw image.

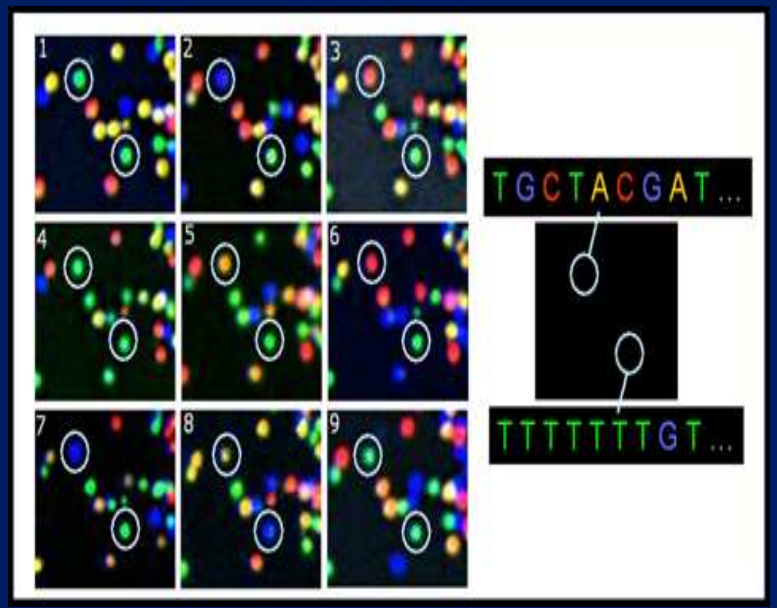

Figure 4: Base calling.

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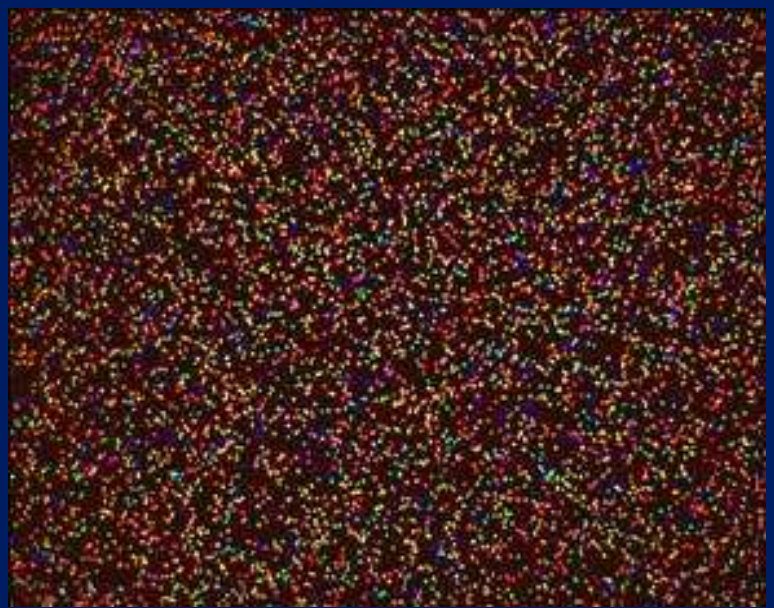

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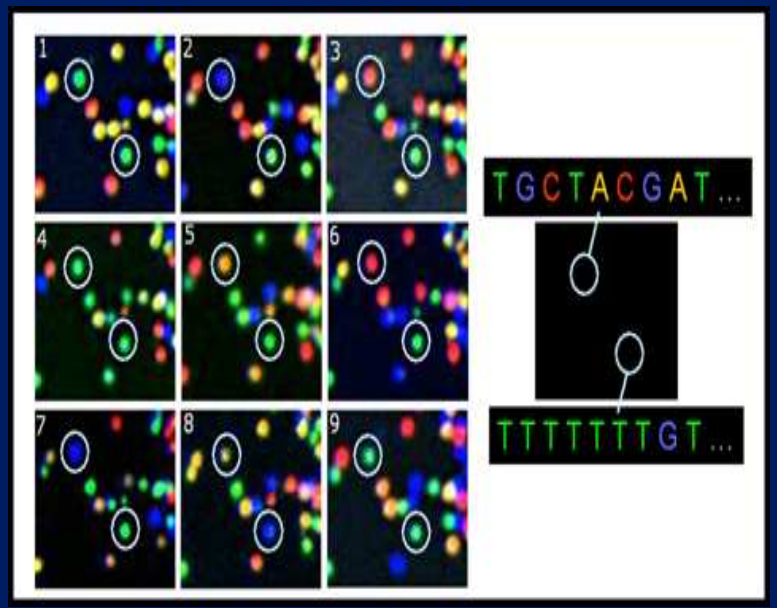

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Output:

- Long list of short DNA sequences.
- Quality scores per base.

Analysis pipelines

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5. Annotation.

Pre-alignment

We use the Trimmomatic / FASTX toolkit for data cleaning.

- Remove linker sequences.
- Clip low quality reads at the end of the read.
- Judge the read that is left over.

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The FASTQC toolkit is used for quality control (both before and after the data cleaning step).

- GC content.
- GC distribution.
- Quality scores distribution.
- ...

Pre-alignment

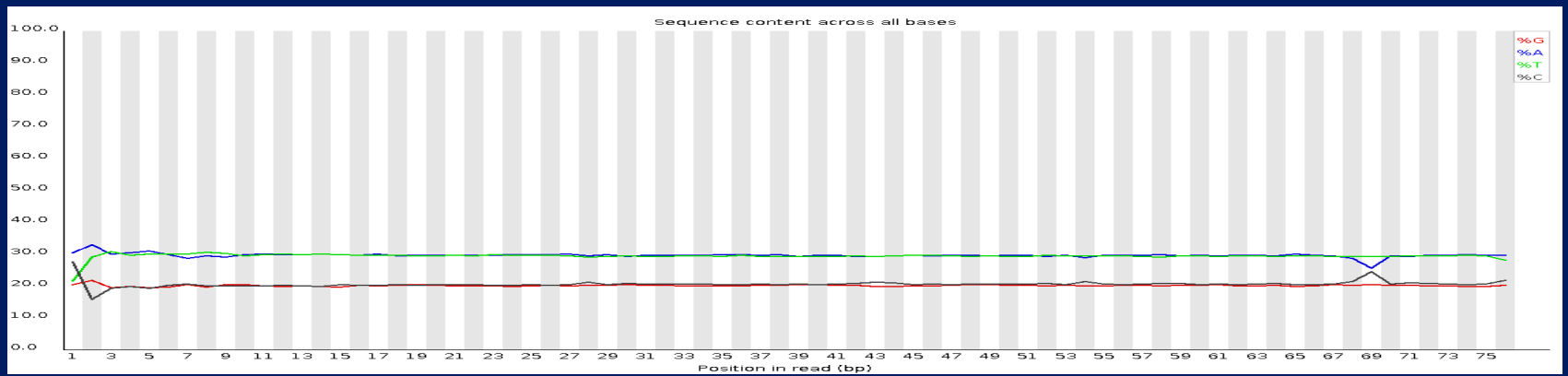

Figure 5: Per base sequence content.

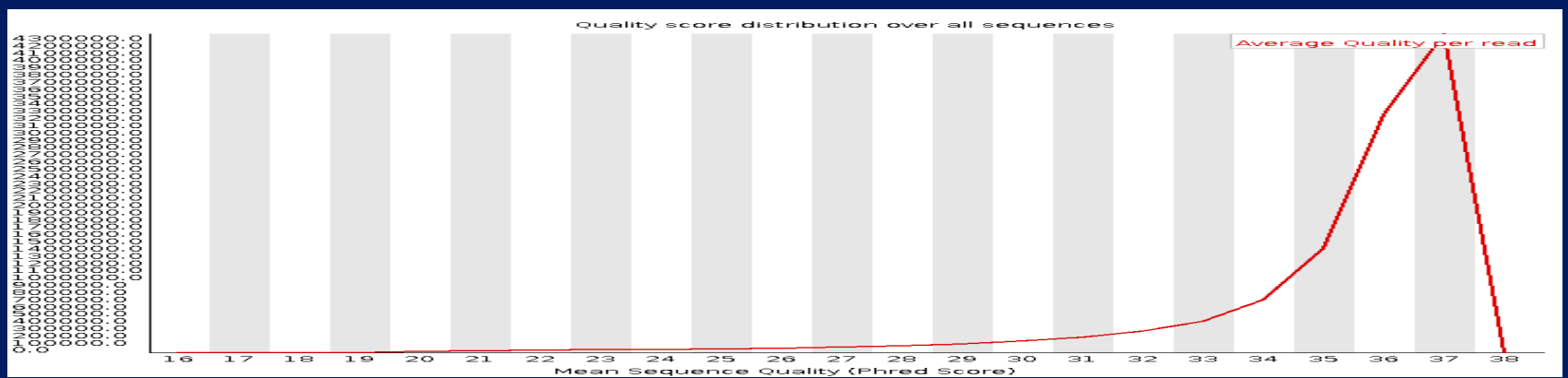

Figure 6: Per sequence quality.

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Some features:

- Base quality recalibration.
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Stampy: A statistical algorithm for sensitive and fast mapping of Illumina sequence reads.

Some features:

- Base quality recalibration.
  - First map 1% of the input.
  - Recalibrate the Fastq quality scores.
  - Redo the alignment with the recalibrated scores.
- Uses BWA for the hard work.
  - Switches to its accurate built in aligner when BWA fails.

Burrows-Wheeler Aligner (BWA) is a short read aligner that allows small insertions and deletions.

Alignment

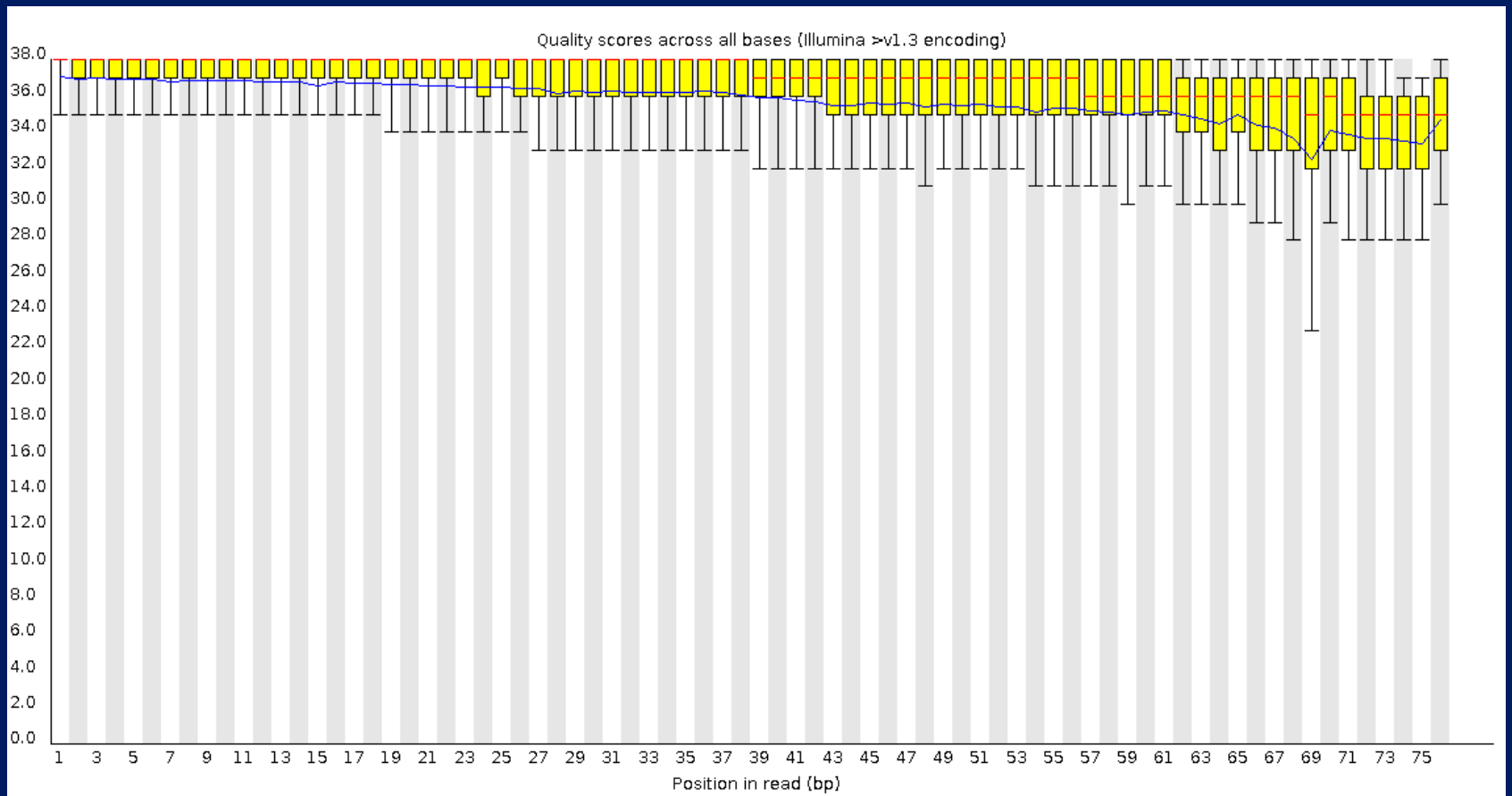

Figure 7: Base quality recalibration.

Variant calling

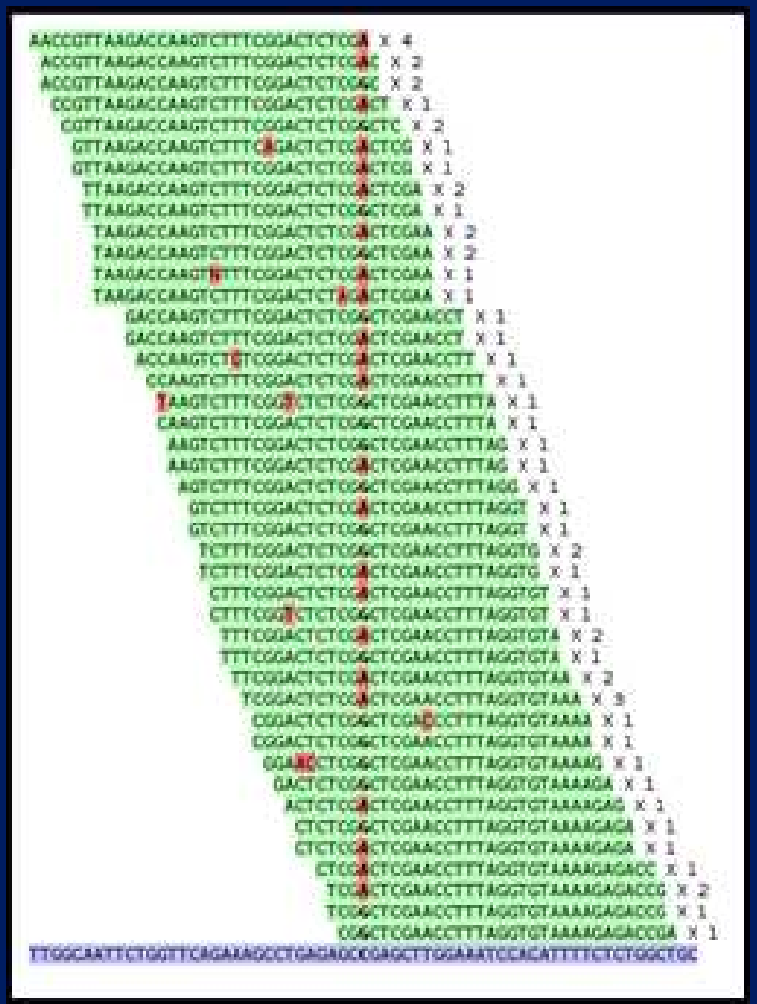

Figure 8: Variant calling.

Variant calling

Variant calling is done by Samtools, BCFtools / VCFutils.

The output of most modern aligners is in *Sequence Alignment / Map* (SAM) format.

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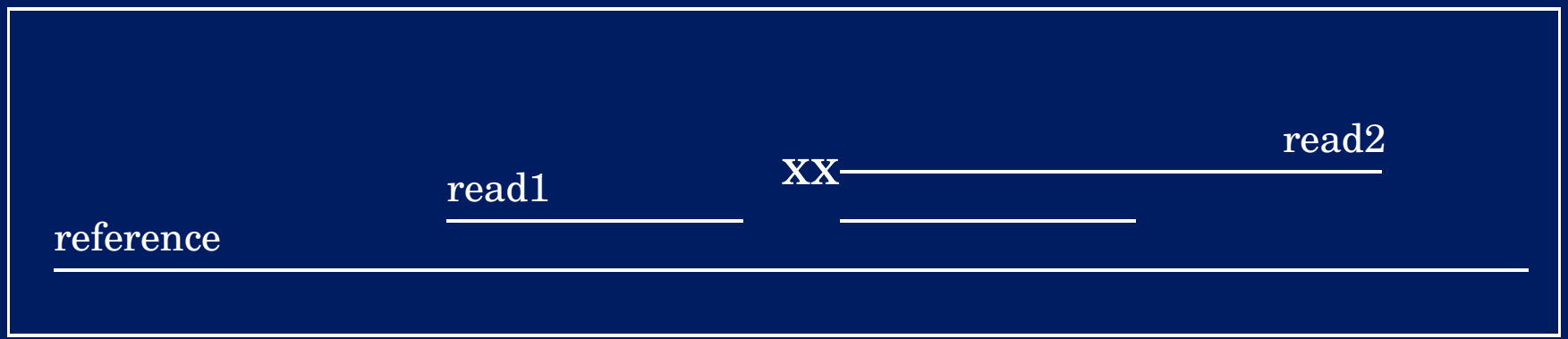
Mainly file format conversions.

- SAM → BAM.
- BAM → BAM.sorted.
- BAM.sorted → BAM.sorted.index.
- BAM.sorted → mpileup (BAQ realignment).
- BAM.sorted → BCF.
- BCF → VCF.

We end up with a list in *Variant Call Format* (VCF).

Variant calling

Base Alignment Quality (BAQ) realignment:
Remove SNPs around indels.



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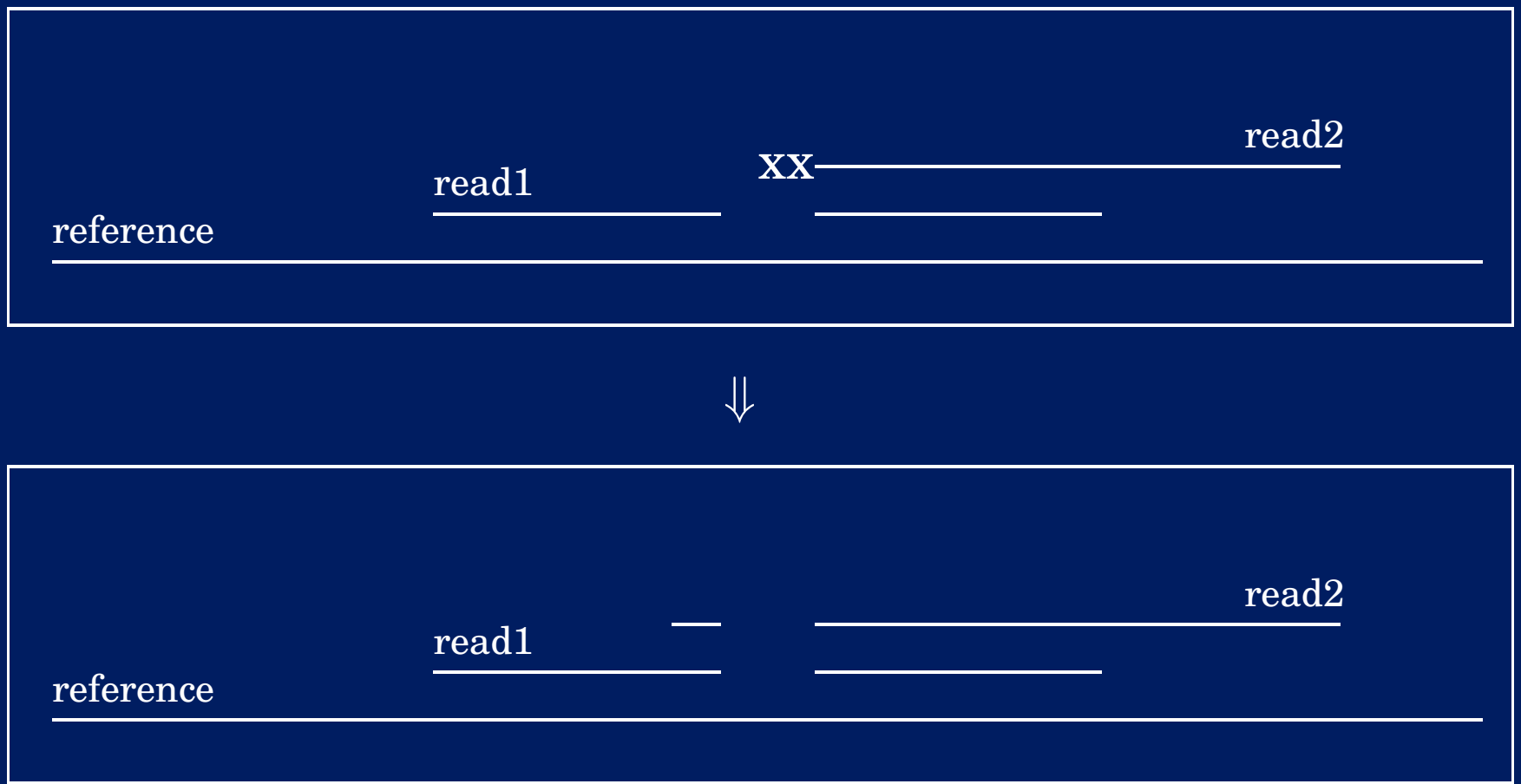

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- Minimum coverage threshold.
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- Around 2.1 human full genome.
- Around 2.8 to 3.0 human exome.

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- Is it in a regulatory region?
- …

Pipelines

Combining all these tools in a pipeline:

```
1  bwa aln -t 8 $reference $i > $i.sai
2  bwa samse $reference $i.sai $i > $i.sam
3  samtools view -bt $reference -o $i.bam $i.sam
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Listing 1: Shell script

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Listing 1: Shell script

```
1  %.sai: %.fq
2    $(BWA) aln -t $(THREADS) $(call MKREF, $@) $< > $@
3
4  %.sam: %.sai %.fq
5    $(BWA) samse $(call MKREF, $@) $^ > $@
6
7  %.bam: %.sam
8    $(SAMTOOLS) view -bt $(call MKREF, $@) -o $@ $<
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Listing 2: Makefile

Advanced annotation and filtering.

Description of the data.

Exome sequencing output:

- List of variants (VCF format).
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We need the coverage information to calculate the frequencies correctly.

- We need to see who *could* have seen a variant.

Advanced annotation and filtering.

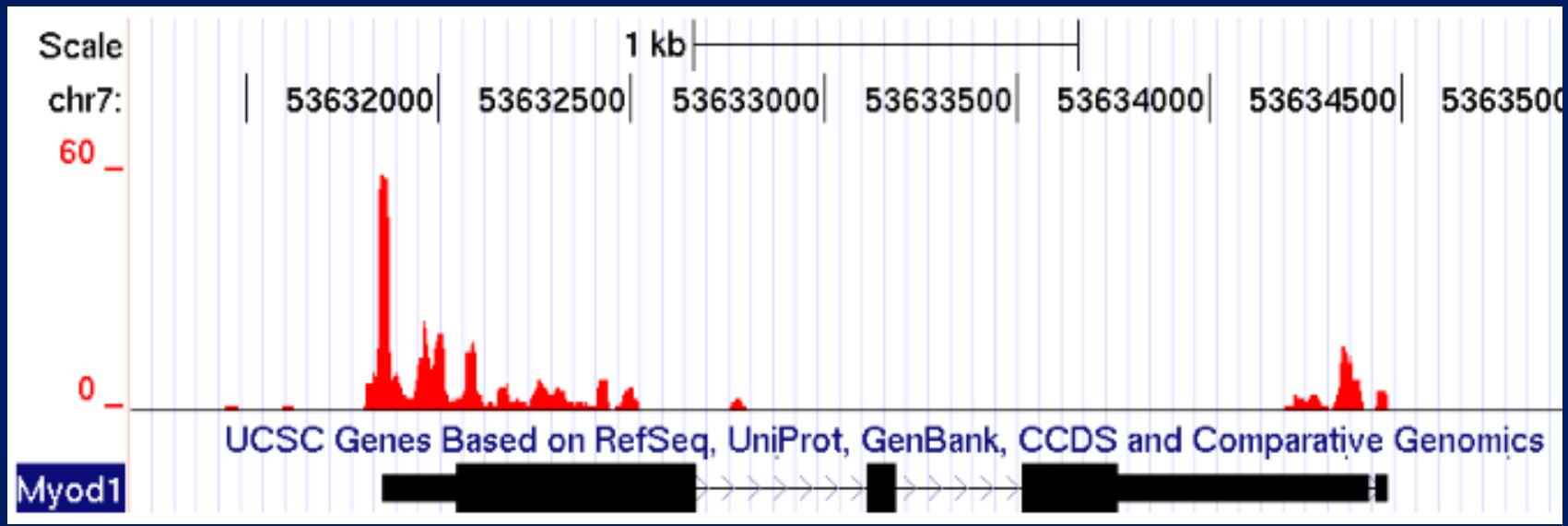

Figure 9: Coverage / depth histogram.

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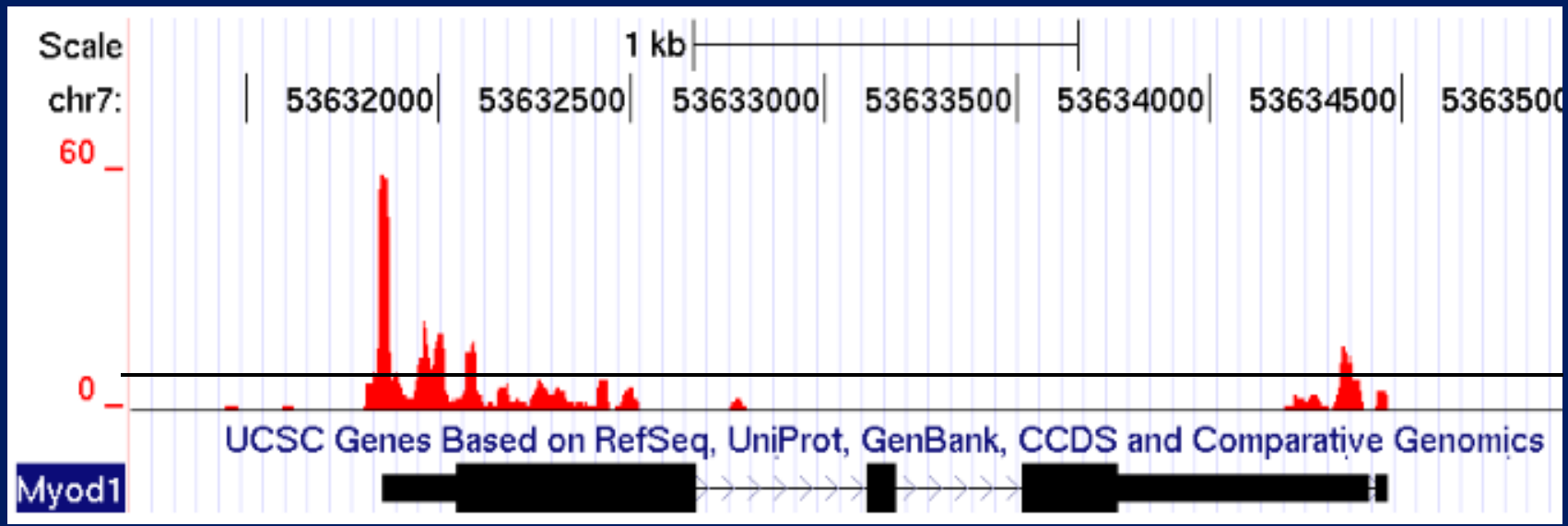

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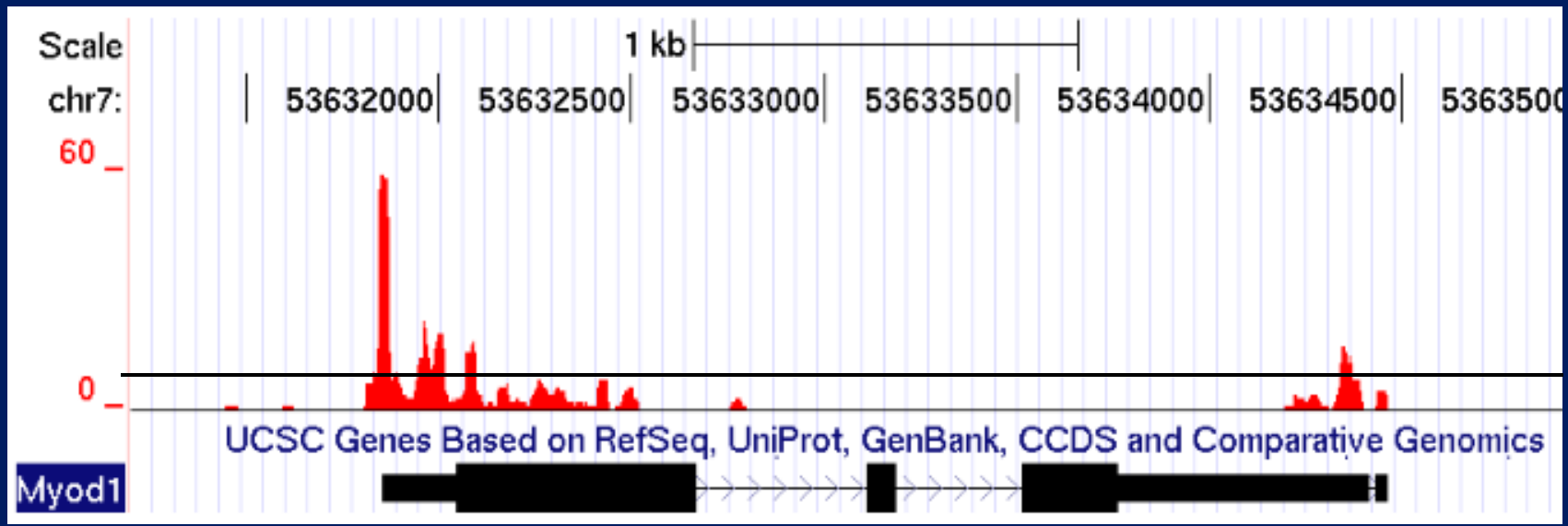

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Figure 10: Coverage summary.

National variant database

Proposed setup:

For privacy reasons:

- No browsing.
- Pre-defined interface.
- Encrypted connection.
- Authentication.

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General design:

- A SOAP interface for interaction with pipelines.
- SOAP over HTTPS (SSL) for encryption.
- Trivial authentication.
    - Send username / password in each call.

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Prototype running in Utrecht:

- MySQL database.
- SOAP interface.

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The exome data can be enriched with other datasets, not available anywhere else:

- Latest 1000 Genomes Project results.
- Genome of the Netherlands (GoNL).
- RefSeq false positives.

National variant database

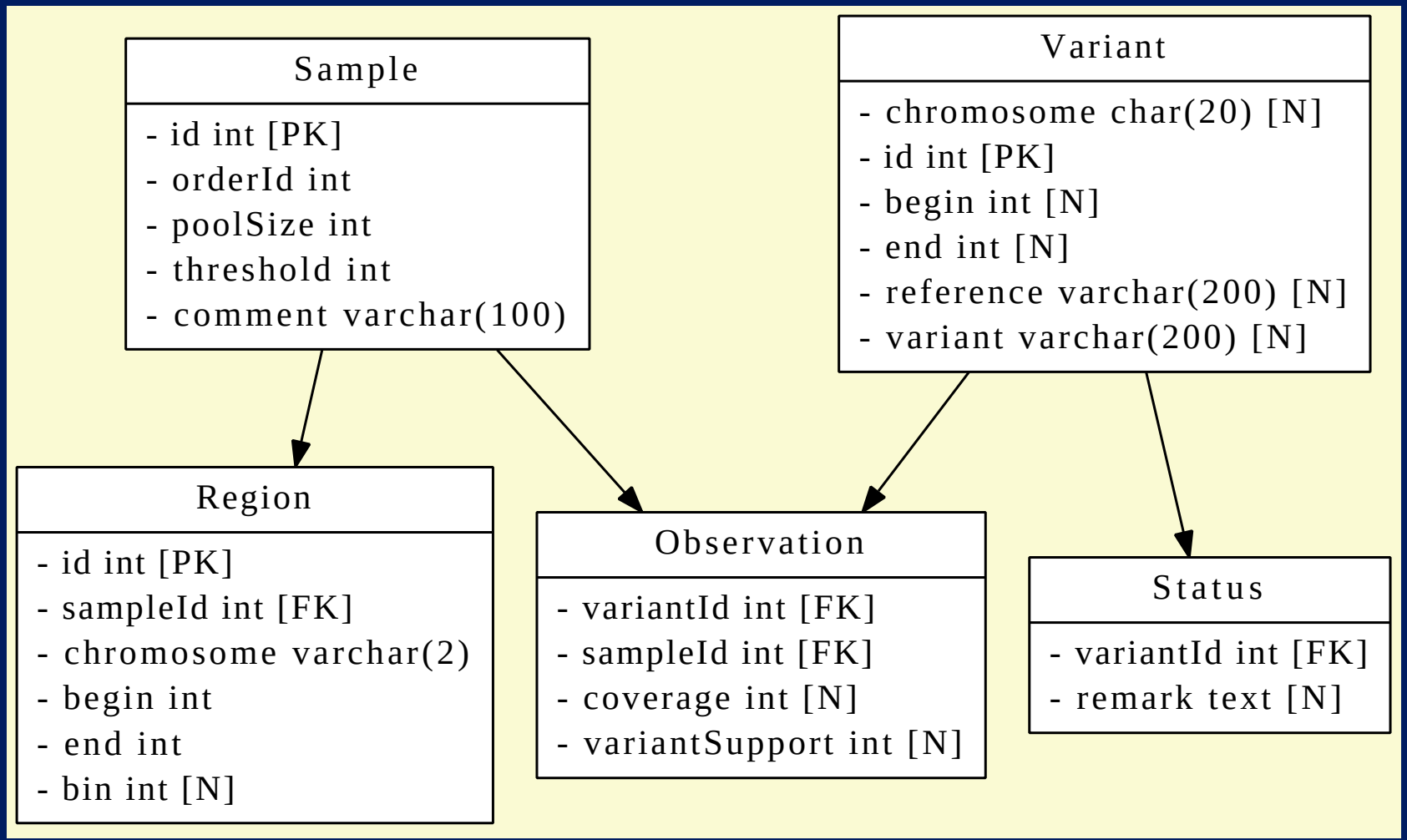

Figure 11: Database schema.

National variant database

Client perspective:

In principle, one program that handles all:

- Upload a VCF and a BED.
- Server responds with an ID.
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Client characteristics:

- Around 30 lines of code.
- Programming language independent.
- Operating system independent.

National variant database

Server perspective:

Uploading:

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Annotation:

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- If the annotation for this ID is finished, return the result.

Note that if the annotation part is finished, the result can already be returned.

National variant database

Column	Name	Description
1	CHROM	Name of the chromosome.
2	POS	Position on the chromosome.
3	REF	Reference allele.
4	ALT	Genotype.
5	Frequency	Frequency of this genotype.
6	Occurrences	Number of hits.
7	IDs ($>$ 100)	If the frequency is low: a list of sample IDs.
8	IDs ($<$ 100)	A list with hits in reserved samples; 1000 genomes, GoNL, HGMD, etc.

Table 1: Output format.

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Stakeholder meeting.

- The proposed interface.
- More sensitive issues.
  - Who gets access?
  - What if someone submits the same data twice?
  - Can we get all variants in a gene?
    - Maybe only for public samples.

Questions?

Acknowledgements:

Christian Gilissen
Victor Guryev
Frans-Paul Ruzius
Nienke Wieskamp
Hailiang Mei
Johan den Dunnen

https://www.mutalyzer.nl/svn/NGSdata/branches/dvd/