LEIDEN UNIVERSITY MEDICAL CENTER

Exome sequencing with GAPSS3

(General annotation Pipeline for Second-generation Sequencers)

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Introduction

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

- PCR.
- On array capture.
- In-solution capture.

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

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These regions are then *sequenced*.

- Illumina Genome Analyzer II (GAII).
- Illumina HiSeq 2000.

Introduction

Figure 1: GAII.

Figure 2: HiSeq 2000.

Paired end, high throughput, cheap.

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1. Pre-alignment.
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3. Variant calling.
    - Description of difference with the reference.
4. Filtering.
    - Variant calling has a high false positive rate.
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.
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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

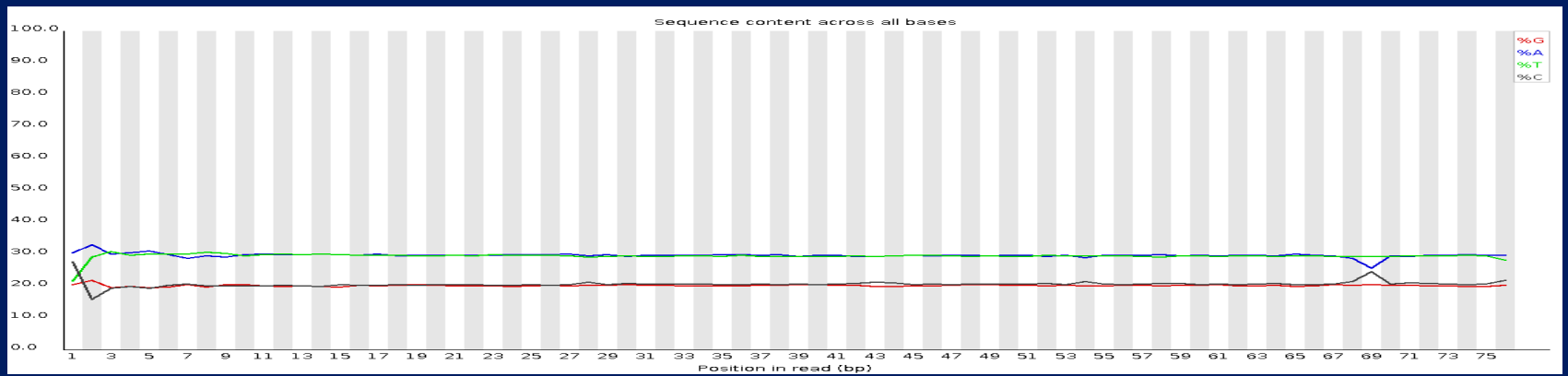

Figure 3: Per base sequence content.

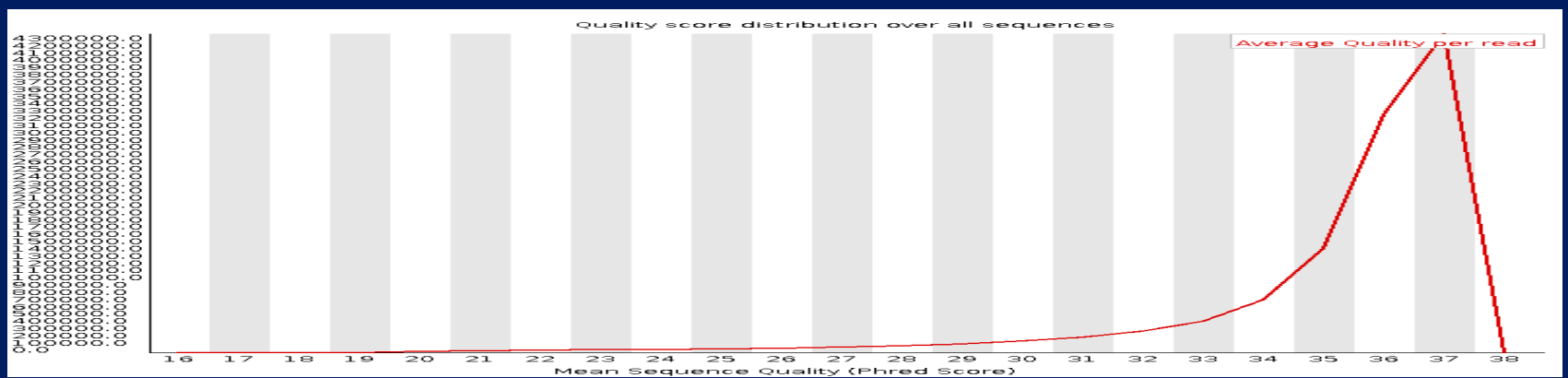

Figure 4: Per sequence quality.

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Some features:

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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.

Variant calling

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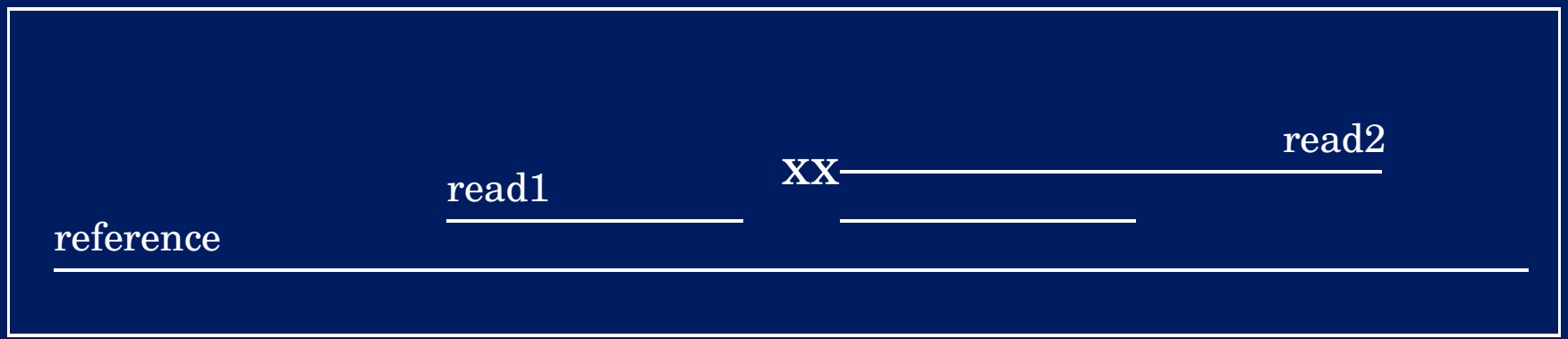
Mainly file format conversions.

- SAM $\rightarrow$ BAM.
- BAM $\rightarrow$ BAM.sorted.
- BAM.sorted $\rightarrow$ BAM.sorted.index.
- BAM.sorted $\rightarrow$ mpileup (BAQ realignment).
- BAM.sorted $\rightarrow$ BCF.
- BCF $\rightarrow$ 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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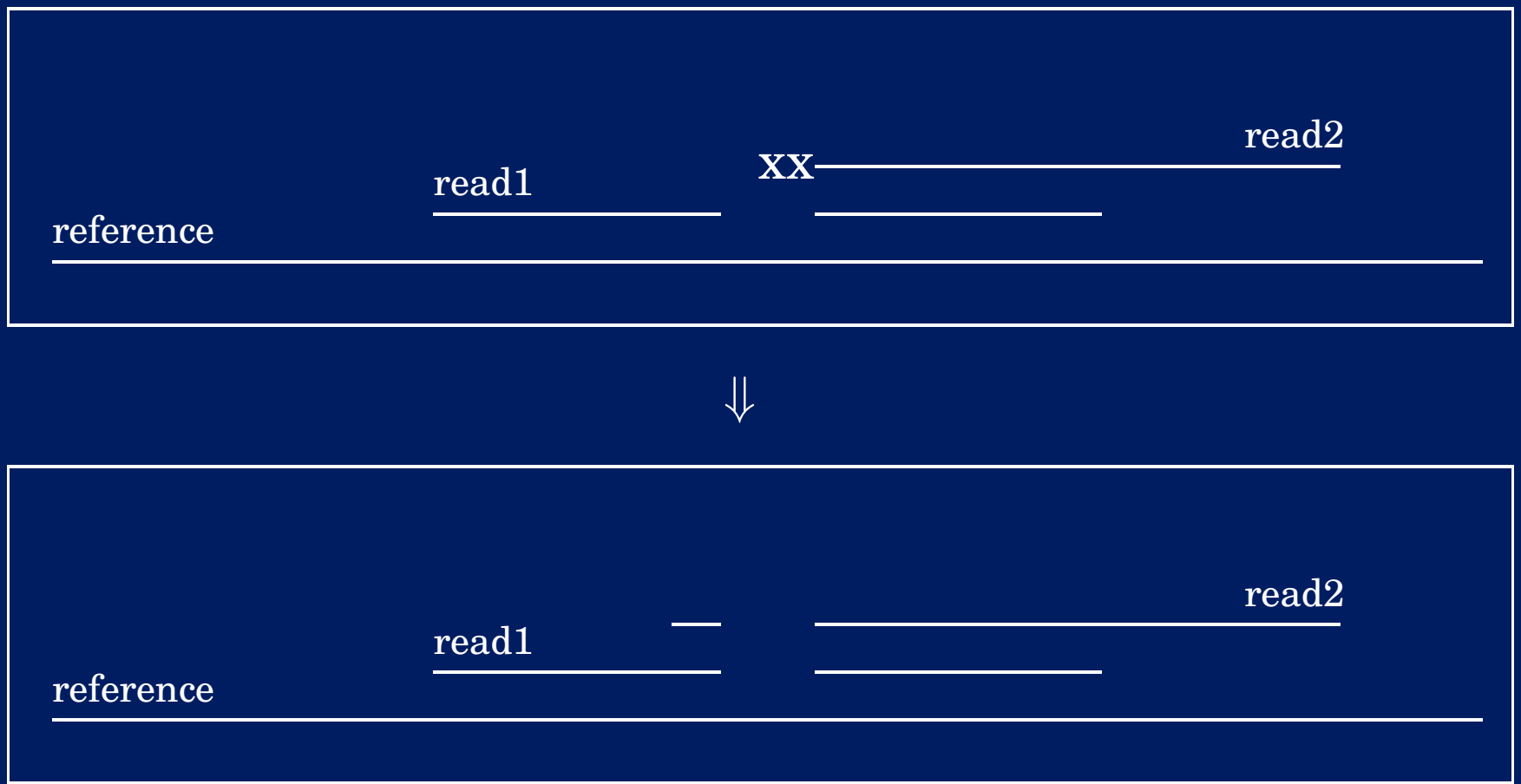

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  - Is it in the 5'/3' UTR of a gene?
  - …
- Is it in a regulatory region?
- …

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 $@ $<
```

Listing 2: Makefile

Pipelines

Name	Manual	Options
bwa	490	±40
samtools	616	±70
fastx	?	±30

Table 1: Selection of used tools

The number of parameters is impressive, but the number of combinations is incredible.

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Sometimes, people want to tweak these parameters, which is not very practical without a graphical interface.

Graphical interfaces

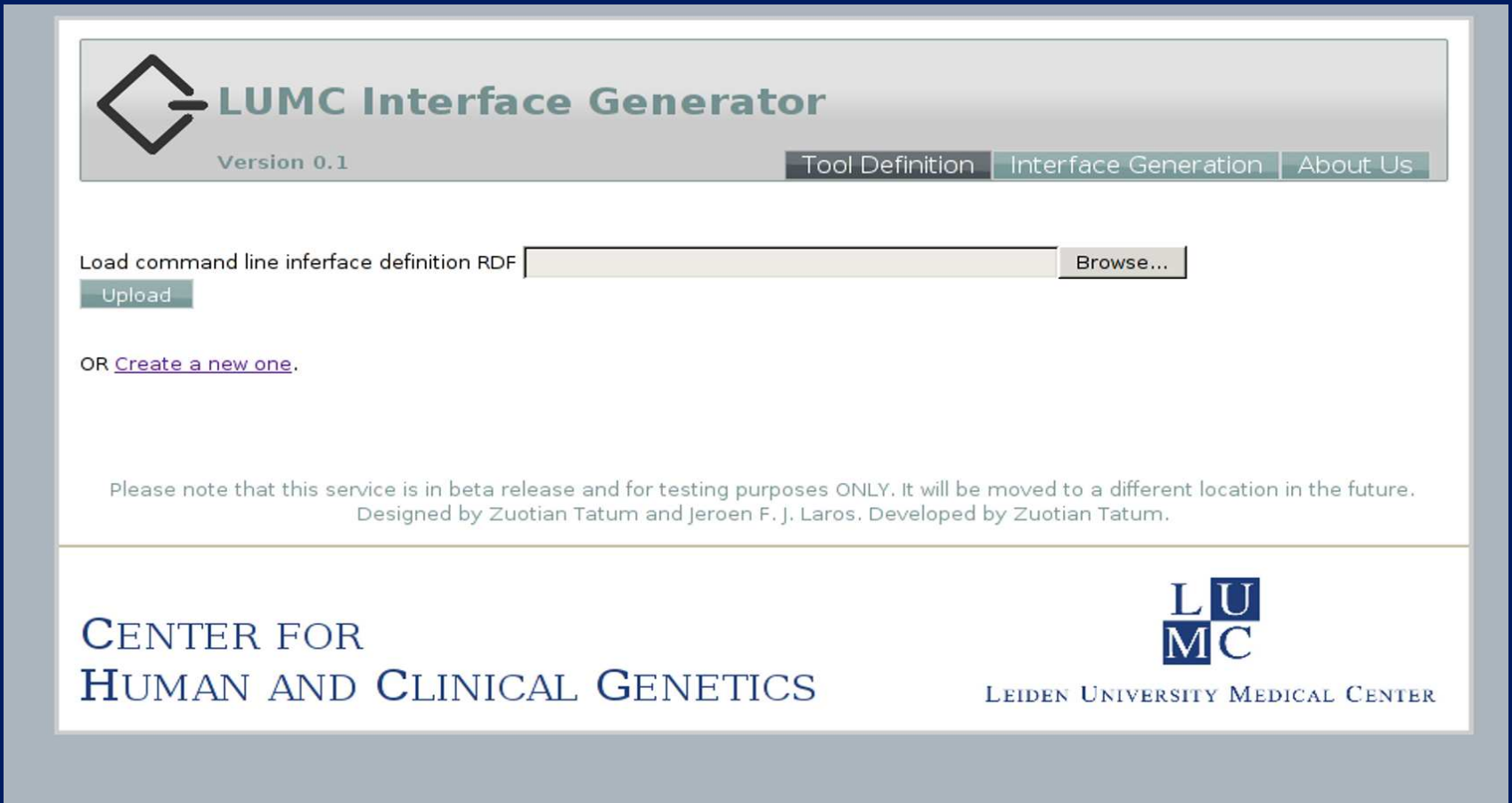

Figure 5: Interface generator

Graphical interfaces

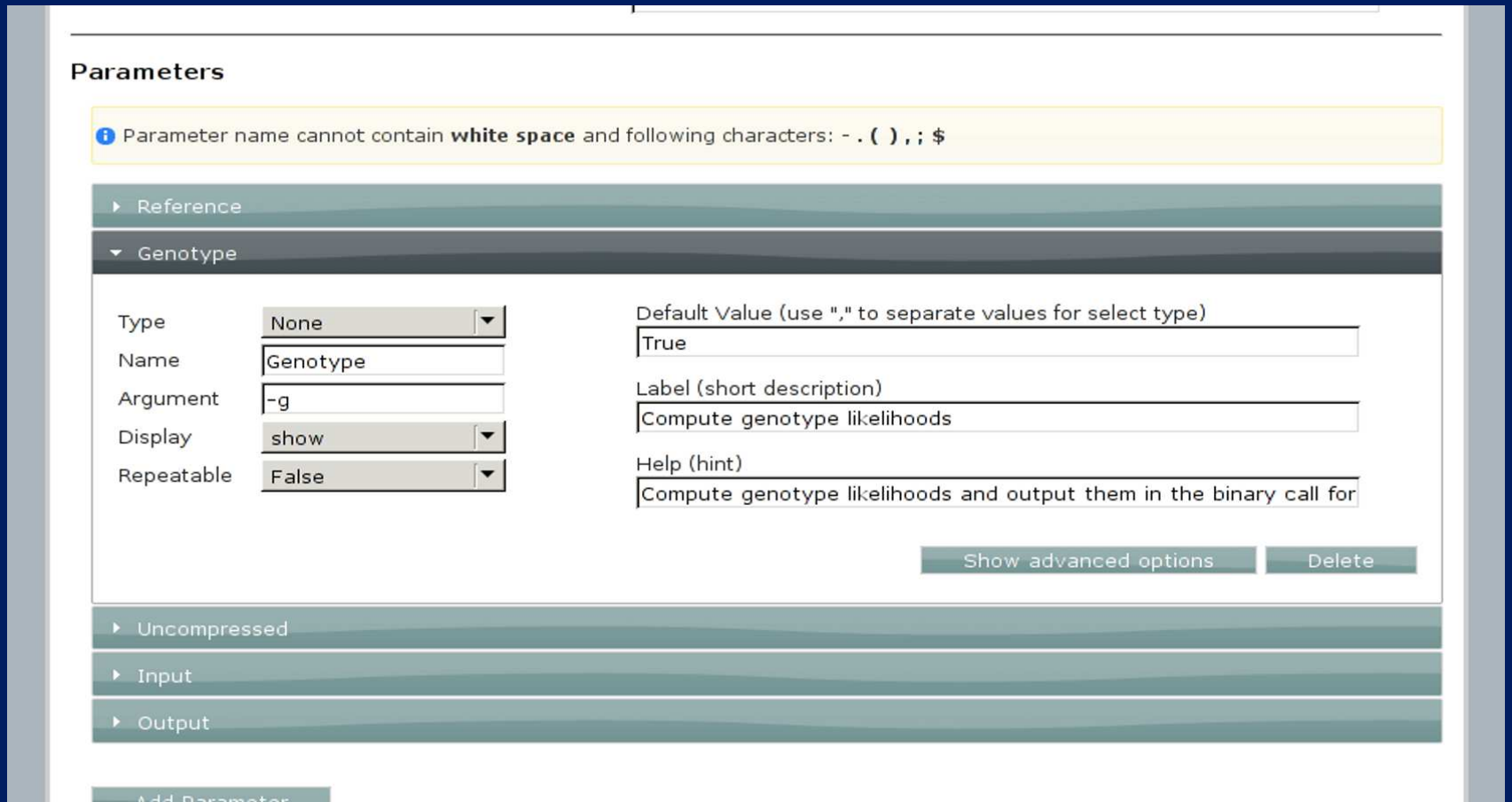

Figure 6: Using the interface generator

Graphical interfaces

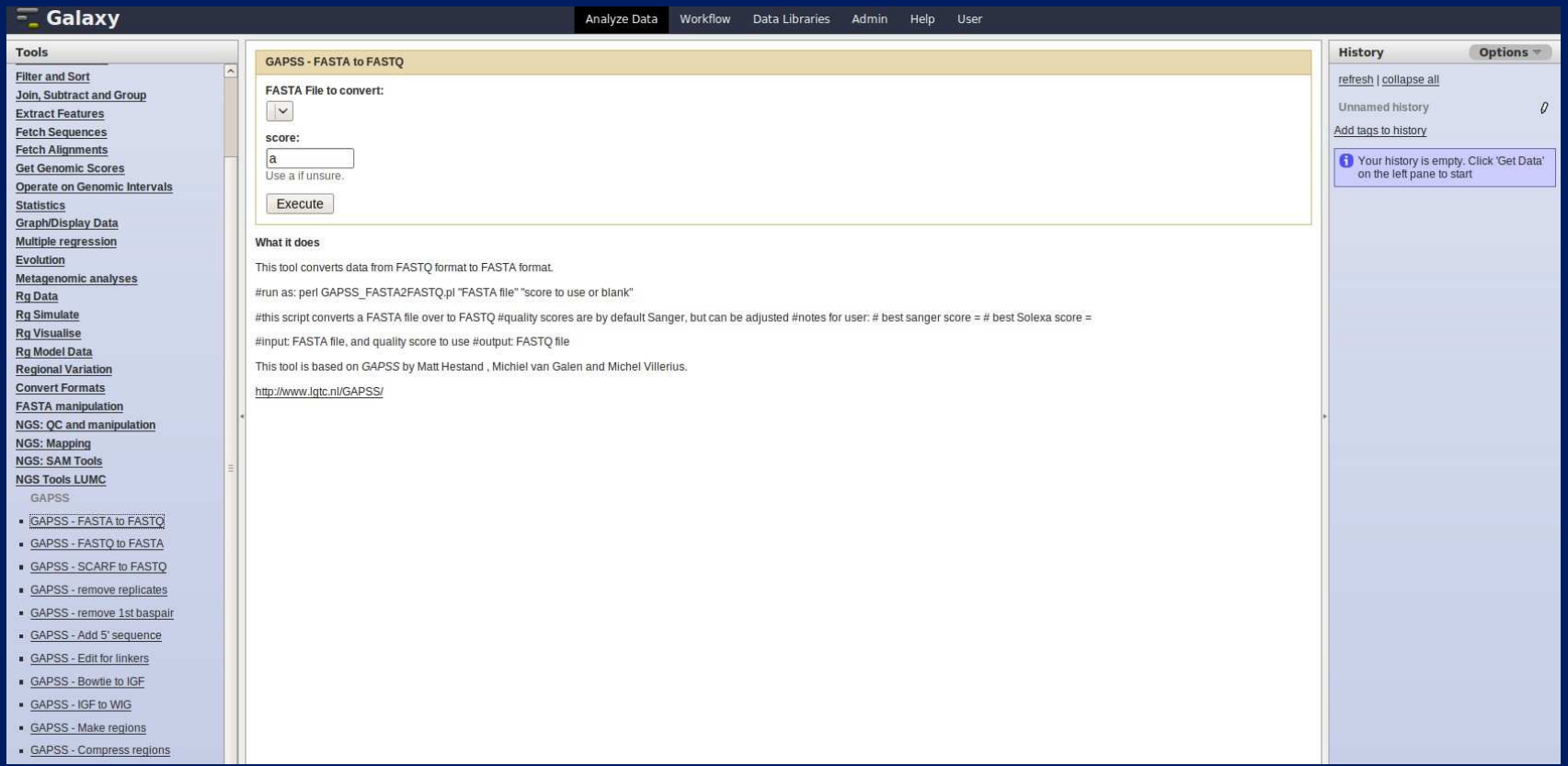

Figure 7: Galaxy

http://galaxy.nbic.nl/

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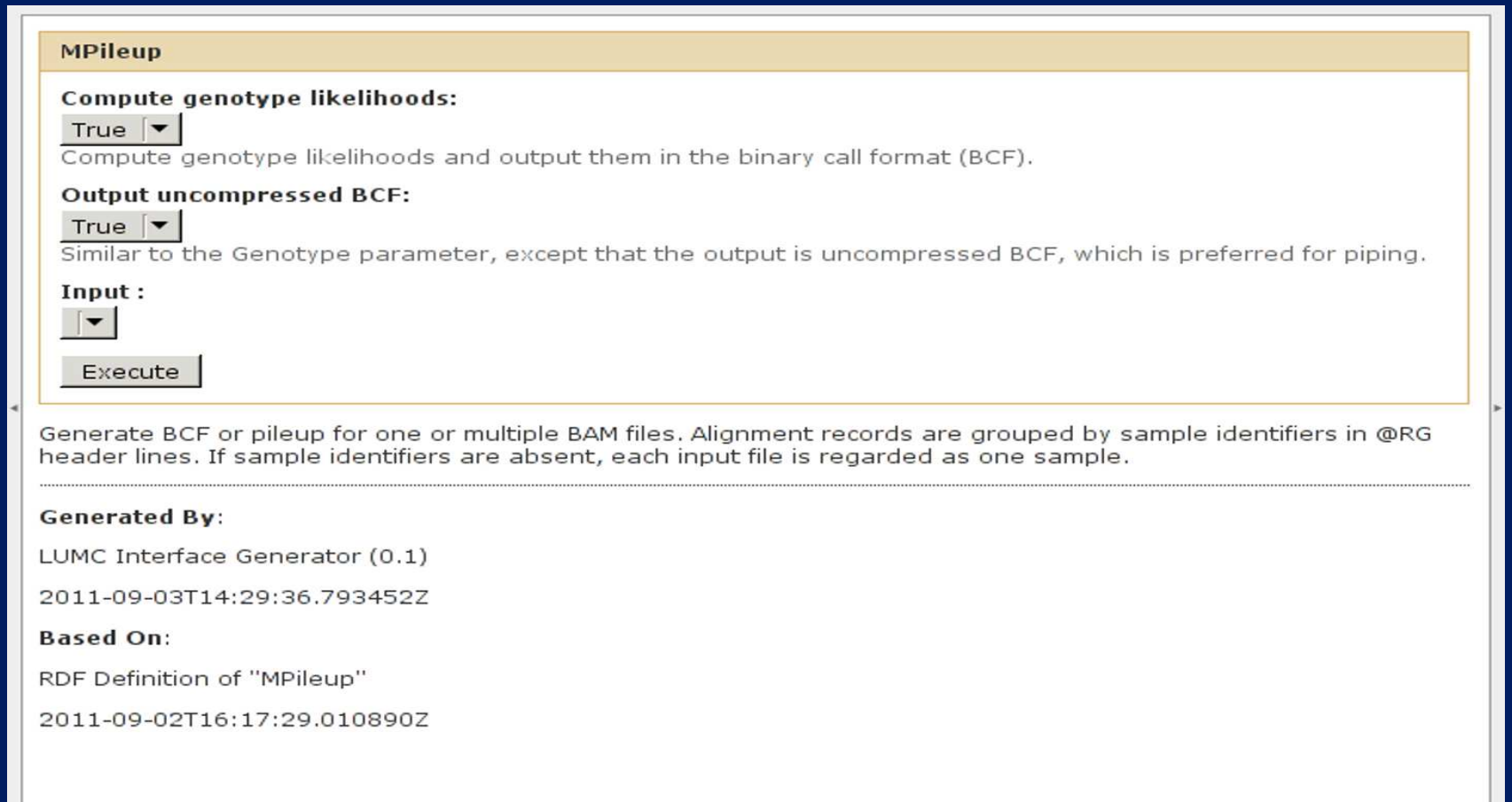

Figure 8: User friendly interface with Galaxy

Questions?

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https://www.mutalyzer.nl/projects/GAPSS3/
http://www.lgtc.nl/