



LEIDEN UNIVERSITY MEDICAL CENTER

# **Exome sequencing with GAPSS3**

**(General annotation Pipeline for Second-generation Sequencers)**

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

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Overview of an in-solution 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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5. Annotation.

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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.
- ...

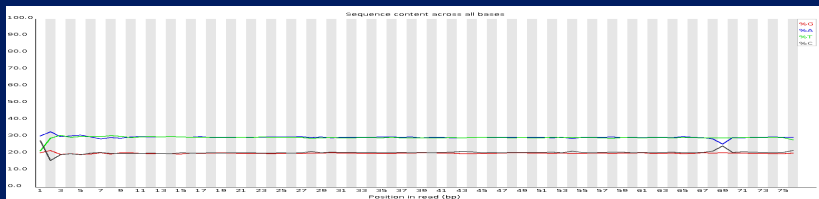


Figure 3: Per base sequence content.

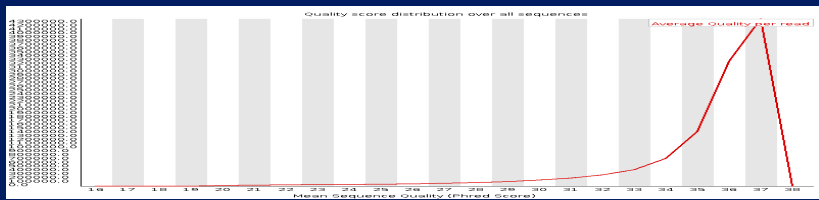


Figure 4: Per sequence quality.

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Some features:

- Base quality recalibration.
  - First map 1% of the input.
  - Recalibrate the Fastq quality scores.
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Some features:

- Base quality recalibration.
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  - 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

Variant calling is done by Samtools, BCFtools / VCFutils.

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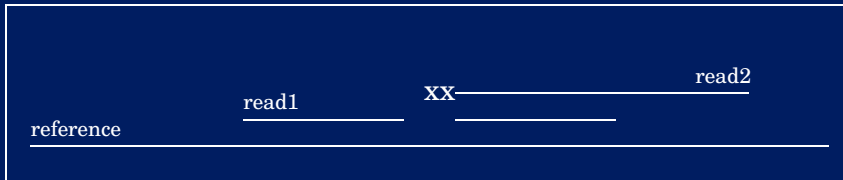
Mainly file format conversions.

- **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).

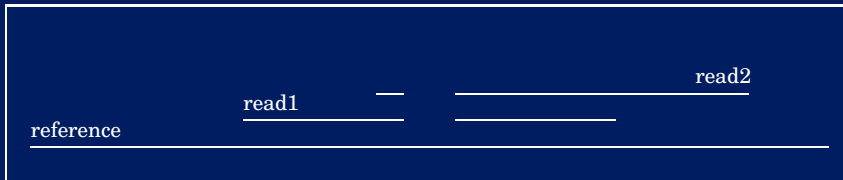
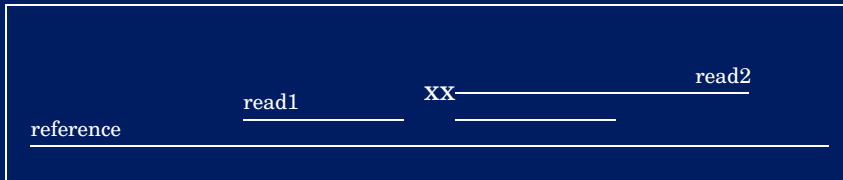
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- Is it in a regulatory region?
- ...

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
```

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

Name	Manual	Options
<b>bwa</b>	490	$\pm 40$
<b>samtools</b>	616	$\pm 70$
<b>fastx</b>	?	$\pm 30$

Table 1: Selection of used tools

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

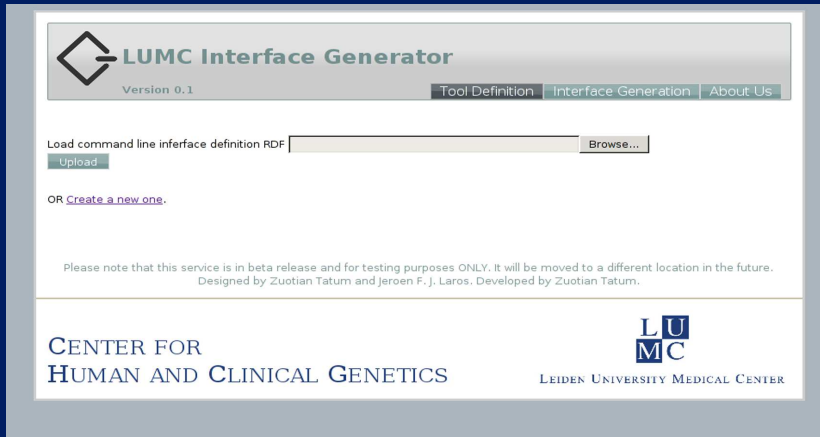
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Sometimes, people want to tweak these parameters, which is not very practical without a graphical interface.

# Graphical interfaces



The screenshot shows the LUMC Interface Generator web application. At the top left is the LUMC logo. The main header contains the text "LUMC Interface Generator" and "Version 0.1". To the right of the header are three navigation tabs: "Tool Definition", "Interface Generation", and "About Us". Below the header, there is a form with the label "Load command line interface definition RDF" and a "Browse..." button. A green "Upload" button is positioned below the form. Below the form, there is a link that says "OR [Create a new one.](#)". A disclaimer text is centered below the link: "Please note that this service is in beta release and for testing purposes ONLY. It will be moved to a different location in the future. Designed by Zuotian Tatum and Jeroen F. J. Laros. Developed by Zuotian Tatum." At the bottom of the page, the text "CENTER FOR HUMAN AND CLINICAL GENETICS" is on the left, and the LUMC logo and "LEIDEN UNIVERSITY MEDICAL CENTER" are on the right.

Figure 5: Interface generator



# Graphical interfaces

**Parameters**

**i** Parameter name cannot contain **white space** and following characters: - . ( ) , ; \$

- ▶ Reference
- ▼ Genotype
 

Type	None	Default Value (use "," to separate values for select type)	True
Name	Genotype	Label (short description)	Compute genotype likelihoods
Argument	-g	Help (hint)	Compute genotype likelihoods and output them in the binary call for
Display	show	<input type="button" value="Show advanced options"/> <input type="button" value="Delete"/>	
Repeatable	False		
- ▶ Uncompressed
- ▶ Input
- ▶ Output

[Add Parameter](#)

Figure 6: Using the interface generator

The screenshot shows the Galaxy web interface. At the top, there is a navigation bar with 'Galaxy' on the left and 'Analyze Data', 'Workflow', 'Data Libraries', 'Admin', 'Help', and 'User' on the right. A left-hand sidebar contains a 'Tools' menu with various categories like 'Filter and Sort', 'Statistics', 'Evolution', and 'NGS: QC and manipulation'. The main content area is titled 'GAPSS - FASTA to FASTQ' and contains a form for 'FASTA File to convert:' with a dropdown menu, a 'score:' input field, and an 'Execute' button. Below the form, there is a 'What it does' section with a description of the tool's function and a URL: <http://www.gtc.nl/GAPSS/>. On the right side, there is a 'History' panel with 'refresh' and 'collapse all' buttons, and a message: 'Your history is empty. Click "Get Data" on the left pane to start.'

Figure 7: Galaxy

<http://galaxy.nbic.nl/>

**Mpileup**

**Compute genotype likelihoods:**  
 ▼  
 Compute genotype likelihoods and output them in the binary call format (BCF).

**Output uncompressed BCF:**  
 ▼  
 Similar to the Genotype parameter, except that the output is uncompressed BCF, which is preferred for piping.

**Input :**  
 ▼

---

**Generated By:**  
 LUMC Interface Generator (0.1)  
 2011-09-03T14:29:36.793452Z

**Based On:**  
 RDF Definition of "Mpileup"  
 2011-09-02T16:17:29.010890Z

Generate BCF or pileup for one or multiple BAM files. Alignment records are grouped by sample identifiers in @RG header lines. If sample identifiers are absent, each input file is regarded as one sample.

Figure 8: User friendly interface with Galaxy

NGS data analysis, 6th edition

15/16

Tuesday, 11 September 2012

## Acknowledgements

Michiel van Galen  
Martijn Vermaat  
Zuotian Tatum  
Yu-Ching Lai  
Michel Villerius  
Jaap van der Heijden  
Bradley ten Broeke  
Johan den Dunnen

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