Variant calling in Exome-seq data using Varscan

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U900 INSERM - Mines ParisTech - Institut Curie

ITMO Roscoff – 11/19/2013

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Dataset

- Public data: exome sequenced by the International HapMap Project
- Single-end reads of 100bp, Illumina Genome Analyzer IIx
- RNA-seq data of this exome available (Pickrell *et al.*, Nature, 2010)

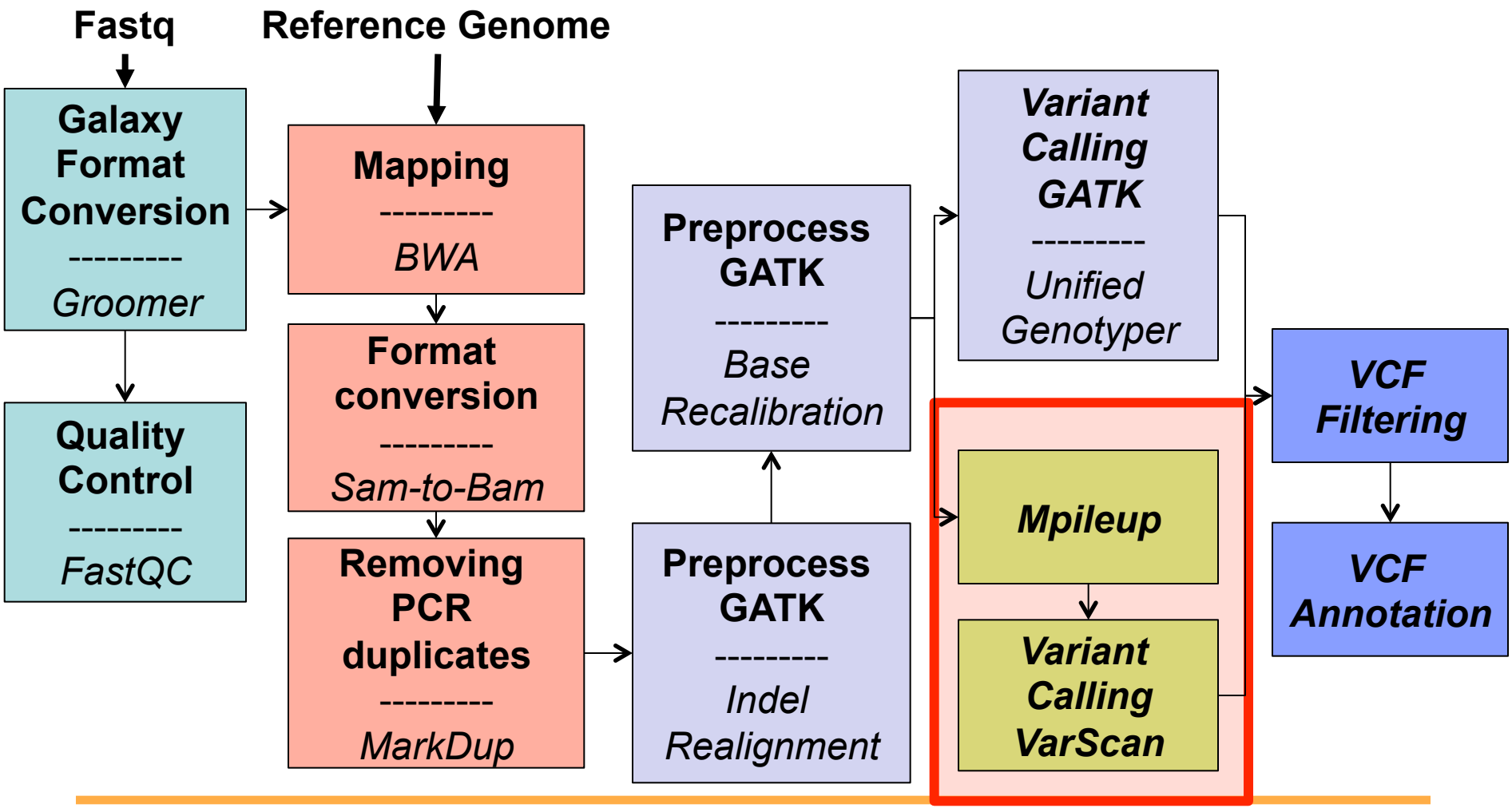
Objectives of the workshop:

- Variant calling, filtering and annotation in exome-seq data
- Observing the potential impact of these variants by looking at the corresponding RNA-seq data

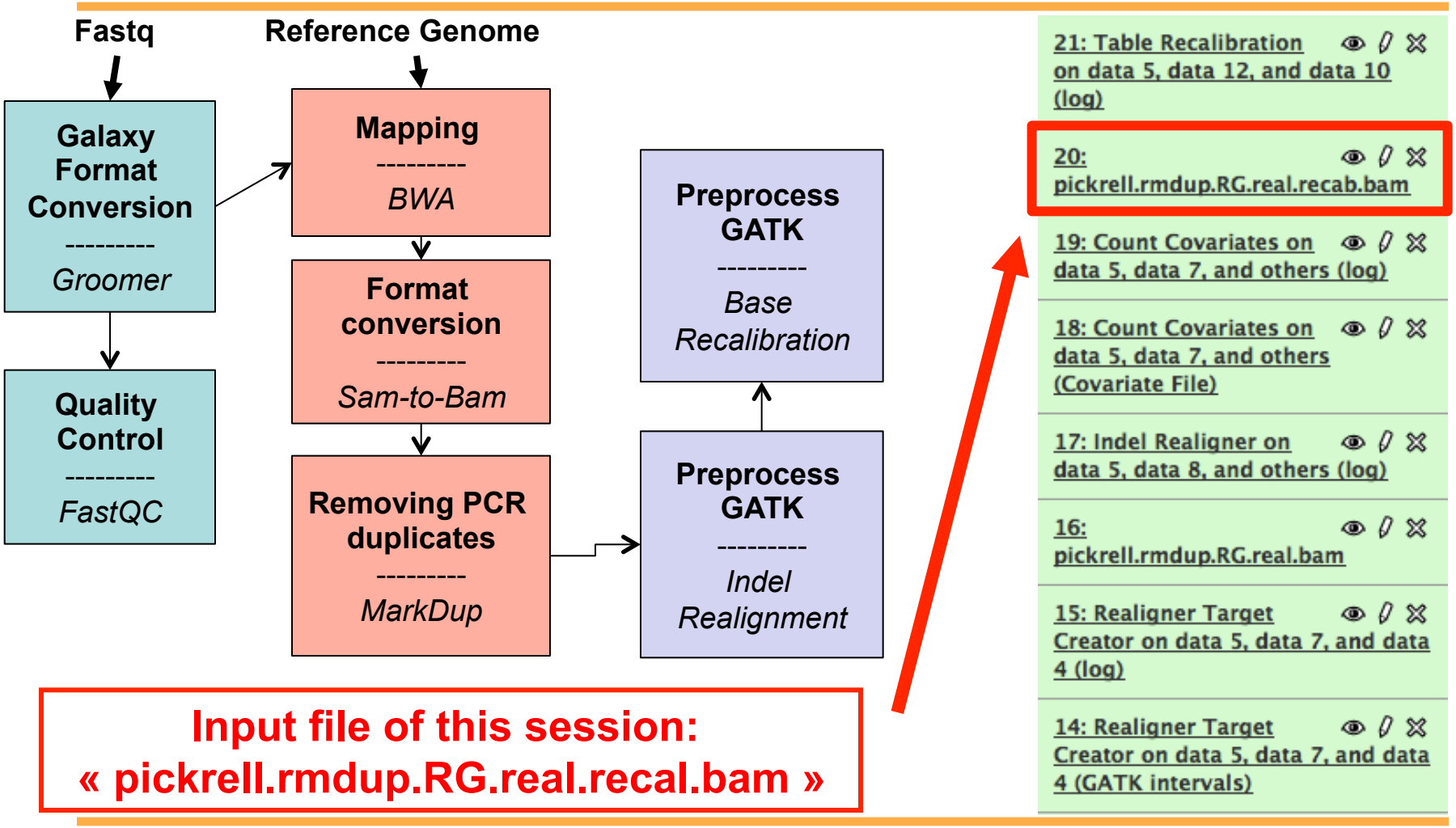
Objectif of this session :

➢ Variant detection using Varscan

Workflow


Fastq
Reference Genome
Galaxy Format Conversion
Groomer
Quality Control
FastQC
Mapping
BWA
Format conversion
Sam-to-Bam
Removing PCR duplicates
MarkDup
Preprocess GATK
Indel Realignment
Preprocess GATK
Base Recalibration
Variant Calling GATK
Unified Genotyper
Mpileup
Variant Calling VarScan
VCF Filtering
VCF Annotation

Galaxy: summary of the previous steps



Input file of this session:
« pickrell.rmdup.RG.real.recal.bam »

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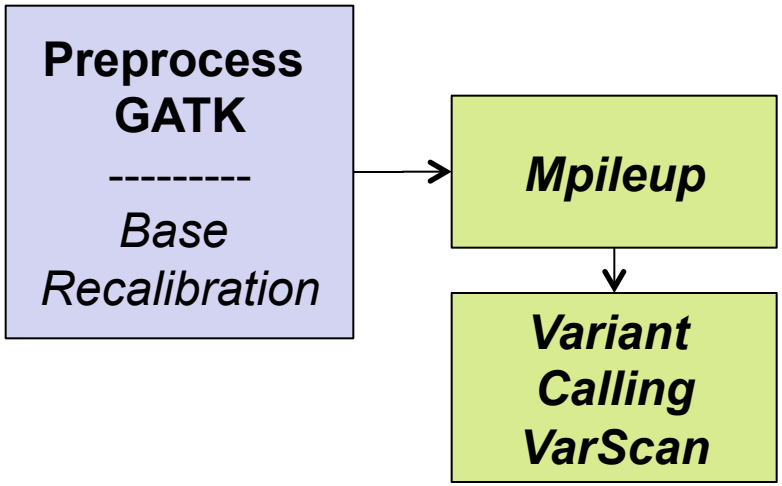
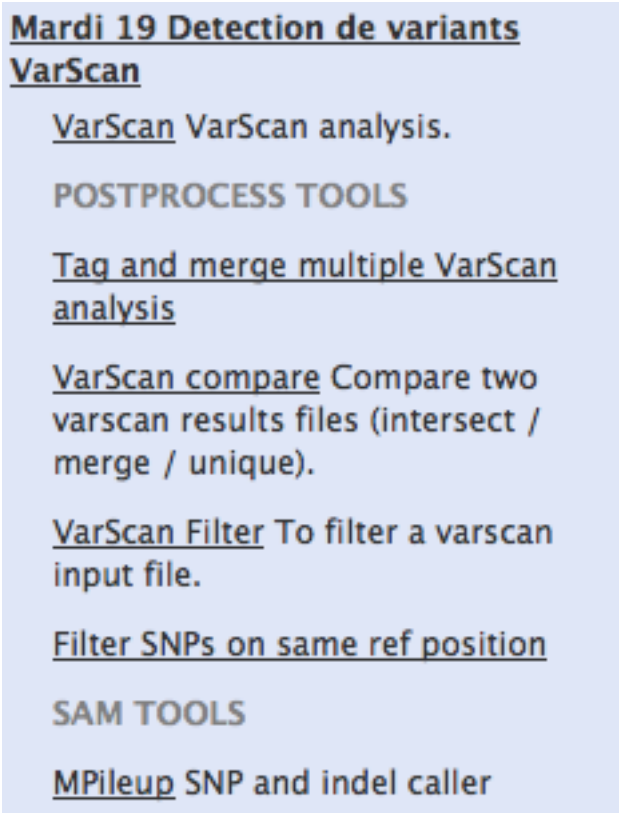
Tools

All the tools are in the left panel: **Mardi 19 Détection de variants VarScan**





Pileup

- **Pileup format:** describes the base-pair information at each position

Reference base

Base qualities

chr12	112888238	A	108	.$, $.,,.,,..,,,.,..,.,..,.,,,.,,,.,..,,..,,.,,.,.,.,,,.,.,,,.,.,.,.,,.,,.,.,., ,,,.,.,.,.,,,.,.,.,.,,,,,.,,.,.,..,,,,,,.,.,,,,^F,	=4 =???????@??@?@@ @=@ ??@ ? @? ? ??< ? ??@???????? ? @??? ? ??@?? A???@@ ?@@???AB????= ? @ @@??@@? @ A 00
chr12	112888239	C	108	. $t,.,,.T,tT,.,T.,.,t.tTtt.tTT,t.T,tTt.tT,T,,.tT tTttt.,.,Tt.ttt.,T,.,.tT,,T,T,.tT,,t,TttTtT,T.,t t,,T,T,tttt^F.^F,	936 78??6??6 45<875? ??? ?@6 @6???<? =6 66= ? ???? 6=7??=???<8@7=7=? 77?7? 8 ??78?7????? 8 <8??88 9?8 ?0048

Number of reads covering the site (total depth)

Read bases:

. / , = match on forward/reverse strand

ACGTN / acgtn = mismatch on forward/reverse strand

`-\+[0-9]+[ACGTNacgtn]+‘ indicates an indel

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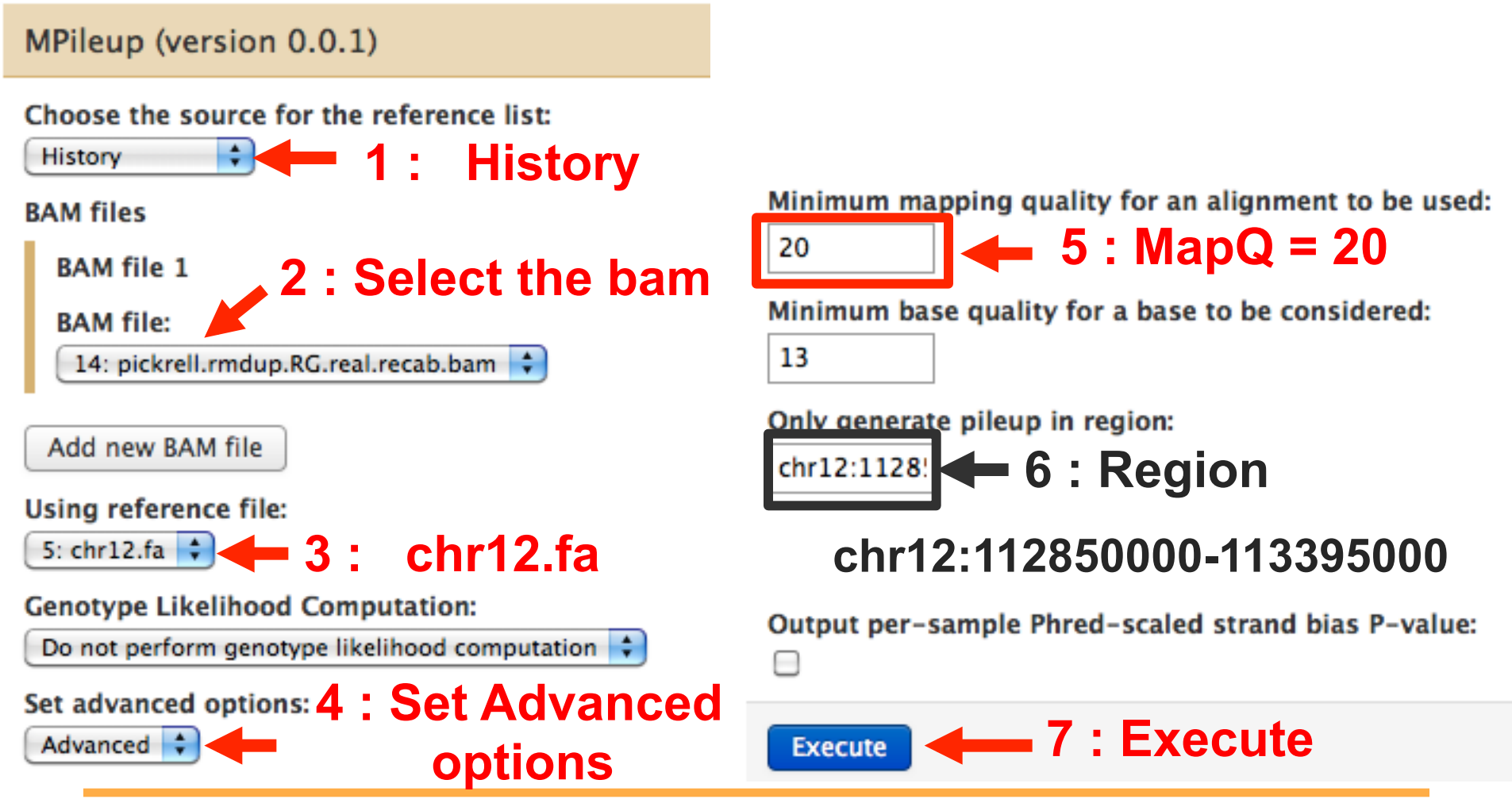
Mpileup



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VarScan

- Mutation caller written in **Java** (no installation required) working with **Pileup files** of Targeted, **Exome**, and Whole-Genome sequencing data

- **Multi-platforms**: Illumina, SOLiD, Life/PGM, Roche/454

- Detection of different kinds of variants (SNVs/Indels) :
  - Germline variants in individual samples
  - Multi-sample variants **shared or private** in multi-sample datasets

- VarScan specificity is to be able to work with **Tumor/Normal pairs**:
  - Somatic and germline mutation, LOH events in tumor-normal pairs
  - Somatic copy number alterations (CNAs) in tumor-normal exome data

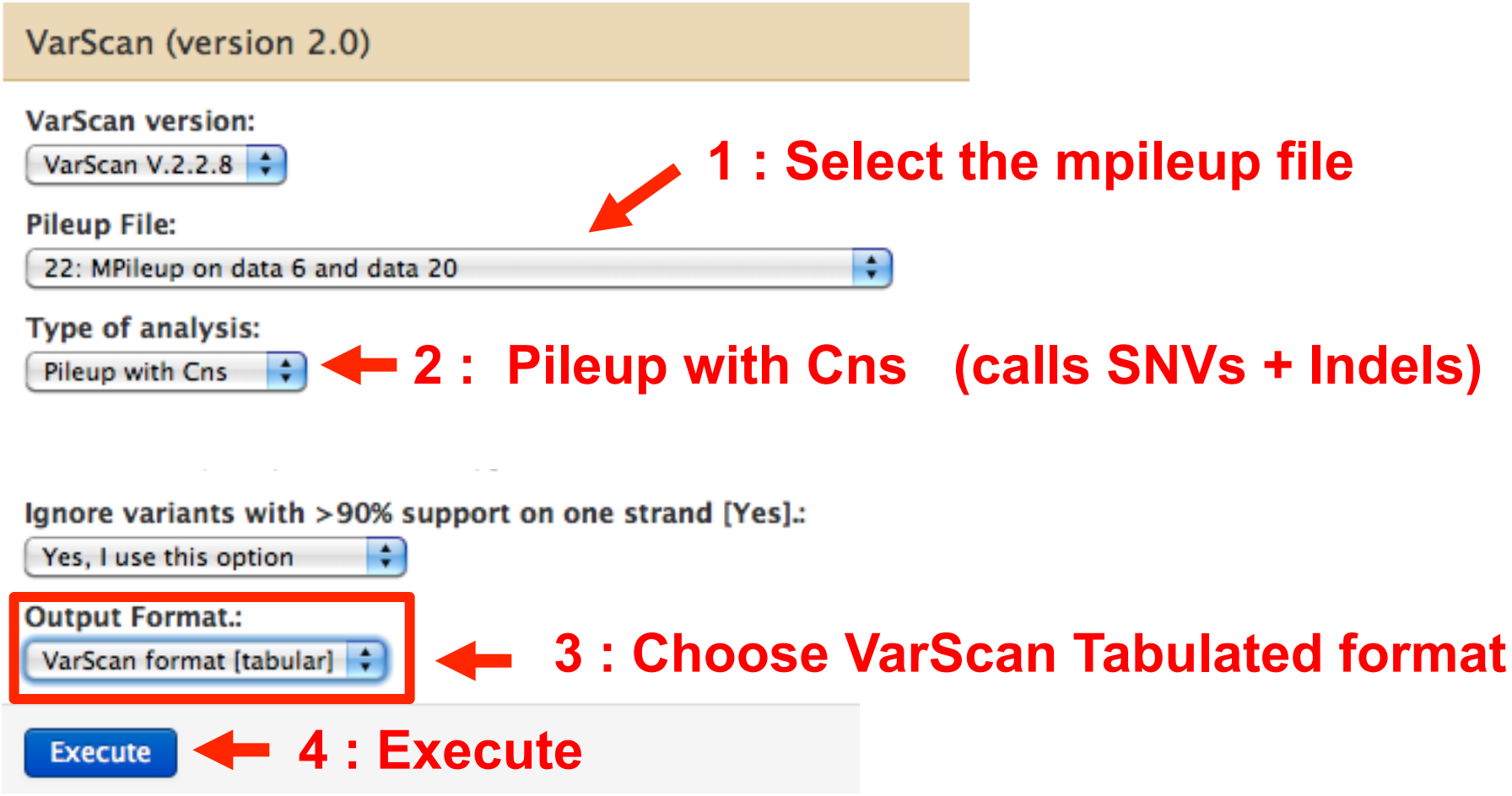
VarScan

- Most published variant callers use **Bayesian statistics** (a probabilistic framework) to detect variants and assess confidence in them (*e.g.:* GATK)

- VarScan uses a robust **heuristic/statistic** approach to call variants that meet desired thresholds for read depth, base quality, variant allele frequency, and statistical significance

- In Stead *et* al. (2013), they compared 3 different **somatic callers** : MuTect, Strelka, VarScan2
  - **VarScan2 performed best** overall with sequencing depths of 100x, 250x, 500x and 1000x required to accurately identify variants present at 10%, 5%, 2.5% and 1% respectively

VarScan


VarScan (version 2.0)
VarScan version:
VarScan V.2.2.8
1 : Select the mpileup file
Pileup File:
22: MPileup on data 6 and data 20
Type of analysis:
Pileup with Cns
2 : Pileup with Cns (calls SNVs + Indels)
Ignore variants with >90% support on one strand [Yes].:
Yes, I use this option
Output Format.:
VarScan format [tabular]
3 : Choose VarScan Tabulated format
Execute
4 : Execute

VarScan VCF Format

- 2 types: **VCF** and **Tabulated** Formats - **VarScan Specific**
  - ➢ VCF output might not work with some filtering or annotating tools

- **VarScan VCF format**: classic VCF header (#) but specific variant lines

#CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO	FORMAT	GENO
chr12	250239	.	A	G	20	PASS	ADP=104;WT=0; **HET=1**;HOM=0; NC=0	GT:GQ:SDP:DP:RD:AD: FREQ: PVAL:RBQ:ABQ: RDF:RDR:ADF:ADR	0/1:153:111:104:61:43: 41,35%: 4,5644E-16:38:32: 48:13:36:7

ADP = Average per-sample depth of bases with Phred score = 20
WT = Number of samples called reference (wild-type)
HET = Number of samples called heterozygous-variant
HOM = Number of samples called homozygous-variant
NC = Number of samples not called

➔ Useful in multi-sample studies

VarScan VCF Format

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#CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO	FORMAT	GENO
chr12	250239	.	A	G	20	PASS	ADP=104;WT=0;HET=1;HOM=0;NC=0	**GT**:GQ:SDP:**DP**:**RD:AD**:**FREQ**:	**0/1**:153:111:**104**:**61:43**:**41,35%**:
								PVAL:RBQ:ABQ:RDF:RDR:ADF:ADR	4,5644E-16:38:32:48:13:36:7

GT=Genotype (1/1: Homozygous ; 0/1 : Heterozygous) / GQ= Genotype Quality
SDP= Raw Read Depth as reported by SAMtools
DP= Quality Read Depth of bases with Phred score >= 20
RD= Depth of reference-supporting bases
AD= Depth of variant-supporting bases
FREQ= Variant allele frequency

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#CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO	FORMAT	GENO
chr12	250239	.	A	G	20	PASS	ADP=104;WT=0; HET=1;HOM=0; NC=0	GT:GQ:SDP:DP:RD:AD: FREQ: PVAL:RBQ:ABQ: **RDF:RDR:ADF:ADR**	0/1:153:111:104:61:43: 41,35%: 4,5644E-16:38:32: **48:13:36:7**

PVAL= P-value from Fisher's Exact Test (**not computed here : default value)**
RBQ= Average quality of reference-supporting bases
ABQ= Average quality of variant-supporting bases
RDF / RDR= Depth of reference-supporting bases on forward/reverse strand
ADF / ADR = Depth of variant-supporting bases on forward/reverse strand

VarScan VCF Format

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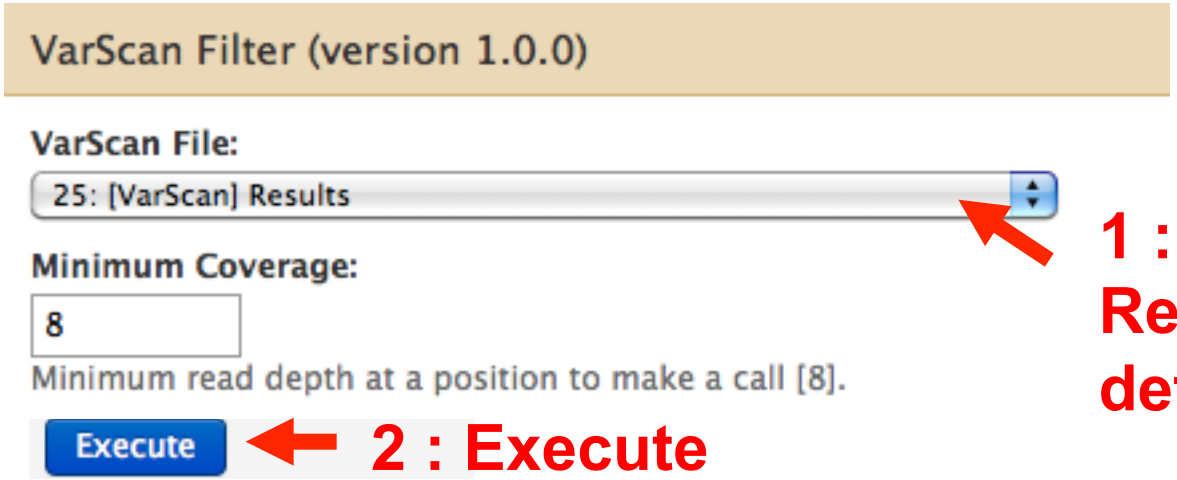
#CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO	FORMAT	GENO
chr12	250239	.	A	G	20	PASS	ADP=104;WT=0; HET=1;HOM=0; NC=0	GT:GQ:SDP:DP:RD:AD: FREQ: PVAL:RBQ:ABQ: **RDF:RDR:ADF:ADR**	0/1:153:111:104:61:43: 41,35%: 4,5644E-16:38:32: **48:13:36:7**

PVAL= P-value from Fisher's Exact Test (**not computed here : default value)**
RBQ= Average quality of reference-supporting bases
ABQ= Average quality of variant-supporting bases
RDF / RDR= Depth of reference-supporting bases on forward/reverse strand
ADF / ADR = Depth of variant-supporting bases on forward/reverse strand

VarScan Tabulated Format

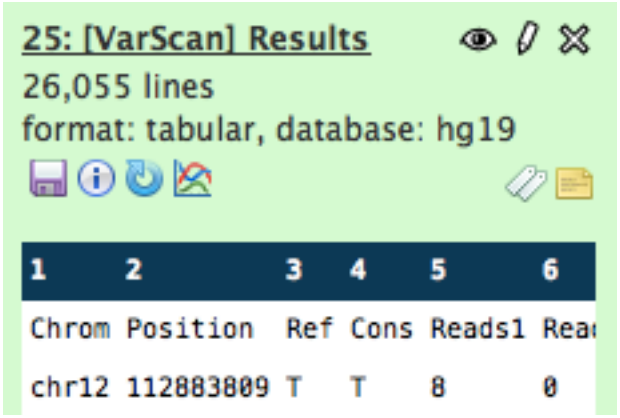
- 2 types: **VCF** and **Tabulated** Formats - **VarScan Specific**
  - Tabulated output works with other VarScan Tools
  - By default on Galaxy, VarScan outputs a line for each base covered by the selected minimum coverage **even if there is no alternative variant**

→ **Pre-process**: use « **VarScan Filter** » to keep only variants

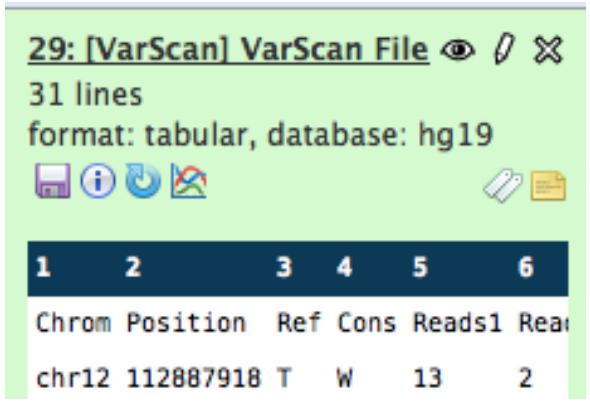


1 : Select the VarScan Results and leave the default parameters

VarScan Tabulated Format



VarScan Filter

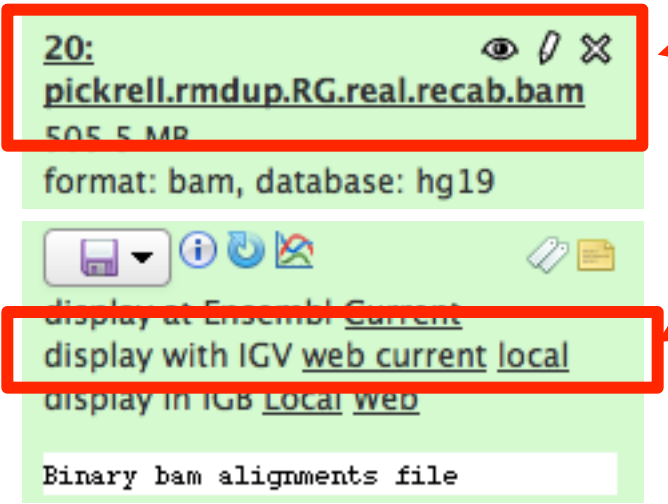


Chrom	Position	Ref	Cons	Reads1	Reads2	VarFreq	Strands 1	Strands 2	Qual1	Qual2	Pvalue	Map Qual1	Map Qual2	R1 +	R1 -	R2 +	Rs2 -	Alt
chr12	113348849	C	Y	31	30	**49.18%**	2	2	27	27	0.98	1	1	19	12	25	5	**T**
chr12	113354329	G	R	72	2	**2.70%**	2	2	31	26	0.98	1	1	48	24	1	1	**A**
chr12	113357193	G	A	2	72	**97.30%**	1	2	28	24	0.98	1	1	2	0	45	27	**A**
chr12	113357209	G	A	0	77	**100%**	0	2	0	29	0.98	0	1	0	0	51	26	**A**

Cons : Consensus Genotype of Variant Called (IUPAC code):

M -> A or C	Y -> C or T	D -> A or G or T	W -> A or T	V -> A or C or G
R -> A or G	K -> G or T	B -> C or G or T	S -> C or G	H -> A or C or T

Variants visualization with IGV



1: Click on the name to open the options

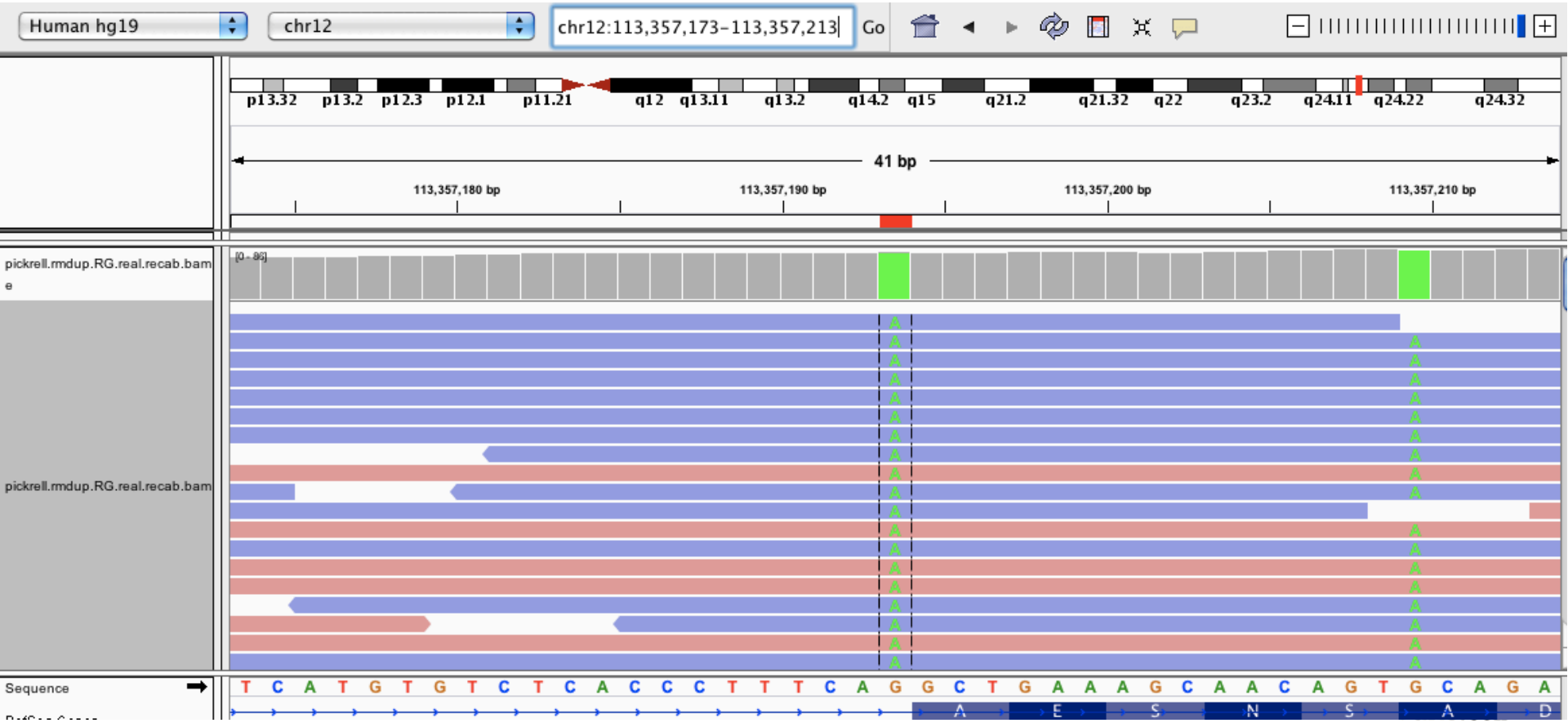
2: Click on « Local » to open it on IGV (if already open, otherwise « Web current »)

- Look at those two variants:

Chrom	Position	Ref	Cons	Reads 1	Reads 2	VarFreq	Strands 1	Strands 2	Qual 1	Qual2	Pval	Map Qual1	Map Qual2	R1 +	R1 -	R2 +	Rs2 -	Alt
chr12	113357193	G	A	2	72	97.30%	1	2	28	24	0.98	1	1	2	0	45	27	A
chr12	112888239	C	Y	52	56	51.85%	2	2	24	28	0.98	1	1	20	32	24	32	T

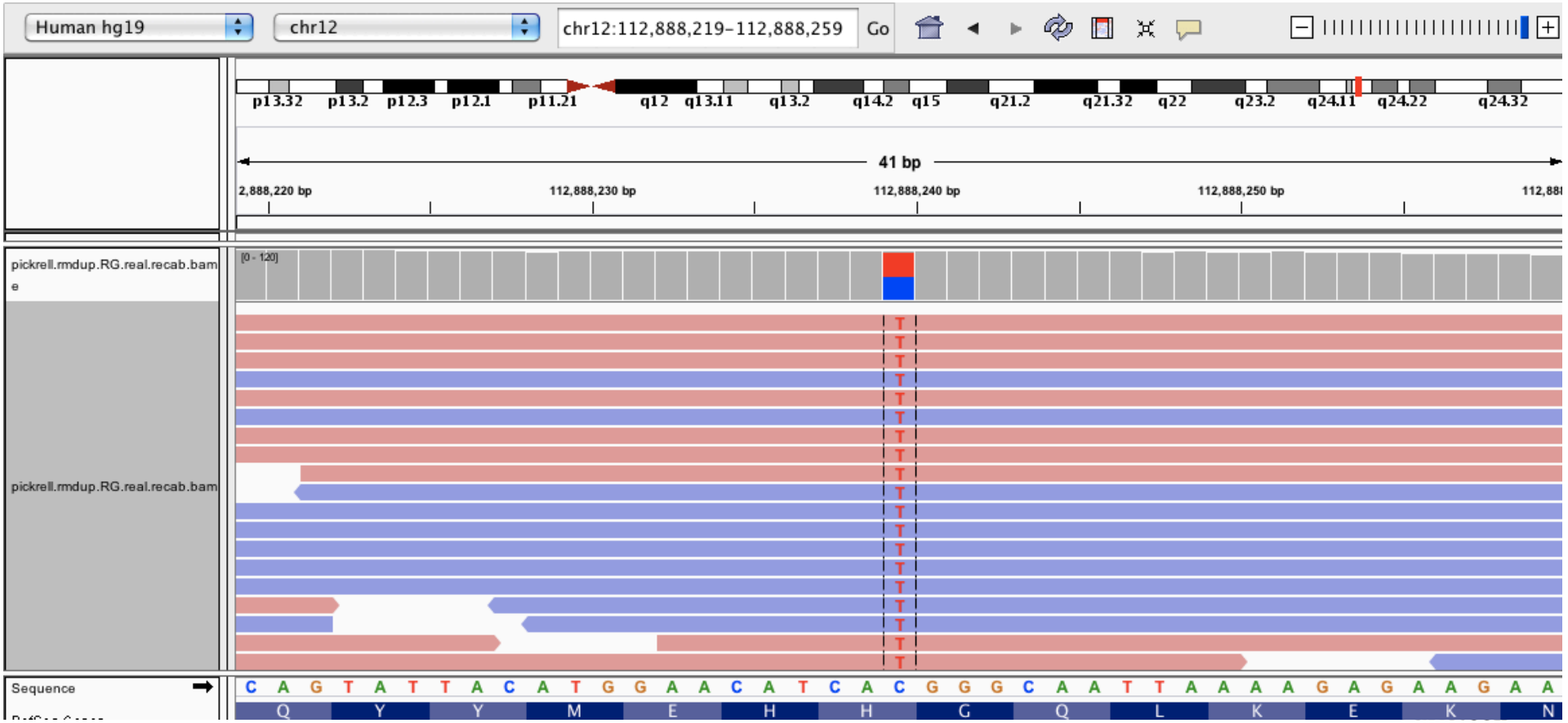
Variants visualization with IGV

Chrom	Position	Ref	Cons	Reads 1	Reads 2	VarFreq	Strands 1	Strands 2	Qual 1	Qual2	Pval	Map Qual1	Map Qual2	R1 +	R1 -	R2 +	Rs2 -	Alt
chr12	113357193	G	A	2	72	97.30%	1	2	28	24	0.98	1	1	2	0	45	27	A

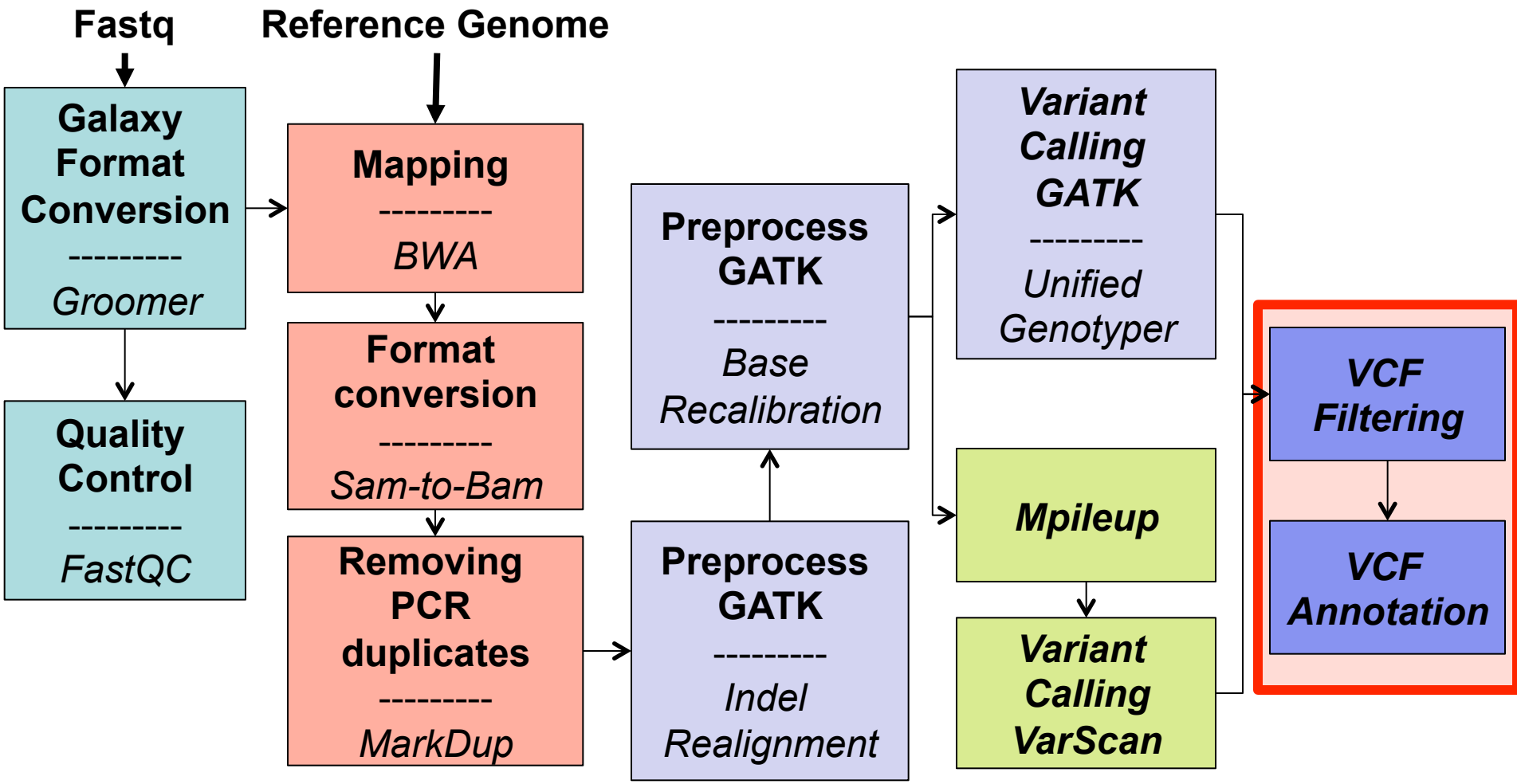


Variants visualization with IGV

Chrom	Position	Ref	Cons	Reads 1	Reads 2	VarFreq	Strands 1	Strands 2	Qual 1	Qual2	Pval	Map Qual1	Map Qual2	R1 +	R1 -	R2 +	Rs2 -	Alt
chr12	112888239	C	Y	52	56	51.85%	2	2	24	28	0.98	1	1	20	32	24	32	T



Next Step


Fastq
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