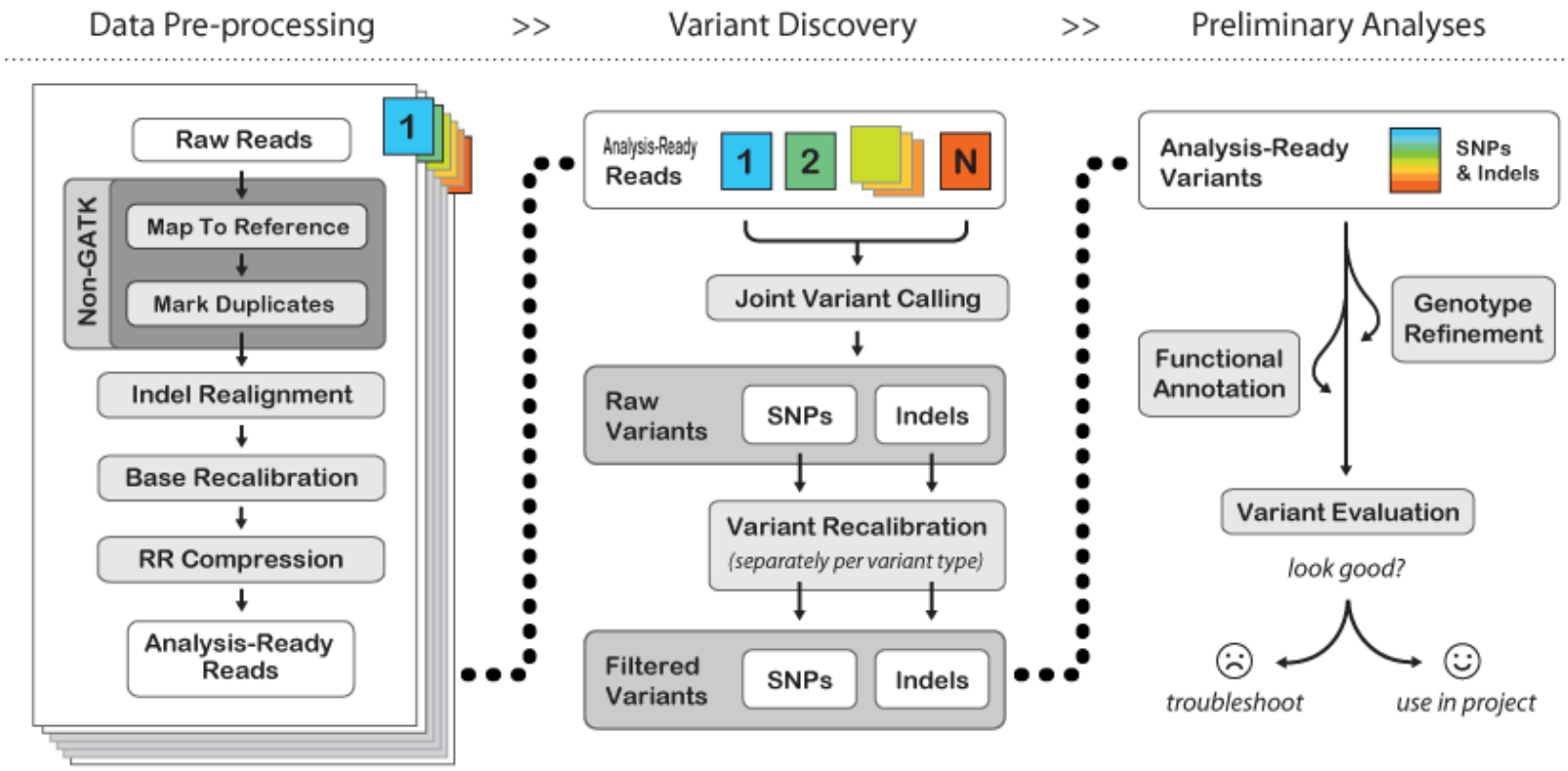
Variants

Martin Morgan (mtmorgan@fhcrc.org)
Fred Hutchinson Cancer Research Center
Seattle, WA

4 February 2014

Work flows

1. Experimental design – tumor / normal pairs; cell lines; ...
2. Sequencing – DNA or Exome
3. Alignment & other pre-processing steps
4. Variant discovery & preliminary analysis
5. **Variant evaluation, annotation, biological and experimental context**


Data Pre-processing
>>
Variant Discovery
>>
Preliminary Analyses
Raw Reads
1
Non-GATK
Map To Reference
Mark Duplicates
Indel Realignment
Base Recalibration
RR Compression
Analysis-Ready Reads
Analysis-Ready Reads
1
2
N
Joint Variant Calling
Raw Variants
SNPs
Indels
Variant Recalibration
(separately per variant type)
Filtered Variants
SNPs
Indels
Analysis-Ready Variants
SNPs & Indels
Genotype Refinement
Functional Annotation
Variant Evaluation
look good?
troubleshoot
use in project

Variant Call Format (VCF) files

- Specification
- Header documenting file content
- CHROMosome, POSition, IDentifier of each variant
- REFerence and ALTernate allele sequence.
- INFOrmation on variants
- FORMAT of sample genotype information, followed by each genotype

VCF content: location

#CHROM	POS	ID	REF	ALT	QUAL	FILTER	...
20	14370	rs6054257	G	A	29	PASS	...
20	17330	.	T	A	3	q10	...
20	1110696	rs6040355	A	G,T	67	PASS	...
20	1230237	.	T	.	47	PASS	...
20	1234567	microsat1	GTC	G,GTCT	50	PASS	...

Lines: good SNP, poor quality SNP, multipe variants, called monomorphic, indel

VCF content: variant INFO

```
#CHROM POS      ... INFO                                    ...
20     14370    ... NS=3;DP=14;AF=0.5;DB;H2                 ...
20     17330    ... NS=3;DP=11;AF=0.017                     ...
20     1110696  ... NS=2;DP=10;AF=0.333,0.667;AA=T;DB       ...
20     1230237  ... NS=3;DP=13;AA=T                         ...
20     1234567  ... NS=3;DP=9;AA=G                          ...
```

Information supporting the SNP: NS, # samples with data; DP, total depth; AF, ancestral frequency; DB, dbSNP membership; H2, HapMap 2 membership.

VCF content: Genotype FORMAT and samples

...	POS	...	FORMAT	NA00001	NA00002
...	14370	...	GT:GQ:DP:HQ	0|0:48:1:51,51	1|0:48:8:51,51
...	17330	...	GT:GQ:DP:HQ	0|0:49:3:58,50	0|1:3:5:65,3
...	1110696	...	GT:GQ:DP:HQ	1|2:21:6:23,27	2|1:2:0:18,2
...	1230237	...	GT:GQ:DP:HQ	0|0:54:7:56,60	0|0:48:4:51,51
...	1234567	...	GT:GQ:DP	0/1:35:4	0/2:17:2

Genotype inforamation in three samples. FORMAT specifies the order and type of information: GT, Genotype, '|' phased, vs. '/' unphased; GQ, quality; DP, read depth; HQ, haplotype quality.

VCF Header

```
##fileformat=VCFv4.2
##fileDate=20090805
##source=myImputationProgramV3.1
##reference=file:///seq/references/1000GenomesPilot-NCBI36.fasta
##contig=<ID=20,length=62435964,assembly=B36,md5=f126cdf8a6e0c7f
##phasing=partial
##INFO=<ID=DP,Number=1,Type=Integer,Description="Total Depth">
##INFO=<ID=AF,Number=A,Type=Float,Description="Allele Frequency"
...
##FILTER=<ID=q10,Description="Quality below 10">
##FILTER=<ID=s50,Description="Less than 50% of samples have data
...
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=GQ,Number=1,Type=Integer,Description="Genotype Qual
```

VCF files

- Very complicated data.
- Content of INFO, FORMAT fields very flexible, depends entirely on up-stream processing.
- Often interested in only part of the file – specific genomic ranges, INFO or FORMAT fields, samples.