Community built analyses that run everywhere with bcbio

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http://bit.ly/pgp-analysis

26 June 2018

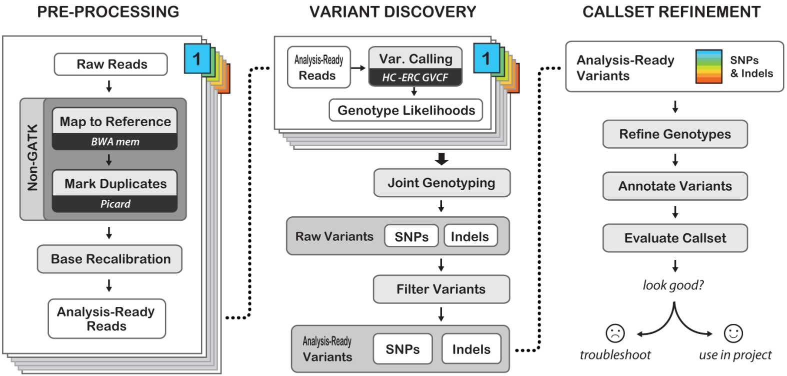
Outline

- Barriers to building analysis pipelines
- bcbio: open source community development
- Common Workflow Language (CWL): assembly language for workflows
- Practical CWL with bcbio: HPC, Cloud, DNAnexus, Arvados, SevenBridges
- Personal Genome Project n=1 analysis example
- GA4GH: Automating validation and multi-platform testing

Takeaways

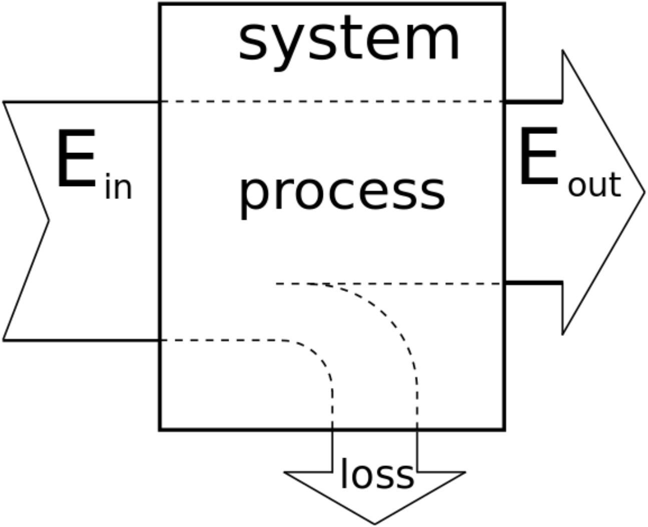
- Science = collaboration and re-use
- bcbio with interoperable workflow abstractions
- How to run bcbio analyses where you want them
- Interpreting variant calling outputs
- We can build better things together

You want to build a variant calling pipeline



Best Practices for Germline SNPs and Indels in Whole Genomes and Exomes - June 2016

https://software.broadinstitute.org/gatk/best-practices/



https://commons.wikimedia.org/wiki/File:Efficiency_diagram_by_Zureks.svg

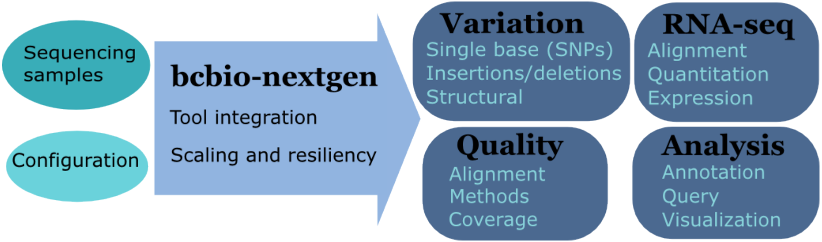
Barriers to implementing yourself

- Changing tools
- Feature support burden
- Multi-platform interoperability
- Validation

Build open source communities

https://galaxyproject.org/events/gccbosc2018/collaboration/

Overview



Sequencing samples

Configuration

bcbio-nextgen

Tool integration

Scaling and resiliency

Variation

Single base (SNPs)
Insertions/deletions
Structural

RNA-seq

Alignment
Quantitation
Expression

Quality

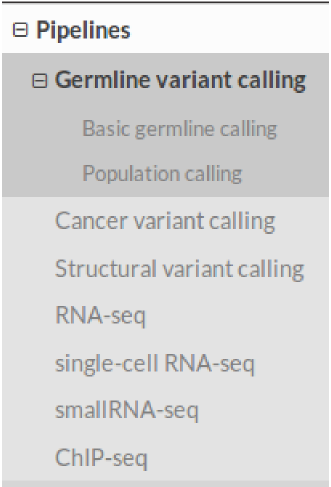
Alignment
Methods
Coverage

Analysis

Annotation
Query
Visualization

https://github.com/chapmanb/bcbio-nextgen

Supported analysis types



⊟ Pipelines

⊟ Germline variant calling

Basic germline calling

Population calling

Cancer variant calling

Structural variant calling

RNA-seq

single-cell RNA-seq

smallRNA-seq

ChIP-seq

https://bcbio-nextgen.readthedocs.org/en/latest/contents/pipelines.html

We made a pipeline – so what?

> *There have been a number of previous efforts to create publicly available analysis pipelines for high throughput sequencing data. Examples include Omics-Pipe, bcbio-nextgen, TREVA and NGSane. These pipelines offer a comprehensive, automated process that can analyse raw sequencing reads and produce annotated variant calls. However, the main audience for these pipelines is the research community. Consequently, there are many features required by clinical pipelines that these examples do not fully address. Other groups have focused on improving specific features of clinical pipelines. The Churchill pipeline uses specialised techniques to achieve high performance, while maintaining reproducibility and accuracy. However it is not freely available to clinical centres and it does not try to improve broader clinical aspects such as detailed quality assurance reports, robustness, reports and specialised variant filtering. The Mercury pipeline offers a comprehensive system that addresses many clinical needs: it uses an automated workflow system (Valence) to ensure robustness, abstract computational resources and simplify customisation of the pipeline. Mercury also includes detailed coverage reports provided by ExCID, and supports compliance with US privacy laws (HIPAA) when run on DNANexus, a cloud computing platform specialised for biomedical users. Mercury offers a comprehensive solution for clinical users, however it does not achieve our desired level of transparency, modularity and simplicity in the pipeline specification and design. Further, Mercury does not perform specialised variant filtering and prioritisation that is specifically tuned to the needs of clinical users.*

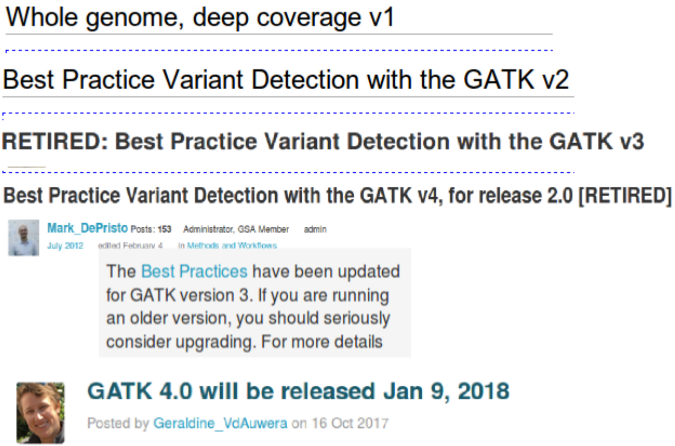
http://www.genomemedicine.com/content/7/1/68

Sustainability

A piece of software is being sustained if people are using it, fixing it, and improving it rather than replacing it.

http://software-carpentry.org/blog/2014/08/sustainability.html

Complex, rapidly changing baseline functionality



Whole genome, deep coverage v1

Best Practice Variant Detection with the GATK v2

RETIRED: Best Practice Variant Detection with the GATK v3

Best Practice Variant Detection with the GATK v4, for release 2.0 [RETIRED]

Mark_DePristo Posts: 153 Administrator, GSA Member admin
July 2012 edited February 4 in Methods and Workflows

The Best Practices have been updated for GATK version 3. If you are running an older version, you should seriously consider upgrading. For more details

GATK 4.0 will be released Jan 9, 2018

Posted by Geraldine_VdAuwera on 16 Oct 2017

Feature support burden

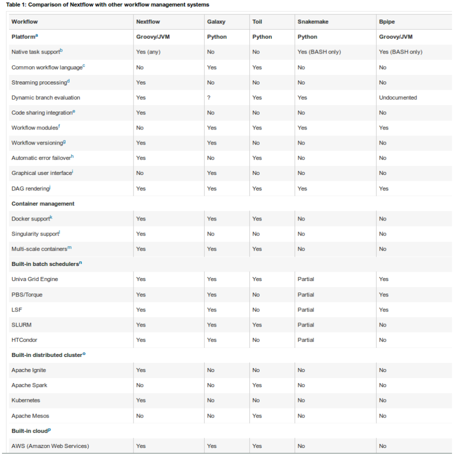
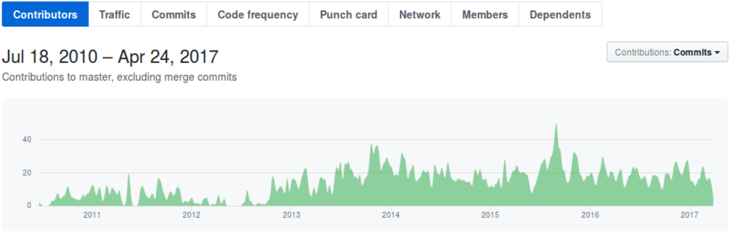


Table 1: Comparison of Nextflow with other workflow management systems

Workflow	Nextflow	Galaxy	Toil	Snakemake	Bpipe
Platform[a]	**Groovy/JVM**	**Python**	**Python**	**Python**	**Groovy/JVM**
Native task support[b]	Yes (any)	No	No	Yes (BASH only)	Yes (BASH only)
Common workflow language[c]	No	Yes	Yes	No	No
Streaming processing[d]	Yes	No	No	No	No
Dynamic branch evaluation	Yes	?	Yes	Yes	Undocumented
Code sharing integration[e]	Yes	No	No	No	No
Workflow modules[f]	No	Yes	Yes	Yes	Yes
Workflow versioning[g]	Yes	Yes	No	No	No
Automatic error failover[h]	Yes	No	Yes	No	No
Graphical user interface[i]	No	Yes	No	No	No
DAG rendering[j]	Yes	Yes	Yes	Yes	Yes
Container management					
Docker support[k]	Yes	Yes	Yes	No	No
Singularity support[l]	Yes	No	No	No	No
Multi-scale containers[m]	Yes	Yes	Yes	No	No
Built-in batch schedulers[n]					
Univa Grid Engine	Yes	Yes	Yes	Partial	Yes
PBS/Torque	Yes	Yes	No	Partial	Yes
LSF	Yes	Yes	No	Partial	Yes
SLURM	Yes	Yes	Yes	Partial	No
HTCondor	Yes	Yes	No	Partial	No
Built-in distributed cluster[o]					
Apache Ignite	Yes	No	No	No	No
Apache Spark	No	No	Yes	No	No
Kubernetes	Yes	No	No	No	No
Apache Mesos	No	No	Yes	No	No
Built-in cloud[p]					
AWS (Amazon Web Services)	Yes	Yes	Yes	No	No

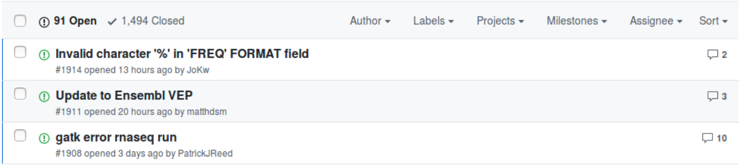
http://www.nature.com/nbt/journal/v35/n4/full/nbt.3820.html

Community: sustainability and support



Jul 18, 2010 – Apr 24, 2017

Contributions to master, excluding merge commits



□ 91 Open ✓ 1,494 Closed

□ Invalid character '%' in 'FREQ' FORMAT field — 2
#1914 opened 13 hours ago by JoKw

□ Update to Ensembl VEP — 3
#1911 opened 20 hours ago by matthdsm

□ gatk error rnaseq run — 10
#1908 opened 3 days ago by PatrickJReed

https://github.com/chapmanb/bcbio-nextgen

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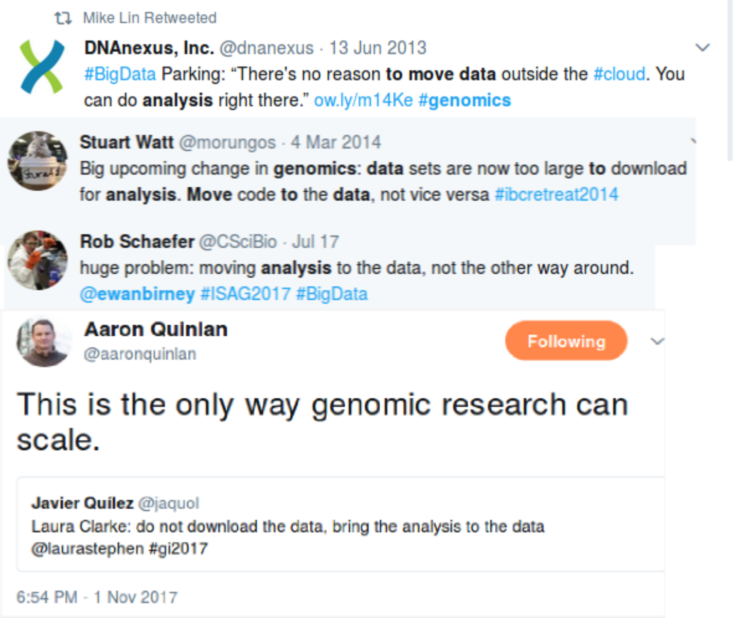
Infrastructure Goals

- Local machines
- Clusters: SLURM, SGE, Torque, PBS, LSF
- Clouds: Amazon, Google, Azure
- Clinical environments
- User interface for researchers
- Integrate with LIMS
- Accessible to the general public



Mike Lin Retweeted

DNAnexus, Inc. @dnanexus · 13 Jun 2013

#BigData Parking: “There's no reason **to move data** outside the #cloud. You can do **analysis** right there.” ow.ly/m14Ke **#genomics**

Stuart Watt @morungos · 4 Mar 2014

Big upcoming change in **genomics**: **data** sets are now too large **to** download for **analysis**. **Move** code **to** the **data**, not vice versa #ibcretreat2014

Rob Schaefer @CSciBio · Jul 17

huge problem: moving **analysis** to the data, not the other way around. **@ewanbirney** #ISAG2017 #BigData

Aaron Quinlan

@aaronquinlan

This is the only way genomic research can scale.

Javier Quilez @jaquol

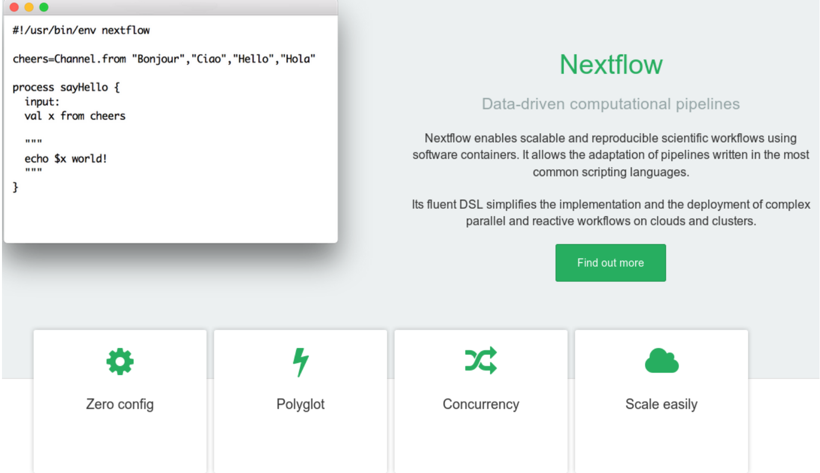
Laura Clarke: do not download the data, bring the analysis to the data @laurastephen #gi2017

6:54 PM - 1 Nov 2017

Why do we transfer data around?

- Lots of work to setup and configure an analysis
- Hard to port scalable analysis to new environment

Many great workflow systems: Nexflow



```
#!/usr/bin/env nextflow

cheers=Channel.from "Bonjour","Ciao","Hello","Hola"

process sayHello {
  input:
  val x from cheers

  """
  echo $x world!
  """
}
```

Nextflow

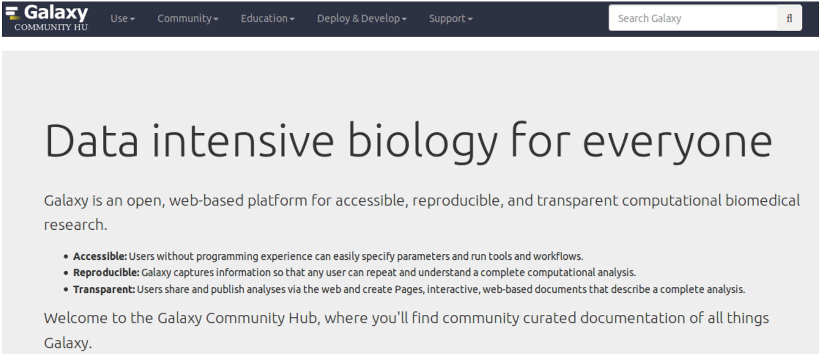
Data-driven computational pipelines

Nextflow enables scalable and reproducible scientific workflows using software containers. It allows the adaptation of pipelines written in the most common scripting languages.

Its fluent DSL simplifies the implementation and the deployment of complex parallel and reactive workflows on clouds and clusters.

http://nextflow.io/

Many great workflow systems: Galaxy



Data intensive biology for everyone

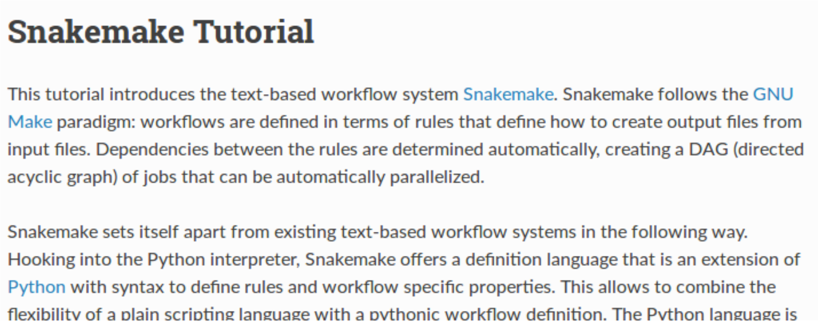
Galaxy is an open, web-based platform for accessible, reproducible, and transparent computational biomedical research.

- **Accessible:** Users without programming experience can easily specify parameters and run tools and workflows.
- **Reproducible:** Galaxy captures information so that any user can repeat and understand a complete computational analysis.
- **Transparent:** Users share and publish analyses via the web and create Pages, interactive, web-based documents that describe a complete analysis.

Welcome to the Galaxy Community Hub, where you'll find community curated documentation of all things Galaxy.

http://galaxyproject.org/

Many great workflow systems: Snakemake



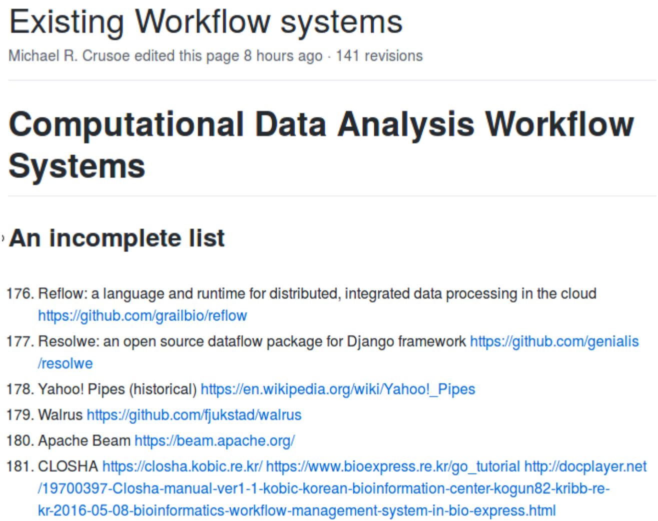
Snakemake Tutorial

This tutorial introduces the text-based workflow system Snakemake. Snakemake follows the GNU Make paradigm: workflows are defined in terms of rules that define how to create output files from input files. Dependencies between the rules are determined automatically, creating a DAG (directed acyclic graph) of jobs that can be automatically parallelized.

Snakemake sets itself apart from existing text-based workflow systems in the following way. Hooking into the Python interpreter, Snakemake offers a definition language that is an extension of Python with syntax to define rules and workflow specific properties. This allows to combine the flexibility of a plain scripting language with a pythonic workflow definition. The Python language is

https://snakemake.readthedocs.io

But, many workflow systems



Existing Workflow systems

Michael R. Crusoe edited this page 8 hours ago · 141 revisions

Computational Data Analysis Workflow Systems

An incomplete list

176. Reflow: a language and runtime for distributed, integrated data processing in the cloud https://github.com/grailbio/reflow
177. Resolwe: an open source dataflow package for Django framework https://github.com/genialis/resolwe
178. Yahoo! Pipes (historical) https://en.wikipedia.org/wiki/Yahoo!_Pipes
179. Walrus https://github.com/fjukstad/walrus
180. Apache Beam https://beam.apache.org/
181. CLOSHA https://closha.kobic.re.kr/ https://www.bioexpress.re.kr/go_tutorial http://docplayer.net/19700397-Closha-manual-ver1-1-kobic-korean-bioinformation-center-kogun82-kribb-re-kr-2016-05-08-bioinformatics-workflow-management-system-in-bio-express.html

https://github.com/common-workflow-language/common-workflow-language/wiki/Existing-Workflow-systems

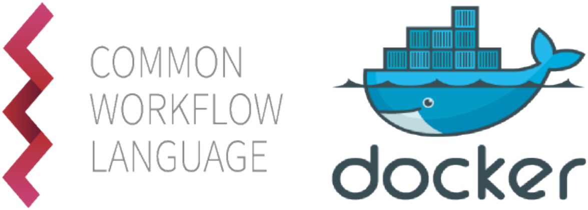
We'll never agree on one system

- Advantages and disadvantages to each
- Familiarity and teaching
- Personal preference

So we can't easily share workflows

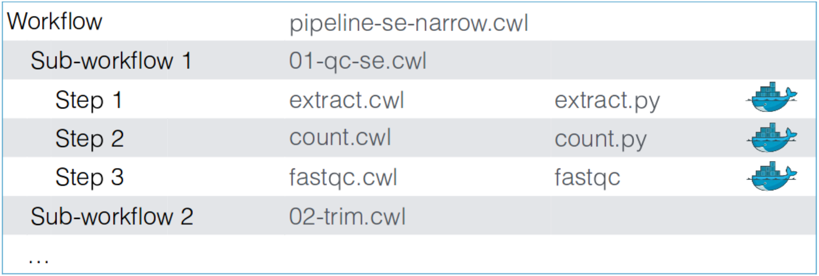
- Single workflow system allows coordinated groups
- Create barrier to sharing externally
- Hard to mix and match components between workflow environments
- How can we do better?

Better abstractions = more interoperability



https://bcbio-nextgen.readthedocs.io/en/latest/contents/cwl.html

Common Workflow Language (CWL)



Workflow	pipeline-se-narrow.cwl		
Sub-workflow 1	01-qc-se.cwl		
Step 1	extract.cwl	extract.py	🐳
Step 2	count.cwl	count.py	🐳
Step 3	fastqc.cwl	fastqc	🐳
Sub-workflow 2	02-trim.cwl		
...			

http://www.commonwl.org/

https://f1000research.com/slides/5-1617

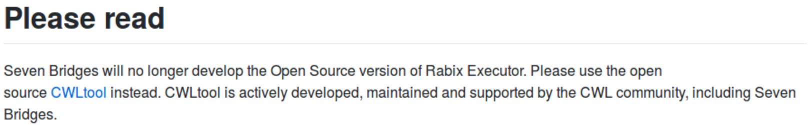
Workflow Description Language (WDL)

http://openwdl.org/

Why use a workflow abstraction?

- Integrate with multiple platforms
  - Toil – local
  - Cromwell – HPC, Cloud, local
  - Arvados
  - DNAnexus
  - Seven Bridges + Cancer Genomics Cloud
- Stop maintaining bcbio specific infrastructure
- Focus on hard biological problems

Advantages of CWL: platform resiliency

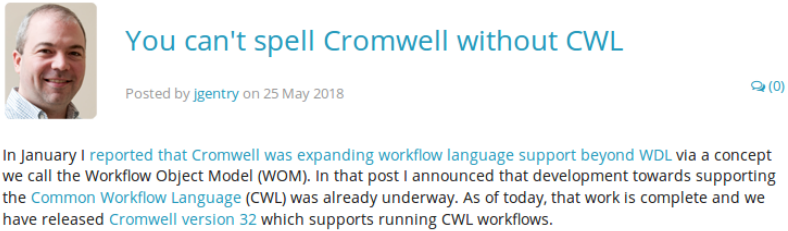


Please read

Seven Bridges will no longer develop the Open Source version of Rabix Executor. Please use the open source CWLtool instead. CWLtool is actively developed, maintained and supported by the CWL community, including Seven Bridges.

https://github.com/rabix/bunny

Advantages of CWL: platform resiliency



You can't spell Cromwell without CWL

Posted by jgentry on 25 May 2018

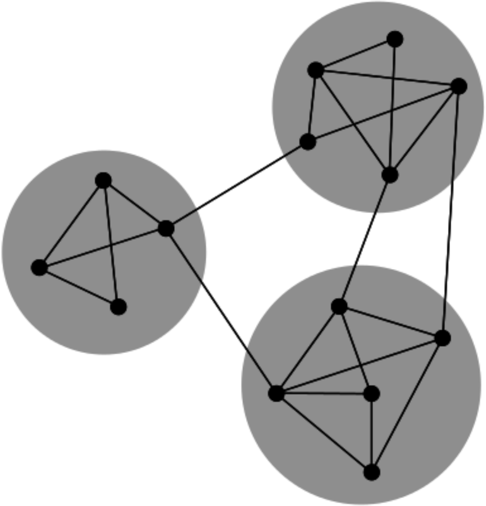
In January I reported that Cromwell was expanding workflow language support beyond WDL via a concept we call the Workflow Object Model (WOM). In that post I announced that development towards supporting the Common Workflow Language (CWL) was already underway. As of today, that work is complete and we have released Cromwell version 32 which supports running CWL workflows.

https://github.com/broadinstitute/cromwell/

Unique goals with CWL

- Multiple concurrent production environments
  - HPC
  - External vendors (DNAnexus, SevenBridges, Arvados)
  - Direct on Cloud (AWS, GCE, Azure)
- Coordinated release and update process
  - Workflow
  - Tools in containers
  - Reference data

Connections



By jham3 - Own work, CC BY-SA 3.0,
https://commons.wikimedia.org/w/index.php?curid=17125894

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CWL in bcbio

- Start with high level configuration file
- Generate CWL
- Run, on any infrastructure that supports CWL
  - Generated CWL
  - Docker or local bcbio installation
  - Genome data

https://bcbio-nextgen.readthedocs.io/en/latest/contents/cwl.html

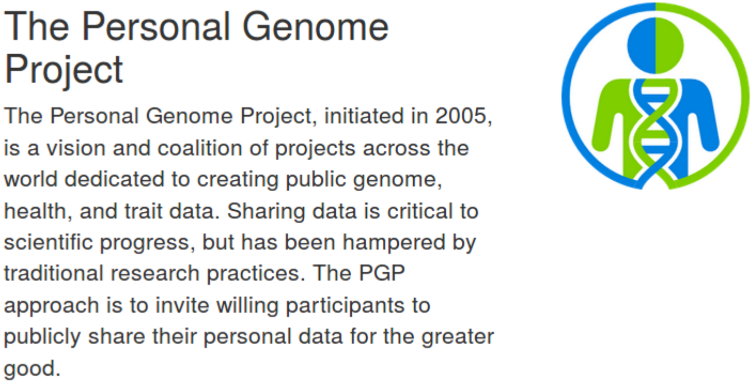
bcbio-vm: CWL wrapper

- bcbio-like interface integrating with external tools
- Install wrapper plus supported runners

```
conda install -c conda-forge -c bioconda bcbio-nextgen-vm
```

https://github.com/chapmanb/bcbio-nextgen-vm
https://bioconda.github.io/

Practical example: Personal Genome Project

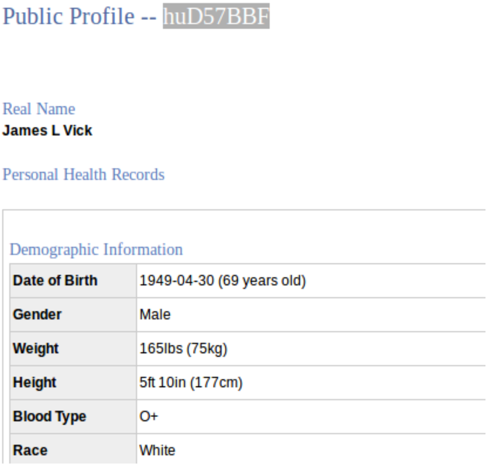


The Personal Genome Project

The Personal Genome Project, initiated in 2005, is a vision and coalition of projects across the world dedicated to creating public genome, health, and trait data. Sharing data is critical to scientific progress, but has been hampered by traditional research practices. The PGP approach is to invite willing participants to publicly share their personal data for the greater good.

http://www.personalgenomes.org/us

Whole genome sequencing data plus metadata



Public Profile -- huD57BBF

Real Name

James L Vick

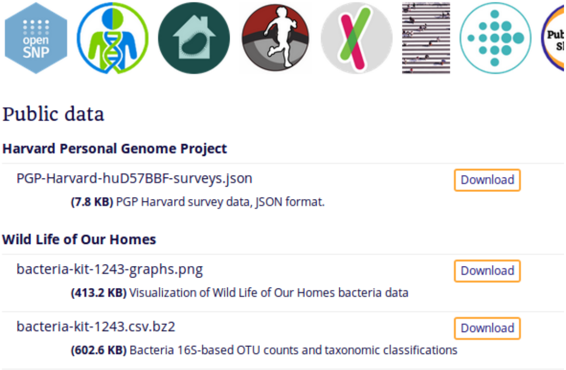
Personal Health Records

Demographic Information

Date of Birth	1949-04-30 (69 years old)
Gender	Male
Weight	165lbs (75kg)
Height	5ft 10in (177cm)
Blood Type	O+
Race	White

https://my.pgp-hms.org/profile/huD57BBF

Rich set of associated data



Public data

Harvard Personal Genome Project

PGP-Harvard-huD57BBF-surveys.json — Download

(7.8 KB) PGP Harvard survey data, JSON format.

Wild Life of Our Homes

bacteria-kit-1243-graphs.png — Download

(413.2 KB) Visualization of Wild Life of Our Homes bacteria data

bacteria-kit-1243.csv.bz2 — Download

(602.6 KB) Bacteria 16S-based OTU counts and taxonomic classifications

https://www.openhumans.org/member/jameslvick/

Template: describe your analysis

```
- files: huD57BBF.bam
  description: huD57BBF
  analysis: variant
  genome_build: hg38
  algorithm:
    aligner: bwa
    variantcaller: gatk-haplotype
    svcaller: [manta, lumpy, cnvkit]
    hlacaller: optitype
```

https://github.com/bcbio/bcbio_validation_workflows

Local filesystem environment

```
local:
  ref: biodata/collections
  inputs:
    - biodata/regions
    - biodata/pgp
resources:
  default:
    cores: 8
    memory: 3500M
    jvm_opts: [-Xms750m, -Xmx3500m]
```

Equivalent on a remote platform

```
arvados:
  reference: su92l-4zz18-3p00f79y4p535ia
  input: [su92l-4zz18-ihm3wrgyuwcmsx1]
resources:
  default: {cores: 16, memory: 3500M,
            jvm_opts: [-Xms1g, -Xmx3500m]}
```

Build Common Workflow Language description

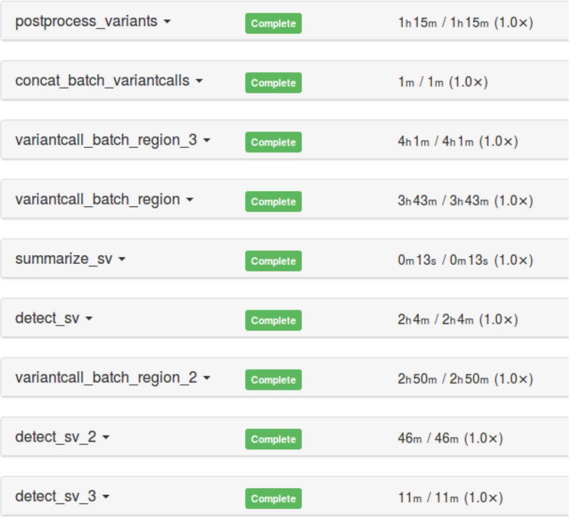
```
bcbio_vm.py cwl --systemconfig bcbio_system-arvados.yaml \
  pgp_sv_hla.yaml
```

Launch analysis

```
bcbio_vm.py cwlrun arvados pgp_sv_hla-workflow -- \
  --project-uuid su92l-j7d0g-eoibug3nrwg8ysj
```

https://workbench.su92l.arvadosapi.com/projects/su92l-j7d0g-eoibug3nrwg8ysj

Arvados pipeline run



postprocess_variants ▾	Complete	1h15m / 1h15m (1.0×)
concat_batch_variantcalls ▾	Complete	1m / 1m (1.0×)
variantcall_batch_region_3 ▾	Complete	4h1m / 4h1m (1.0×)
variantcall_batch_region ▾	Complete	3h43m / 3h43m (1.0×)
summarize_sv ▾	Complete	0m13s / 0m13s (1.0×)
detect_sv ▾	Complete	2h4m / 2h4m (1.0×)
variantcall_batch_region_2 ▾	Complete	2h50m / 2h50m (1.0×)
detect_sv_2 ▾	Complete	46m / 46m (1.0×)
detect_sv_3 ▾	Complete	11m / 11m (1.0×)

Generate CWL for local or HPC run

```
bcbio_vm.py cwl --systemconfig bcbio_system-local.yaml \
  pgp_sv_hla.yaml
```

Run multicore on single machine with Toil

```
bcbio_vm.py cwlrun toil pgp_sv_hla-workflow
```

http://toil.readthedocs.io

Run distributed on SLURM cluster with Cromwell

```
bcbio_vm.py cwlrun cromwell pgp_sv_hla-workflow \
  --no-container \
  -q your_queue -s slurm -r timelimit=0-12:00
```

http://cromwell.readthedocs.io

Run on DNAnexus platform

```
dnanexus:
  project: PGP
  ref:
    project: bcbio_resources
    folder: /reference_genomes
  inputs:
    - /data/input
resources:
  default:
    cores: 8
    memory: 3500M
    jvm_opts: [-Xms750m, -Xmx3500m]
```

https://platform.dnanexus.com

DNAnexus: upload configuration

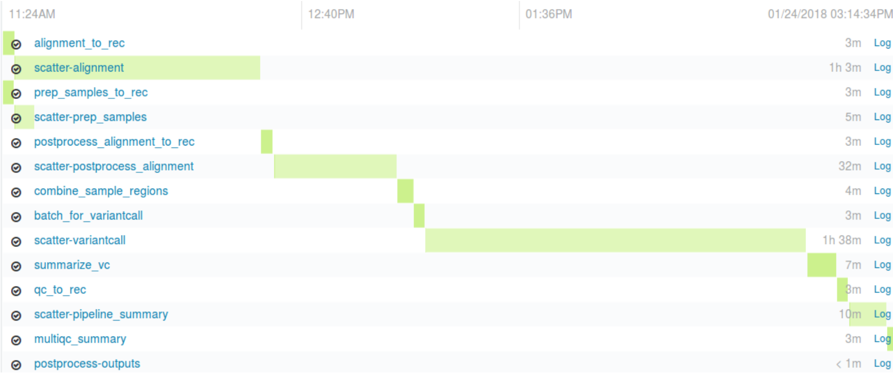
```
PNAME=pgp_sv_hla
TEMPLATE=svcall
PROJECT=PGP

dx select $PROJECT
dx mkdir -p $PNAME
for F in $TEMPLATE.yaml $PNAME.csv bcbio_system.yaml
do
        dx rm -a /$PNAME/$F || true
        dx upload --path /$PNAME/ $F
done
```

DNAnexus: run bcbio CWL applet

```
dx run bcbio_resources:/applets/bcbio-run-workflow \
  -iyaml_template=/$PNAME/$TEMPLATE.yaml \
  -isample_spec=/$PNAME/$PNAME.csv \
  -isystem_configuration=/$PNAME/bcbio_system.yaml \
  -ioutput_folder=/$PNAME/dx-cwl-run
```

https://github.com/bcbio/bcbio-dnanexus-wrapper

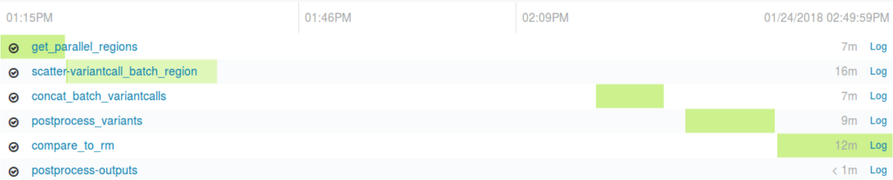
DNAnexus monitoring: align, variant call, QC



11:24AM 12:40PM 01:36PM 01/24/2018 03:14:34PM

✓	alignment_to_rec	3m	Log
✓	scatter-alignment	1h 3m	Log
✓	prep_samples_to_rec	3m	Log
✓	scatter-prep_samples	5m	Log
✓	postprocess_alignment_to_rec	3m	Log
✓	scatter-postprocess_alignment	32m	Log
✓	combine_sample_regions	4m	Log
✓	batch_for_variantcall	3m	Log
✓	scatter-variantcall	1h 38m	Log
✓	summarize_vc	7m	Log
✓	qc_to_rec	3m	Log
✓	scatter-pipeline_summary	10m	Log
✓	multiqc_summary	3m	Log
✓	postprocess-outputs	< 1m	Log

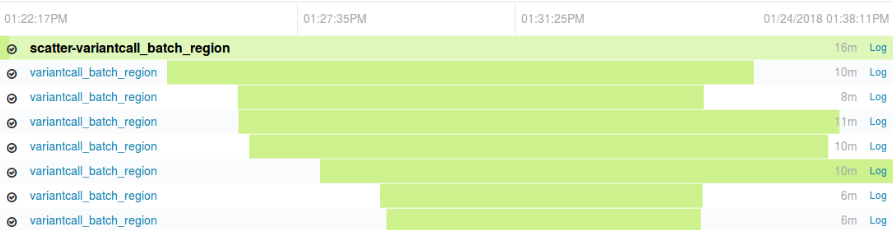
Subworkflow parallelization: per sample or batch



01:15PM 01:46PM 02:09PM 01/24/2018 02:49:59PM

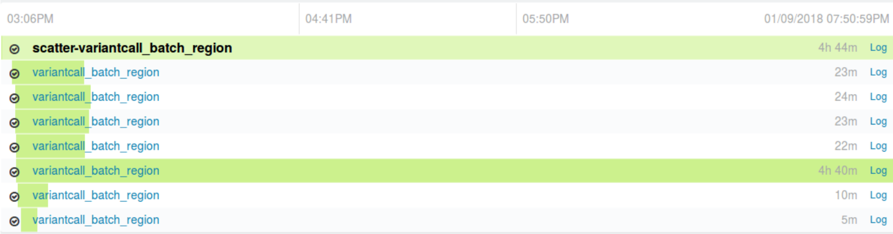
get_parallel_regions	7m	Log
scatter-variantcall_batch_region	16m	Log
concat_batch_variantcalls	7m	Log
postprocess_variants	9m	Log
compare_to_rm	12m	Log
postprocess-outputs	< 1m	Log

Variant calling parallelization: per region



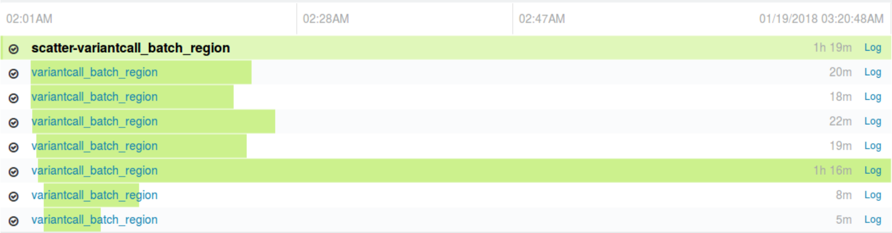
scatter-variantcall_batch_region	16m	Log
variantcall_batch_region	10m	Log
variantcall_batch_region	8m	Log
variantcall_batch_region	11m	Log
variantcall_batch_region	10m	Log
variantcall_batch_region	10m	Log
variantcall_batch_region	6m	Log
variantcall_batch_region	6m	Log

Region problem: long tail jobs



03:06PM	04:41PM	05:50PM	01/09/2018 07:50:59PM
scatter-variantcall_batch_region		4h 44m	Log
variantcall_batch_region		23m	Log
variantcall_batch_region		24m	Log
variantcall_batch_region		23m	Log
variantcall_batch_region		22m	Log
variantcall_batch_region		4h 40m	Log
variantcall_batch_region		10m	Log
variantcall_batch_region		5m	Log

Region improvement: multicore Spark parallelization



02:01AM 02:28AM 02:47AM 01/19/2018 03:20:48AM

✓	**scatter-variantcall_batch_region**	1h 19m	Log
✓	variantcall_batch_region	20m	Log
✓	variantcall_batch_region	18m	Log
✓	variantcall_batch_region	22m	Log
✓	variantcall_batch_region	19m	Log
✓	variantcall_batch_region	1h 16m	Log
✓	variantcall_batch_region	8m	Log
✓	variantcall_batch_region	5m	Log

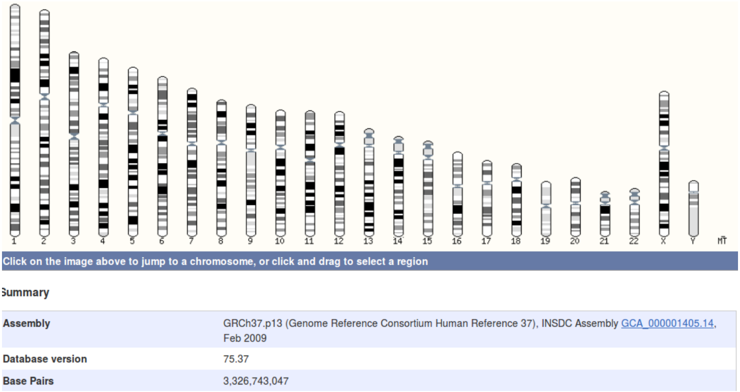
Outline

- Barriers to building analysis pipelines
- bcbio: open source community development
- Common Workflow Language (CWL): assembly language for workflows
- Practical CWL with bcbio: HPC, Cloud, DNAnexus, Arvados, SevenBridges
- **Personal Genome Project n=1 analysis example**
- GA4GH: Automating validation and multi-platform testing

Looking at data – interpreting the results

- Overview of variant calling
- Example of bcbio outputs in PGP data, n=1 analysis
- Small variants
- HLA calls
- Structural variants

Human whole genome sequencing

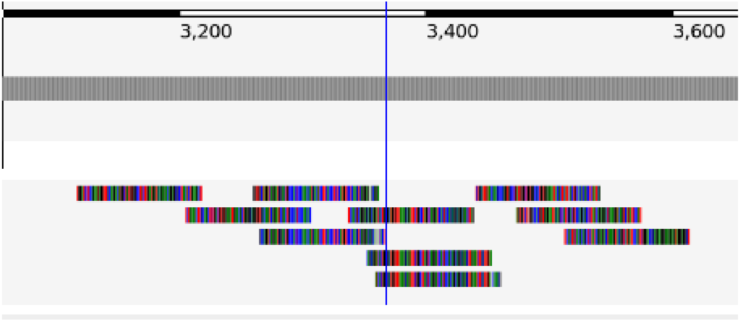

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y
MT
Click on the image above to jump to a chromosome, or click and drag to select a region

Summary

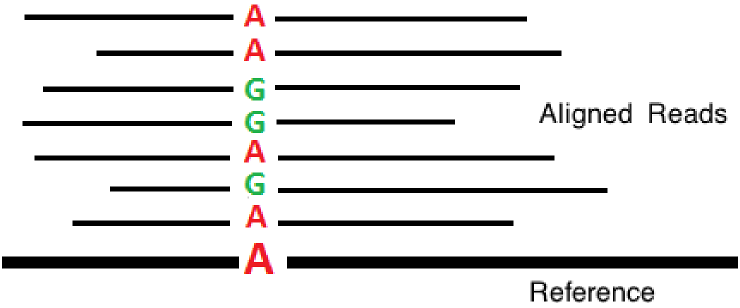
Assembly	GRCh37.p13 (Genome Reference Consortium Human Reference 37), INSDC Assembly GCA_000001405.14, Feb 2009
Database version	75.37
Base Pairs	3,326,743,047

http://ensembl.org/Homo_sapiens/Location/Genome

High throughput sequencing

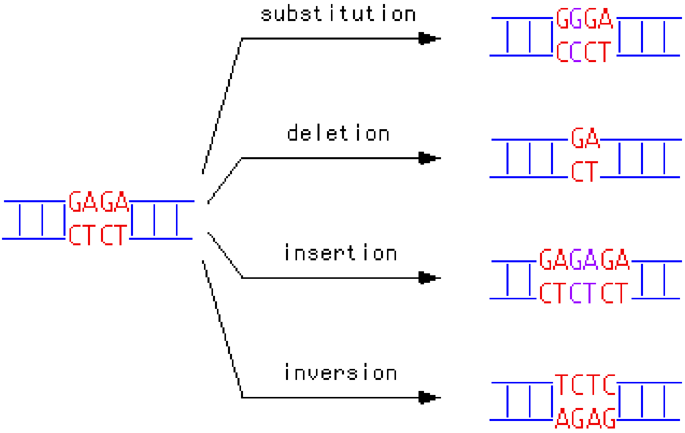


Variant calling



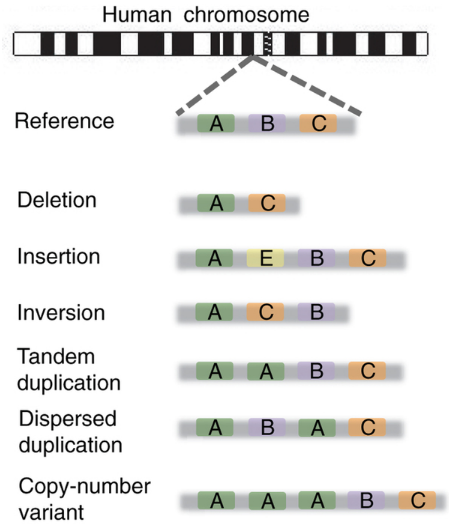
http://en.wikipedia.org/wiki/SNV_calling_from_NGS_data

SNPs and Indels



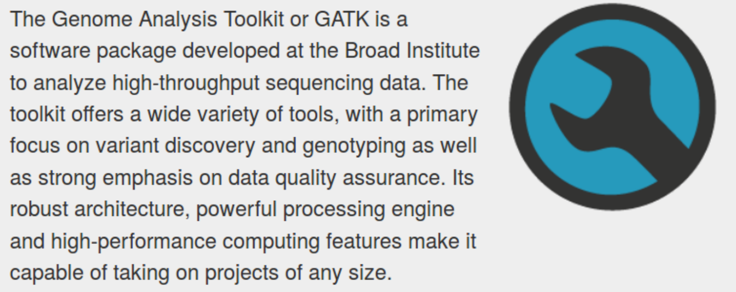
http://carolguze.com/text/442-2-mutations.shtml

Structural variations



http://www.nature.com/nmeth/journal/v9/n2/full/nmeth.1858.html

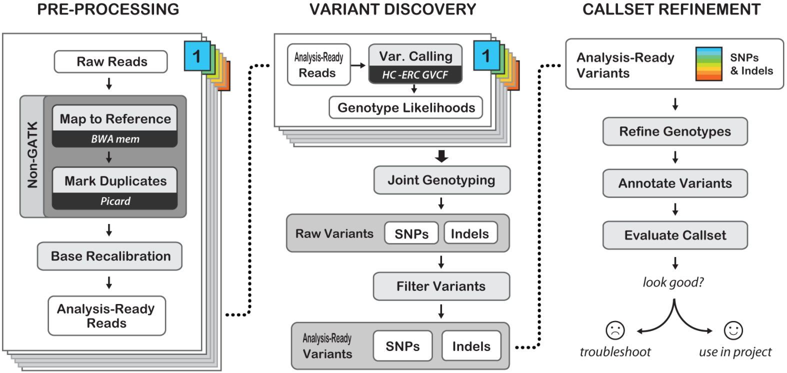
Genome Analysis Toolkit (GATK)



The Genome Analysis Toolkit or GATK is a software package developed at the Broad Institute to analyze high-throughput sequencing data. The toolkit offers a wide variety of tools, with a primary focus on variant discovery and genotyping as well as strong emphasis on data quality assurance. Its robust architecture, powerful processing engine and high-performance computing features make it capable of taking on projects of any size.

https://www.broadinstitute.org/gatk/

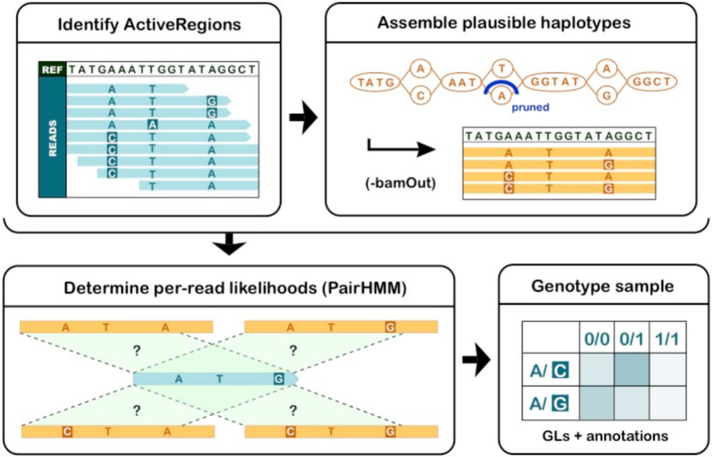
GATK Best Practices



Best Practices for Germline SNPs and Indels in Whole Genomes and Exomes - June 2016

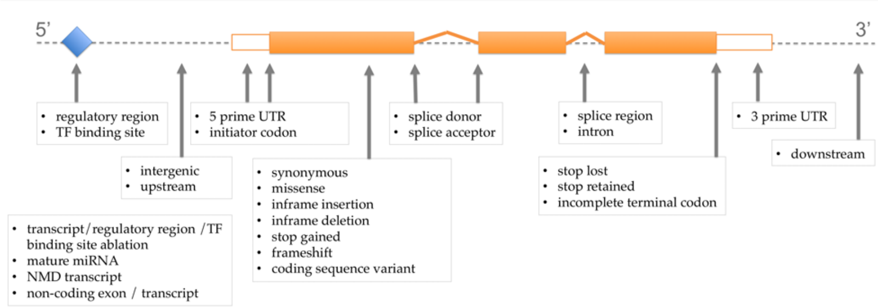
https://software.broadinstitute.org/gatk/best-practices/

HaplotypeCaller



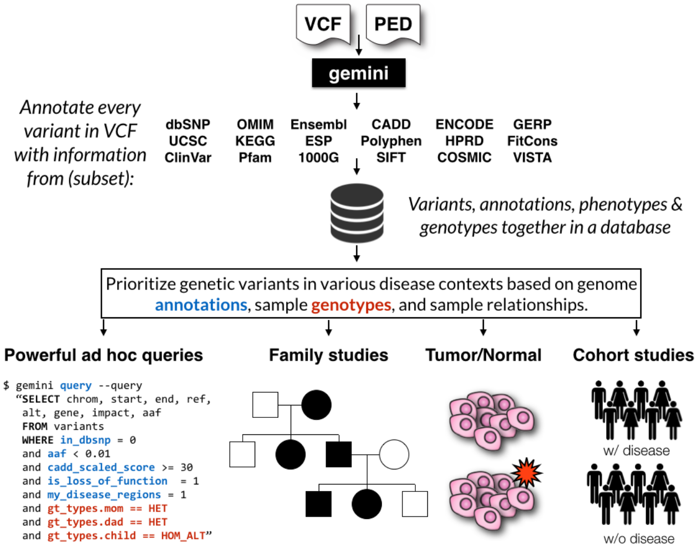
http://gatkforums.broadinstitute.org/discussion/5464/workshop-presentations-2015-uk-4-20-24

Effects prediction



http://www.ensembl.org/info/genome/variation/predicted_data.html

Annotation and analysis – GEMINI



Annotate every variant in VCF with information from (subset):

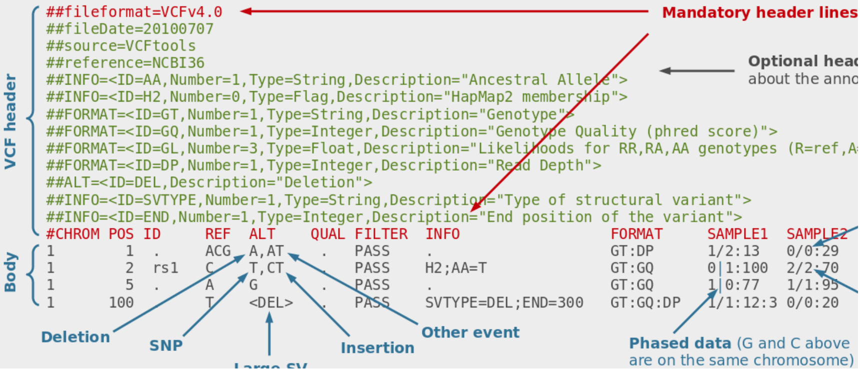
Variants, annotations, phenotypes & genotypes together in a database

Prioritize genetic variants in various disease contexts based on genome **annotations**, sample **genotypes**, and sample relationships.

```
$ gemini query --query
  “SELECT chrom, start, end, ref,
   alt, gene, impact, aaf
   FROM variants
   WHERE in_dbsnp = 0
   and aaf < 0.01
   and cadd_scaled_score >= 30
   and is_loss_of_function  = 1
   and my_disease_regions = 1
   and gt_types.mom == HET
   and gt_types.dad == HET
   and gt_types.child == HOM_ALT”
```

https://github.com/arq5x/gemini

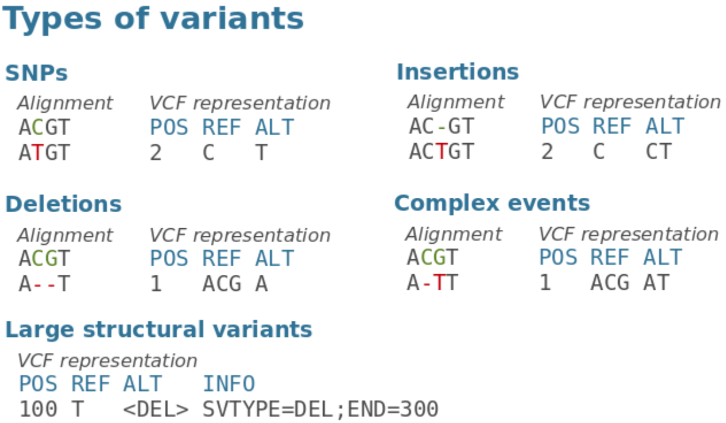
VCF – overview



```
##fileformat=VCFv4.0
##fileDate=20100707
##source=VCFtools
##reference=NCBI36
##INFO=<ID=AA,Number=1,Type=String,Description="Ancestral Allele">
##INFO=<ID=H2,Number=0,Type=Flag,Description="HapMap2 membership">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=GQ,Number=1,Type=Integer,Description="Genotype Quality (phred score)">
##FORMAT=<ID=GL,Number=3,Type=Float,Description="Likelihoods for RR,RA,AA genotypes (R=ref,A
##FORMAT=<ID=DP,Number=1,Type=Integer,Description="Read Depth">
##ALT=<ID=DEL,Description="Deletion">
##INFO=<ID=SVTYPE,Number=1,Type=String,Description="Type of structural variant">
##INFO=<ID=END,Number=1,Type=Integer,Description="End position of the variant">
#CHROM POS ID     REF  ALT    QUAL FILTER  INFO                 FORMAT     SAMPLE1   SAMPLE2
1        1 .      ACG  A,AT     .  PASS    .                    GT:DP      1/2:13    0/0:29
1        2 rs1    C    T,CT     .  PASS    H2;AA=T              GT:GQ      0|1:100   2/2:70
1        5 .      A    G        .  PASS    .                    GT:GQ      1|0:77    1/1:95
1      100 .      T    <DEL>    .  PASS    SVTYPE=DEL;END=300   GT:GQ:DP   1/1:12:3  0/0:20
```

http://vcftools.sourceforge.net/VCF-poster.pdf

VCF – representations



Types of variants

SNPs

Alignment	*VCF representation*		
ACGT	POS	REF	ALT
ATGT	2	C	T

Insertions

Alignment	*VCF representation*		
AC-GT	POS	REF	ALT
ACTGT	2	C	CT

Deletions

Alignment	*VCF representation*		
ACGT	POS	REF	ALT
A--T	1	ACG	A

Complex events

Alignment	*VCF representation*		
ACGT	POS	REF	ALT
A-TT	1	ACG	AT

Large structural variants

VCF representation

```
POS REF ALT   INFO
100 T   <DEL> SVTYPE=DEL;END=300
```

http://vcftools.sourceforge.net/VCF-poster.pdf

Learning to read VCFs

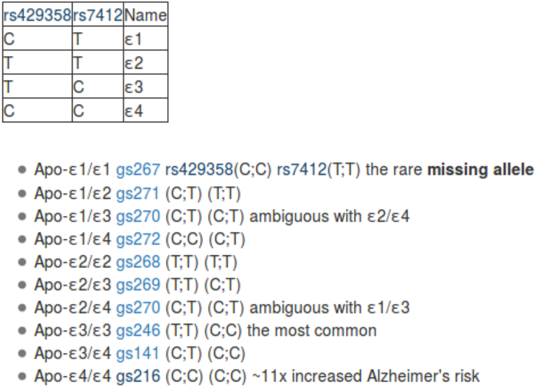
- Step by step guide from Broad

https://www.broadinstitute.org/gatk/guide/article?id=1268

- Specification

http://samtools.github.io/hts-specs/

- ApoE https://www.snpedia.com/index.php/APOE
- Two variants, on chromosome 19, that impact risk of Alzheimer's disease and cholesterol metabolism



rs429358	rs7412	Name
C	T	ε1
T	T	ε2
T	C	ε3
C	C	ε4

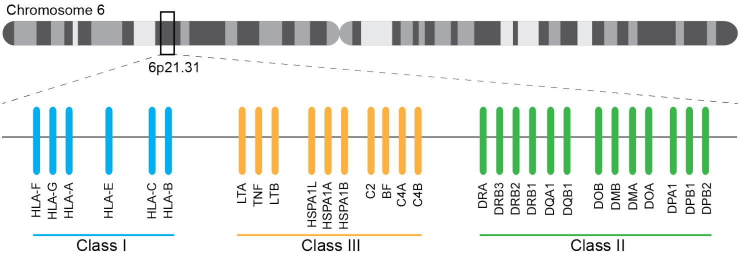
- Apo-ε1/ε1 gs267 rs429358(C;C) rs7412(T;T) the rare **missing allele**
- Apo-ε1/ε2 gs271 (C;T) (T;T)
- Apo-ε1/ε3 gs270 (C;T) (C;T) ambiguous with ε2/ε4
- Apo-ε1/ε4 gs272 (C;C) (C;T)
- Apo-ε2/ε2 gs268 (T;T) (T;T)
- Apo-ε2/ε3 gs269 (T;T) (C;T)
- Apo-ε2/ε4 gs270 (C;T) (C;T) ambiguous with ε1/ε3
- Apo-ε3/ε3 gs246 (T;T) (C;C) the most common
- Apo-ε3/ε4 gs141 (C;T) (C;C)
- Apo-ε4/ε4 gs216 (C;C) (C;C) ~11x increased Alzheimer's risk

ApoE analysis

```
$ tabix huD57BBF-gatk-haplotype.vcf.gz
    chr19:44908684-44908684
chr19   44908684        rs429358        T       C
1116.80    PASS
ANN=C|missense_variant|MODERATE|APOE|c.388T>C|p.Cys130Arg
GT:AD:DP:GQ:MMQ:PL      1/1:0,26:26:78:60:1145,78,0
$ tabix huD57BBF-gatk-haplotype.vcf.gz
     chr19:44908822-44908822
```

http://bit.ly/pgp-analysis

Major histocompatibility complex (MHC) – HLAs



http://www.ebi.ac.uk/ipd/imgt/hla/

http://sciscogenetics.com/technology/human-leukocyte-antigen-complex/

HLA typing

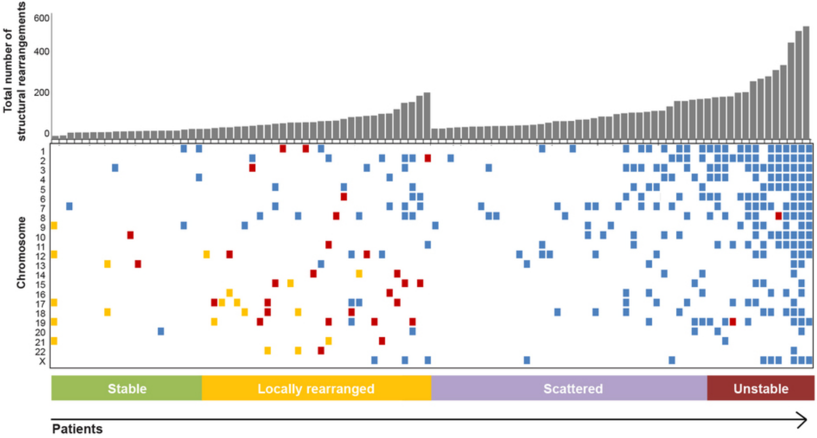
- 1000 genomes: build 38 + IMGT/HLA-3.18.0
- bwa mem extracts HLA reads
- Map reads only to HLA sequences
- OptiType: Call HLA types

https://github.com/lh3/bwa/blob/master/README-alt.md\#hla-typing
https://github.com/FRED-2/OptiType

HLA outputs

```
HLA-A*11:01;HLA-A*24:02
HLA-B*27:05;HLA-B*55:01
HLA-C*07:02;HLA-C*07:02
```

Structural variants critical – pancreatic cancer example



http://www.nature.com/nature/journal/v518/n7540/full/nature14169.html

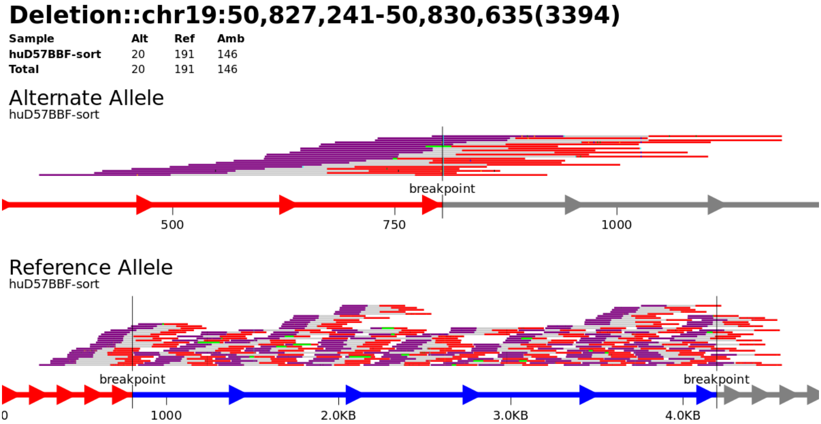
Tools used

- Manta: https://github.com/Illumina/manta
  Split and paired end reads
- Lumpy: https://github.com/arq5x/lumpy-sv
  Split and paired ends reads
- CNVkit: https://github.com/etal/cnvkit
  Read depth based

Example deletion call – 3 callers

```
chr19   50827242        MantaDEL:67020:0:1:0:0:0
T   <DEL>   658.0 PASS
END=50830636;SVTYPE=DEL;SVLEN=-3394;
ANN=<DEL>|bidirectional_gene_fusion|HIGH|AC011523.2&KLK15|
ENSG00000267968&ENSG00000174562|gene_variant|
GT:FT:GQ:PL:PR:SR       0/1:PASS:504:708,0,501:18,16:23,12
```

Viewing deletion – svviz

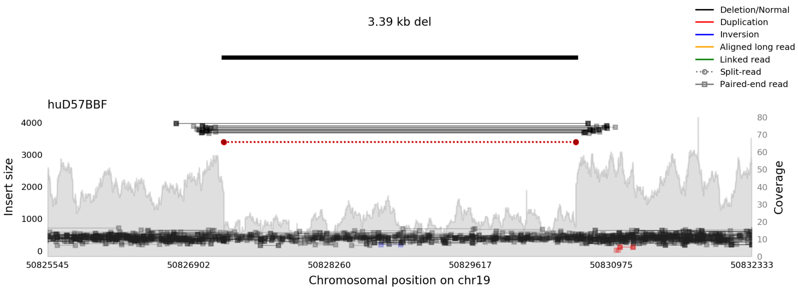


Deletion::chr19:50,827,241-50,830,635(3394)

Sample	Alt	Ref	Amb
huD57BBF-sort	20	191	146
Total	20	191	146

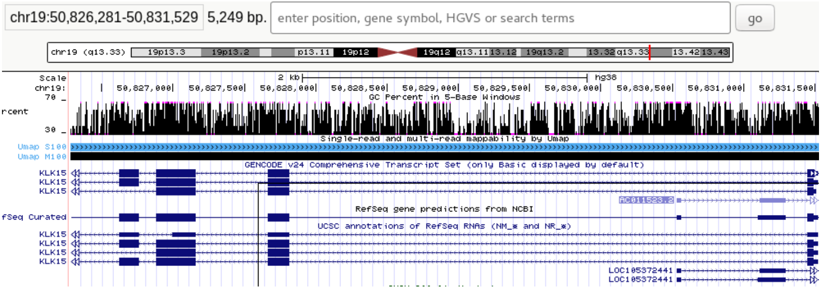
http://svviz.readthedocs.io

Viewing deletion – SV-plaudit



https://github.com/jbelyeu/SV-plaudit

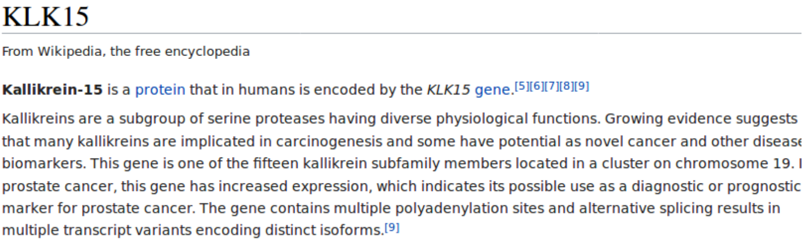
Genomic region with deletion – KLK15



chr19:50,826,281-50,831,529 5,249 bp.

http://genome.ucsc.edu/cgi-bin/hgTracks?db=hg38

KLK15 known function



KLK15

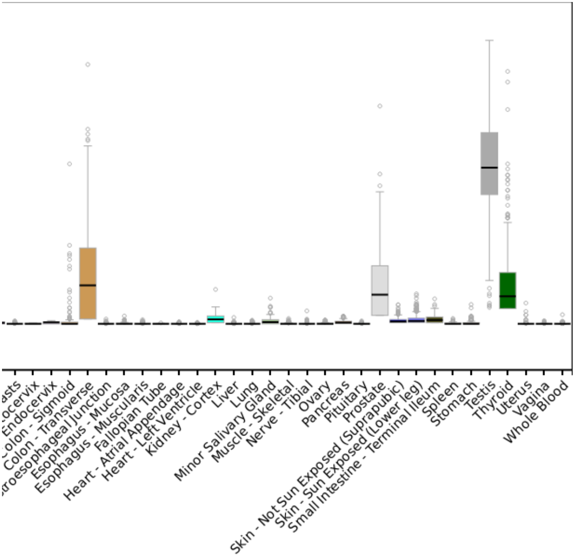
From Wikipedia, the free encyclopedia

Kallikrein-15 is a protein that in humans is encoded by the *KLK15* gene.[5][6][7][8][9]

Kallikreins are a subgroup of serine proteases having diverse physiological functions. Growing evidence suggests that many kallikreins are implicated in carcinogenesis and some have potential as novel cancer and other disease biomarkers. This gene is one of the fifteen kallikrein subfamily members located in a cluster on chromosome 19. I prostate cancer, this gene has increased expression, which indicates its possible use as a diagnostic or prognostic marker for prostate cancer. The gene contains multiple polyadenylation sites and alternative splicing results in multiple transcript variants encoding distinct isoforms.[9]

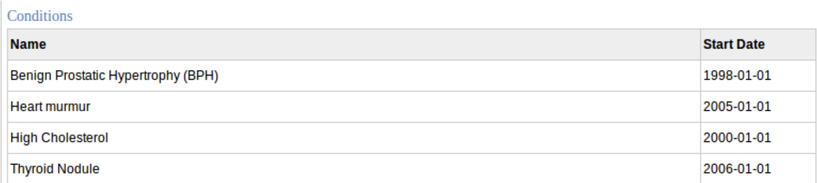
https://en.wikipedia.org/wiki/KLK15

Tissue specific gene expression



https://www.gtexportal.org/home/gene/ENSG00000174562.9

Self reported conditions



Conditions

Name	Start Date
Benign Prostatic Hypertrophy (BPH)	1998-01-01
Heart murmur	2005-01-01
High Cholesterol	2000-01-01
Thyroid Nodule	2006-01-01

`https://my.pgp-hms.org/profile/huD57BBF`

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- Personal Genome Project n=1 analysis example
- **GA4GH: Automating validation and multi-platform testing**

Validation: key component of bcbio

- Pre-built workflows with known outputs
- Cover multiple cases: germline, somatic, low frequency, FFPE, structural variants
- Large collections of diverse workflows

https://github.com/bcbio/bcbio_validation_workflows

Value of validation

- Integration tests for pipelines
- Unbiased algorithm comparisons
- Baseline for improving methods
- Automated tests for platforms

Reference materials

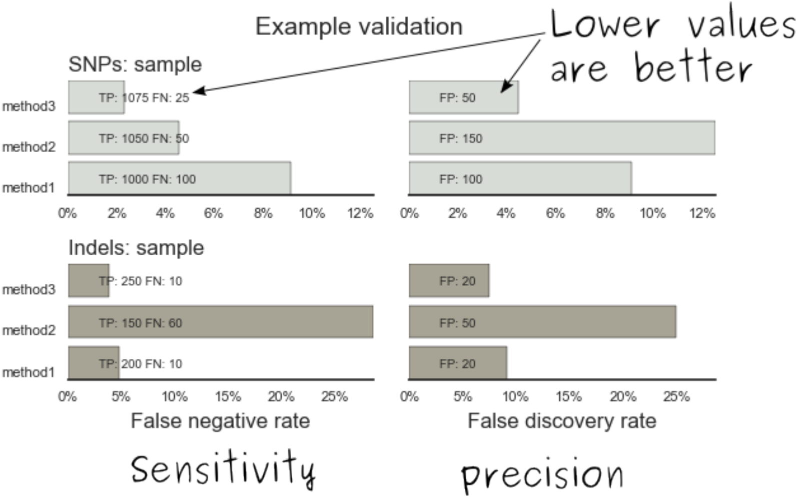
Genome in a Bottle Consortium

Global Alliance for Genomics & Health

ICGC-TCGA DREAM Mutation Calling challenge

http://www.genomeinabottle.org/
http://ga4gh.org/\#/benchmarking-team
https://www.synapse.org/\#!Synapse:syn312572

Validation graphs

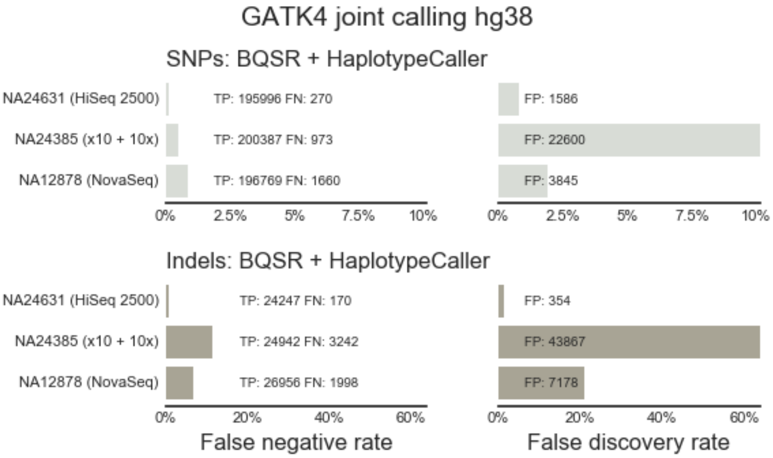


Lower values are better

Sensitivity

precision

NA12878, NA24385, NA24631 GATK4 joint calling



https://github.com/bcbio/bcbio_validations/tree/master/gatk4

Need continuous integration process

- Automate testing across multiple platforms
- Test new workflow definitions
- Test new tools and algorithms
- Transparent process

GA4GH: workflow coordination

https://www.synapse.org/#!Synapse:syn8507133/wiki/415976

GA4GH: next steps

- Automation of validation
- Workflow Execution Service (WES)
- Shared API for running CWL/WDL workflows
- Contributors welcome

https://github.com/ga4gh/workflow-execution-schemas

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Takeaways

- Science = collaboration and re-use
- bcbio with interoperable workflow abstractions
- How to run bcbio analyses where you want them
- Interpreting variant calling outputs
- We can build better things together