Next challenges for the DNA sequencing pipeline

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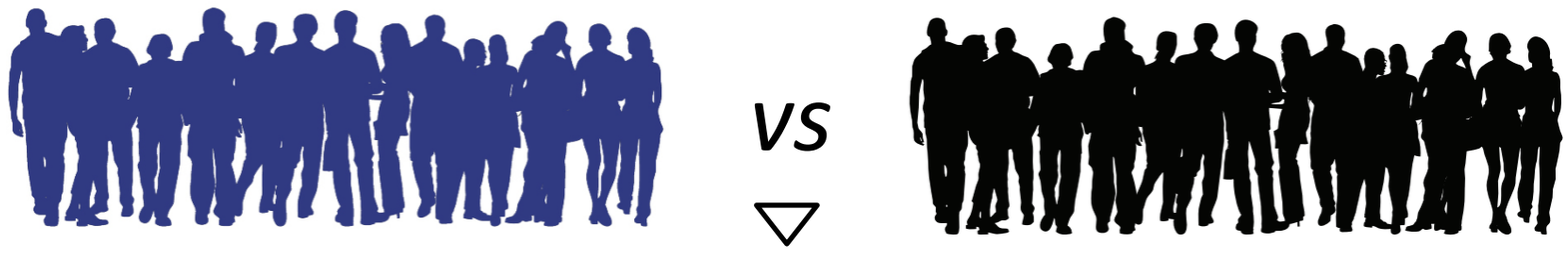
To fully understand **one** genome we need **tens of thousands** of genomes

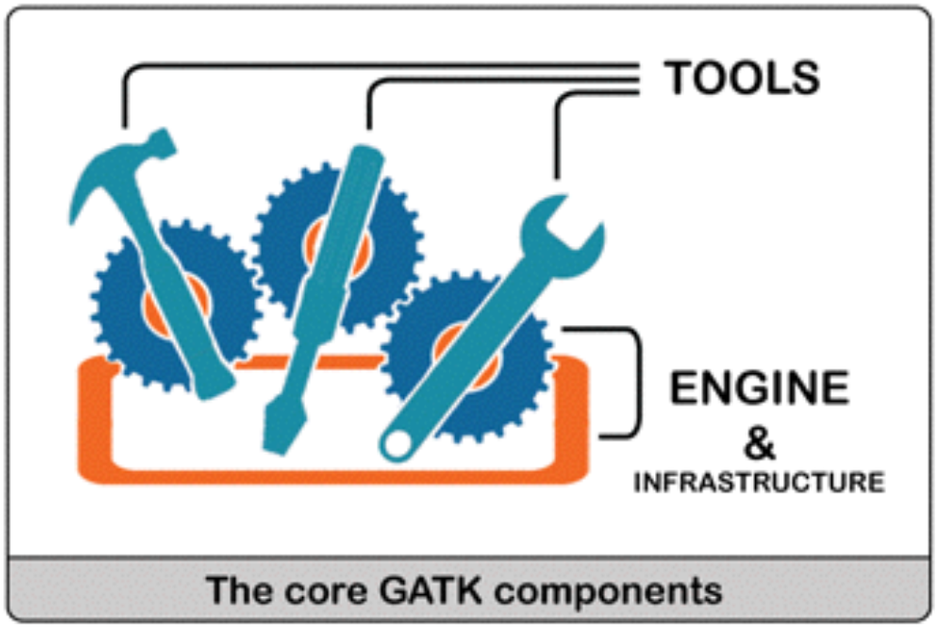
Rare Variant Association Study (RVAS)

Common Variant Association Study (CVAS)



GATK is both a toolkit and a programming framework, enabling NGS analysis by scientists worldwide

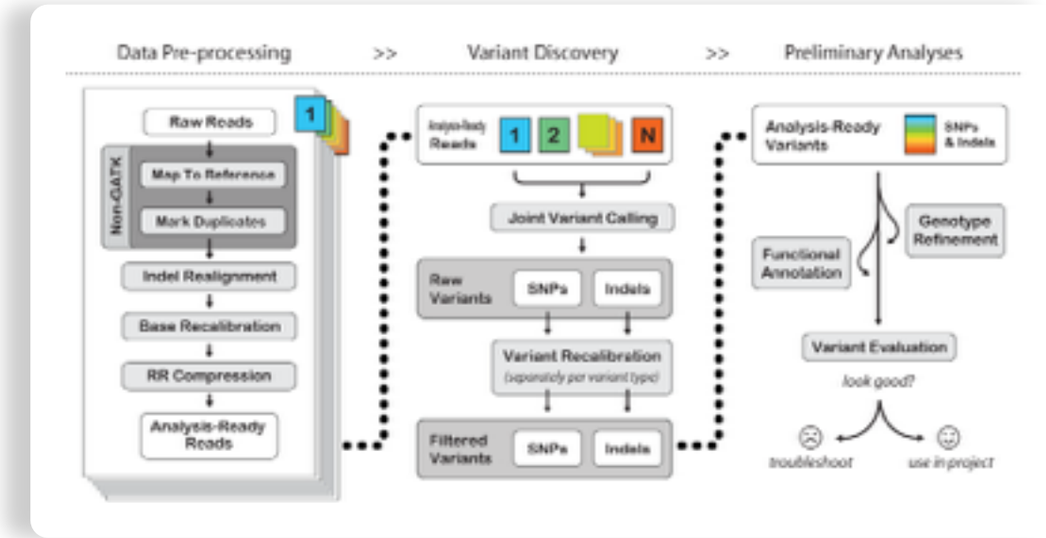
Toolkit & framework packages



The core GATK components

Toolkit

Best practices for variant discovery



Framework

MuTect, XHMM, GenomeSTRiP, ...

Tools developed on top of the GATK framework by other groups

Extensive online documentation & user support forum serving >10K users worldwide

http://www.broadinstitute.org/gatk

About
Overview of the GATK and the people behind it

Guide
Detailed documentation, guidelines and tutorials

Community
Forum for questions and announcements

Events
Materials from live and online events

iSeqTools

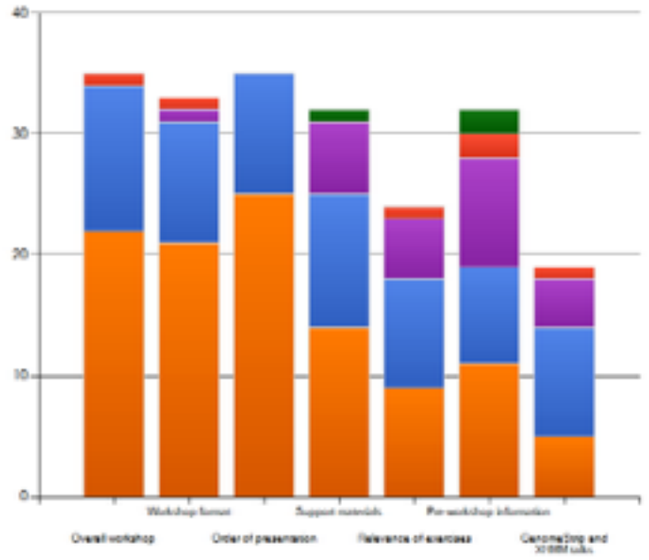
Workshop series educates local and worldwide audiences

Completed:
- Dec 4-5 2012, Boston
- July 9-10 2013, Boston
- July 22-23 2013, Israel
- Oct 21-22 2013, Boston

Planned:
- March 3-5 2014, Thailand
- Oct 18-29 2014, San Diego

Format
- Lecture series (general audience)
- Hands-on sessions (for beginners)

Portfolio of workshop modules
- GATK Best Practices for Variant Calling
- Building Analysis Pipelines with Queue
- Third-party Tools:
  - GenomeSTRiP
  - XHMM



- High levels of satisfaction reported by users in polls
- Detailed feedback helps improve further iterations

Tutorial materials, slide decks and videos all available online through the GATK website, YouTube and iTunesU

BroadE: Overview of GATK & best practices

by **broadinstitute** • 1 week ago • 1 view

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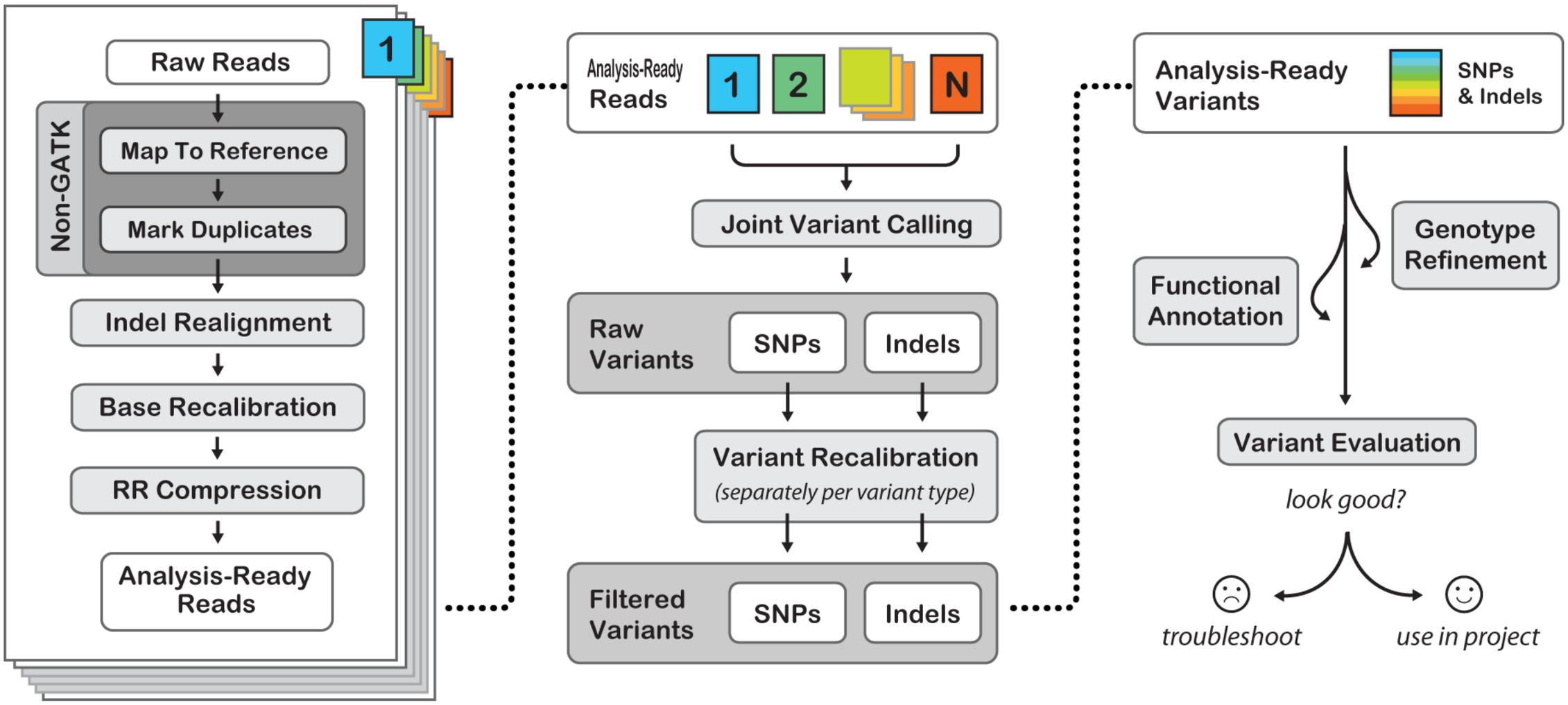
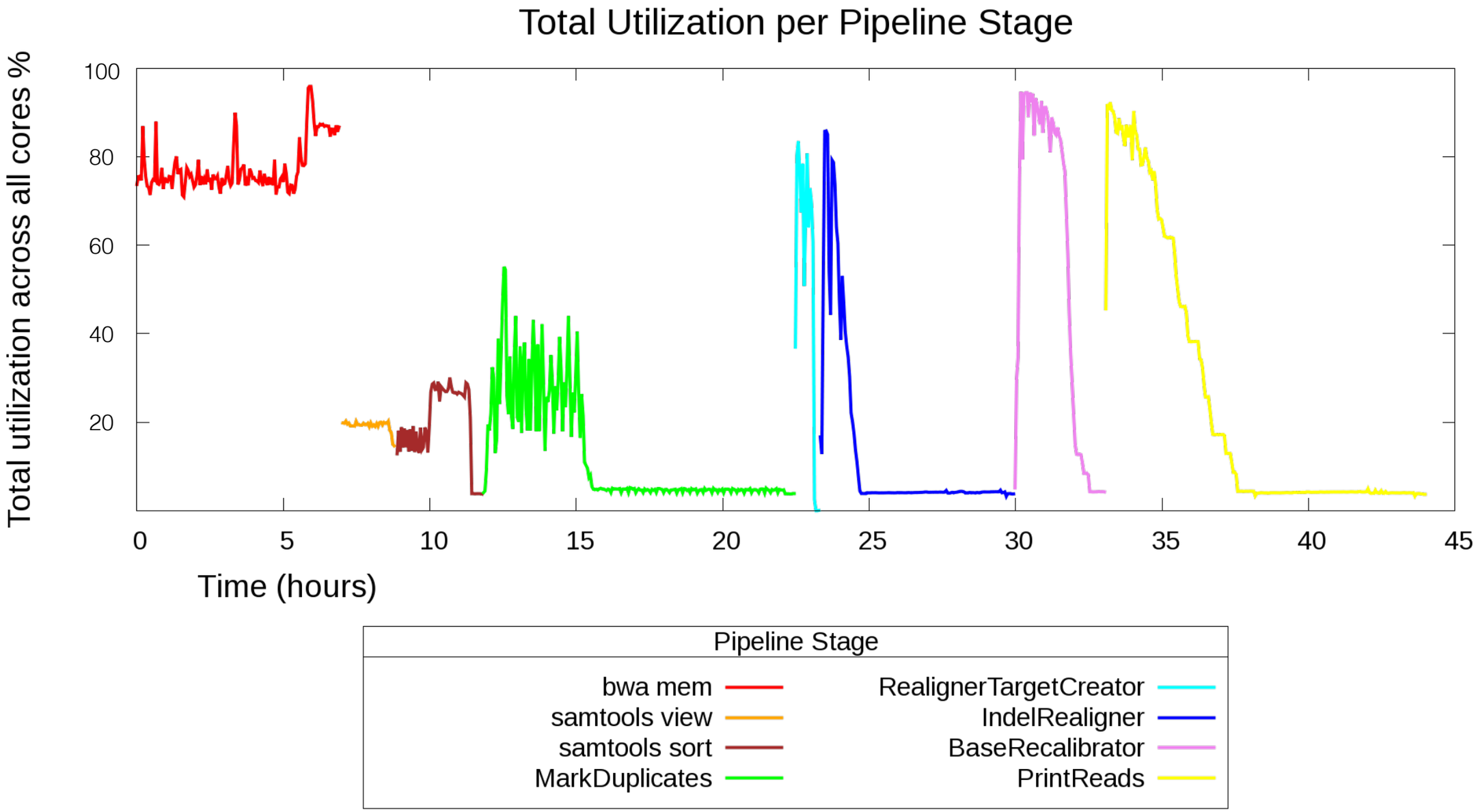
NEW HD

We have defined the best practices for sequencing data processing



Auwera, GA et al. *Current Protocols in Bioinformatics* (2013)

Processing is a big cost on whole genome sequencing



Challenges to scale up the processing pipeline

- Eliminate disk read/writing in between pipeline steps

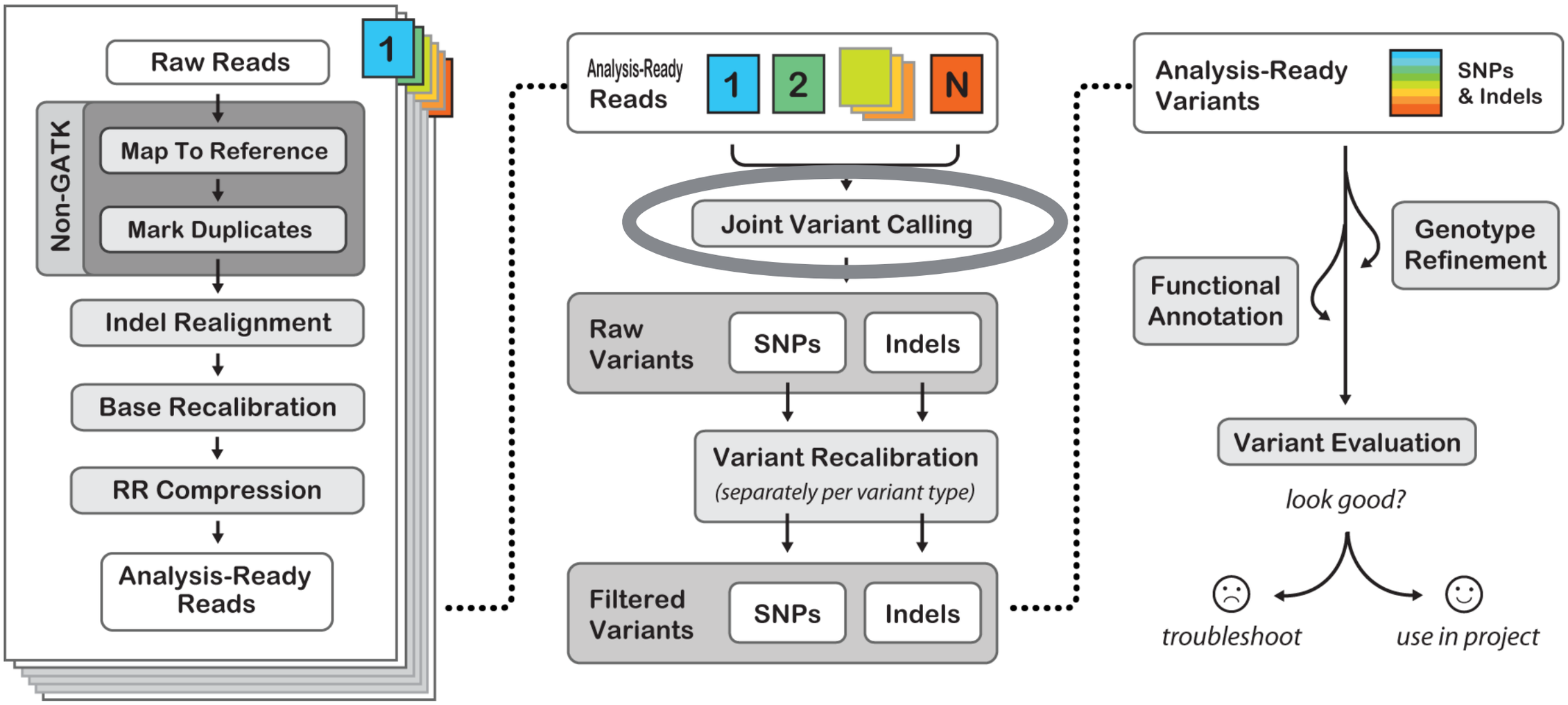
- Reduce time spent doing unnecessary calculations
(e.g. Base Recalibration on *good* data)

- Design high performance native I/O libraries
(*Gamgee*: https://github.com/MauricioCarneiro/gamgee)

- Redesign algorithms with performance in mind

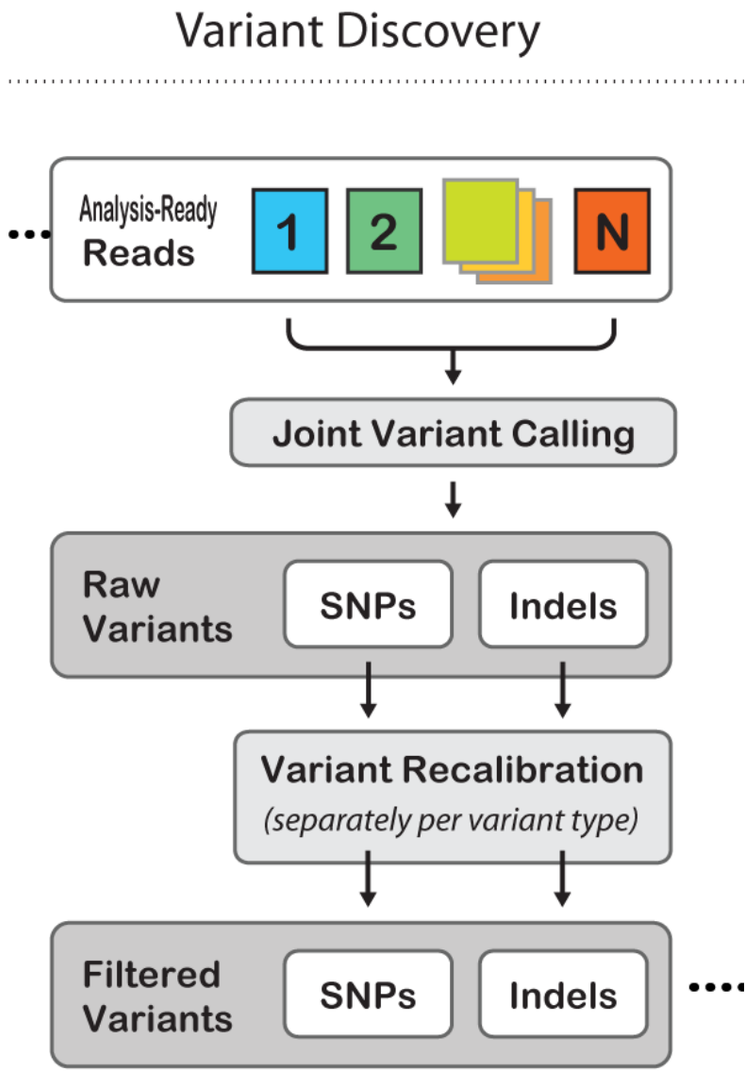
step	threads	time
BWA	24	7
samtools view	1	2
sort + index	1	3
MarkDuplicates	1	11
RealignTargets	24	1
IndelRealigner	24	6.5
BaseRecalibrator	24	1.3
PrintReads + index	24	12.3
Total		**44**

Joint calling is an important step in disease research



Auwera, GA et al. *Current Protocols in Bioinformatics* (2013)

Variant calling is a large-scale bayesian modeling problem



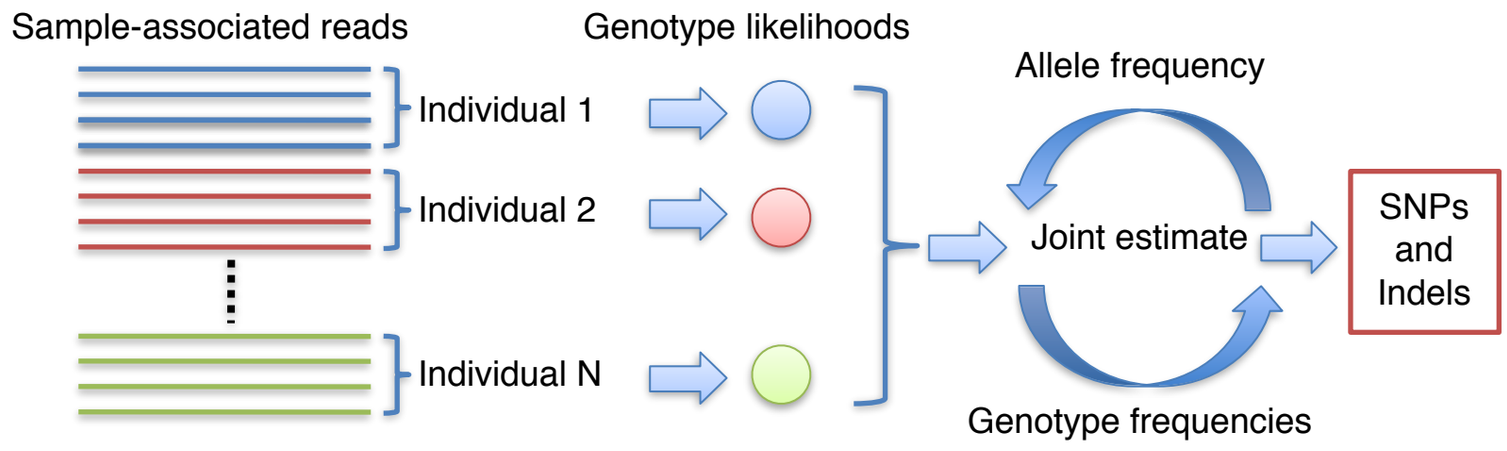
prior Likelihood

$$\Pr\{G|D\} = \frac{\Pr\{G\}\Pr\{D|G\}}{\Sigma_i \Pr\{G_i\}\Pr\{D|G_i\}}, \text{ [Bayes' rule]}$$

Diploid

$$\Pr\{D|G\} = \prod_j \left(\frac{\Pr\{D_j|H_1\}}{2} + \frac{\Pr\{D_j|H_2\}}{2}\right) \text{ where } G = H_1H_2$$

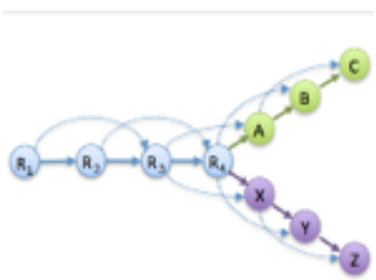
$\Pr\{D|H\}$ is the haploid likelihood function



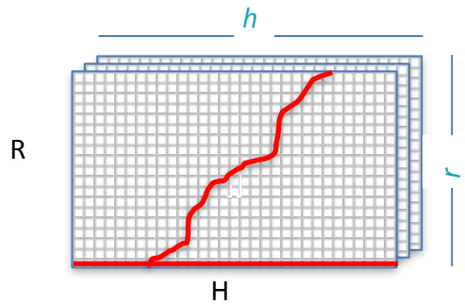
DePristo et al. *Nature Genetics* (2011)

Understanding the GATK's *denovo* assembly based variant caller

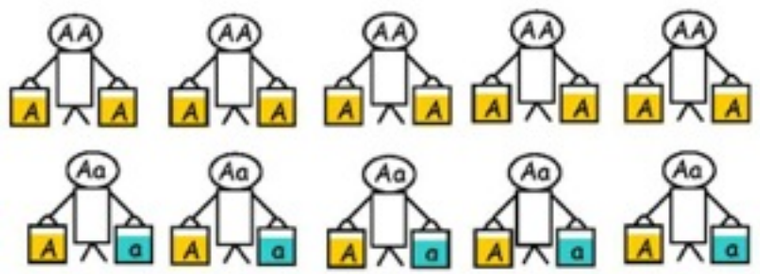
1. **Active region traversal**
identifies the regions that need to be reassembled



2. **Local de-novo assembly**
builds the most likely haplotypes for evaluation



3. **Pair-Hmm evaluation** of all reads against all haplotypes
(scales exponentially)



4. **Genotyping**
using the exact model

Pair-HMM is the biggest culprit for the low performance of the Haplotype Caller

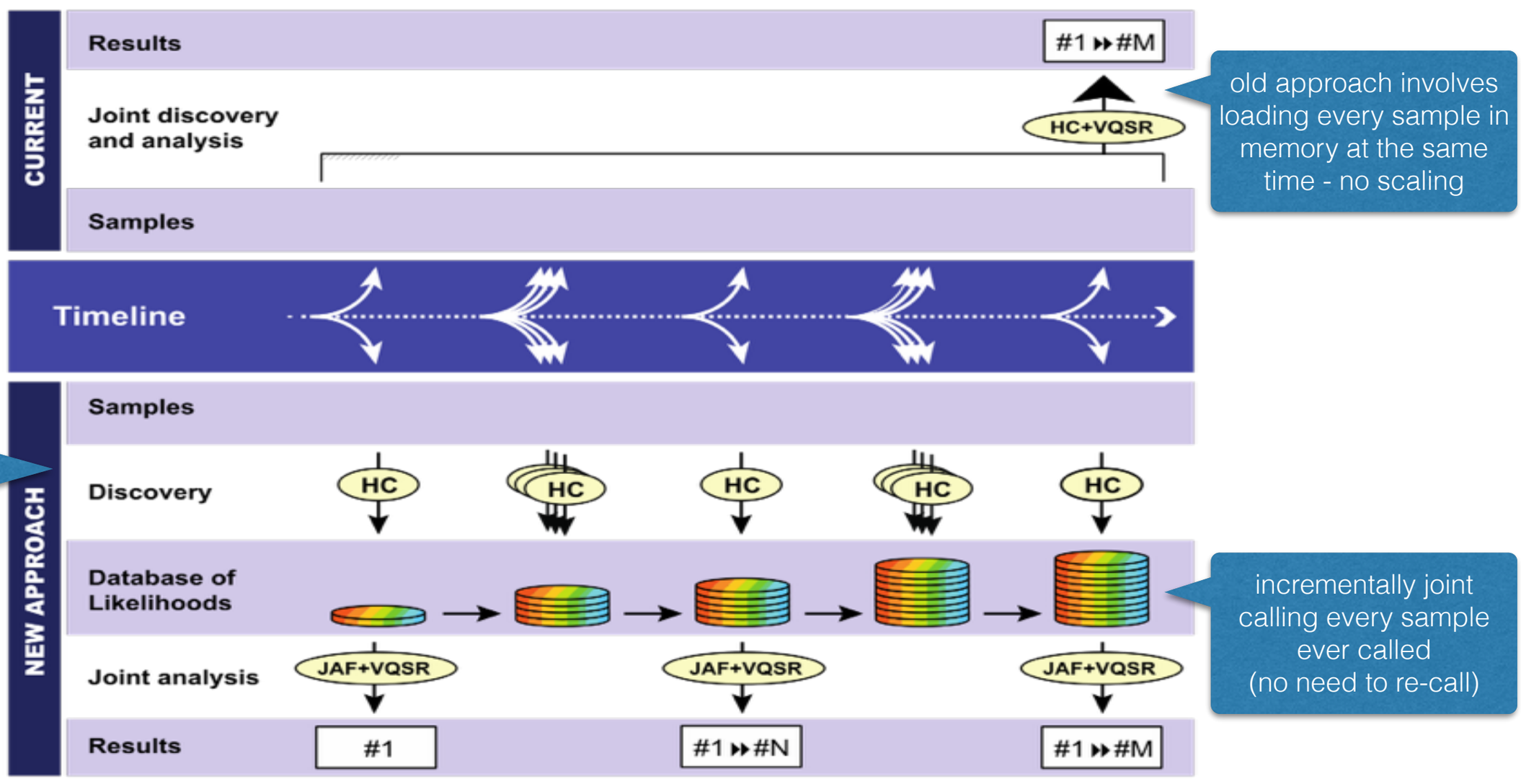
Stage	Time	Runtime %
Assembly	2,598s	13%
Pair-HMM	14,225s	70%
Traversal + Genotyping	3,379s	17%

NA12878 80xWGS performance on a single core
chr20 time: 5.6h
whole genome: 7.6 days

Heterogeneous compute speeds up variant calling significantly

Technology	Hardware	Runtime	Improvement
GPU	NVidia Tesla K40	70	154x
GPU	NVidia GeForce GTX Titan	80	135x
GPU	NVidia GeForce GTX 480	190	56x
GPU	NVidia GeForce GTX 680	274	40x
GPU	NVidia GeForce GTX 670	288	38x
AVX	Intel Xeon 1-core	309	35x
FPGA	Convey Computers HC2	834	13x
-	C++ (baseline)	1,267	9x
-	Java (gatk 2.8)	10,800	-

The reference model enables incremental calling



by separating discovery from joint analysis, we can now jointly call any arbitrary number of samples

But the joint analysis needs better infrastructure to become a trivial step

All case and control samples

~3M variants

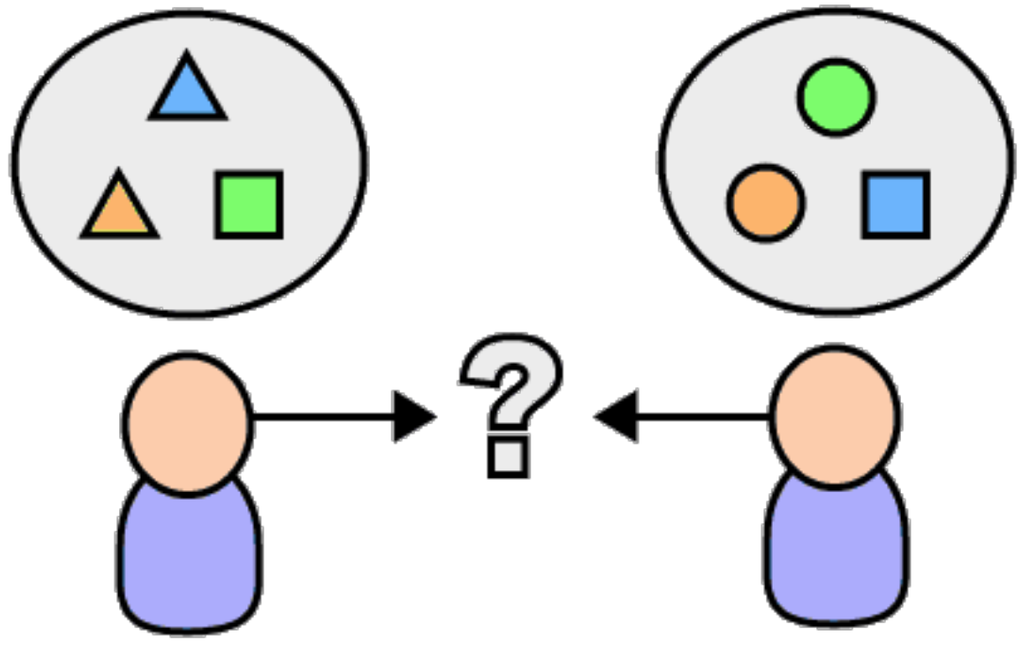
	Site	Variant	Sample 1	Sample 2	…	Sample N
SNP	***1:1000***	***A/C***	***0/0 0,10,100***	***0/1 20,0,200***	…	***0/0 0,100,255***
Indel	1:1050	T/TC	0/0 0,10,100	0/0 0,20,200	…	1/0 255,0,255
SNP	***1:1100***	***T/G***	***0/0 0,10,100***	***0/1 20,0,200***	…	***0/0 0,100,255***
	…	…	…	…	…	…
SNP	***X:1234***	***G/T***	***0/1 10,0,100***	***0/1 20,0,200***	…	***1/1 255,100,0***

Genotypes:
0/0 ref
0/1 het
1/1 hom-alt

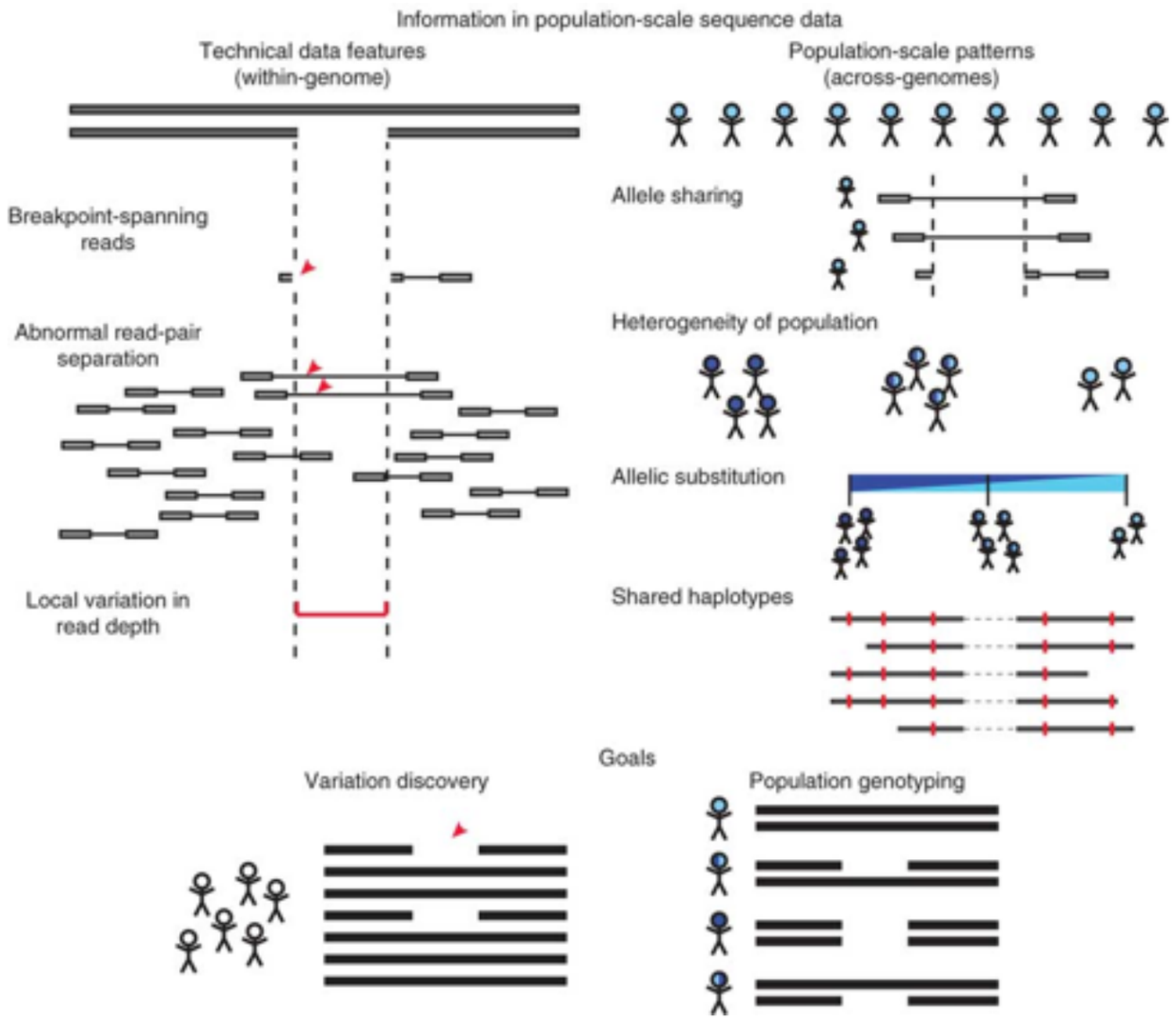
Likelihoods:
A/B/C phred-scaled probability of hom (A), het (B), hom-alt (C) genotypes given NGS data

Analysis pipeline standardization and scaling is the next big challenge

- What happens after variant calling is not standardized.
- Hundreds of completely unrelated tools are chained together with non-reusable scripts.
- Analyses are very often unrepeatable.
- Tools are not generalized and performance does not scale. (typically written in matlab, R, PERL and Python…)
- Most tools are written by one grad student/ postdoc and is no longer maintained.
- Complementary data types are not standardized (e.g. phenotypic data).

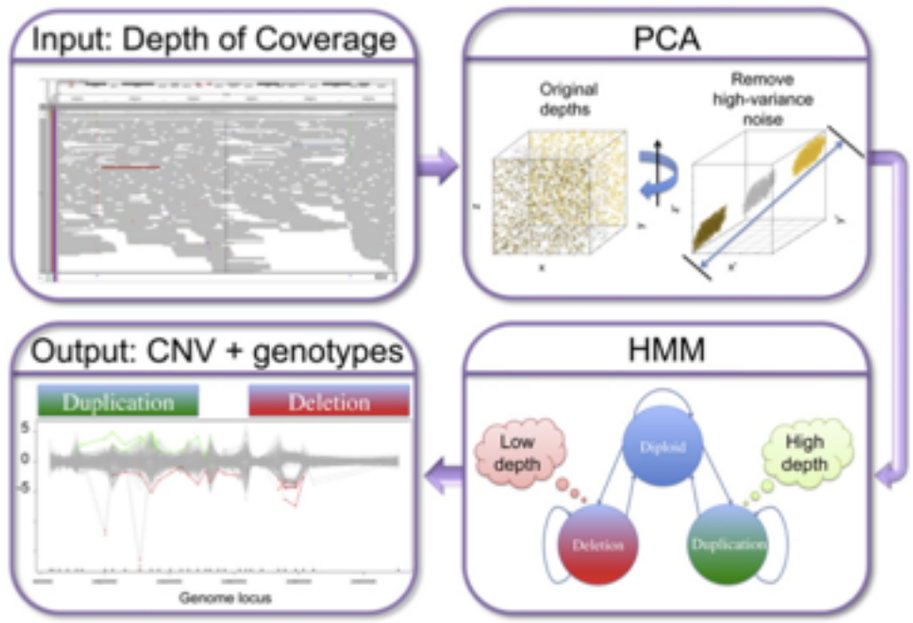


Structural variation is an important missing piece in the analysis pipeline



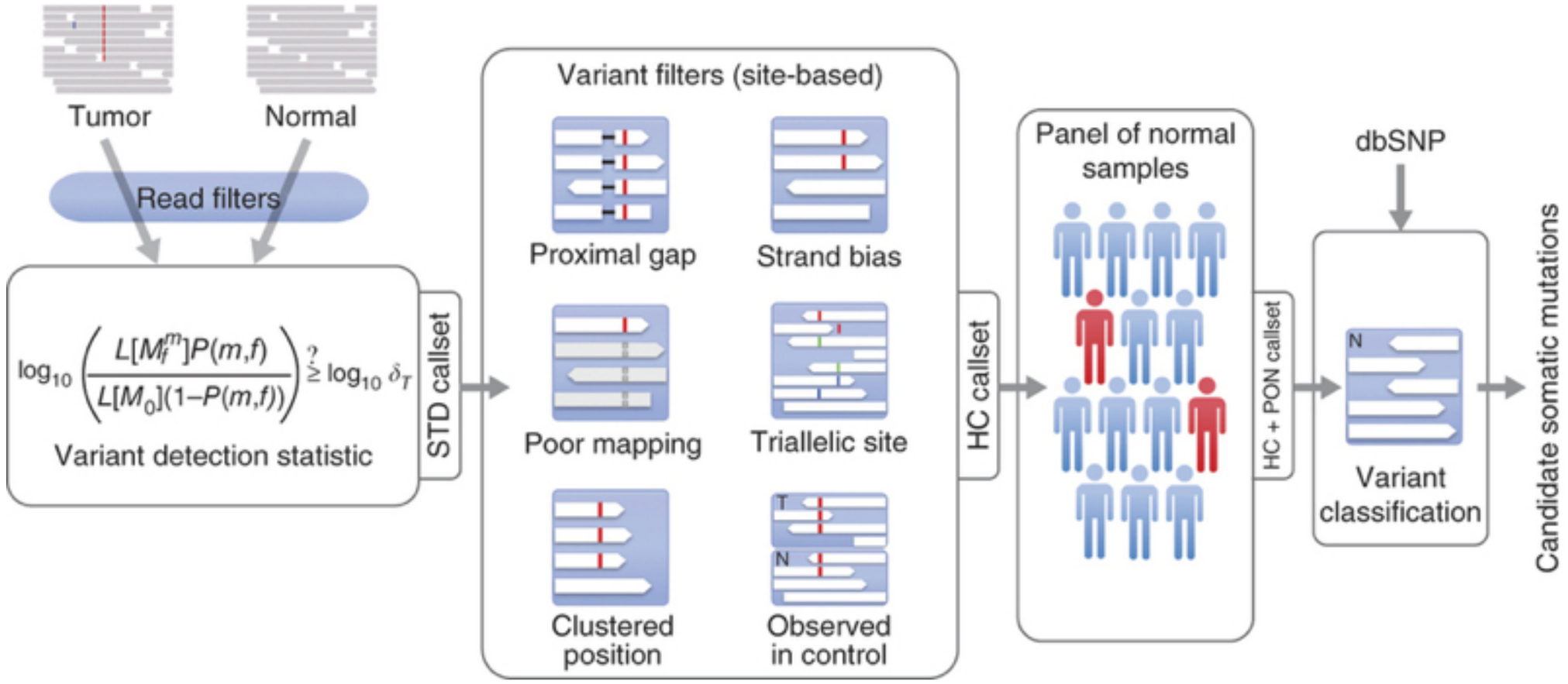
Handsaker, R et al. *Nature Genetics* (2011)

current implementations have confirmed the importance of structural variation calling for complex disease research but have not been *standardized* or *productionized.*



Fromer, M et al. *Nature* (2014)

Cancer studies also need the same rigorous standardization and scalability



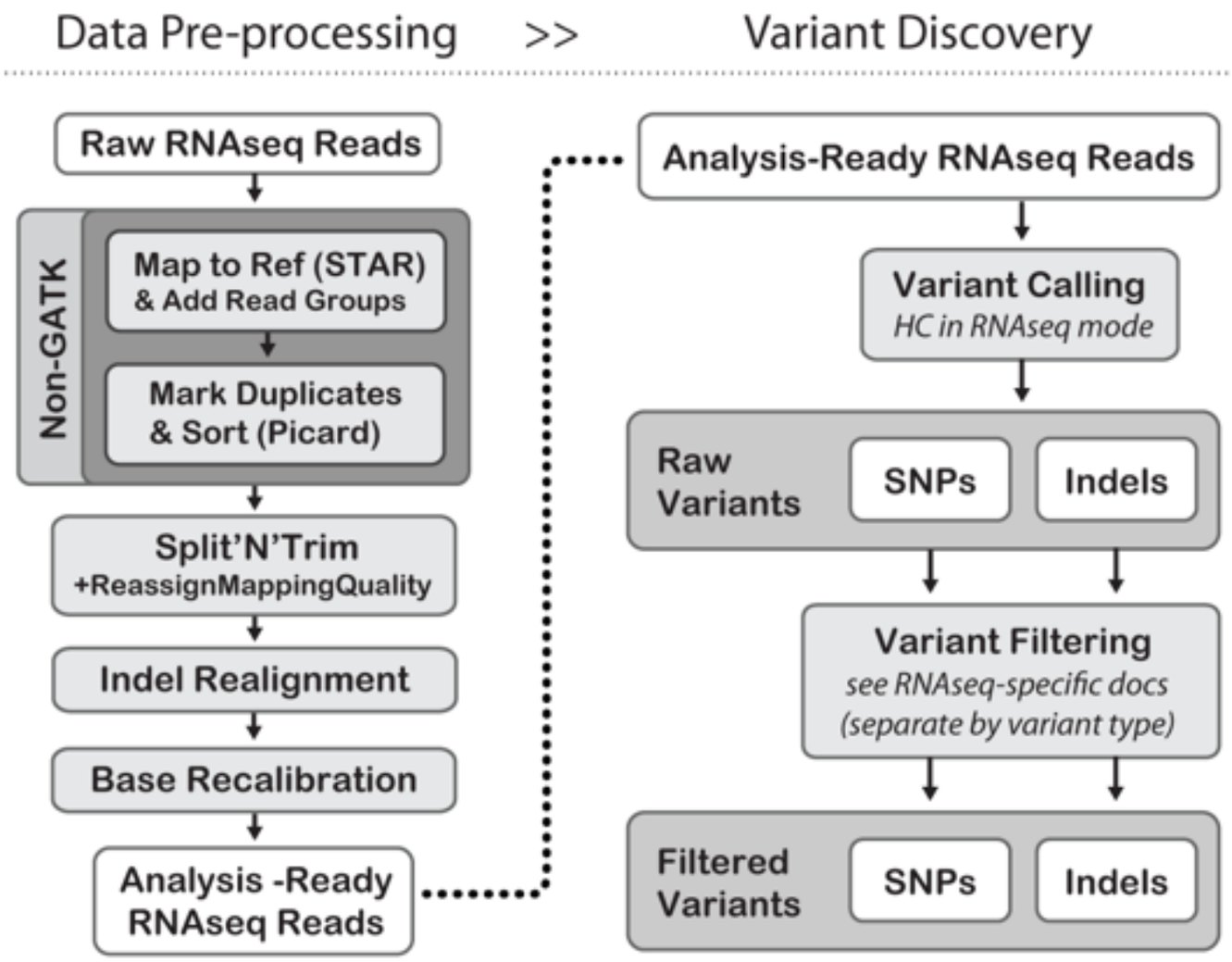
Cibulskis, K et al. *Nature Biotechnology* (2013)

DNA does not tell the whole story — there is RNA too!

We have standardized the RNA-seq pipeline, but now there is a lot of work to do!

Milestone 1:
update GATK tools to make best use of RNA data including contrastive variant calling with DNA. Improve accuracy and overall performance.

Milestone 2:
Build new tools to address the needs



Carneiro, M et al. *In preparation*

There is a lot *we* can contribute to disease research

- Samples must be consistently processed worldwide and the processing pipelines need to scale in performance to reduce overall cost.

- Variants must be jointly called and currently available tools need to provide the necessary performance. *(We solved the scaling problem!)*

- Post variant calling analysis pipelines need to be built from scratch with performance and scalability in mind.

- We need to build new infrastructure to enable the aggregation of the massive wave of data that is coming our way

- RNA-seq and structural variation need to be integrated and standardized for scientists and clinicians to understand the whole picture.

This is the work of many…

the team

Eric Banks
Ryan Poplin
Khalid Shakir
David Roazen
Joel Thibault
Geraldine VanDerAuwera
Ami Levy-Moonshine
Valentin Rubio
Bertrand Haas
Laura Gauthier
Christopher Wheelan
Sheila Chandran

Broad colleagues

Heng Li
Daniel MacArthur
Timothy Fennel
Steven McCarrol
Mark Daly
Sheila Fisher
Stacey Gabriel
David Altshuler

collaborators

Menachem Fromer
Paolo Narvaez
Diego Nehab

“Thank you!”

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