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Somatic SNV Calling

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Outline

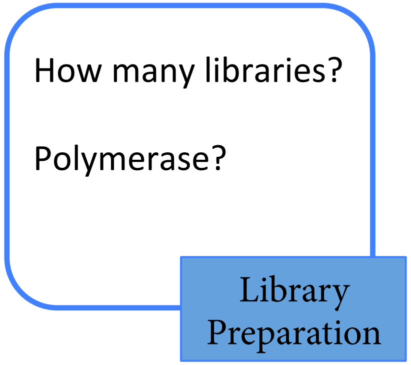
This morning’s session:

- **An example processing pipeline**
- **Some calling tools**
- **How well should you expect a tool to perform?**
- **Some special cases**

Somatic SNV calling

An example processing pipeline

A processing pipeline

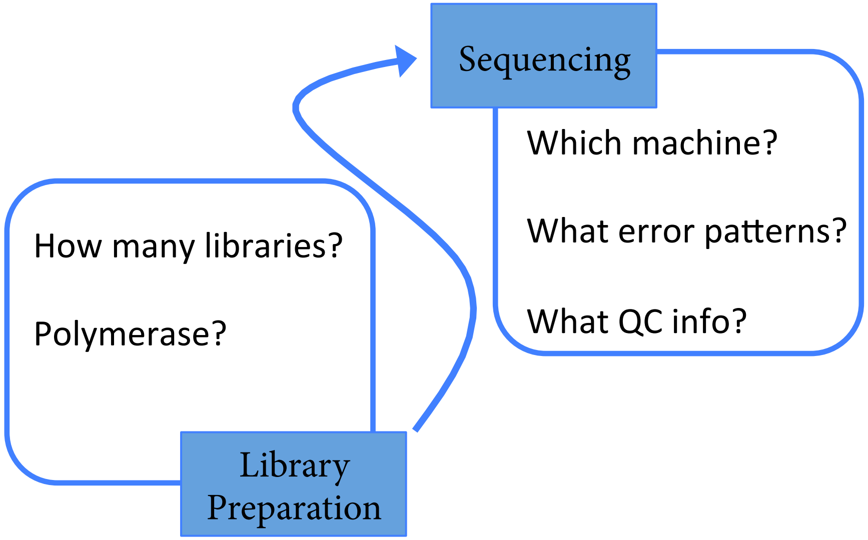


How many libraries?

Polymerase?

Library Preparation

A processing pipeline



Sequencing

Which machine?

What error patterns?

What QC info?

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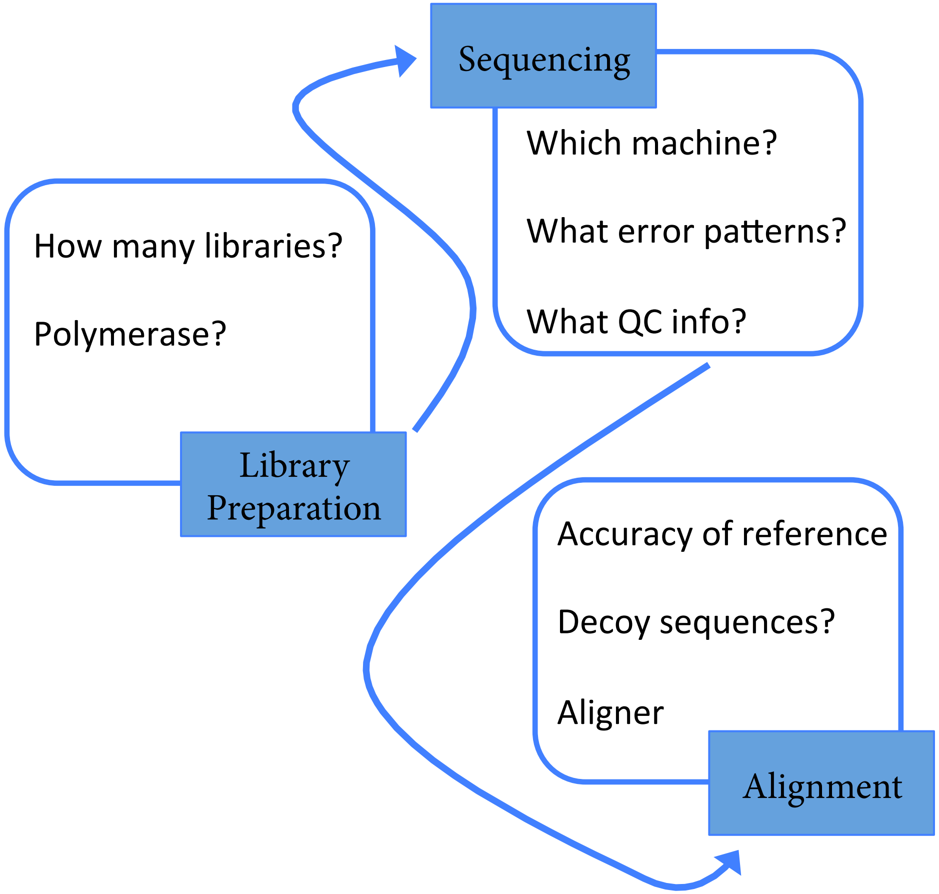

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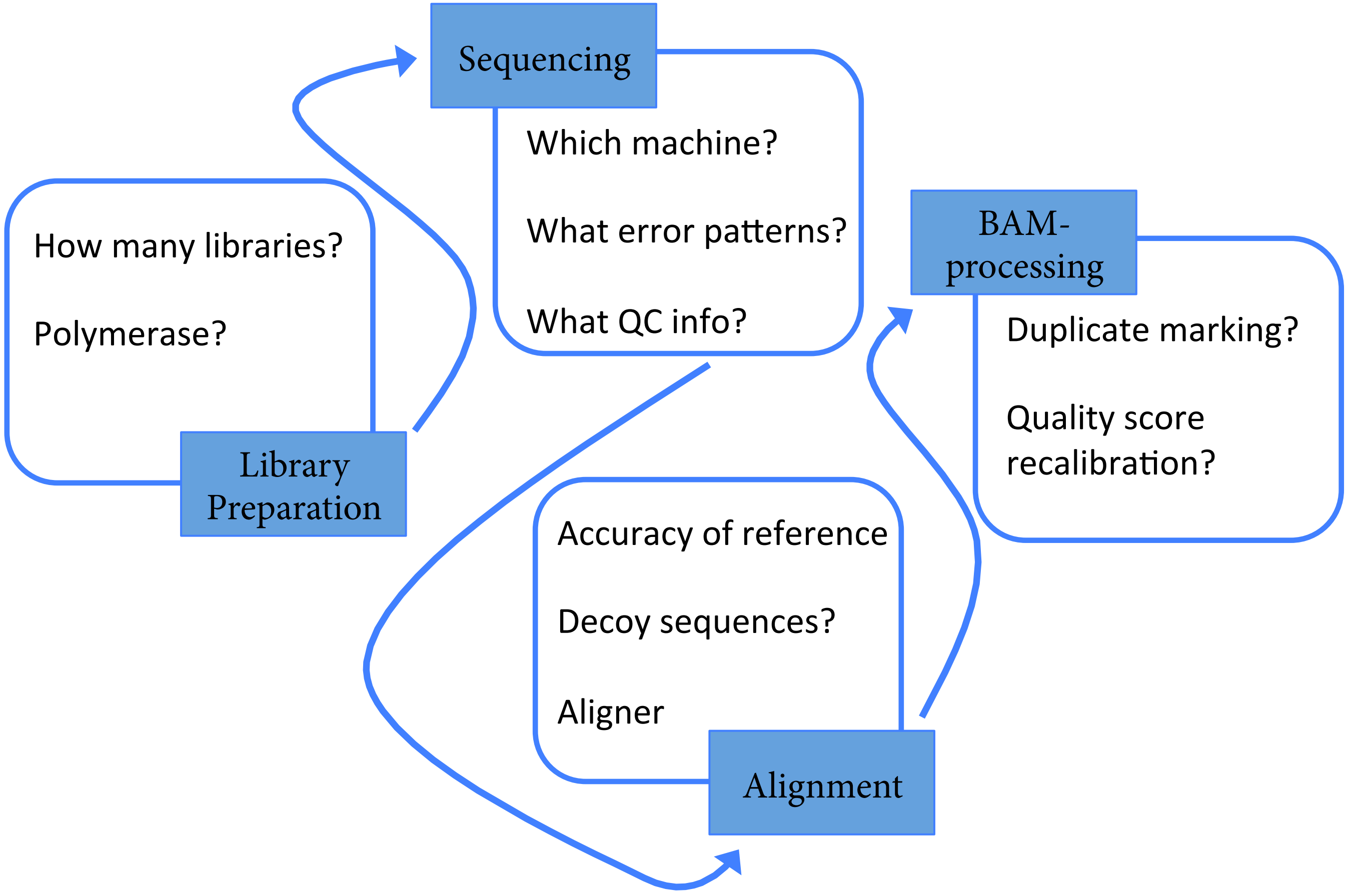
Accuracy of reference

Decoy sequences?

Aligner

A processing pipeline



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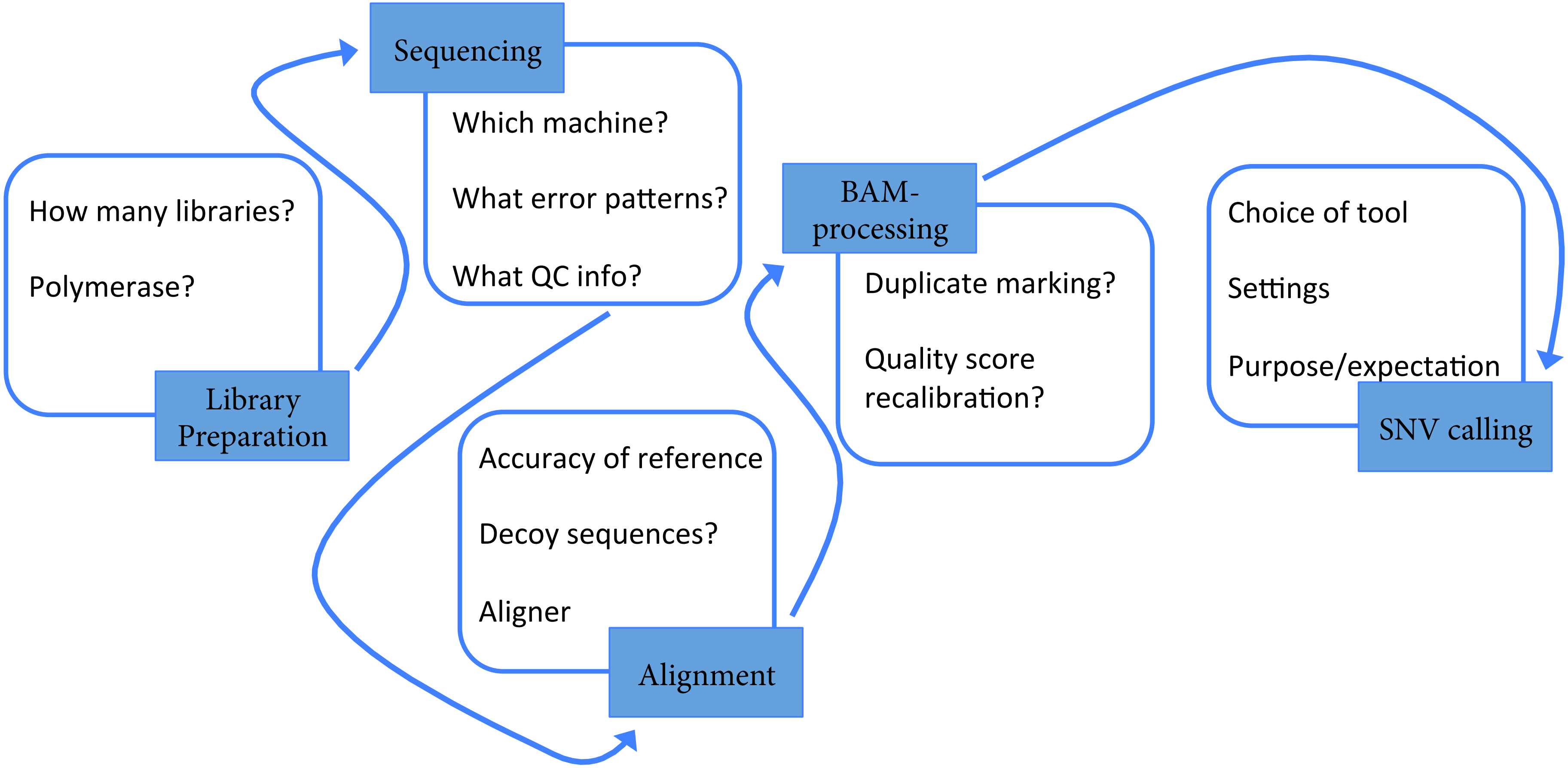
BAM-processing

Duplicate marking?

Quality score recalibration?

A processing pipeline



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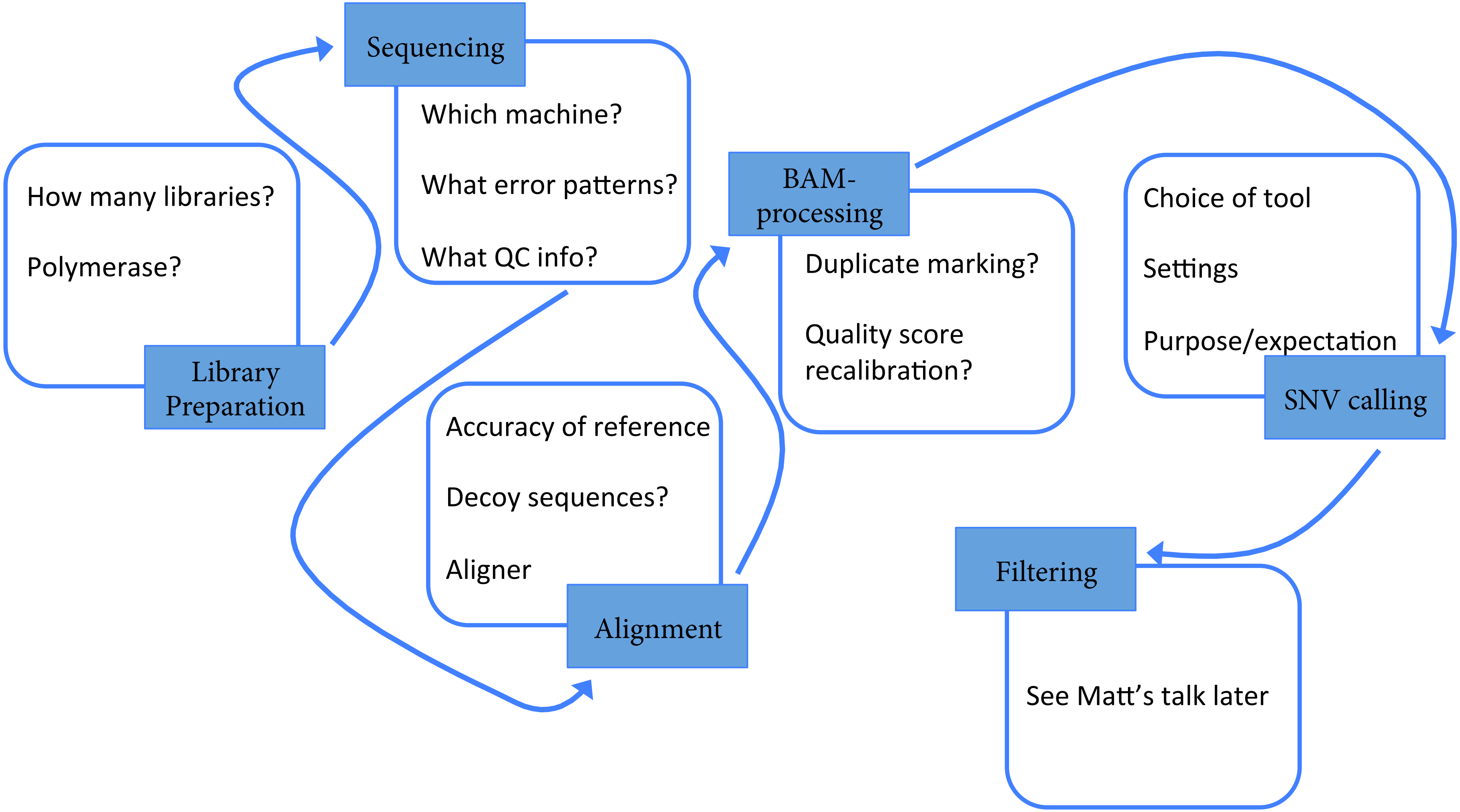
SNV calling

Choice of tool

Settings

Purpose/expectation

A processing pipeline



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Filtering

See Matt’s talk later

Somatic SNV calling

SNV-calling tools

The SNV caller is not the only concern

We use CaVEMan here.

Caveat – full details of CaVEMan are not explicitly reported anywhere, and I am not going to go through the code (java). So all a bit of a black box

- **Seems to have a sensible Bayesian model**
- **Considers base quality, read position, lane, and read orientation**
- **Can make use of copy number profiles**
- **Associated filters**

One could argue that any sensible caller would do the job. The secret is in the filtering.

Other tools

Several tools worth considering:

The detail of

- **MuTect2** – Combines a good quality caller with haplotype reassembly. Built in filters and the ability to take in a panel of normal samples. Can also return indels.
- **VarScan2 (Koboldt 2012)** – Uses a basic statistical test rather than a full Bayesian model, but will probably be followed by filtering anyway. A portable java program.
- **Strelka (Saunders 2012)** – A hierarchical model of allele frequencies. Also returns indels.
- **SMuFin (Moncunill 2014)** – A reference free variant caller with high specificity.

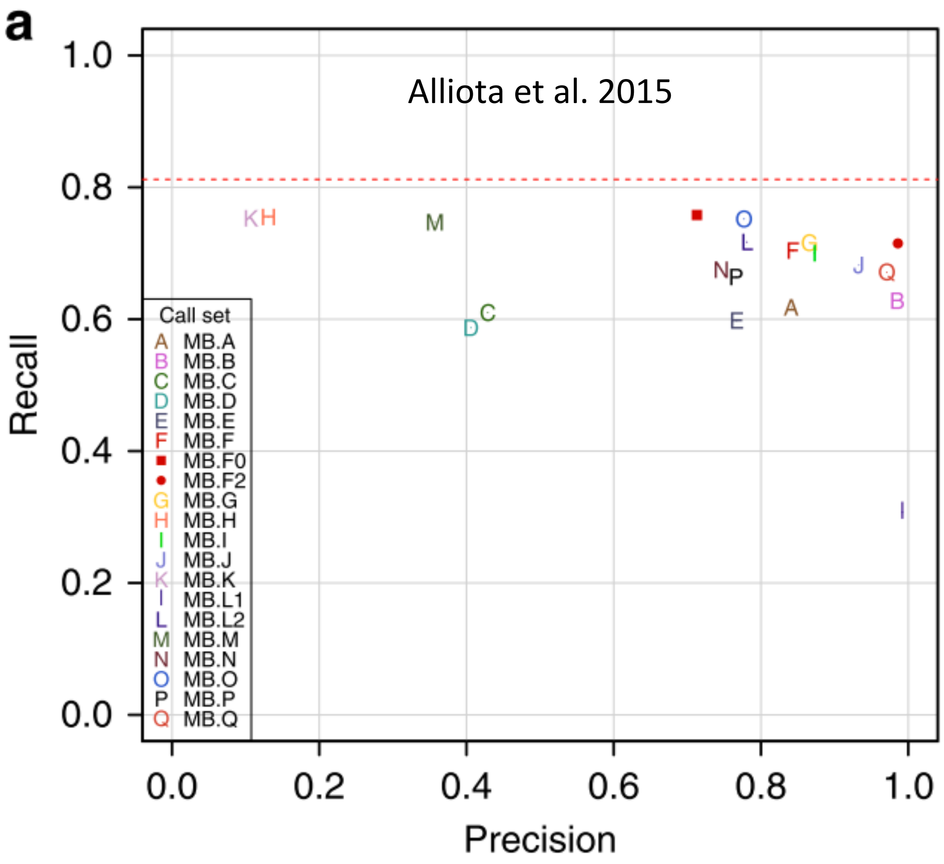
Somatic SNV calling

Anticipated performance

How well should we expect a tool to perform?

Precision/recall is a function of the biology, the sample/data quality, and the calling method.

- **When you see a tool promising a particular precision/recall, how will it perform for you?**
- **The precision and recall are averaged over many SNVs and typically over many samples (although not in this illustration)**
- **The two things affecting precision and recall are the ability to deal with artefacts, and the genuine power of the study**
- **Typically we have the power to detect SNVs that are present in two or more copies in every cell, but those in one copy, or sub-clonal, are more often missed**
- **A sample with relatively more of these recent events will have lower recall/precision**
- **A sample with generally low power will have lower recall/precision**


Alliota et al. 2015

Somatic SNV calling

Some special cases

What if you have multiple tumour samples

Exploring heterogeneity

Datasets that are more that Tumour-Normal are increasingly common. What can be done for variant calling in them?

Theoretically, we can draw strength from related samples to improve our sensitivity.

Still require a filtering regime afterwards.

- **VarScan2 (Koboldt 2012)** offers the ability to call over multiple samples, but doesn’t appear to make the best use of structure in those calls

- **FreeBayes (Garrisson 2012)** can be applied to this task, but the set-up is not optimized for this scenario

- **Platypus (Rimmer 2014)** can be applied to this task. Although it is primarily a germline caller, it does a good job

- **multiSNV (Josephidou 2015)** was designed specifically for the task. It works particularly well in combination with Platypus

What if you have RNA-seq data?

Things get trickier.

We need to stop worrying about recall – there will be a lot missed, and splicing activity and post-transcriptional modifications will introduce artefacts that require new filters.

Nevertheless, there are data to be interrogated…

- **Tophat (Kim et al. 2013) + Isaac (Raczy 2013) variant caller**. Isaac not specifically designed for RNA-seq.

- **MAP-RSeq (Kalari et al. 2014).** Tophat + GATK-based approach**.** Large suite of tools – not a nimble solution.

- **RNASEQR (Chen et al. 2011).** A Bowtie-based approach that takes several passes at the alignment to remove splice-site driven artefacts. Low precision?

- **SNPiR (Piskol et al. 2013).** More expensive aligner to address the problems. Not really designed for somatic variants. See also SNVQ.

- **GLMVC (Sheng et al. 2016).** Specifically for somatic. Addresses cycle bias, but this could be filtered later.

What if you have no matched normal

Obvious strategies:

- **Treat the sample as if it were a normal sample in which you were calling variants. Cellularity allowing, it is probable that somatic events will look like germline heterogeneous SNPs.**

- **Use a relative's, or ethnically-matched, normal sample and run as a T:N pair.**

Either approach will lead to an excess of a couple of million calls, so filtering is required

- **dbSNP**

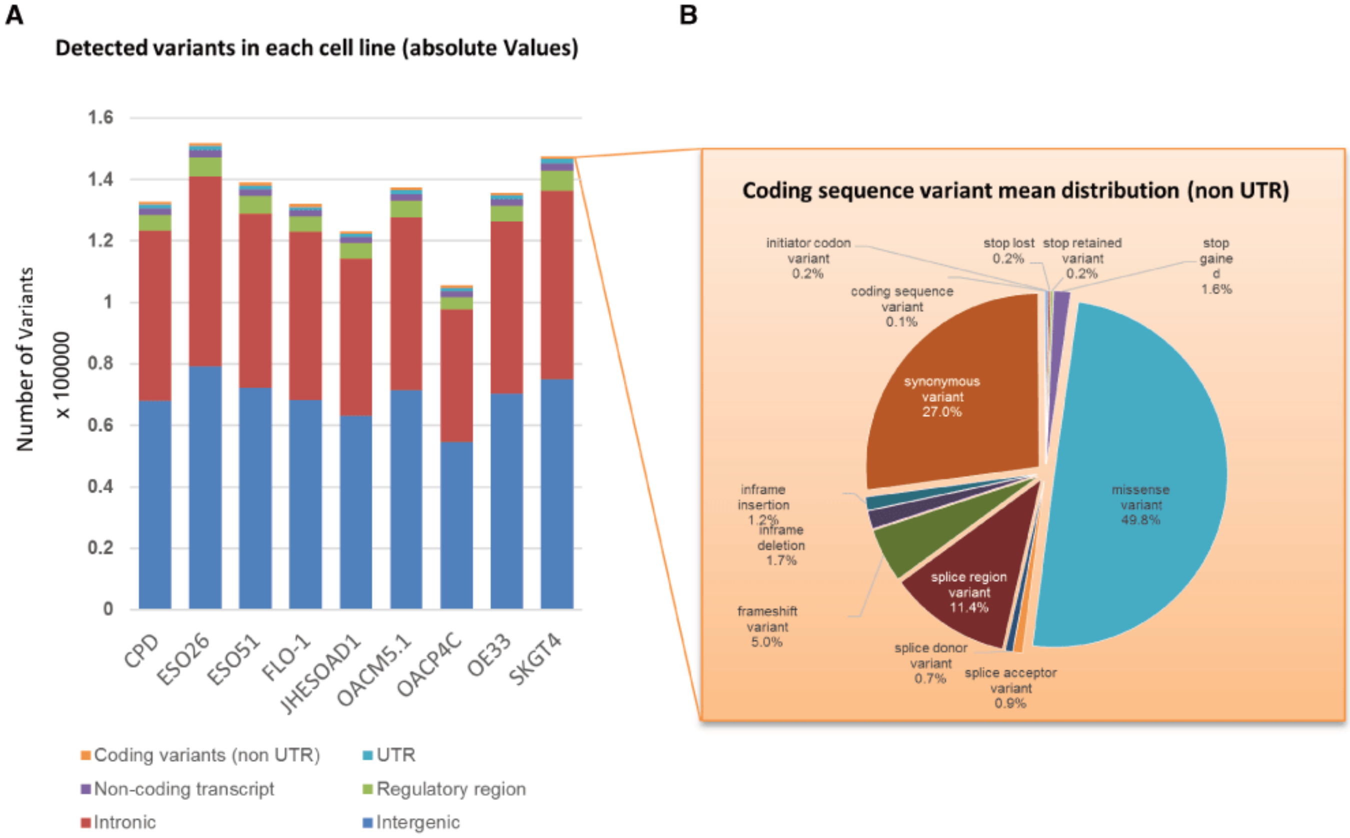
- **Cellularity-driven distinctions in allele-fraction may help**

These should reduce the numbers substantially, but there will still be an excess

What if you have cell-lines

Generally won't have matched normal or cellularity

- **Recently in this situation with OAC cell lines**
- **Clearly too many variants being called**
- **Exonic regions give a feel for the overall performance**



Contino et al. 2016

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