

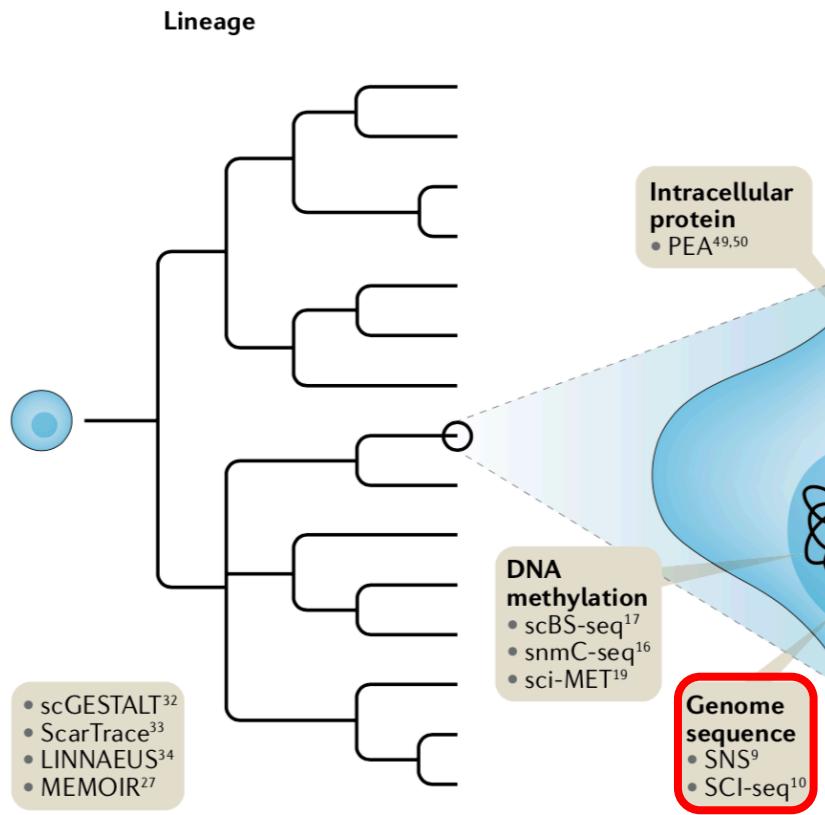
Single-cell genomics data analysis focusing on Strand-seq

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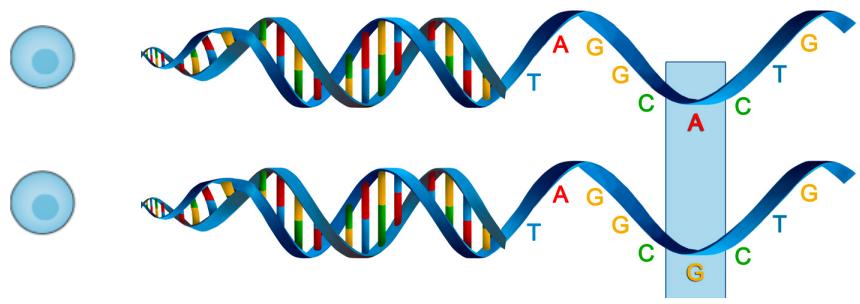
²Korbel Group, Genome Biology, EMBL

Exploration of the genome sequence at the single-cell level

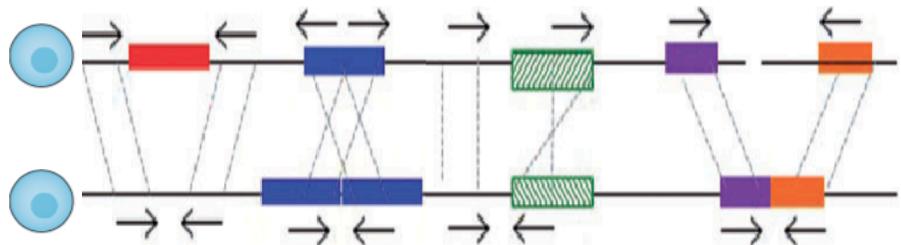


Stuart and Satija.
Nature review genetics, 2019

Single nucleotide variation (SNVs) - scWGS



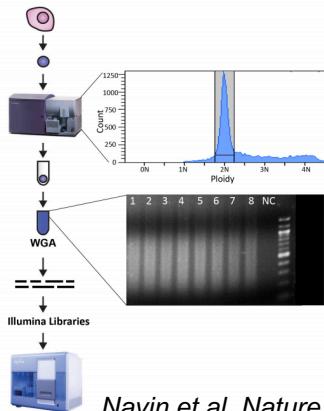
Structural Variation (SVs) – Strand-seq



Overview of the single-cell genome data analysis – scWGS

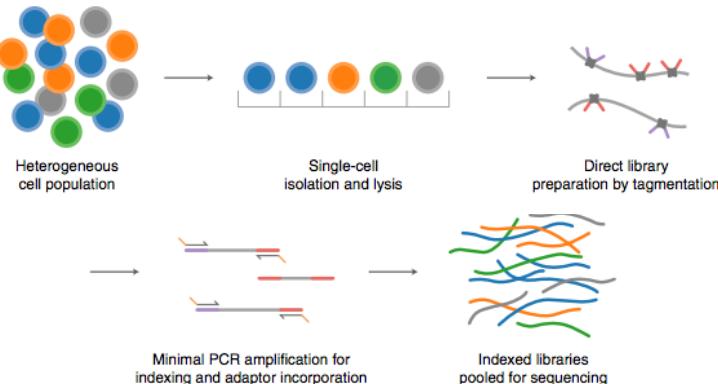
Single nucleotide variation (SNVs) - scWGS

Single-nucleus sequencing (SNS)



Navin et al. *Nature*, 2011

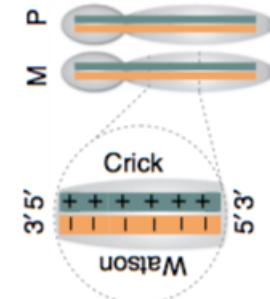
Direct library preparation (DLP)



Zahn et al. *Nat Methods*, 2017

Structural Variation

Strand-seq



Sanders et al. *Nat protocol*, 2017

Step1. Alignment - Finding a correct position of reads: Bowtie2, BWA

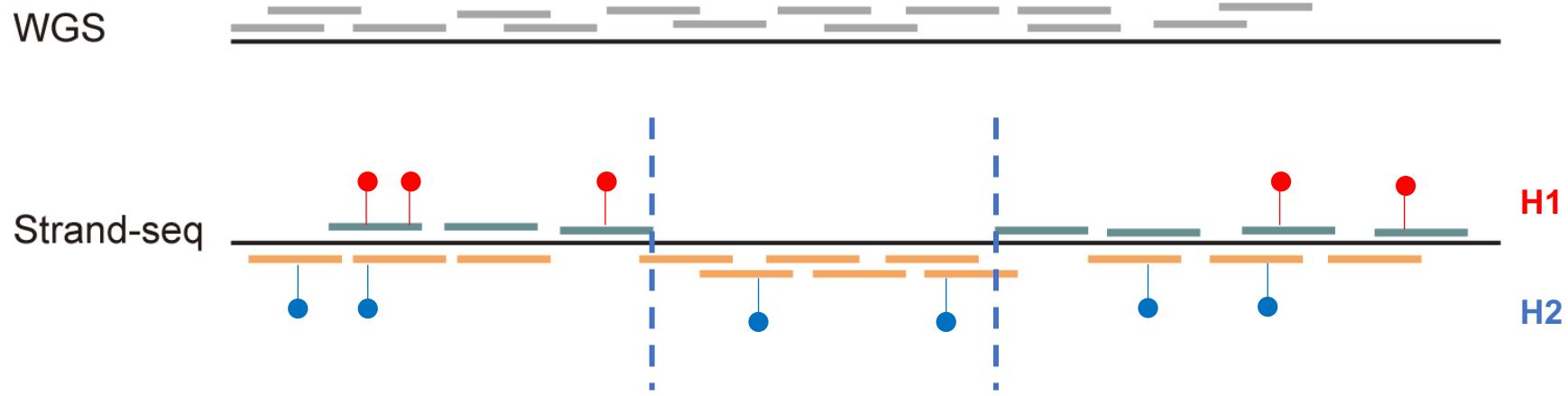
Step2. Remove PCR duplicate: Picard mark duplicate, Biobambam

Step3. Genotyping, Segmentation: Freebayes, GATK, CBS

Step4. Mutation, SV (copy-number variation, inversion, translocation) calling: SCcaller, Monovar

Step5. Single-cell clustering and Phylogenetics: phangorn, TimeScape

Specialties and challenges of the Strand-seq data analysis

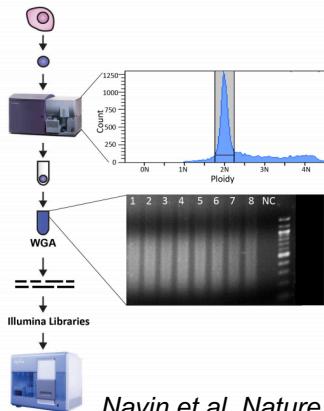


- Sequence orientation is important (Crick or Watson)
- Detection of breakpoint
- Detection of Strand state and haplotypes
- Multiple types of structural variations have their own characteristic patterns

Overview of the single-cell genome data analysis – Strand-seq

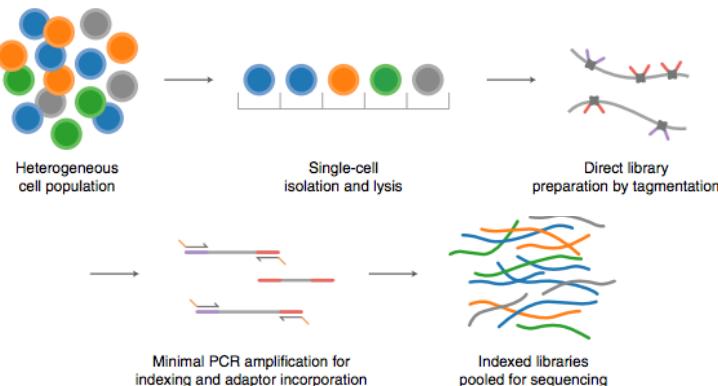
Single nucleotide variation (SNVs) - scWGS

Single-nucleus sequencing (SNS)



Navin et al. *Nature*, 2011

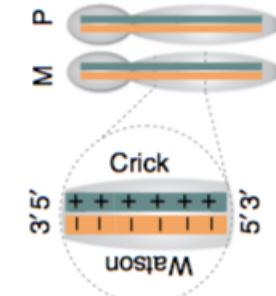
Direct library preparation (DLP)



Zahn et al. *Nat Methods*, 2017

Structural Variation

Strand-seq



Sanders et al. *Nat protocol*, 2017

Step1. Alignment - Finding a correct position of reads: **BWA**, sequence orientation

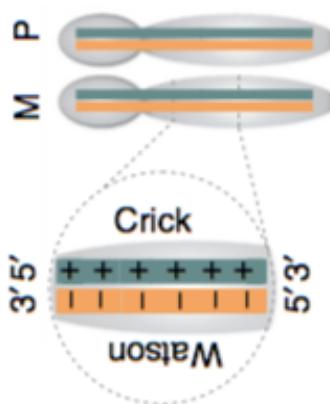
Step2. Remove PCR duplicate: **Biobambam**

Step3. Genotyping, Segmentation: **Freebayes**, **StrandPhaseR**, **BreakpointR**

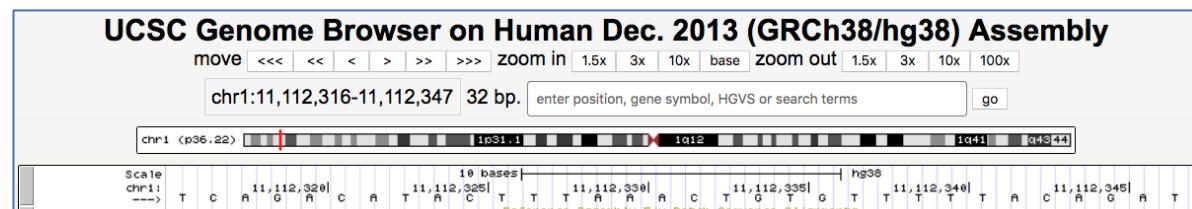
Step4. Mutation, SV (copy-number variation, inversion, translocation) calling: **MosaiCatcher**

Step5. Single-cell clustering and Phylogenetics

Orientation of the reads are the key of Strand-seq data analysis

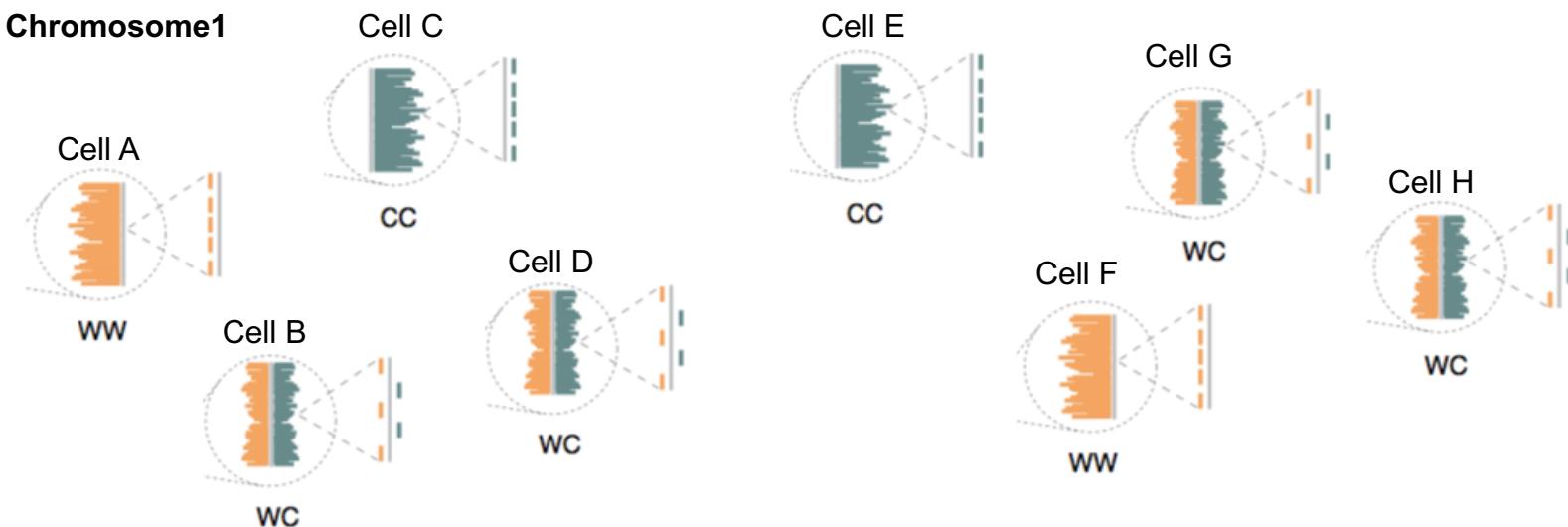


- Crick (C) aligns to the plus (forward) strand of the reference assembly
- Watson (W) aligns to the minus (reverse) strand

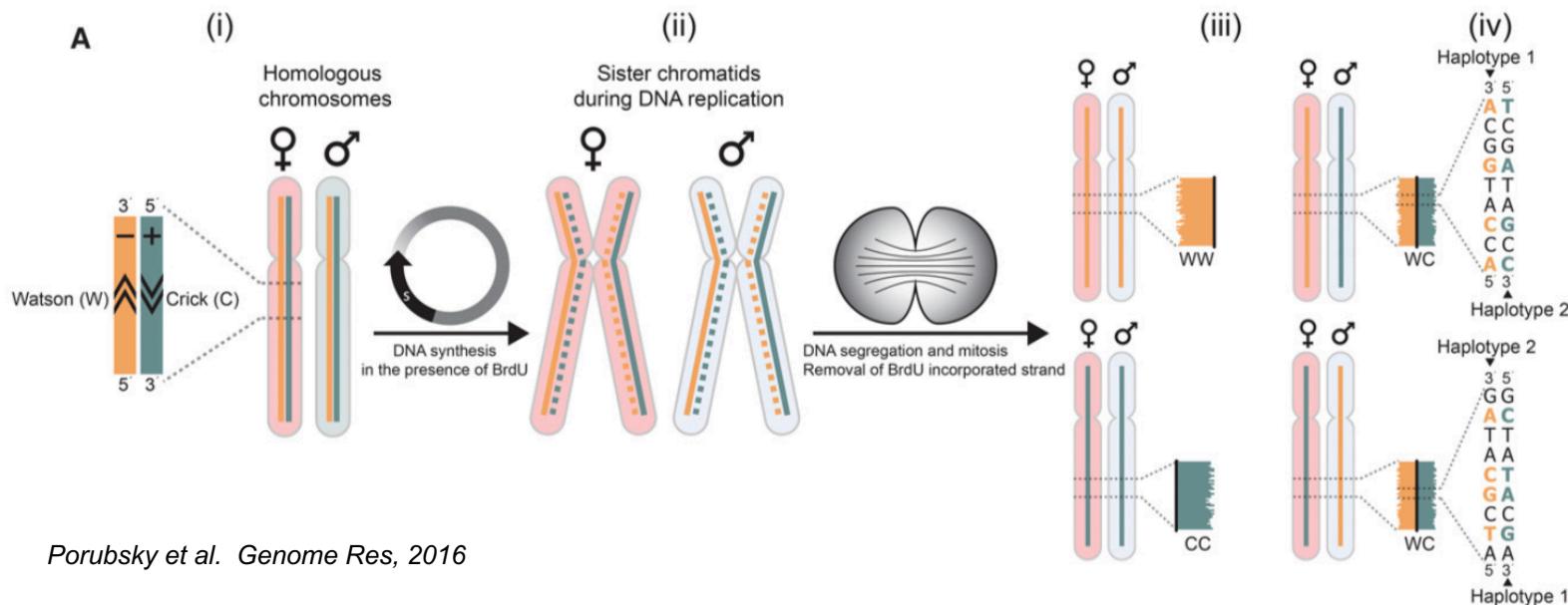


Forward (+)
→ Crick

Sanders et al. *Nature protocol*, 2017



Orientation of the reads gives chromosome length scale haplotype information



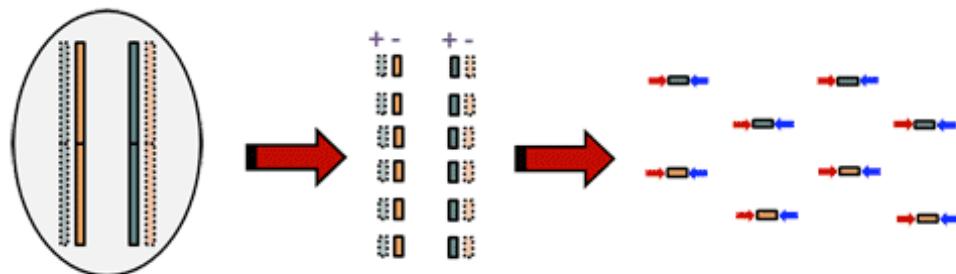
- Why haplotype phasing is important?
- Variant-disease associations
- Mapping regions with loss of heterozygosity
- Inheritance patterns in pedigrees



How can we know the orientation of the sequencing read from the bam file?

Classify reads into Crick and Watson using bamfile and samtools

Crick = A1 read matches + or A2 read matches -
Watson = A1 read matches - or A2 read matches +



<https://sourceforge.net/p/bait/wiki/>

##Crick reads

```
samtools view -f 99 output.bam > A1_C.txt  
samtools view -f 147 output.bam > A2_C.txt
```

##Watson reads

```
samtools view -f 83 output.bam > A1_W.txt  
samtools view -f 163 output.bam > A2_W.txt
```

Decoding SAM flags

This utility makes it easy to identify what are the properties of a read based on its SAM flag value. To see what combination of properties would result in a given SAM flag value, just enter the number in the field below.

SAM Flag: [Explain](#)

[Switch to mate](#) [Toggle first in pair / second in pair](#)

Find SAM flag by property:

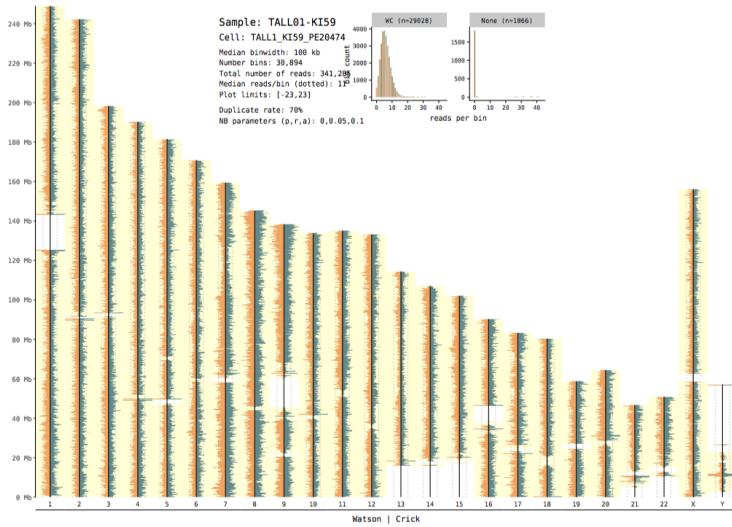
To find out what the SAM flag value would be for a given combination of properties, tick the boxes for those that you'd like to include. The flag value will be shown in the SAM Flag field above.

- read paired
- read mapped in proper pair
- read unmapped
- mate unmapped
- read reverse strand
- mate reverse strand
- first in pair
- second in pair
- not primary alignment
- read fails platform/vendor quality checks
- read is PCR or optical duplicate
- supplementary alignment

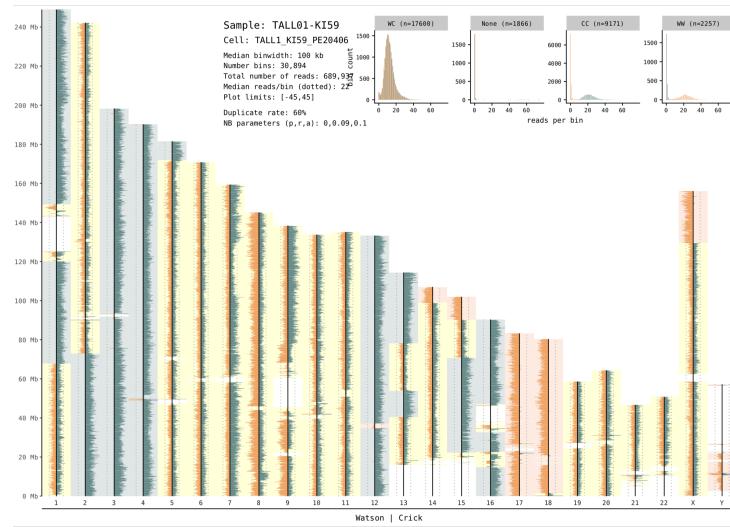
<https://broadinstitute.github.io/picard/explain-flags.html>

Quality check: Which one is the good quality Strand-seq library?

Whole-genome seq

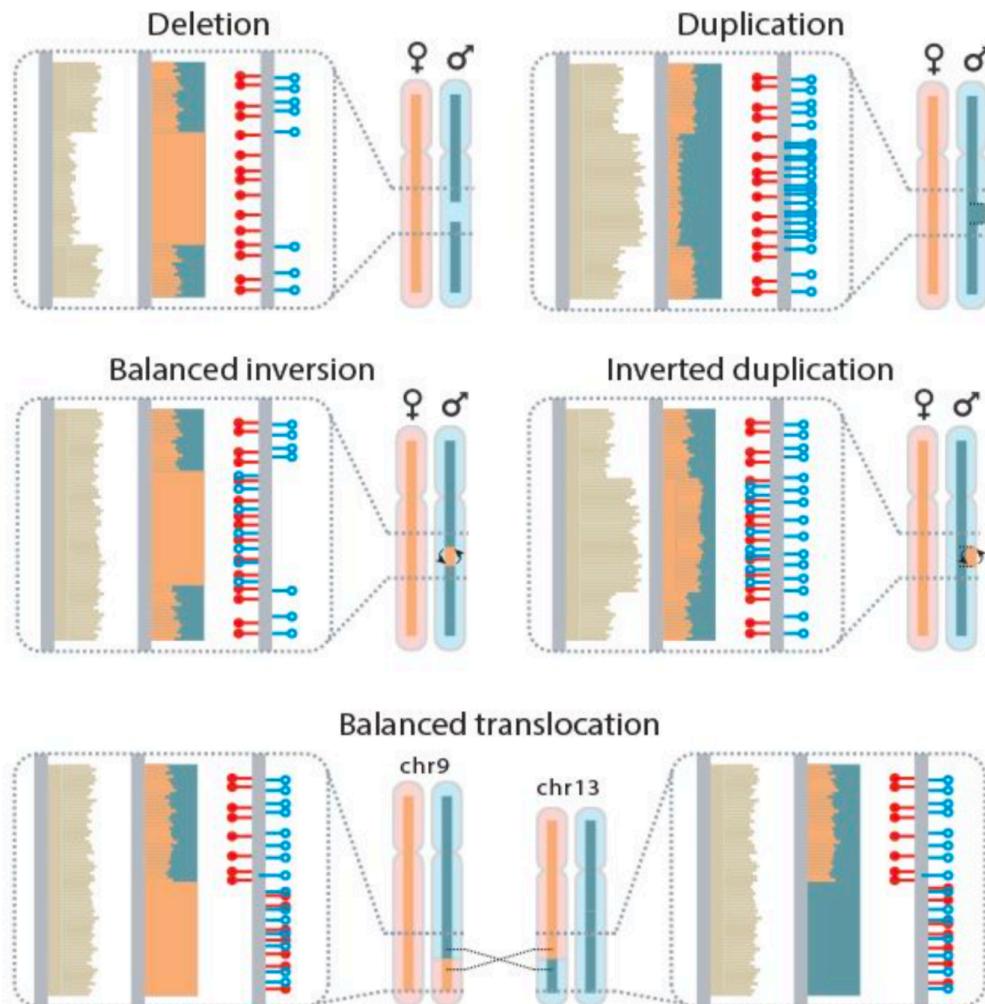


Strand-seq



- In a successful Strand-seq library derived from a diploid cell, expect to see ~50% of the chromosomes exhibiting either a WW or CC state.
- If all chromosomes in the library have a WC inheritance pattern, Strand-seq was probably unsuccessful for that cell.
- By assessing the genomic coverage, level of background and proportion of WW:WC:CC chromosomes for each library, the success rate of the experiment can be assessed and successful libraries selected for further analysis

MosaiCatcher to detect characteristic footprints of SVs from Strand-seq data



- Input: single-cell BAM files
- Workflow management: Snakemake
- Binned read counting (100kb) and normalization
- Assign strand-specific read data into genomic bins
- Detects and haplotype-phases heterozygous SNPs
- Segments the single cell sequence data
- Calculates genotype likelihoods for each segment and single cell

Figure from *scTRIP* manuscript,
submitted, Sanders et al.

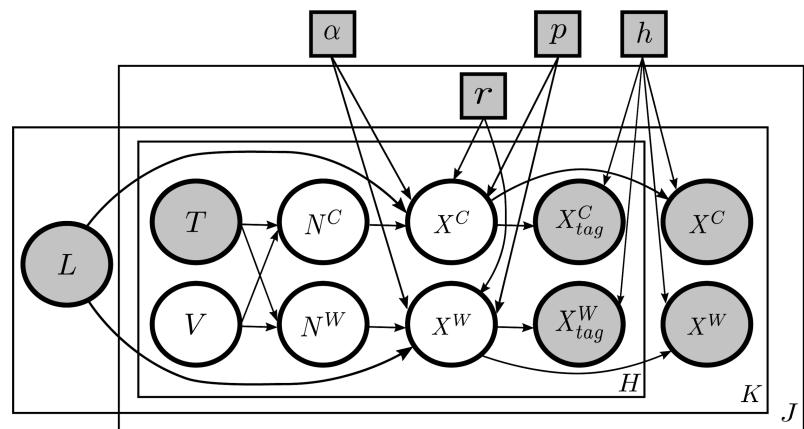
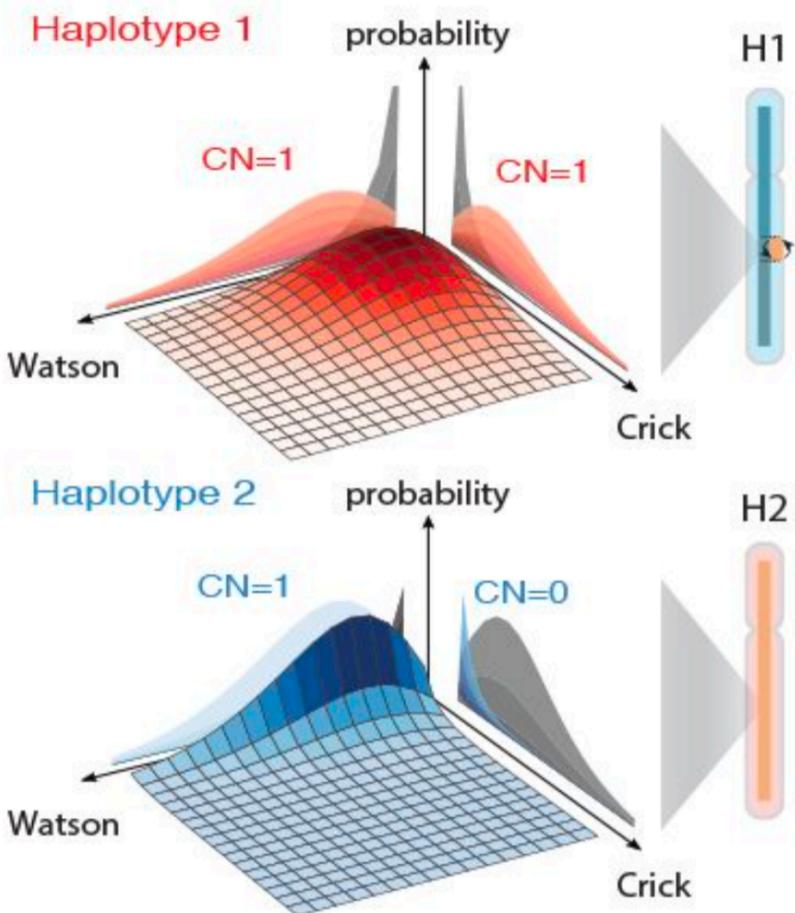
Translation of diagnostic footprint into expected number of copies in W and C

SV diagnostic footprints in a WC ground state							SV diagnostic footprints in a WW ground state						
SV state	Depth	W:C	Haplotype tags		W cov	C cov	SV state	Depth	W:C	Haplotype tags		W cov	C cov
			W	C						W	C		
Reference state	2N	50%	H1	H2	1N	1N	Reference state	2N	100%	H1+H2	-	2N	0N
Deletion of H1	1N	0%	-	H2	0N	1N	Deletion of H1 ³	1N	100%	H2	-	1N	0N
Deletion (homozygous)	0N	-	-	-	0N	0N	Deletion (homozygous)	0N	-	-	-	0N	0N
Duplication of H1	3N	66%	2xH1	H2	2N	1N	Duplication of H1 ³	3N	100%	2xH1+H2	-	3N	0N
Duplication (homozygous)	4N	50%	2xH1	2xH2	2N	2N	Duplication (homozygous)	4N	100%	2xH1+2xH2	-	4N	0N
Inversion of H1	2N	0%	-	H1+H2	0N	2N	Inversion of H1 ³	2N	50%	H2	H1	1N	1N
Inversion (homozygous) ¹	2N	50%	H2	H1	1N	1N	Inversion (homozygous)	2N	0%	-	H1+H2	0N	2N
Inverted duplication of H1 ²	3N	33%	H1	H1+H2	1N	c	Inverted duplication of H1 ³	3N	67%	H1+H2	H1	2N	1N

SV diagnostic footprints in a CW ground state							SV diagnostic footprints in a CC ground state						
SV state	Depth	W:C	Haplotype tags		W cov	C cov	SV state	Depth	W:C	Haplotype tags		W cov	C cov
			W	C						W	C		
Reference state	2N	50%	H2	H1	1N	1N	Reference state	2N	0%	-	H1+H2	0N	2N
Deletion of H1	1N	0%	H2	-	1N	0N	Deletion of H1 ³	1N	0%	-	H2	0N	1N
Deletion (homozygous)	0N	-	-	-	0N	0N	Deletion (homozygous)	0N	-	-	-	0N	0N
Duplication of H1	3N	66%	H2	2xH1	1N	2N	Duplication of H1 ³	3N	0%	-	2xH1+H2	0N	3N
Duplication (homozygous)	4N	50%	2xH2	2xH1	2N	2N	Duplication (homozygous)	4N	0%	-	2xH1+2xH2	0N	4N
Inversion of H1	2N	0%	H1+H2	-	2N	0N	Inversion of H1 ³	2N	50%	H2	H1	1N	1N
Inversion (homozygous) ¹	2N	50%	H1	H2	1N	1N	Inversion (homozygous)	2N	100%	H1+H2	-	2N	0N
Inverted duplication of H1 ²	3N	33%	H1+H2	H1	c	1N	Inverted duplication of H1 ³	3N	33%	H1	H1+H2	1N	2N

- Negative binomial (NB) distribution is the basis for this Bayesian framework (p, r)
- Parameters (p, r) are estimated from the observed read counts
- Each SV diagnostic footprint can be translated into the expected number of copies sequenced in W and C orientation
- These expectations are formalized in Bayesian model

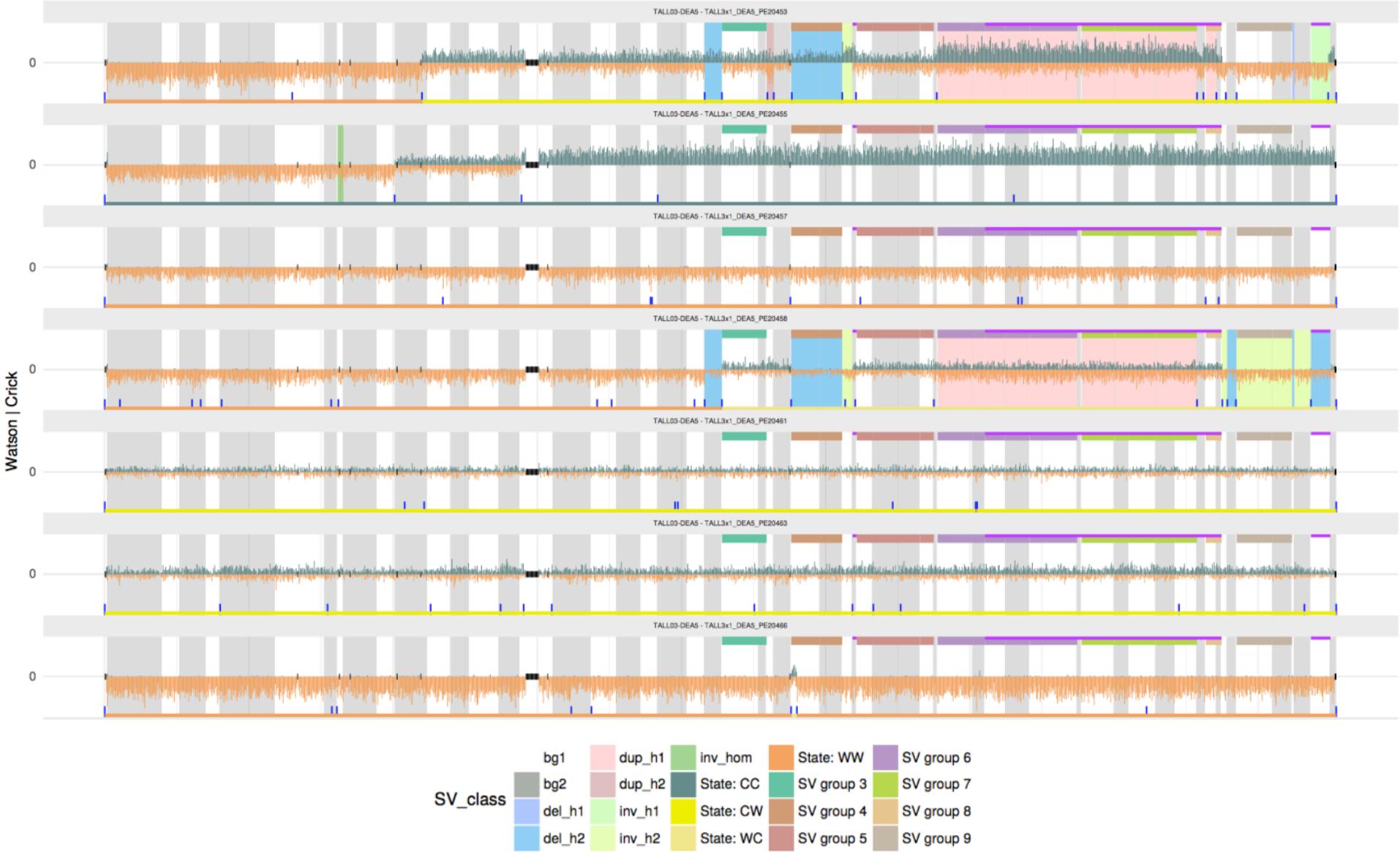
MosaiCatcher uses Bayesian model for SV genotype likelihoods in single cells



- Every haplotype-resolved SV class in a segment together with the ground state define a Watson and Crick copy number used to compute the NB likelihood of observed read counts.
- SV call is accepted if the log odds ratios (of an SV genotype vs. the reference state) was at least 4
- The probability distributions represent an InvDup on H1
- Segments on both strands are seen for haplotype 1 (H1), but H2 is represented on the W strand only

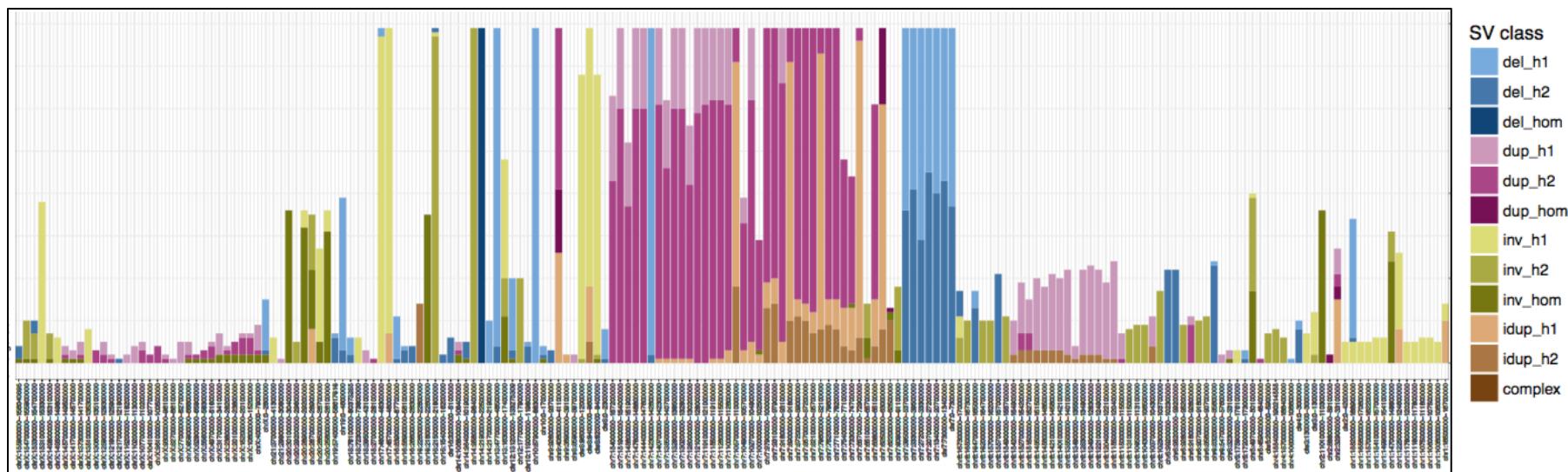
Chromosome plot with SVs called by MosaiCatcher framework

Strand-seq from T-ALL PDX (P1) Chromosome6



Variant allele fraction of SVs called by MosaiCatcher framework

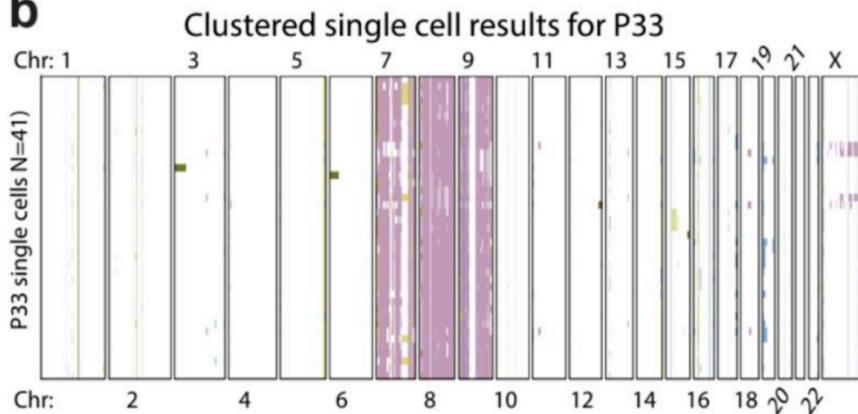
Strand-seq from T-ALL PDX (P1)



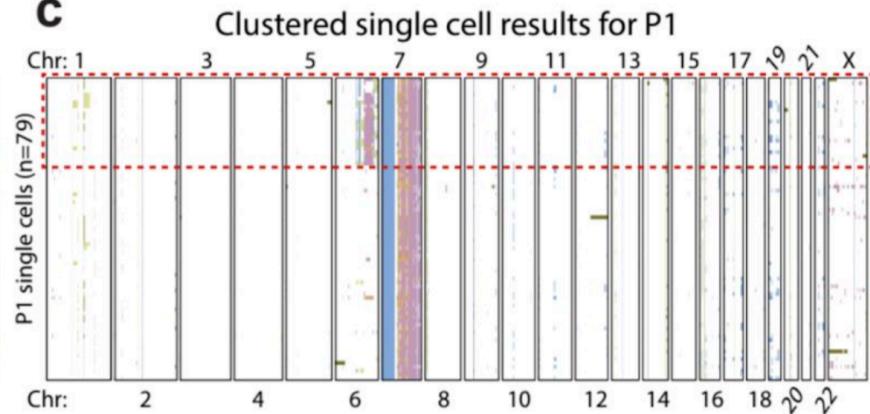
- If the VAF is close to 1, the SVs are expected to be germline variant
- If the VAF is below 1, the SVs are expected to be somatic variant
- If the SVs only detected by one cell, it can be rare SV event, or an SCE (sister chromatid exchange) event
- SCEs happen independently in each single cell, and unlike SVs, SCEs are not transmitted clonally to daughter cells. Hence, changepoints resulting from SCEs are very unlikely to recur at the same position in >1 cell of a sample

Heatmap of single-cells based on SVs called by MosaiCatcher framework

b



c

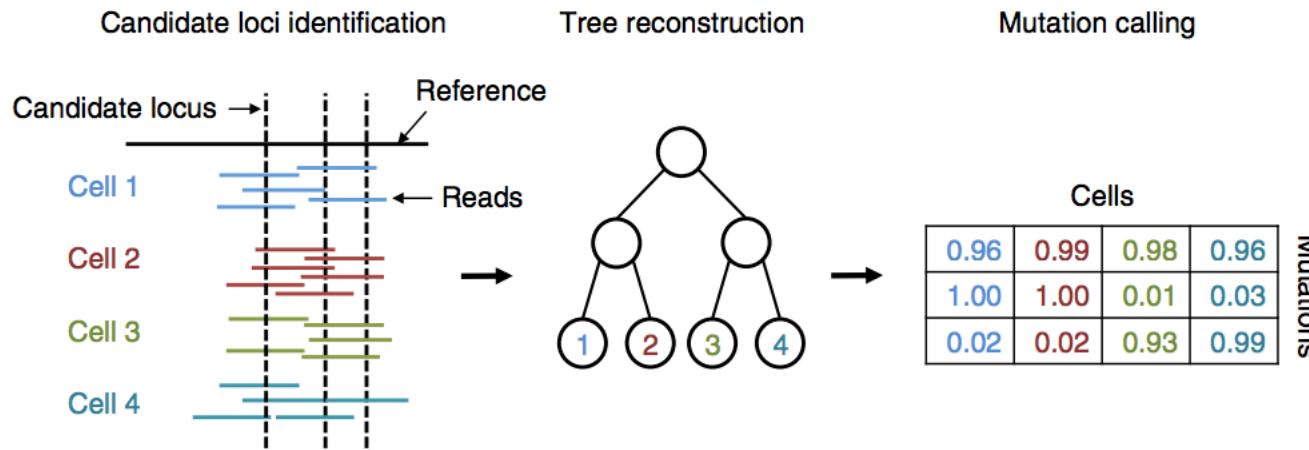


SV class

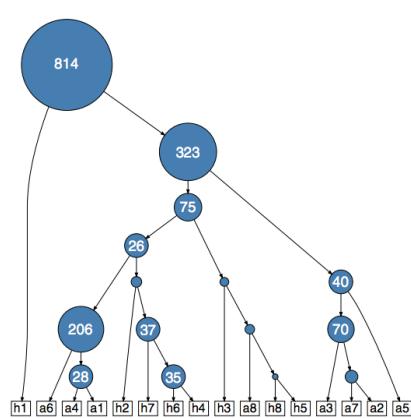
- del_h1
- del_h2
- del_hom
- dup_h1
- dup_h2
- dup_hom
- inv_h1
- inv_h2
- inv_hom
- idup_h1
- idup_h2
- complex

- This heatmap was arranged using Ward's method for hierarchical clustering of SVs genotype likelihoods in two PDX samples
- P33 shows single dominant clone but P1 shows subclonal population in the sample represented by 23 cells

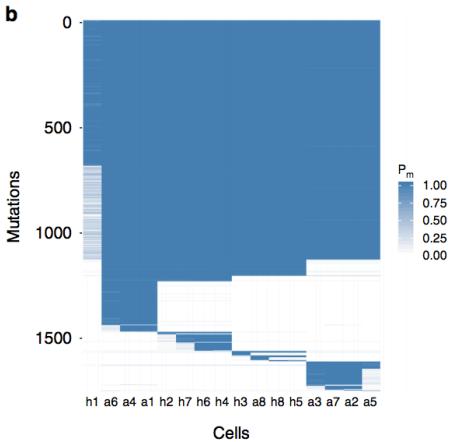
More tools for the clustering and Phylogenetics of single cells - [SCIΦ](#)



a

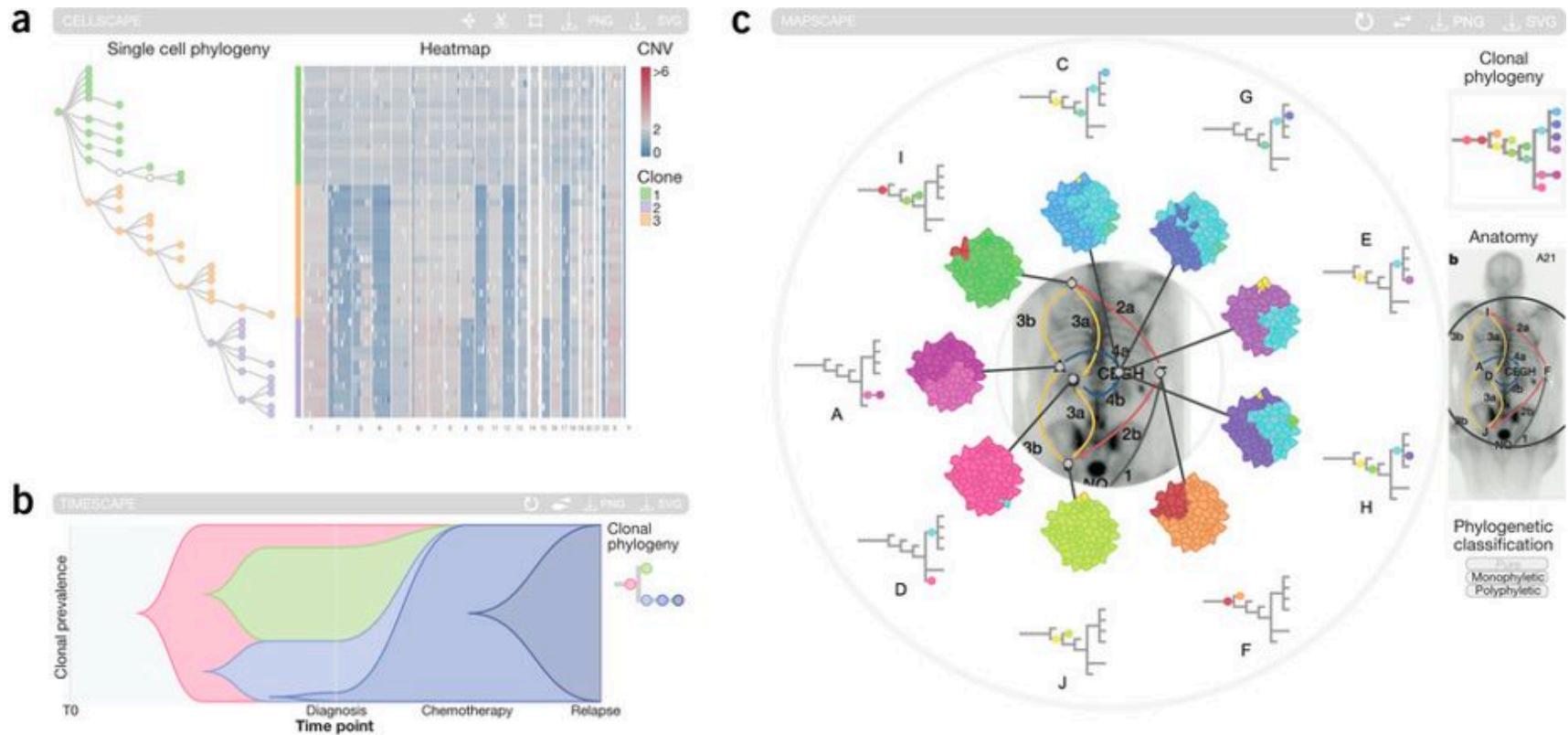


b



- This method jointly calls mutations in individual cells and estimates the tumor phylogeny among these cells.
- Employing a Markov Chain Monte Carlo scheme enables us to reliably call mutations in each single cell even in experiments with high drop-out rates and missing data.

More tools for the clustering and Phylogenetics of single cells – E-scape



Smith et al. *Nat Methods*, 2017 Sohrab P Shah group

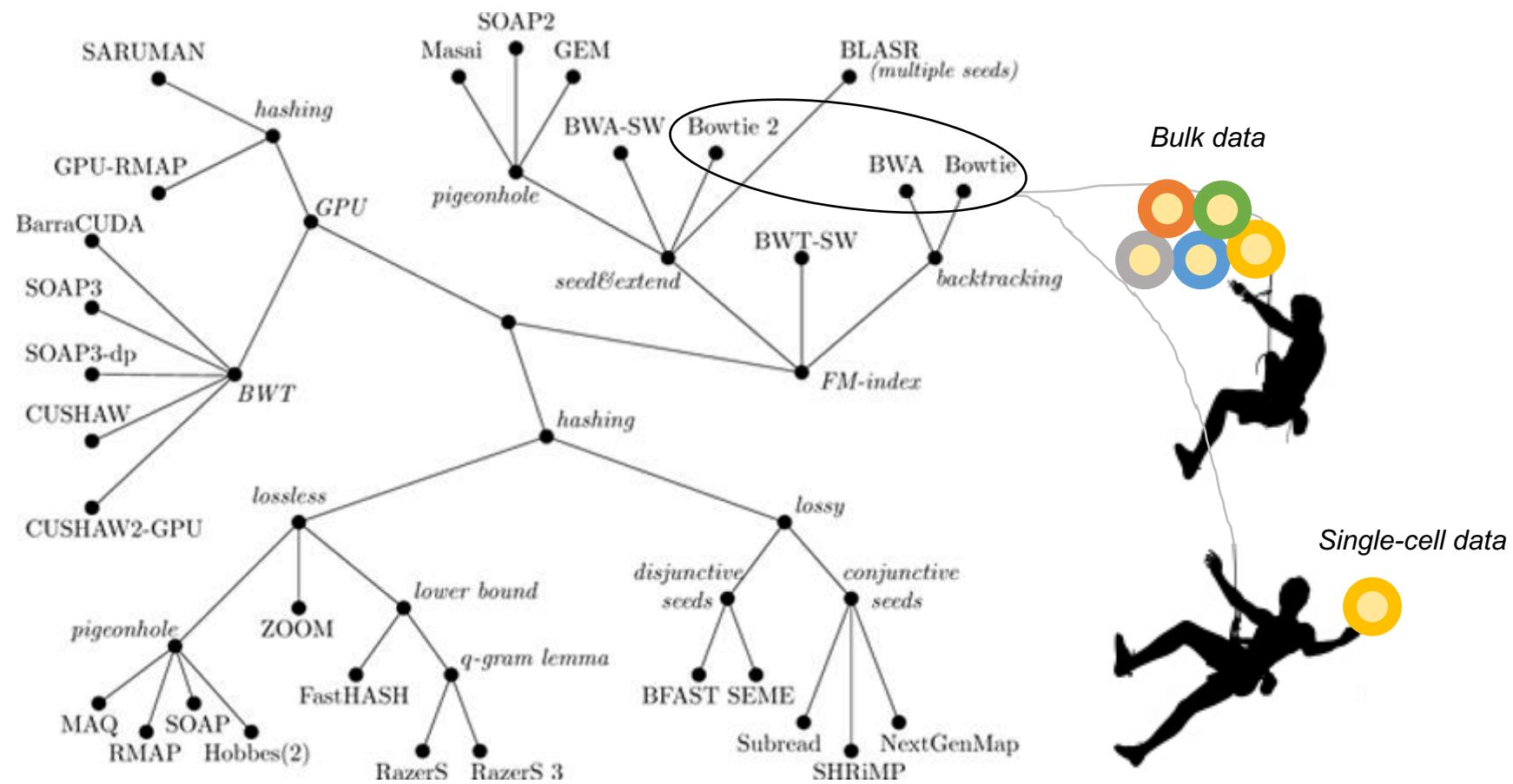
- E-scape consists of three visualization tools deployed as R html widgets
- 1) TimeScape for time series analysis
- 2) MapScape for spatial distribution analysis
- 3) CellScape for single-cell analysis, including analysis of the phylogenetic and population dynamics of cancer – the heatmap depicts genome-wide CNVs across single cells. The tree illustrates the evolutionary relationships between cells.

Summary

- Single-cell whole-genome sequencing and Strand-seq are the representative technologies for the single-cell genomics
 - In addition to genomic position, orientation of the alignment (Crick, Watson), breakpoint, Strand state and phasing is important in Strand-seq analysis
 - Presentation from David
-
- MosaiCatcher is the computational algorithm to detect characteristic footprint of diverse SV classes from the Strand-seq data
 - SCIPhi, and Timescape can be used for the clustering and phylogenetics of single-cell genomics data to understand subclonal structure and tumor evolutionary tree

Thank you for listening

Short DNA Read Mapping: An Algorithmic Tour



Proc IEEE Inst Electr
Electron Eng. 2017

Additionally, for Strand-seq, orientation of the reads need to be considered!

General challenges and approaches of the single-cell genome analysis

- Challenge1: High rates of allelic drop-out as a result of the random non-amplification of one allele at the heterozygous genotype sites
 - Challenge2: False positive artifacts can arise in the DNA amplification when random errors introduced early in the process
 - Challenge3: Uneven amplification across the genome which results in non-uniform coverage and insufficient coverage depth for reliable base calling
-
- SNV caller doesn't transfer information between cells : [**SCcaller**](#) – it detects variants independently for each cell and accounts for local allelic amplification biases. However, it cannot recover mutations from drop-out events or loss of heterozygosity.
 - SNP caller transfers information between cells : [**Monovar**](#) – It addresses the problem of low and uneven coverage in mutation calling by pooling sequencing information across cells while assuming that no dependencies exist across sites

How's the computational workflow different to detect structural variations (SV) ?