BreaKmer: detection of structural variation in targeted massively parallel sequencing data using kmers

Abo, MacConaill et al.

Genomic Structural Variations

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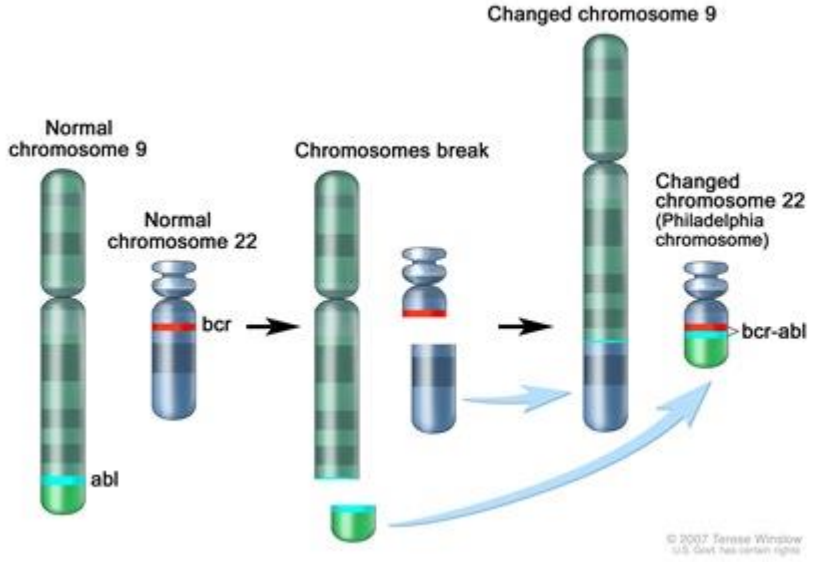

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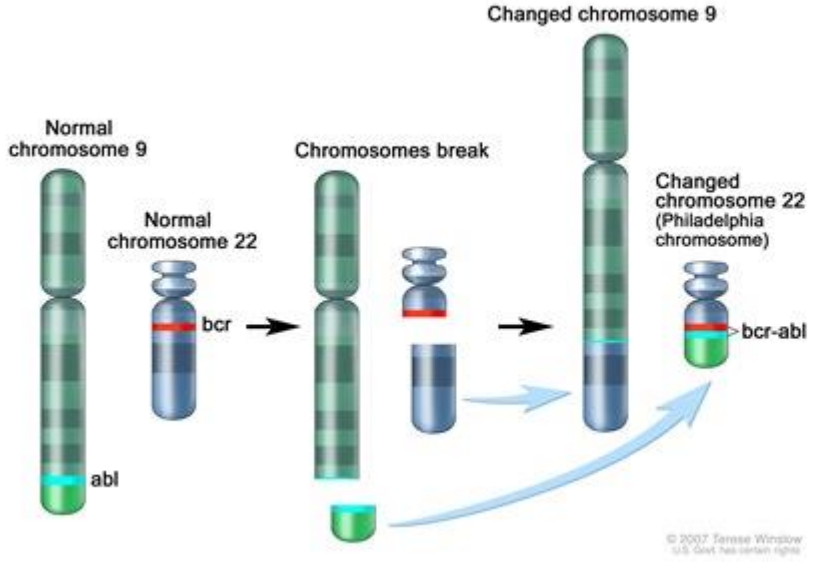
BCR-ABL fusion gene in
Chronic Myeloid Leukemia

Genomic Structural Variations

- SV's are one of the driving mechanisms of cancer



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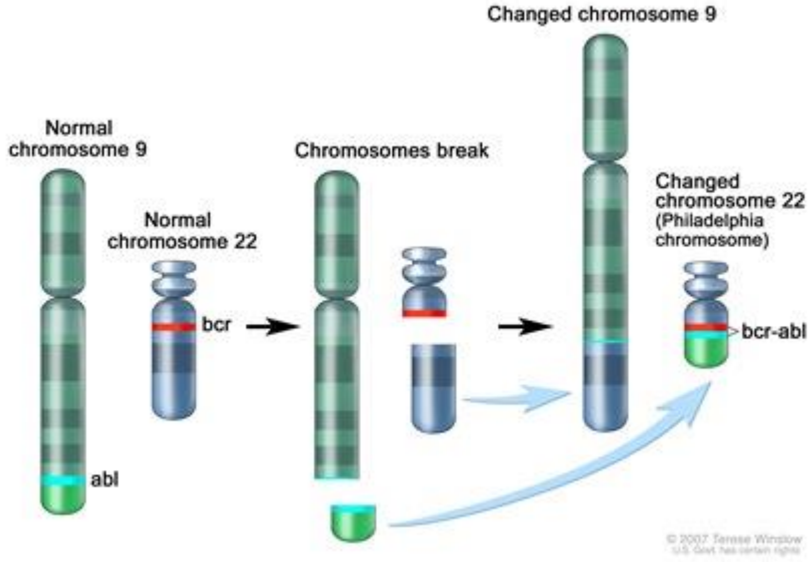
Changed chromosome 9
Normal chromosome 9
Chromosomes break
Normal chromosome 22
Changed chromosome 22 (Philadelphia chromosome)
bcr
bcr-abl
abl
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BCR-ABL fusion gene in Chronic Myeloid Leukemia

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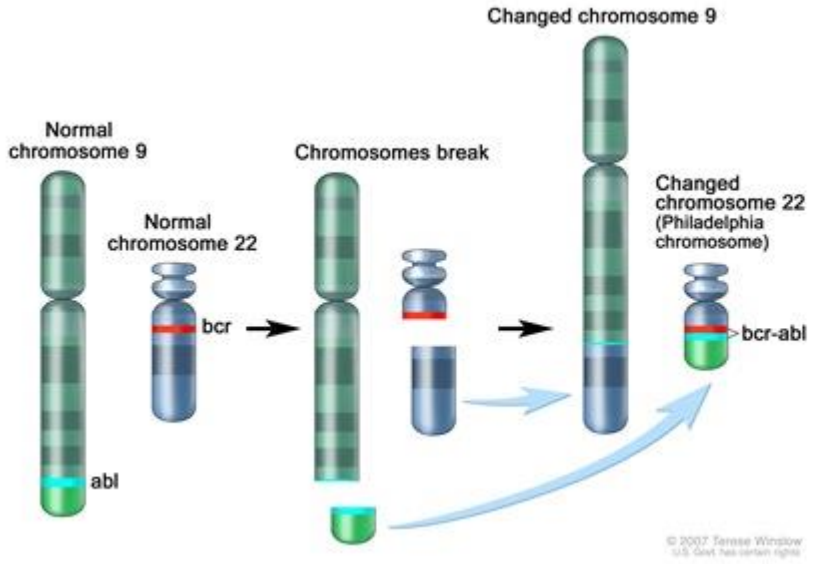

BCR-ABL fusion gene in Chronic Myeloid Leukemia

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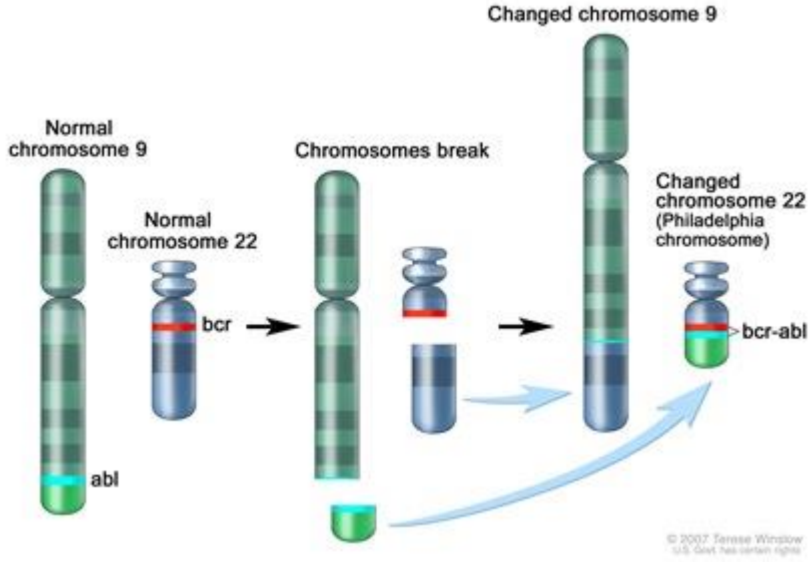
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BreaKmer – A novel method for identifying SV’s

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  - Using all alignment data available: unmatched pairs, mis-aligned reads and discordant reads.
  - Sequence assembly from reads using k-mers is the core.

Discordant and misaligned reads

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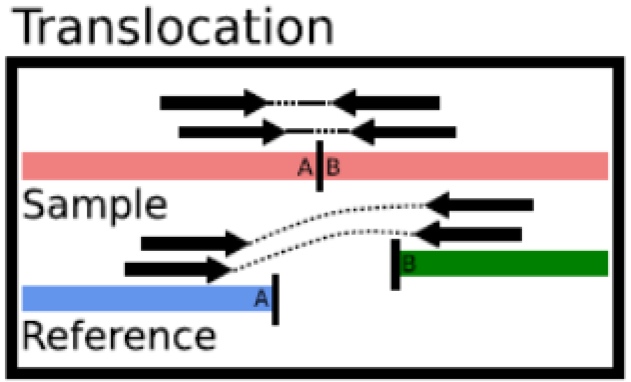

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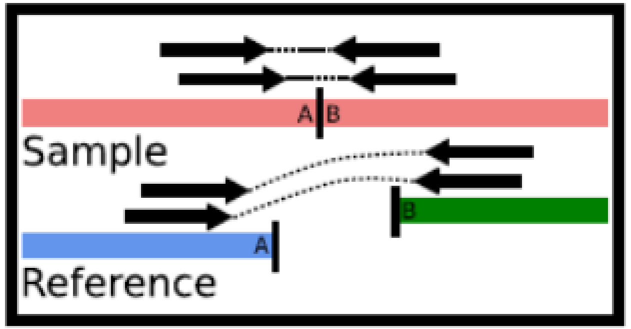
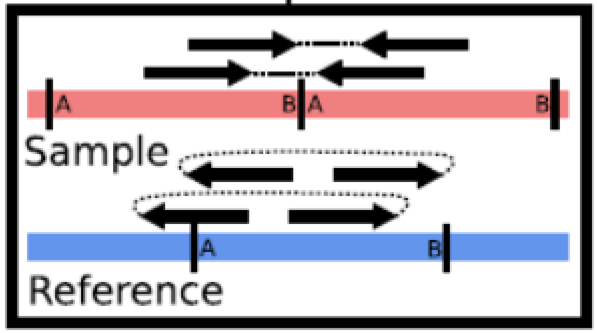
Discordant reads:

Translocation



Tandem duplication



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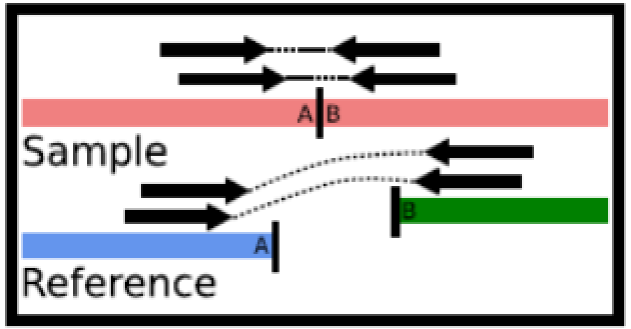

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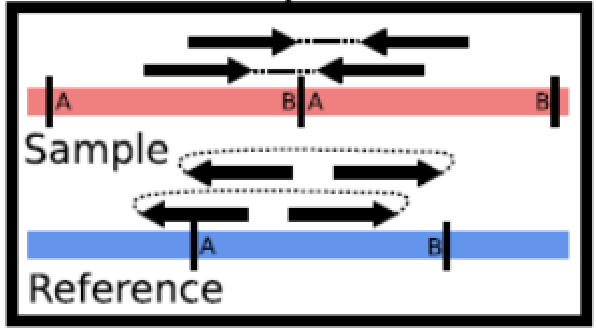
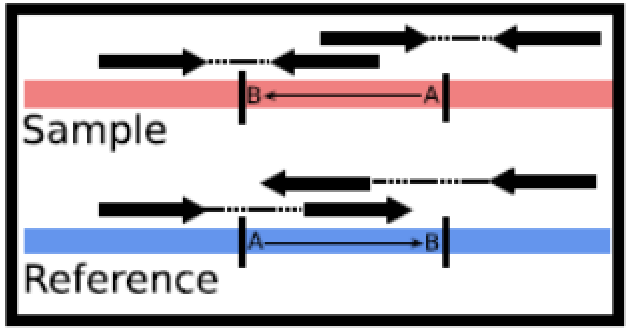


Inversion



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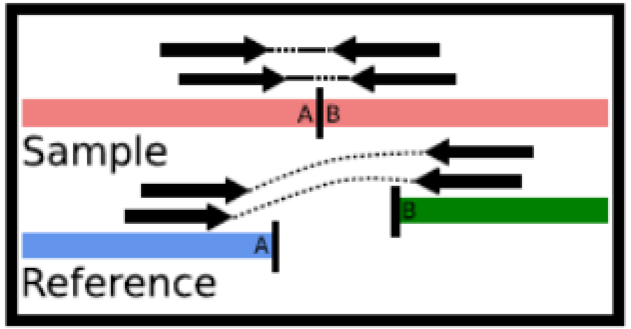

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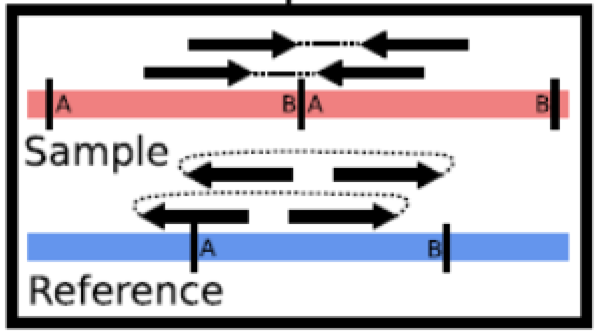

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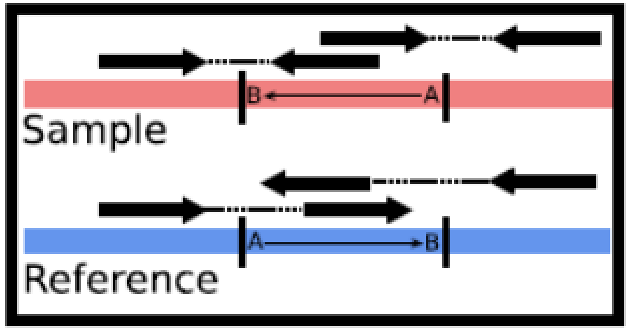

Misaligned reads:

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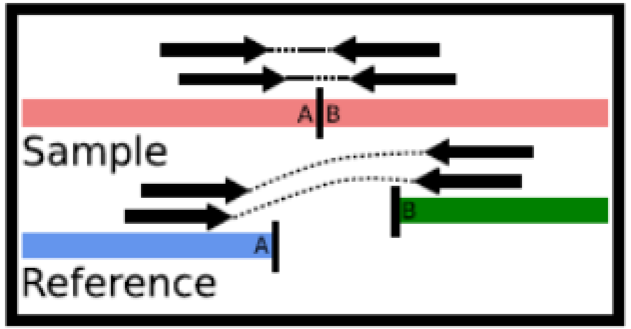

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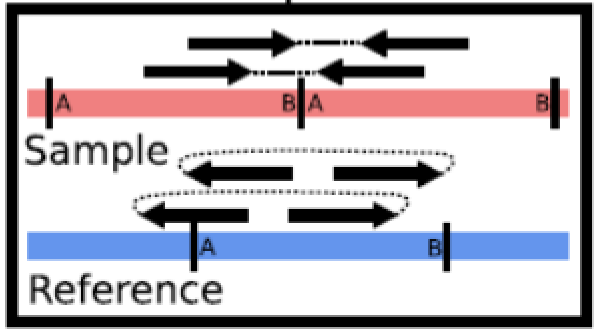

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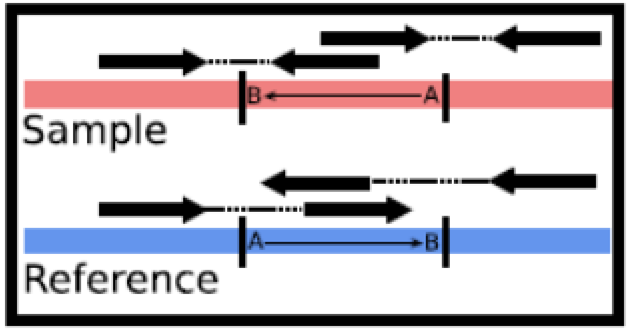

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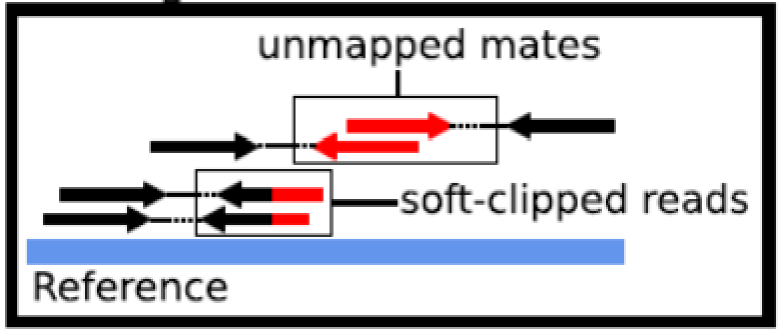

BreaKmer – General outline

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SV Calling

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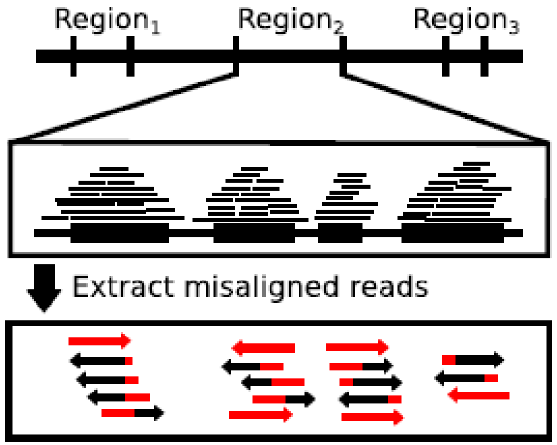

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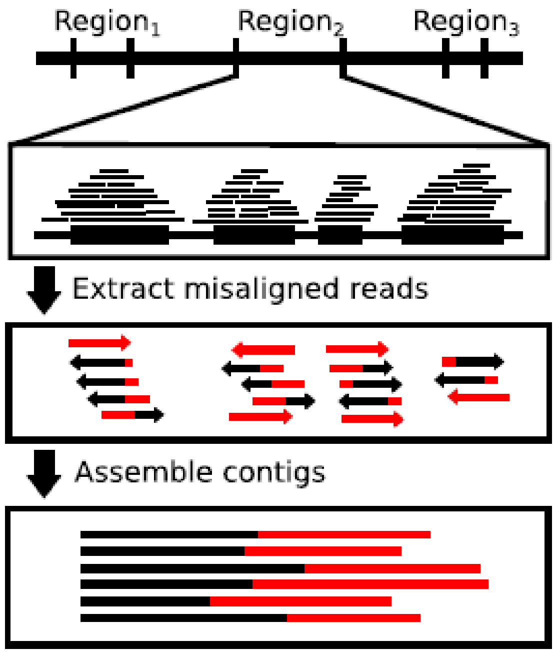

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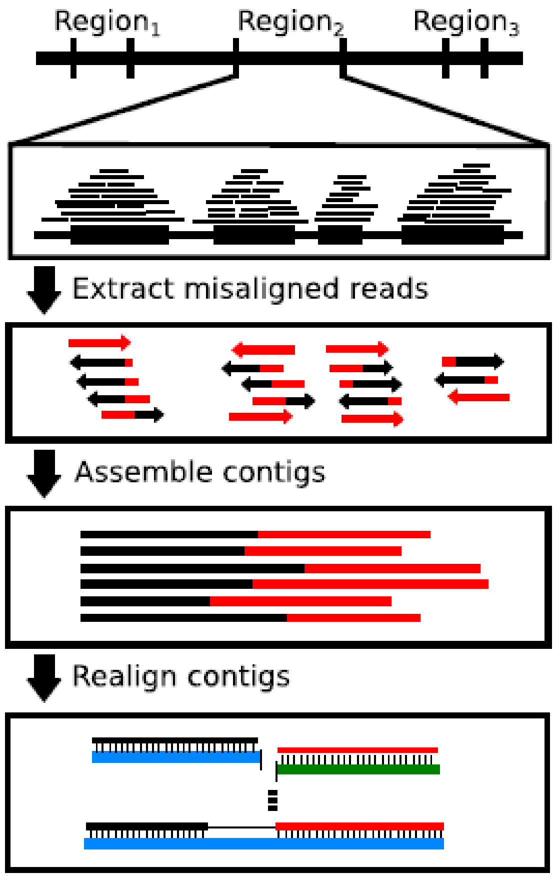

BreaKmer – General outline

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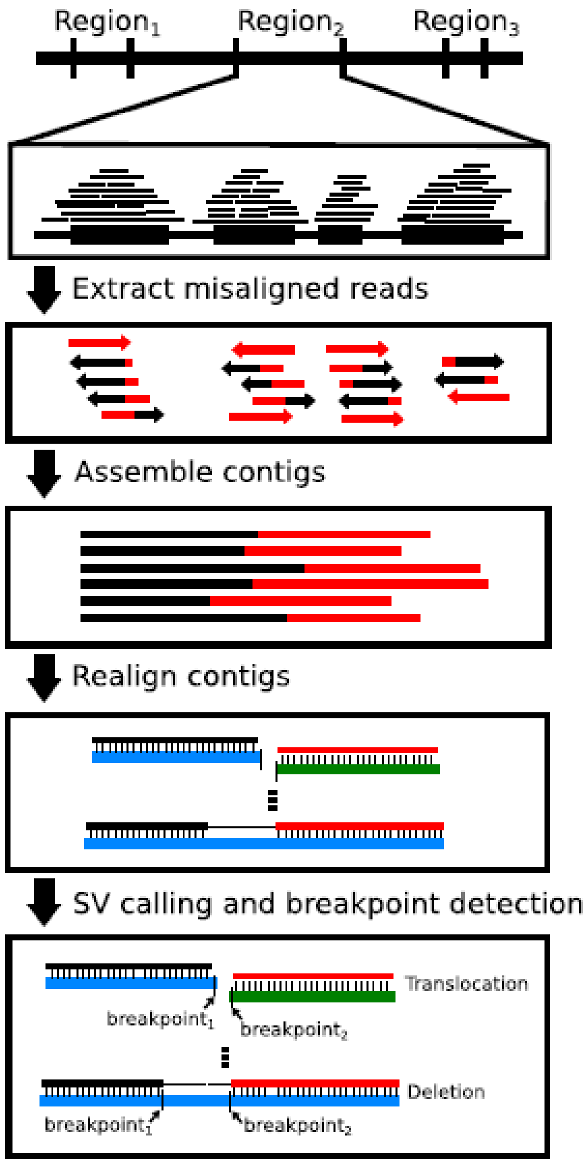

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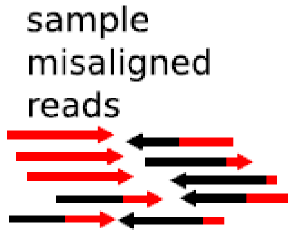

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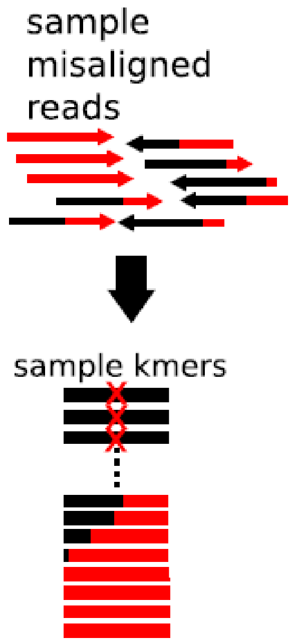

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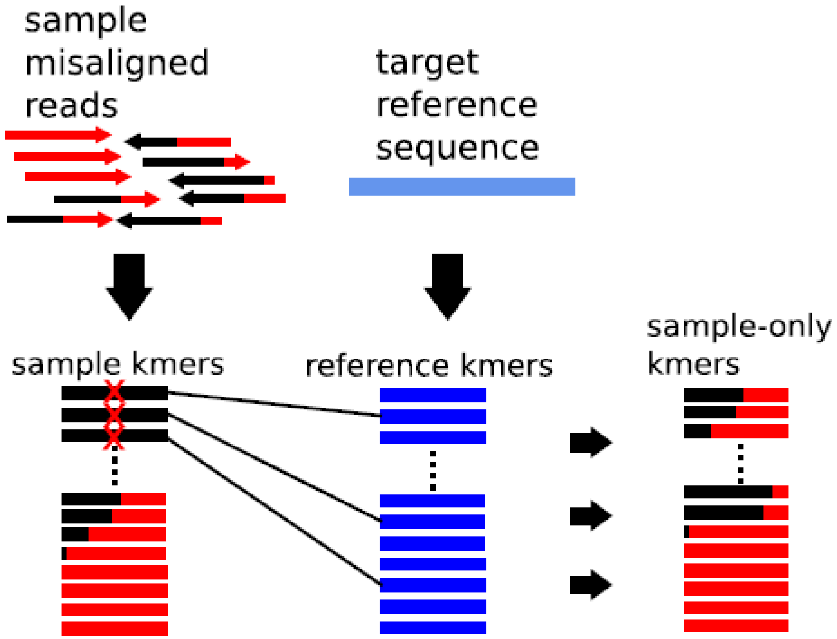

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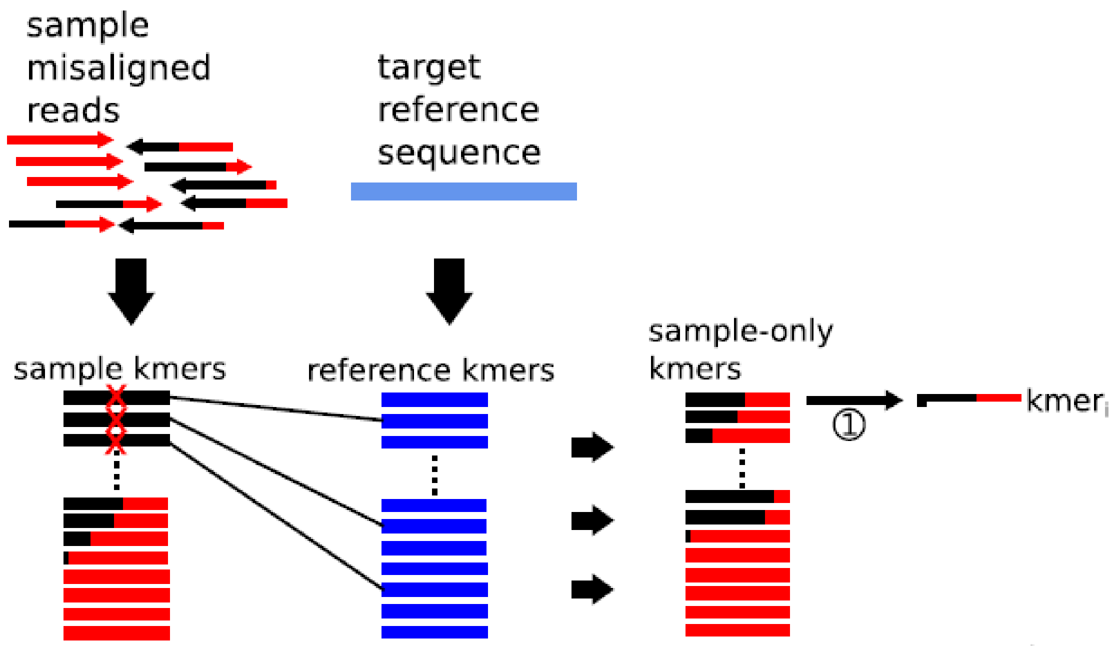

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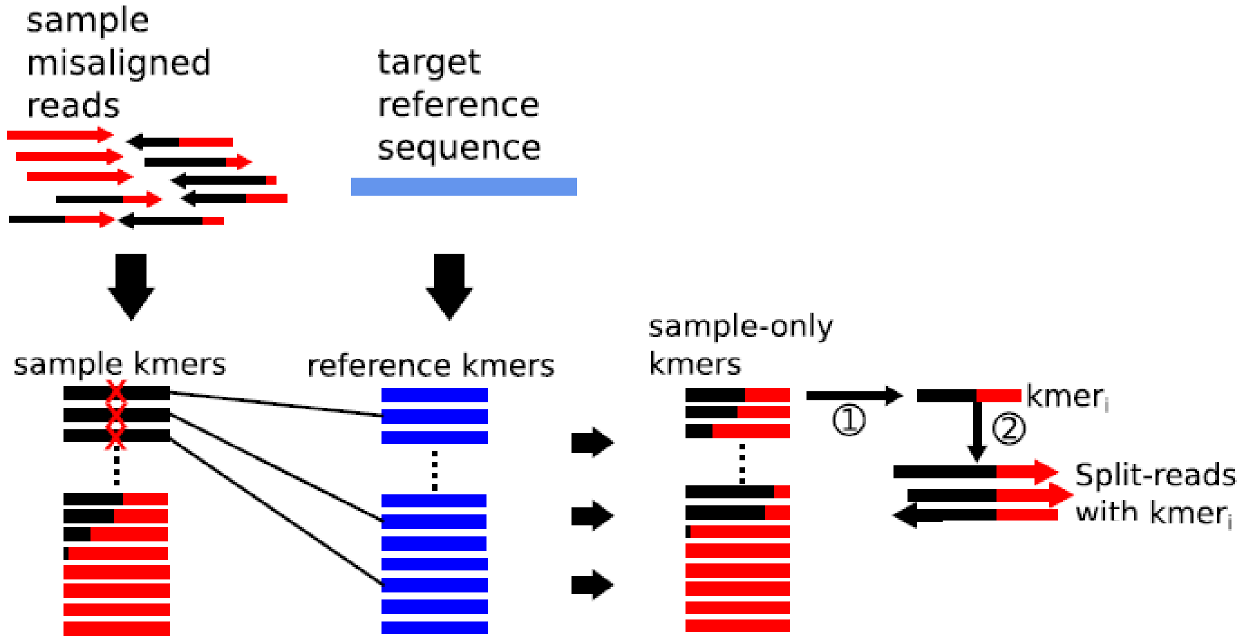

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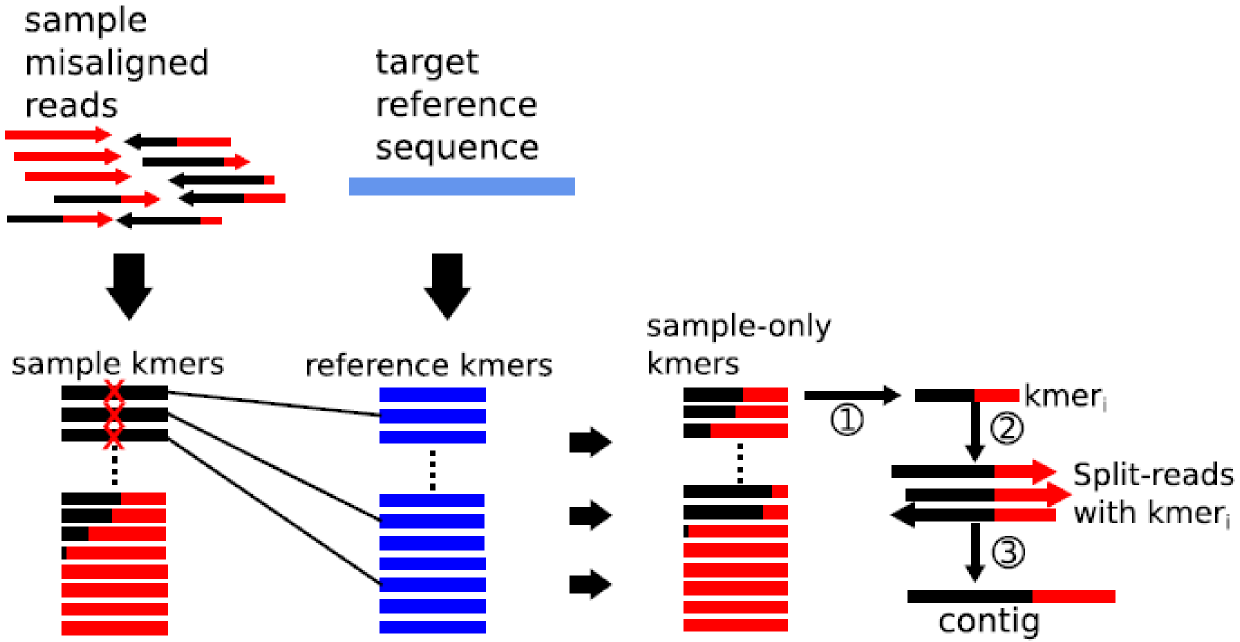

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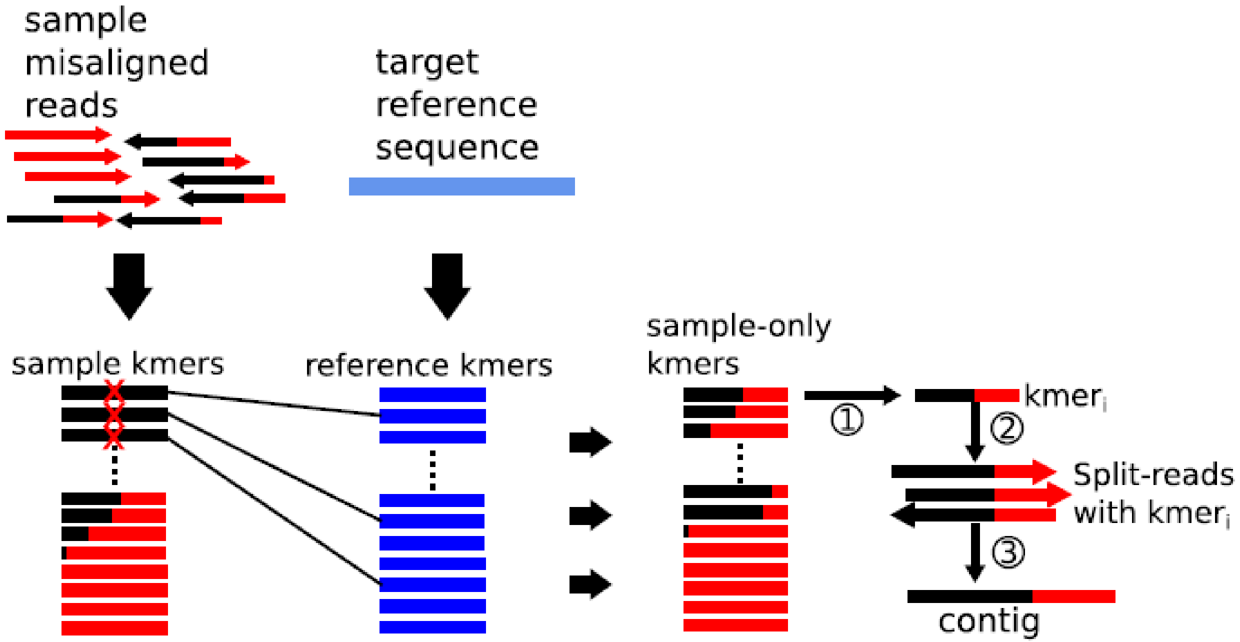

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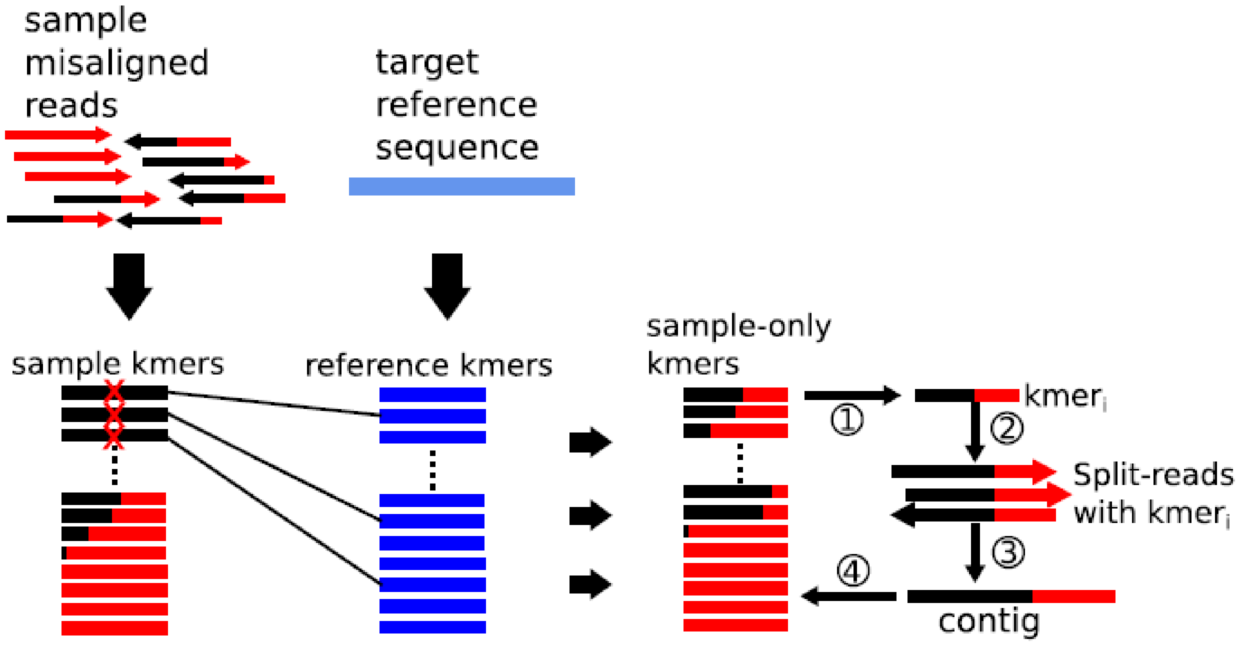

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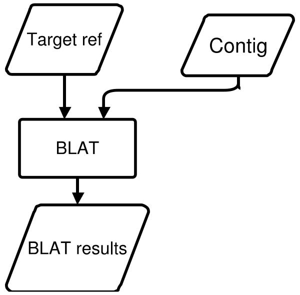

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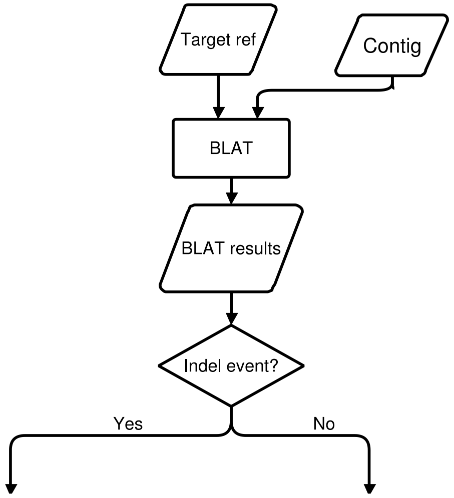

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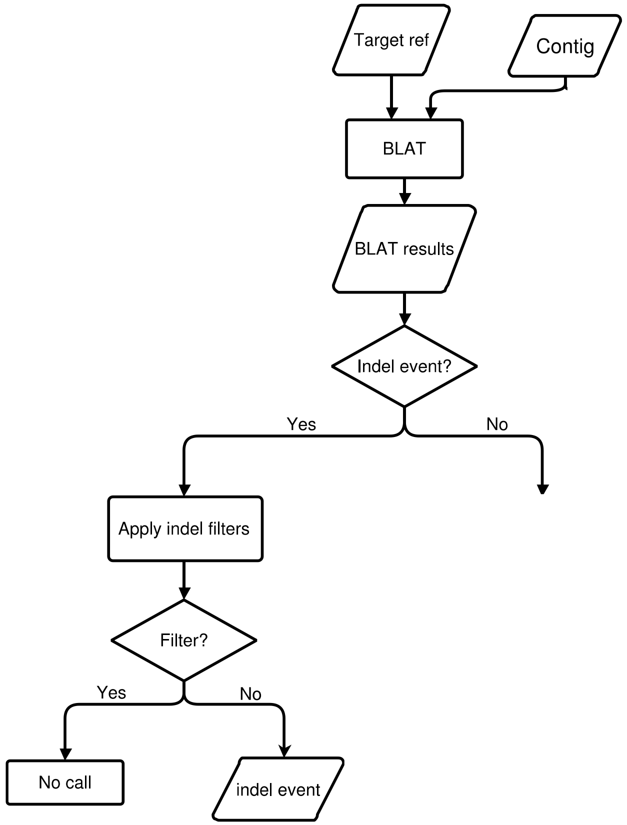

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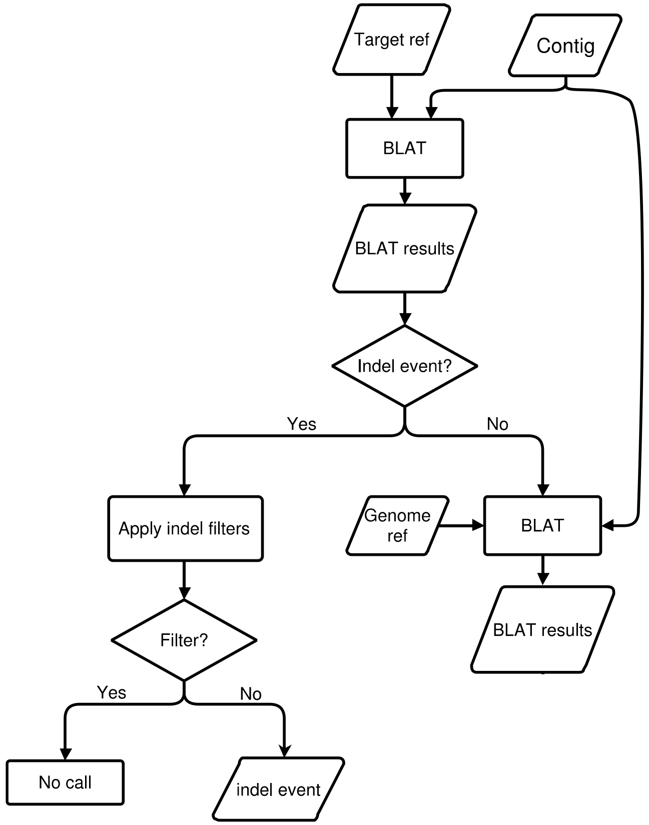

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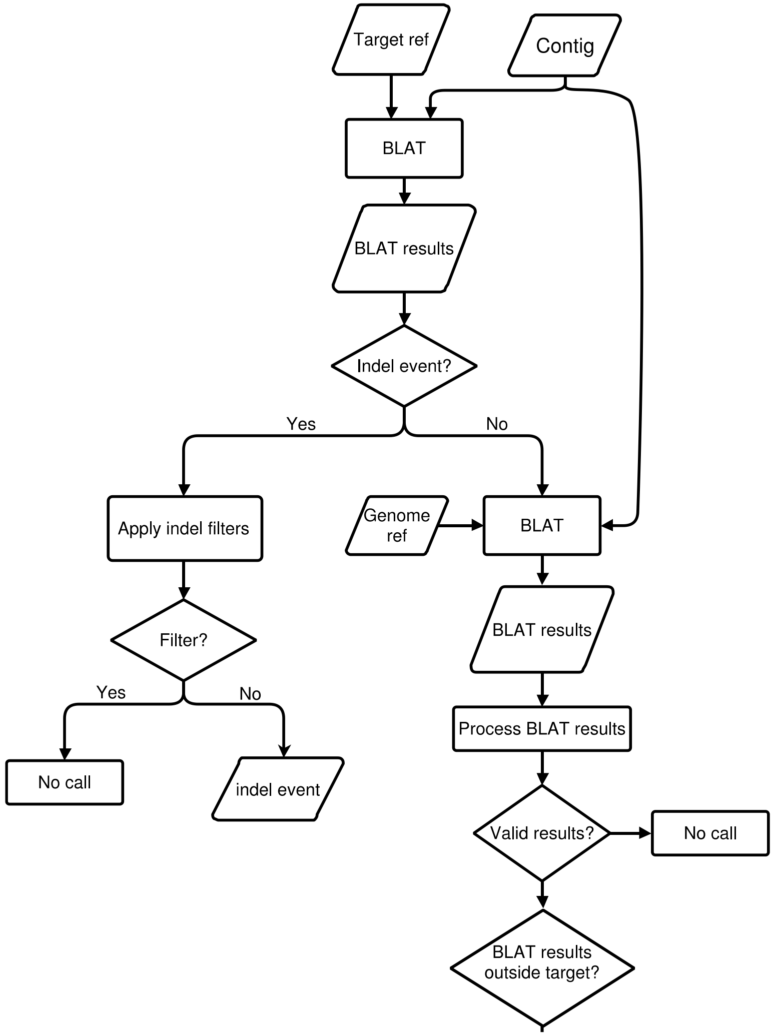

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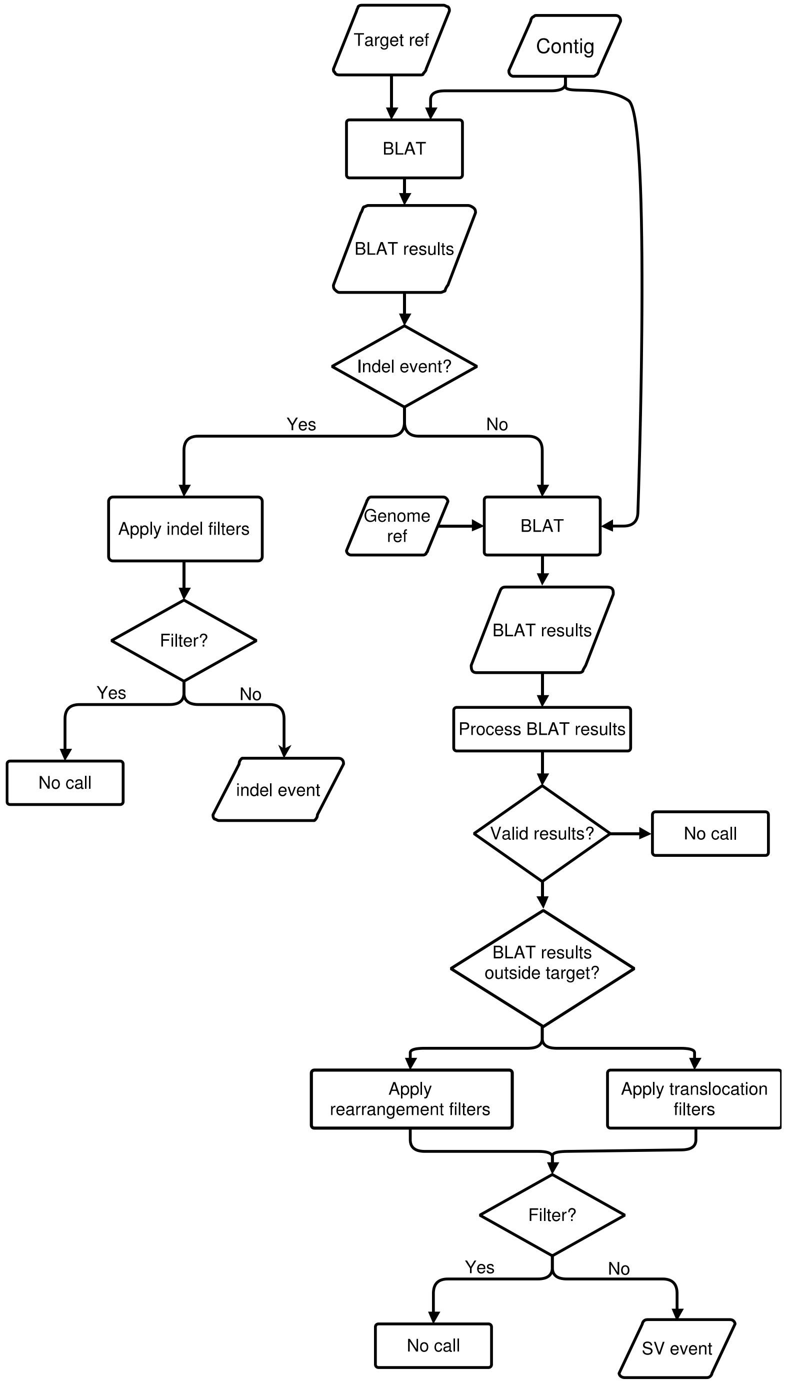
- Apply rearrangement (local) or translocation filters.



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- Comparison to 4 other methods – CREST, Meerkat, BreakDancer, Pindel

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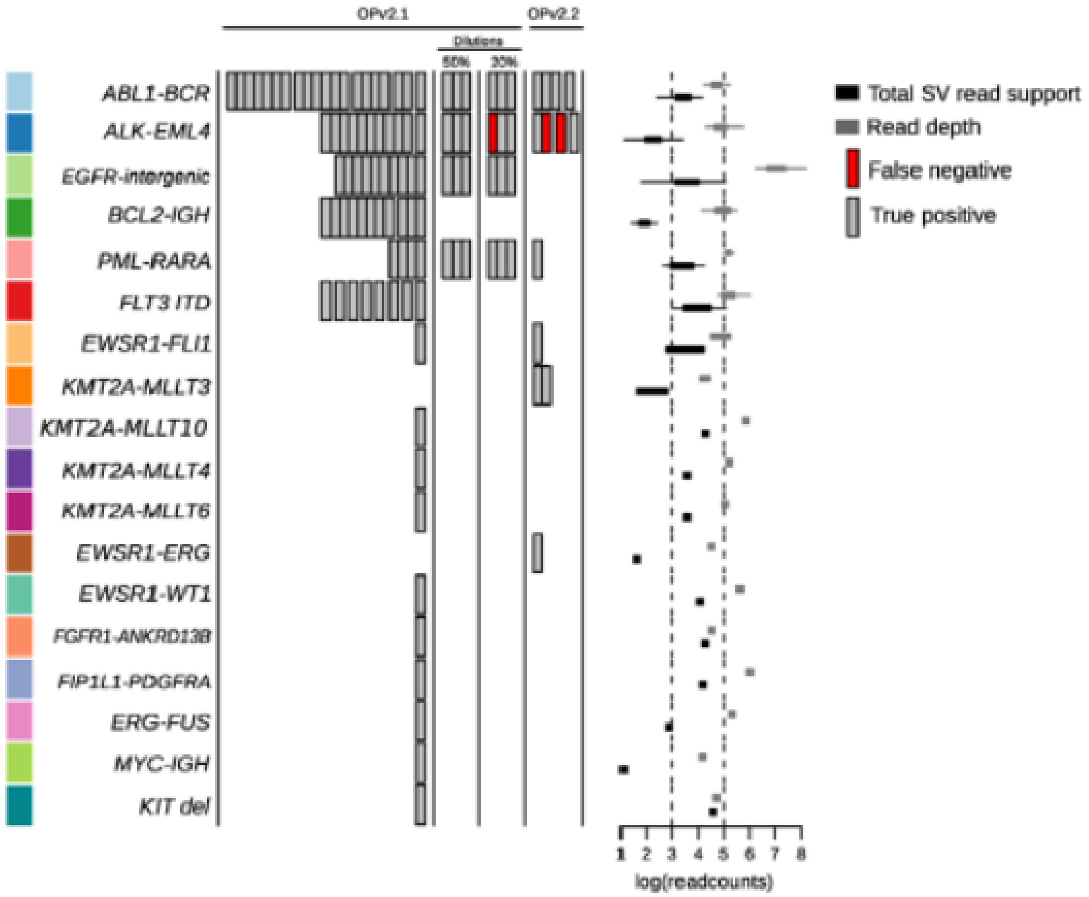
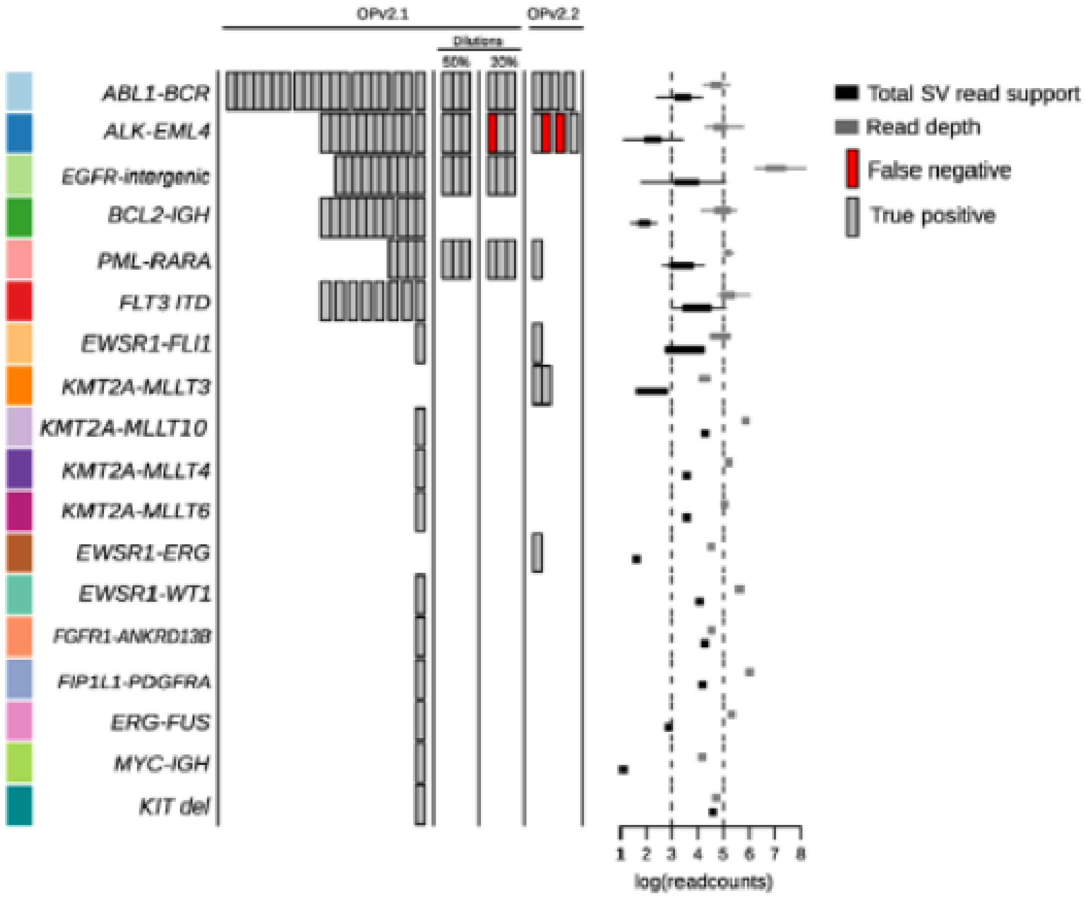

OPv2.1
OPv2.2
Dilutions
50%
20%
ABL1-BCR
ALK-EML4
EGFR-intergenic
BCL2-IGH
PML-RARA
FLT3 ITD
EWSR1-FLI1
KMT2A-MLLT3
KMT2A-MLLT10
KMT2A-MLLT4
KMT2A-MLLT6
EWSR1-ERG
EWSR1-WT1
FGFR1-ANKRD13B
FIP1L1-PDGFRA
ERG-FUS
MYC-IGH
KIT del
Total SV read support
Read depth
False negative
True positive
1 2 3 4 5 6 7 8
log(readcounts)

Results

- 28/29 translocation positive samples were called.



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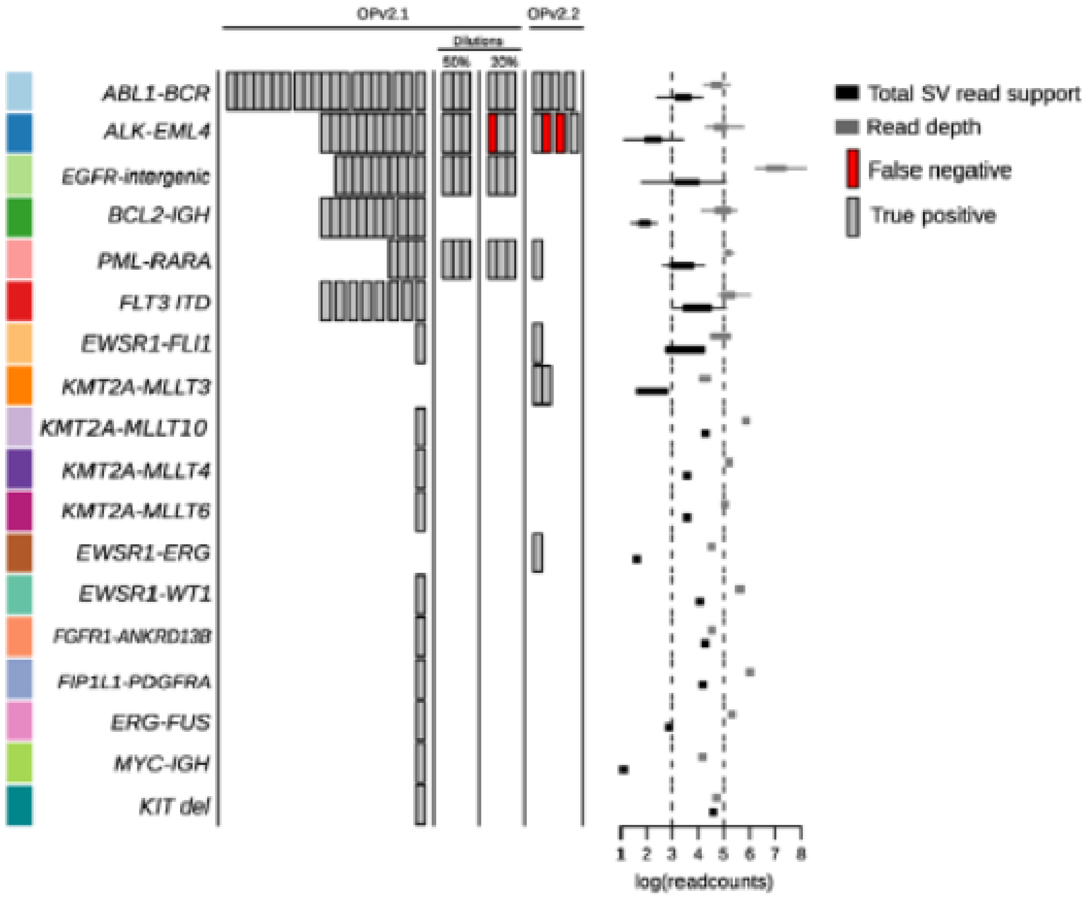

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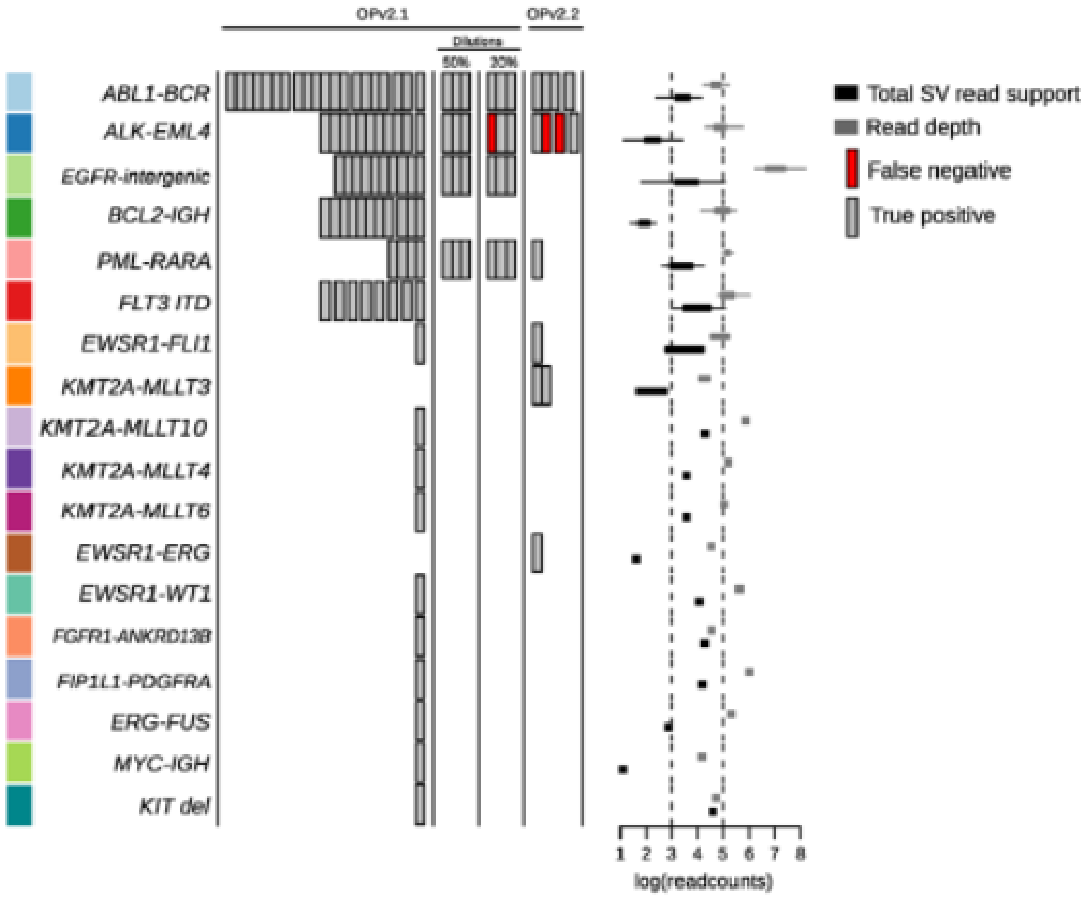

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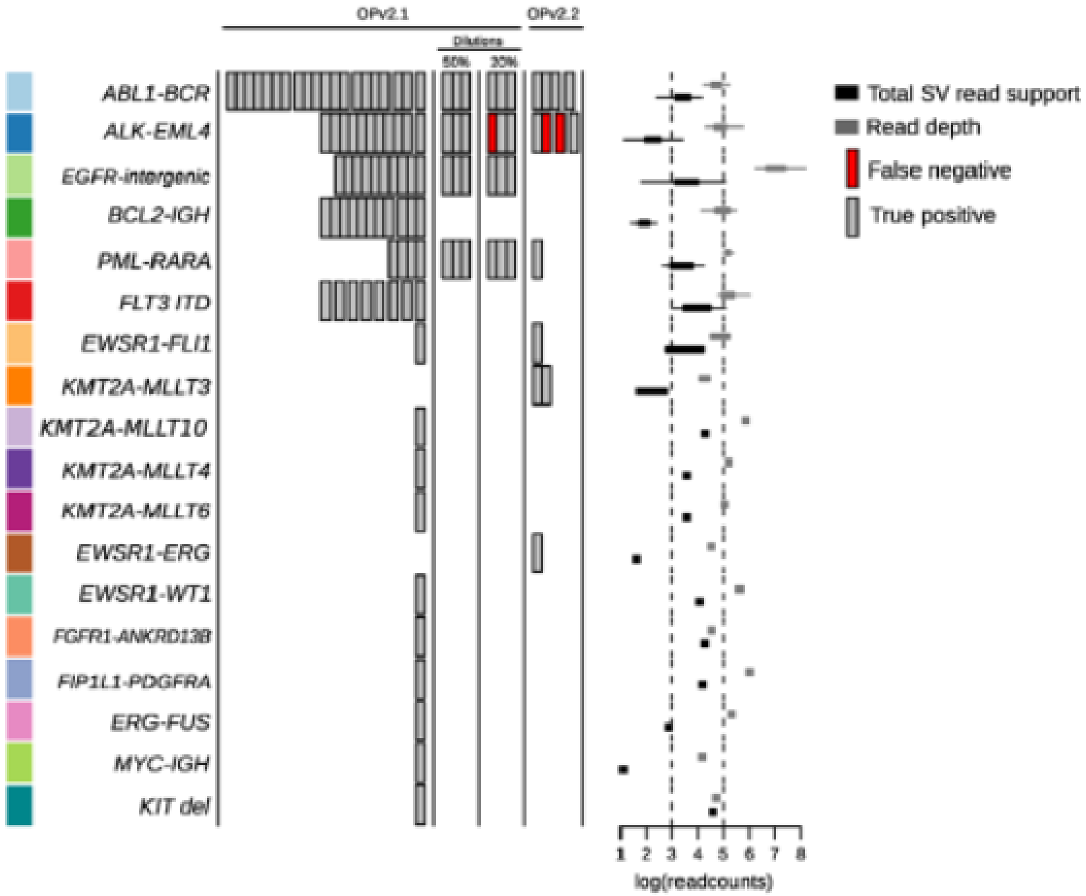

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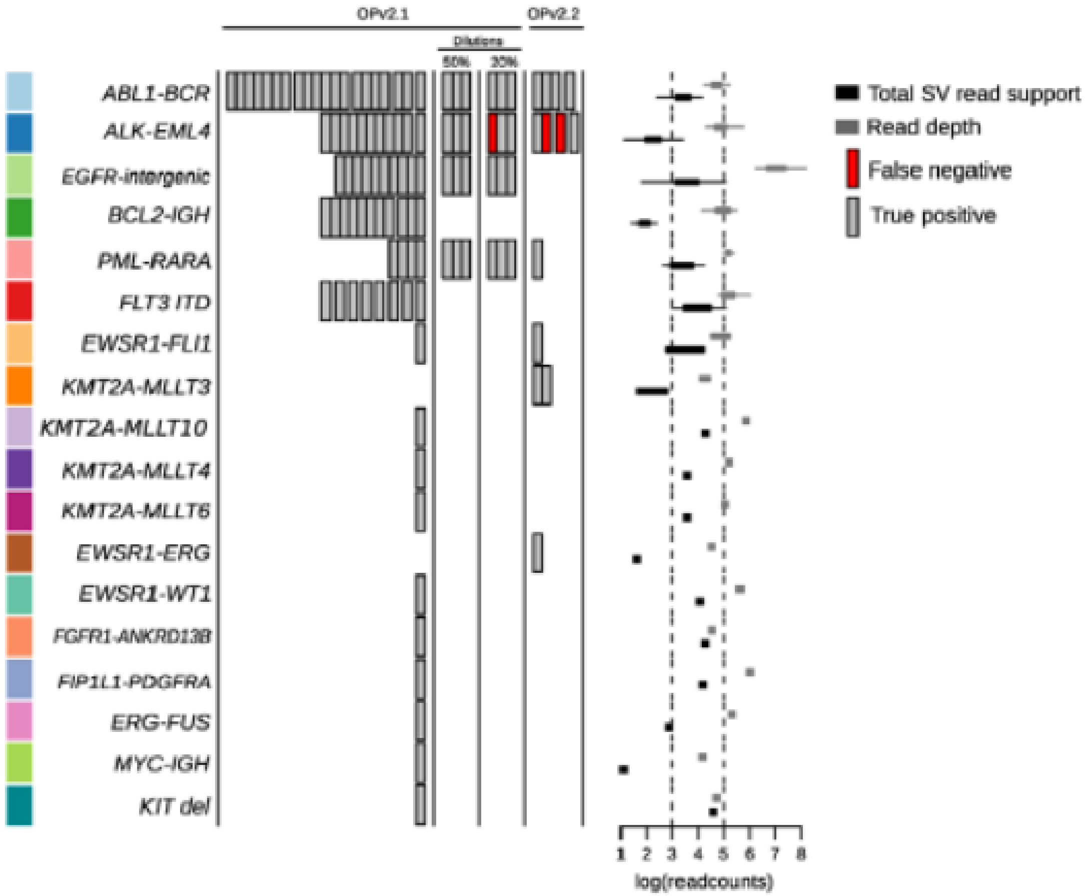
- Overall 97.4% sensitivity in detecting the 38 known events.



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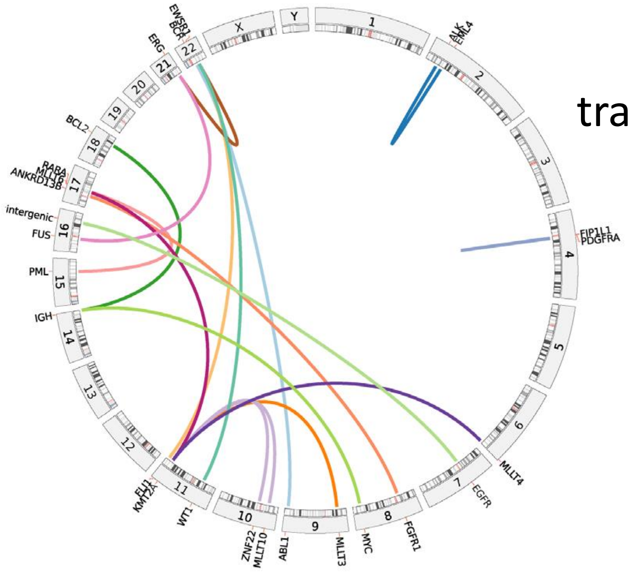
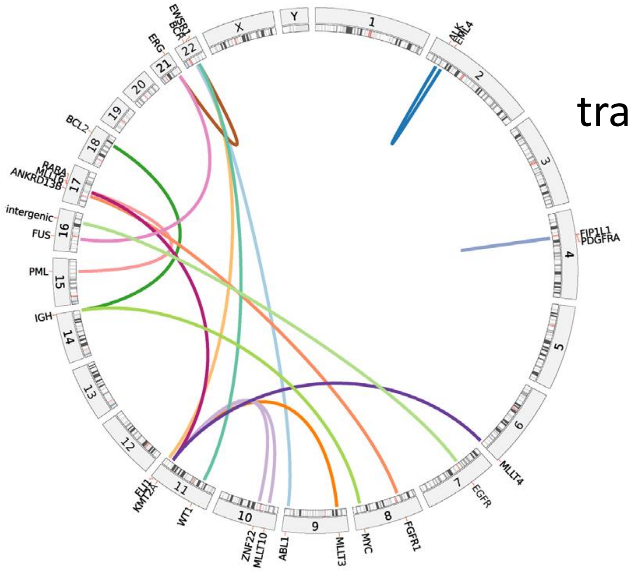


Known translocations

Results

- 21 unknown SV's detected.



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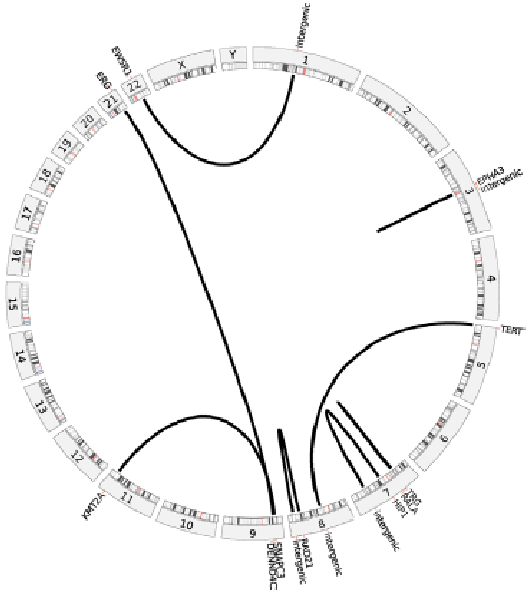

Novel translocations identified by BreaKmer

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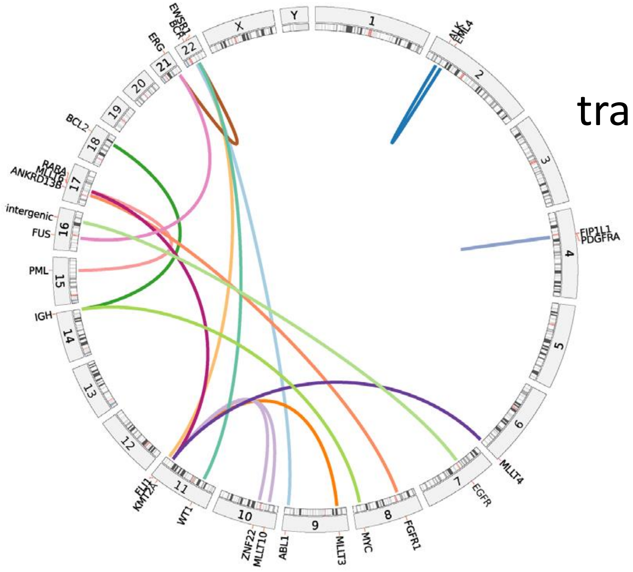

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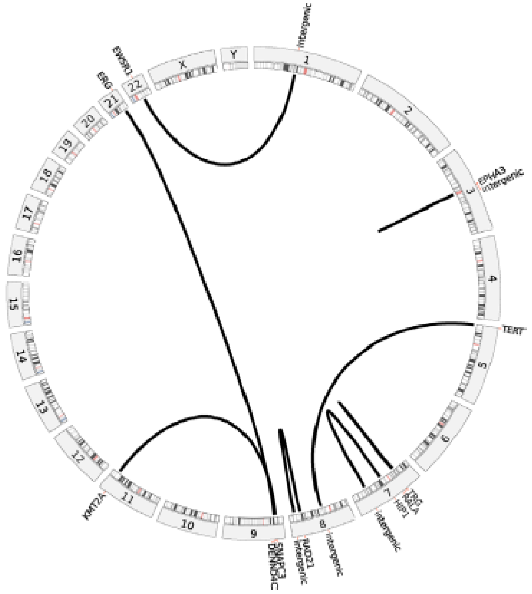

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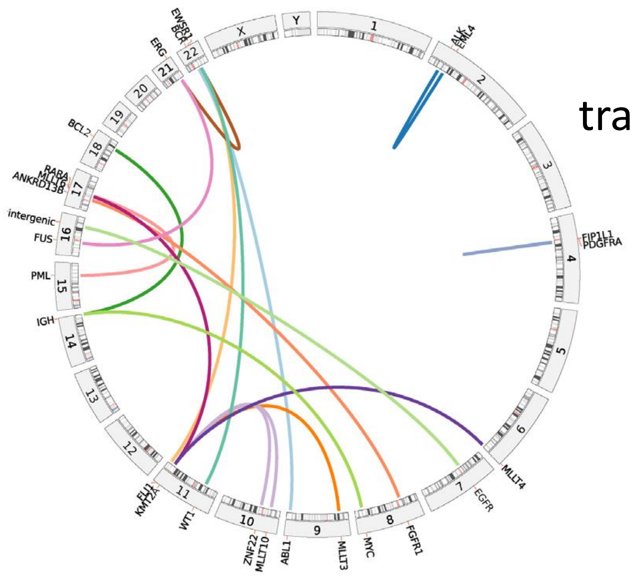
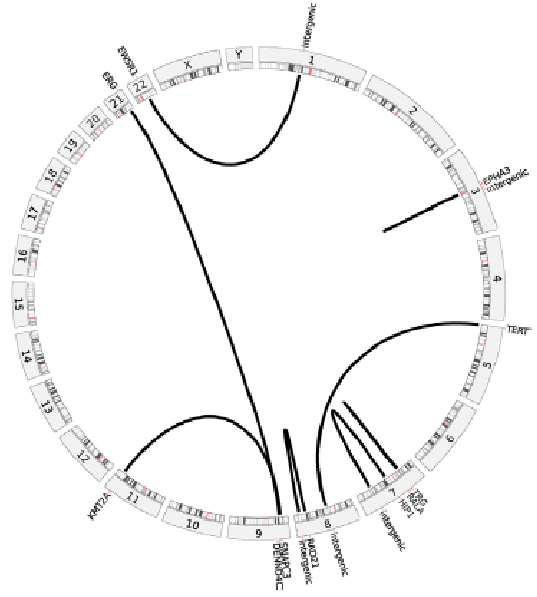
  - 8/9 indels were validated (1 sample didn’t have sufficient DNA)



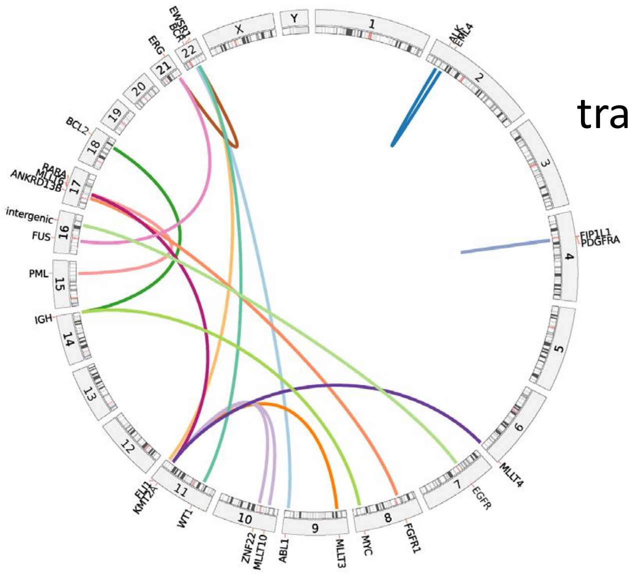
Known translocations



Novel translocations identified by BreaKmer

Results

- 21 unknown SV's detected.
  - 9/11 translocations were validated
  - 8/9 indels were validated (1 sample didn't have sufficient DNA)
- 77.3% predictive value



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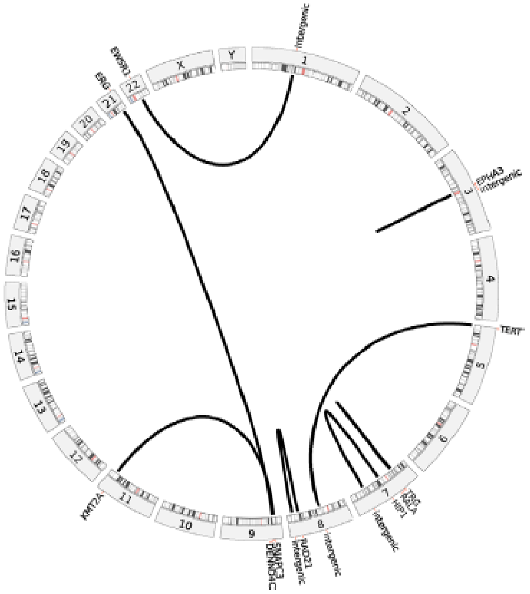

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  - 9/11 translocations were validated
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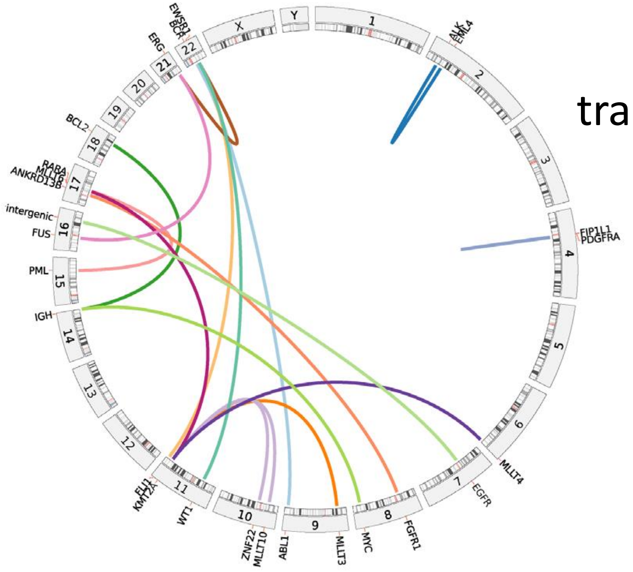
- 77.3% predictive value
- 5 SV’s detected in the 80 non-cancer samples – 3 of them later validated.



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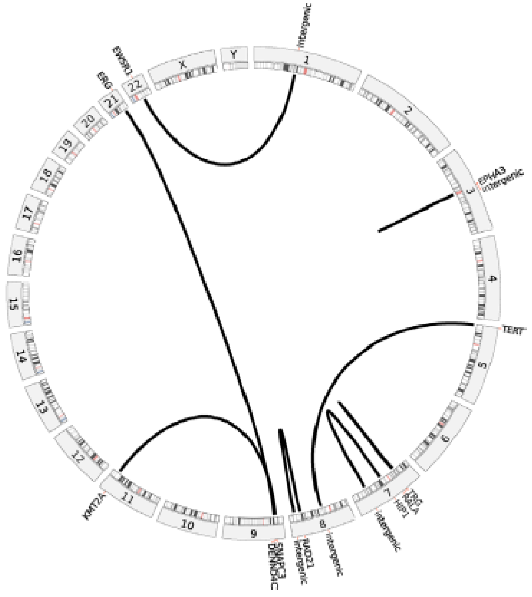

Novel translocations identified by BreaKmer

Comparison to other methods

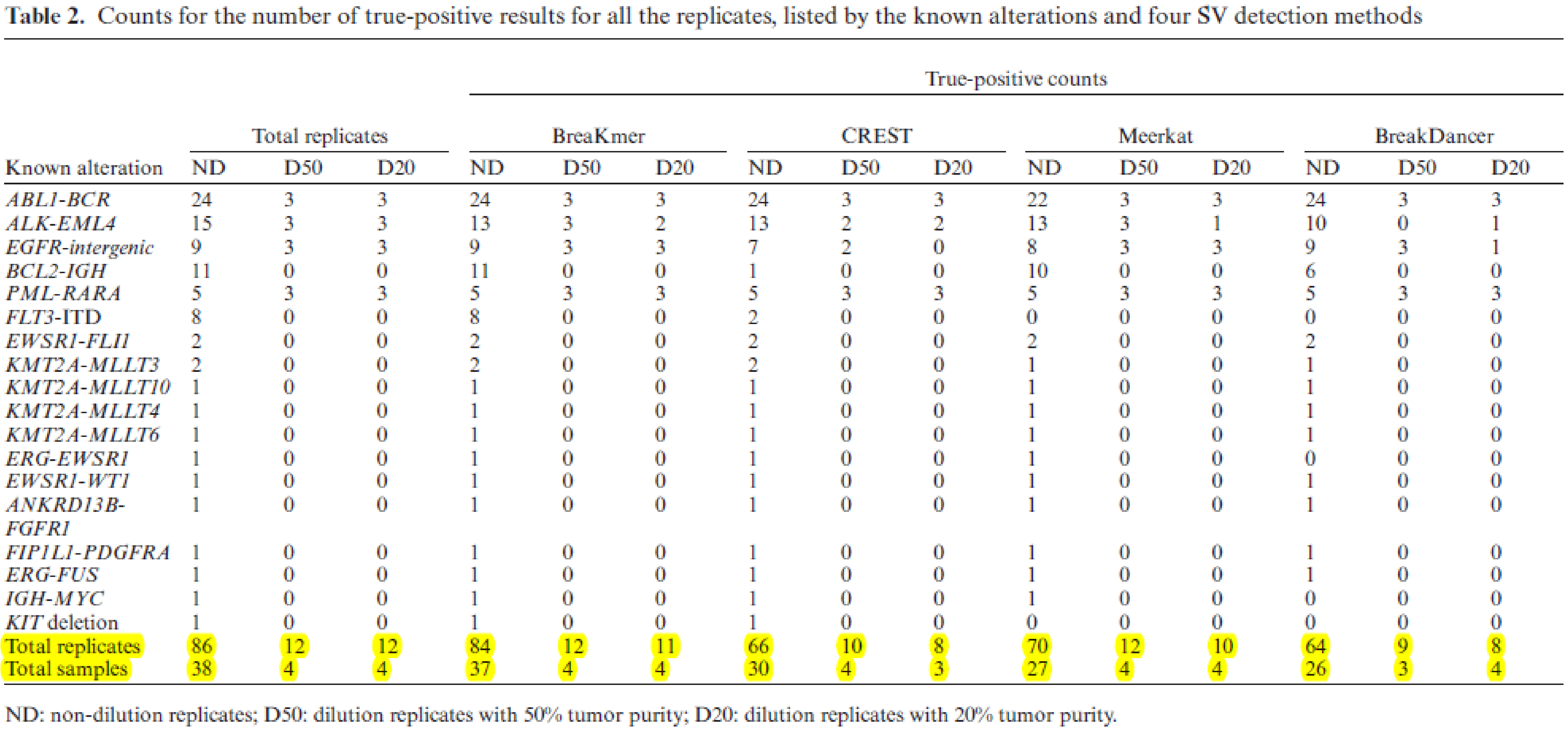

Table 2. Counts for the number of true-positive results for all the replicates, listed by the known alterations and four SV detection methods

				True-positive counts											
	Total replicates			BreaKmer			CREST			Meerkat			BreakDancer		
Known alteration	ND	D50	D20	ND	D50	D20	ND	D50	D20	ND	D50	D20	ND	D50	D20
ABL1-BCR	24	3	3	24	3	3	24	3	3	22	3	3	24	3	3
ALK-EML4	15	3	3	13	3	2	13	2	2	13	3	1	10	0	1
EGFR-intergenic	9	3	3	9	3	3	7	2	0	8	3	3	9	3	1
BCL2-IGH	11	0	0	11	0	0	1	0	0	10	0	0	6	0	0
PML-RARA	5	3	3	5	3	3	5	3	3	5	3	3	5	3	3
FLT3-ITD	8	0	0	8	0	0	2	0	0	0	0	0	0	0	0
EWSR1-FLI1	2	0	0	2	0	0	2	0	0	2	0	0	2	0	0
KMT2A-MLLT3	2	0	0	2	0	0	2	0	0	1	0	0	1	0	0
KMT2A-MLLT10	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0
KMT2A-MLLT4	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0
KMT2A-MLLT6	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0
ERG-EWSR1	1	0	0	1	0	0	1	0	0	1	0	0	0	0	0
EWSR1-WT1	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0
ANKRD13B-FGFR1	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0
FIP1L1-PDGFRA	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0
ERG-FUS	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0
IGH-MYC	1	0	0	1	0	0	1	0	0	1	0	0	0	0	0
KIT deletion	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0
Total replicates	86	12	12	84	12	11	66	10	8	70	12	10	64	9	8
Total samples	38	4	4	37	4	4	30	4	3	27	4	4	26	3	4

ND: non-dilution replicates; D50: dilution replicates with 50% tumor purity; D20: dilution replicates with 20% tumor purity.

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BreaKmer	494	17	9	11
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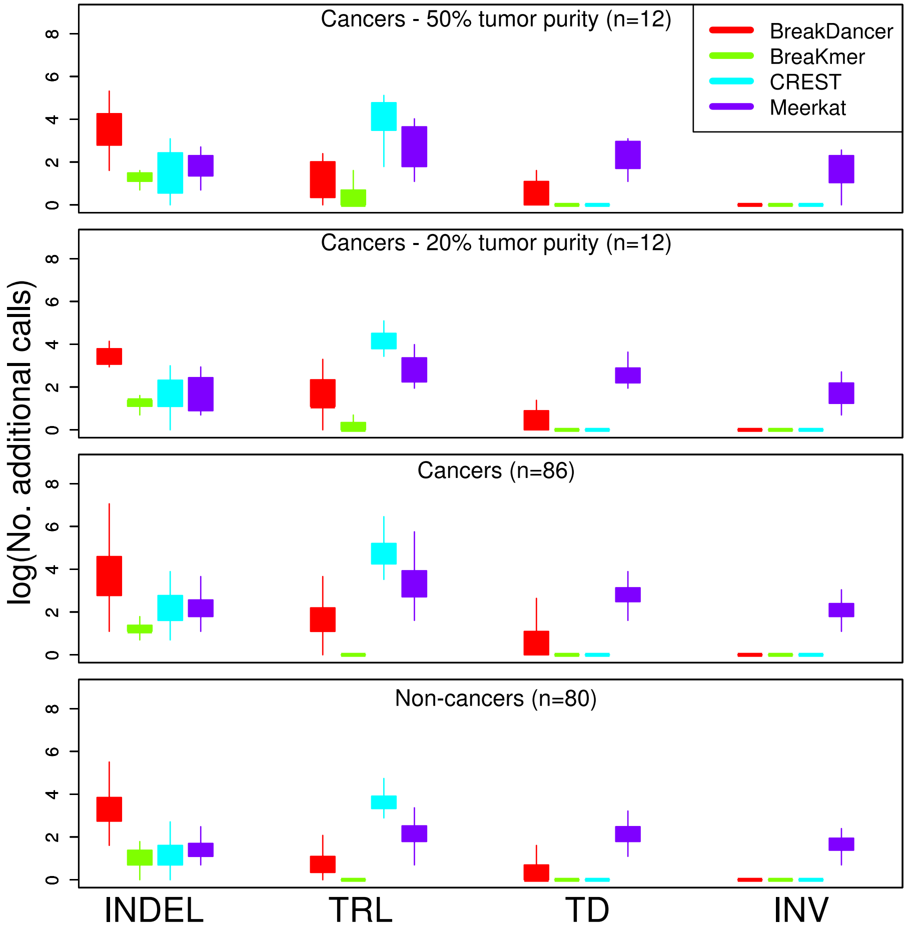

Results

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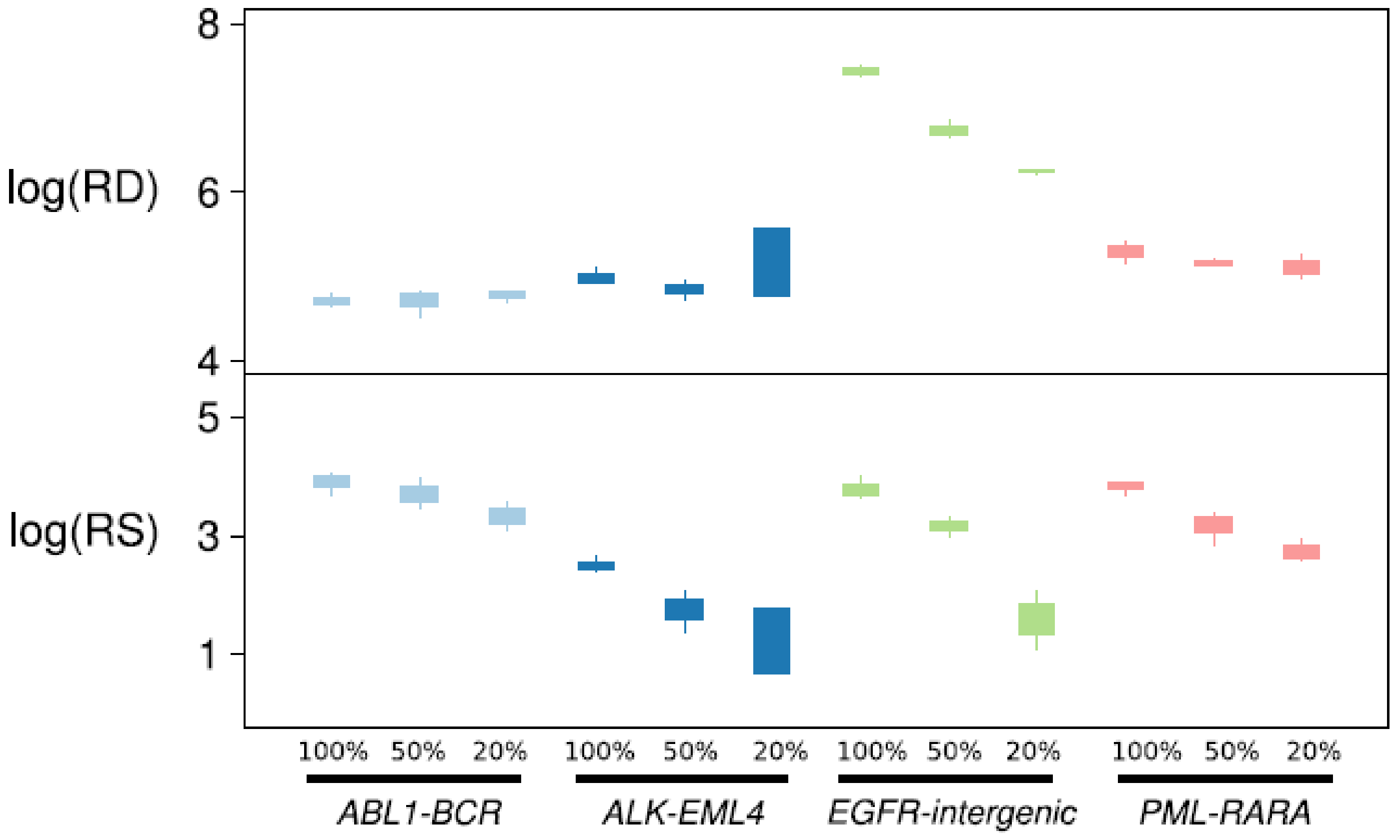
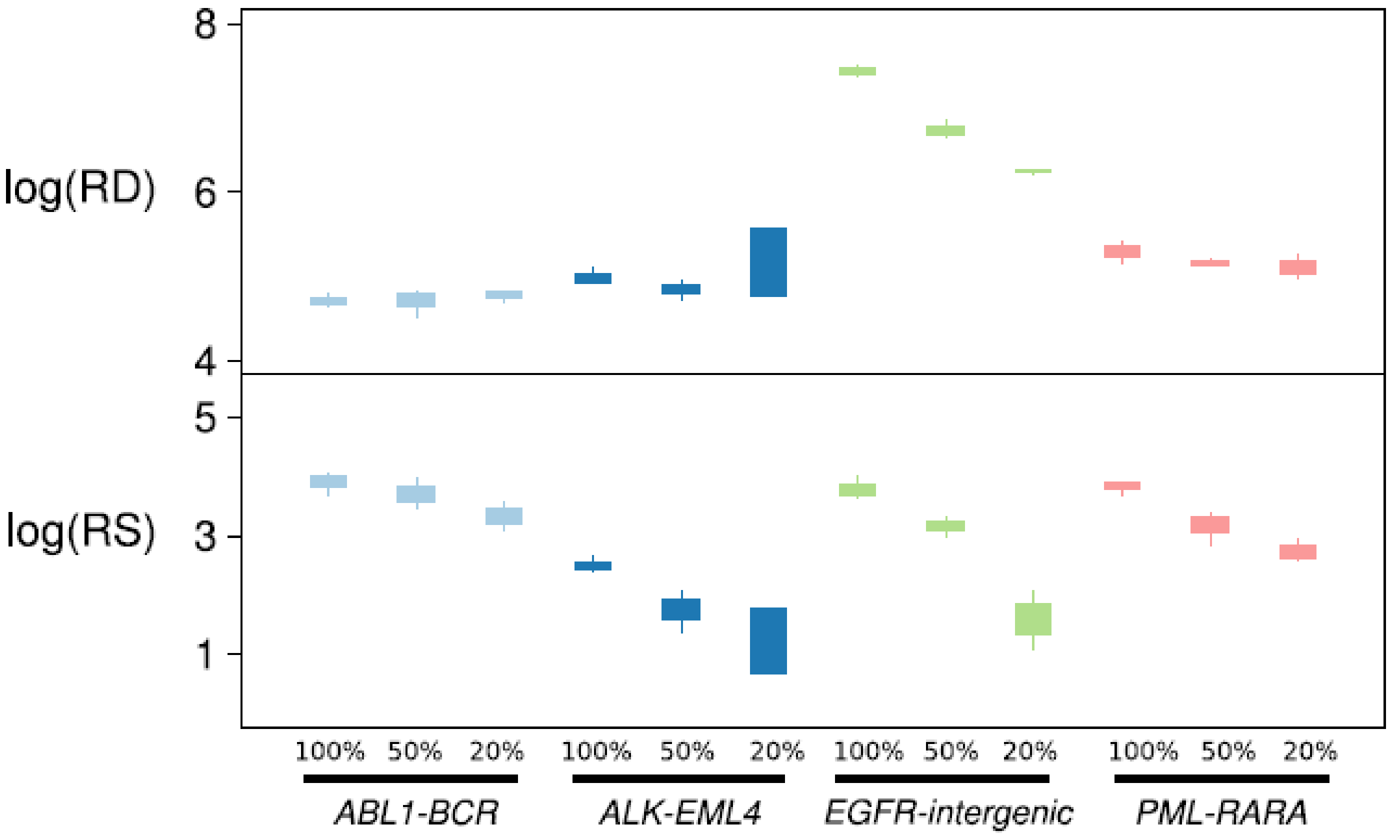


log(RD)
8
6
4
log(RS)
5
3
1
100%
50%
20%
100%
50%
20%
100%
50%
20%
100%
50%
20%
ABL1-BCR
ALK-EML4
EGFR-intergenic
PML-RARA

Results

- Dilution expectedly affects the SV evidence.



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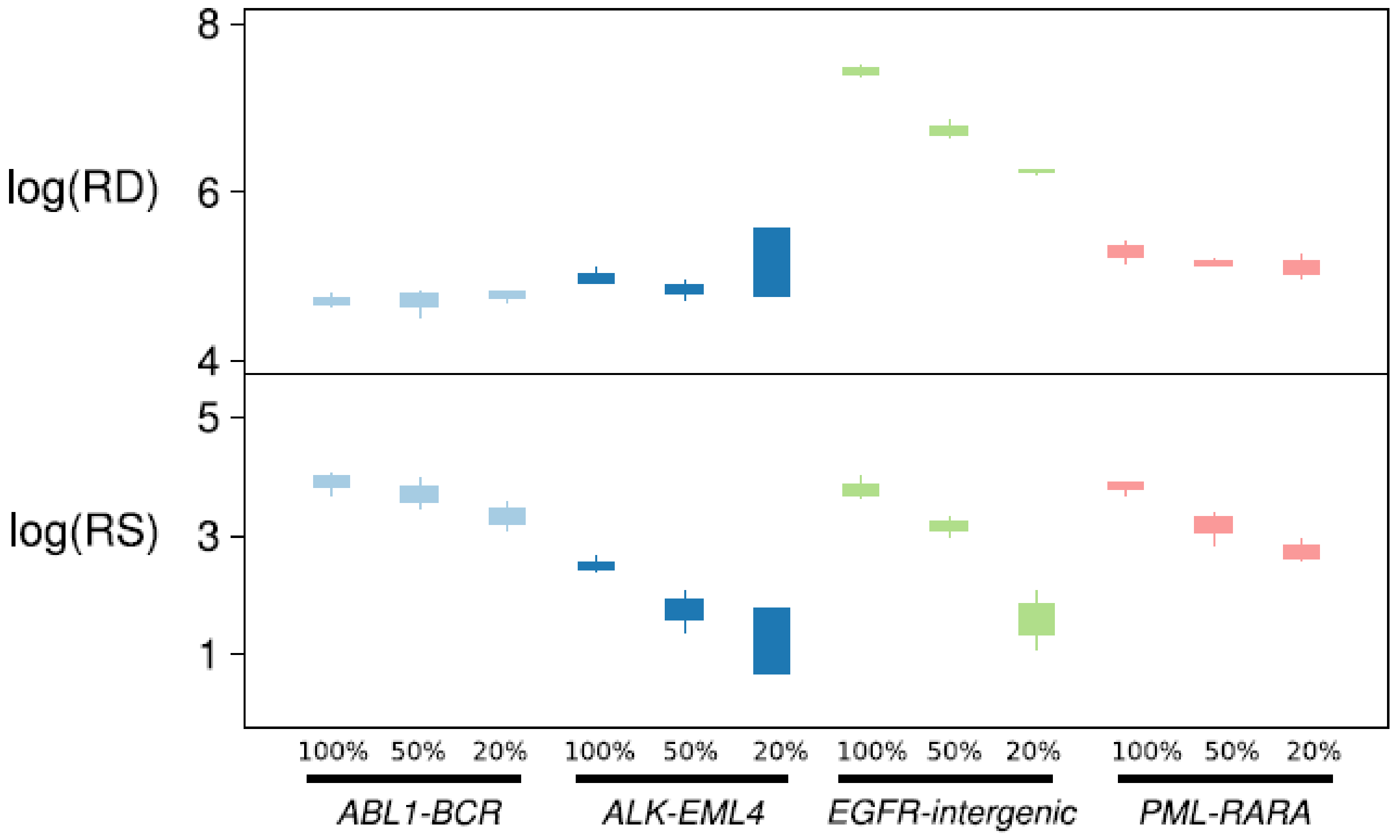
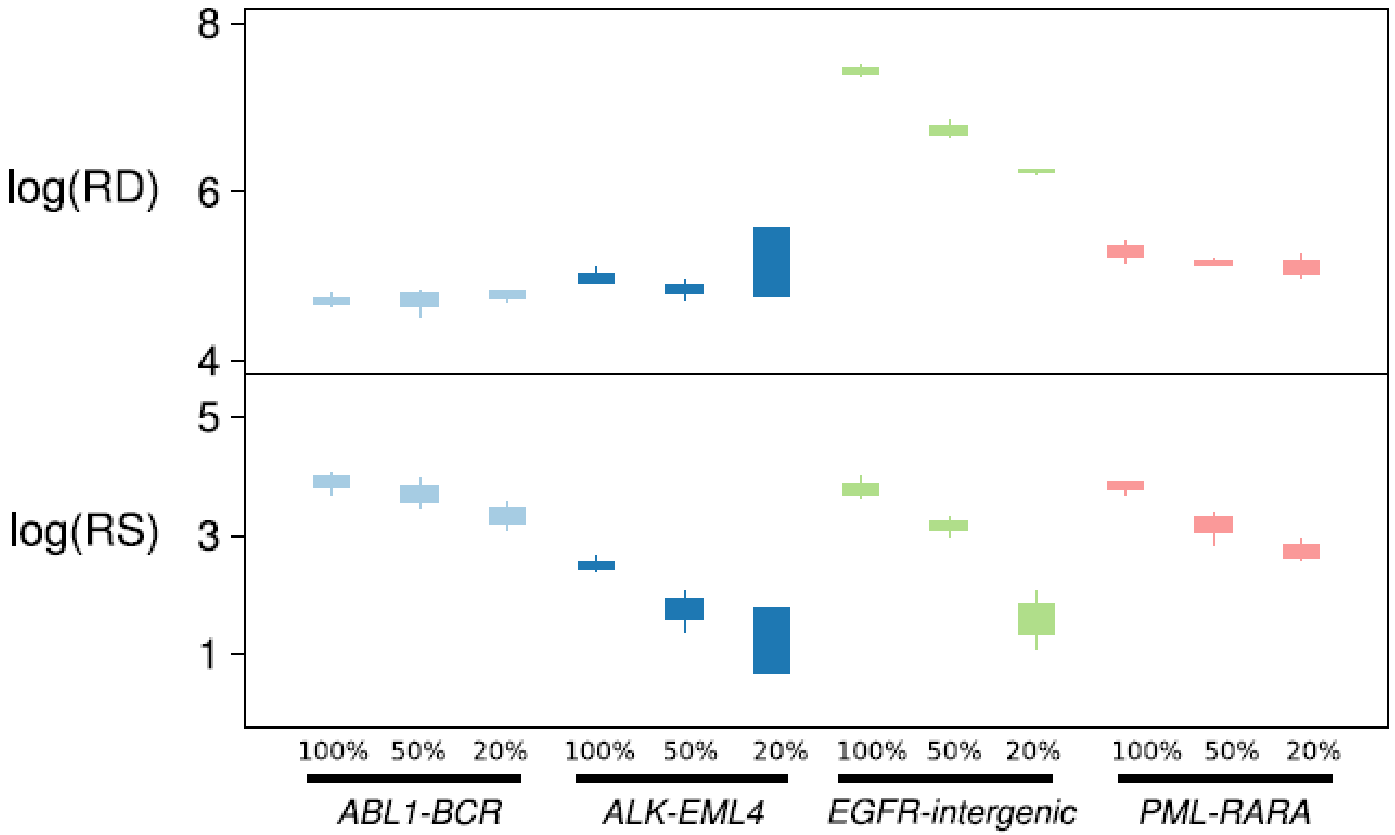


Results

- Dilution expectedly affects the SV evidence.
- Read support lowers as the tumor content in the sample grows smaller.
- (EGFR went through a big somatic amplification which also affected the read depth).



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- Very high sensitivity, reproducibility and predictive results.
- Maybe too good?
- Designed with detecting known SV’s quickly and cheaply as the primary goal.