Supplementary Material

1 Supplementary Data

Filtering steps

Different pipelines were used for the analysis of the RNA-seq data. We applied different manual filters to reduce the number of artifacts and false positive called variants. Filters applied to each of the callers are described in the following sections:

Arriba

1. Column "confidence": Discard low-confidence variants
2. Column "discordant_mates": Discard variants with <5 spanning reads
3. Column "split_reads1 + split_reads2": Discard variants with <3 split reads
4. Manual curation (explained below)

CICERO

1. Column "rating": Discard rating LQ (low quality) variants
2. Column "medal": Keep variants with medal ≥3*
3. Column "readsA + readsB": Discard variants with <5 supporting reads
4. Manual curation (explained below). Moreover, have a look at column "score" (high values are supportive of true positivity).

* Estimated pathogenicity assessment using St. Jude Medal Ceremony

deFuse

1. Column "span_count": Discard variants with <5 spanning reads
2. Column "splitr_count": Discard variants with <3 split reads
3. Column "gene_location1/gene_location2": keep those genes with the breakpoint in the coding region
4. Column "exon_boundaries": Keep Exon_boundaries=YES
5. Manual curation (explained below).

Fusion Catcher

1. Column "Fusion_description": Discard variants with the following tags: Bodymap2, ribosomal, Banned, Pseudogene, lncRNA.
2. Column "Spanning_pairs reads": Discard variants with <5 spanning reads
3. Column "Spanning_unique_reads": Discard variants with <3 split reads

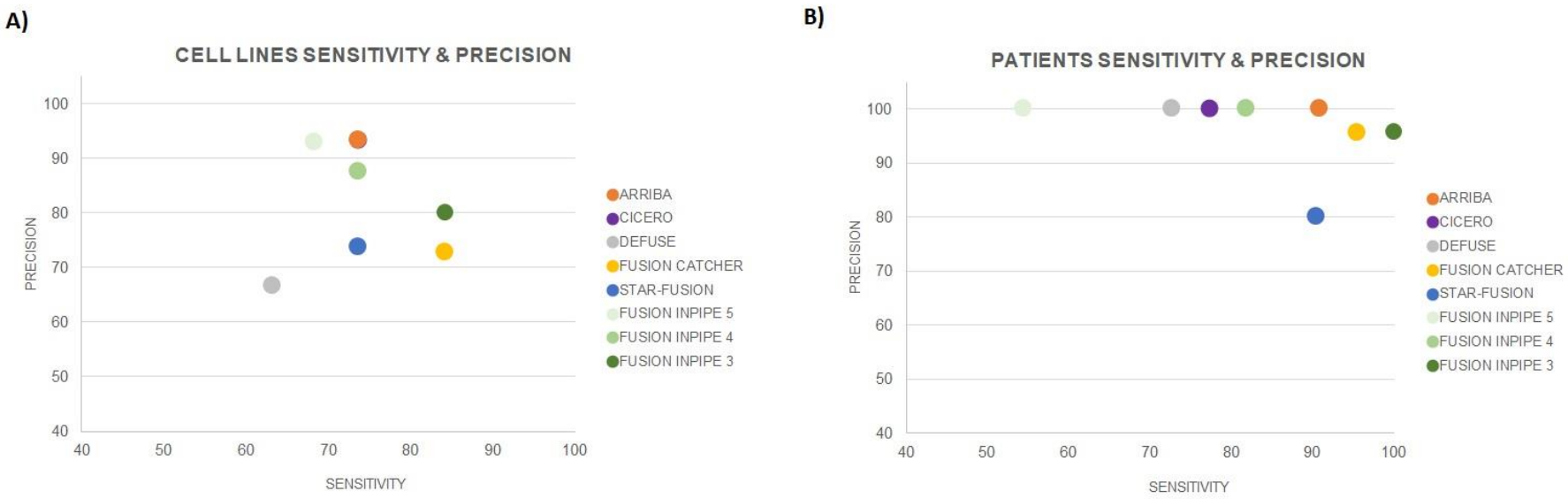
4. Manual curation (explained below). Moreover, review the "Fusion_finding_method" column and keep variants found with more than one aligner (BOWTIE/BLAT/STAR/BOWTIE2).

STAR-Fusion

1. Column "SpanningFrag": Discard variants with <5 spanning reads
2. Column "JunctionReads": Discard variants with <3 split reads
3. Manual curation (explained in the main text).

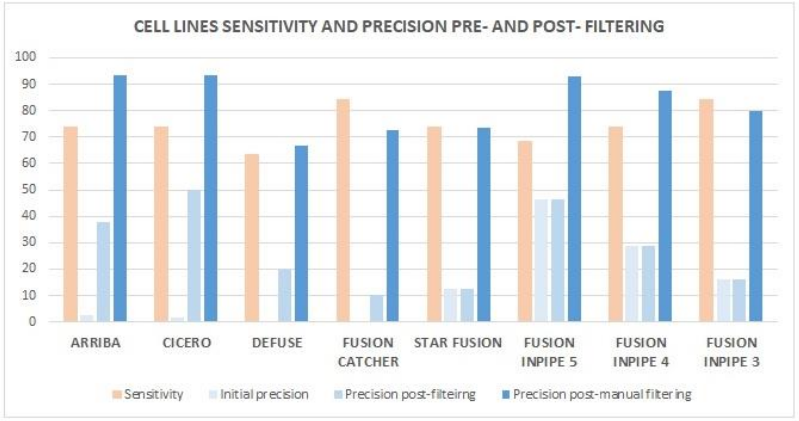
2 Supplementary Figures and Tables

2.1 Supplementary Figures

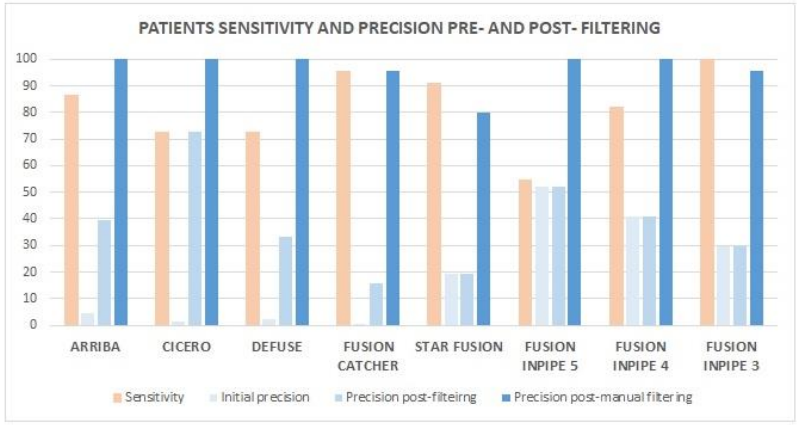


Supplementary figure 1. Sensitivity and precision graphics obtained by the different pipelines for a) cell lines and b) patients.

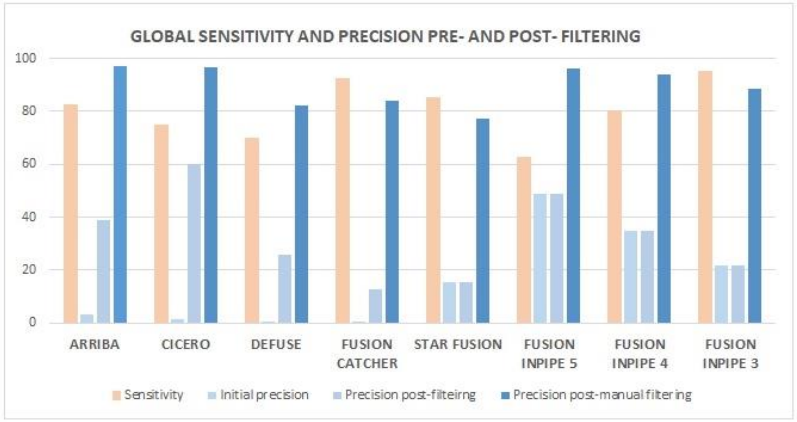
A)



B)



C)



Supplementary figure 2. Differences in precision before and after applying the filters for each pipeline. Orange bars show sensitivity. Data is shown separately for A) Cell lines, B) Patients, and C) Global.

2.2 Supplementary tables

Cell line	Leukemia subtype	Age/Gender	Alteration	
EOL-1 (ERR3003531)	acute myeloid leukemia	33 year/male	*FIP1L1::PDGFRA*	del(4)(q12q12)
HL-60 (ERR3003540)	acute myeloid leukemia	36 year/female	*CYFIP2::PLCG2*	t(5;16)(q33.3;q23.3)
KASUMI-1 (ERR3003548)	acute myeloid leukemia	7 year/male	*RUNX1::RUNX1T1*	t(8;21)(q22;q22)
KG-1 (ERR3003550)	acute myeloid leukemia	59 year/male	*FGFR1OP2::FGFR1*	t(8;12)(p12;p11)
NB-4 (ERR3003575)	acute myeloid leukemia	23 year/female	*PML::RARA*	t(15;17)(q22;q12)
SKNO-1 (ERR3003594)	acute myeloid leukemia	22 year/male	*RUNX1::RUNX1T1*	t(8;21)(q22;q22)
697 (ERR3003513)	B-Cell Precursor-acute lymphoblastic leukemia	12 year/male	*TCF3::PBX1*	t(1;19)(q23;p13)
KOPN-8 (ERR3003554)	B-Cell Precursor-acute lymphoblastic leukemia	3 month/female	*KMT2A::MLLT1*	t(11;19)(q23;p13.3)
REH (ERR3003588)	B-Cell Precursor-acute lymphoblastic leukemia	15 year/female	*ETV6::RUNX1*	t(12;21)(p13;q22)
			RUNX1::PRDM7	t(16;21)(q22;q16)
SEM (ERR3003592)	B-Cell Precursor-acute lymphoblastic leukemia	5 year/female	*KMT2A::AFF1*	t(4;11)(q21;q23)
CCRF-CEM (ERR3003519)	T-cell acute lymphoblastic leukemia	4 year/female	*NKX2.5::BCL11B*	t(5;14)(q35;q32.2)
DND-41 (ERR3003527)	T-cell acute lymphoblastic leukemia	13 year/male	*TLX3::BCL11B*	t(5;14)(q35;q32.2)
HPB-ALL (ERR3003541)	T-cell acute lymphoblastic leukemia	14 year/male	*CBFB::MYLPF*	inv(16)(p13q22) / t(16;16)(p13;q22)
RPMI-8402 (ERR3003591)	T-cell acute lymphoblastic leukemia	16 year/female	*LMO1::TRD*	t(11;14)(p15;q11)
			SIL::TAL1	del(1p32)

Supplementary Table 1. Main characteristics of the different leukemia cell lines used for the analysis.

Sample	Sex	Leukemia subtype	Karyotype	FISH	RT-qPCR
Sample 1	Female	B-ALL	46,XX,?t(7;9),?t(9;12),t(9;22)(q34;q11)(22)/46,XX(3)	97% of the nuclei with *BCR::ABL1* rearranged	*BCR::ABL (P210)*
Sample 2	Male	B-ALL	47, XY,t(9;22)(q34;q11),+17	87% of the nuclei with *BCR::ABL1* rearranged	*BCR::ABL (P190)*
Sample 3	Male	B-ALL	46,XY,del(6)(q?13q?23),add(9)(q34),del(12)(p13), del(13)(q?12q?14)(9)/47,idem,+?10(8)/46,XY(3)	Not performed	*ETV6::RUNX1*
Sample 4	Male	B-ALL	46,XY,del(6)(q13q25)[4]/46,XY[34]	50% of the nuclei with *ETV6::RUNX1* rearranged, 35% of them with deleted *ETV6*	*ETV6::RUNX1* *P2RY8::CRLF2*
Sample 5	Female	B-ALL	46,XX,der(19)t(1;19)(q23;p13)[6]/46,XX[46]	27% of the nuclei with *TCF3* rearranged.	*TCF3::PBX1*
Sample 6	Female	B-ALL	46, XX	98% of the nuclei with *KMT2A* rearranged	*KMT2A::AF4*
Sample 7	Male	AML	46,XY,der(11)(q23)[3]/46,XY[17]	22% of the nuclei with *KMT2A* rearranged	*KMT2A::AF9*
Sample 8	Male	T-ALL	no metaphases	No alterations identified	*STIL::TAL1*
Sample 9	Male	T-ALL	46,XY[5]	No alterations identified	*STIL::TAL1*

Sample 10	Female	AML	47,XX,+8[3]/47,XX,+1[2]/46,XX[5]	Not performed	*RUNX1::CBF2AT3*
Sample 11	Male	B-ALL	no metaphases	86% of the nuclei presented a monoallelic deletion of *JAK2*	*PAX5::NOL4L*
Sample 12	Male	AML	47, XY, del(7)(q?32), inv(16)(p13q22), +22 [17]	Not performed	*CBFB::MYH11*
Sample 13	Female	AML	46,XX,t(15;17)(q24;q21)	Not performed	*PML::RARA*
Sample 14	Male	B-ALL	47,XY,+5[10]/46,XY[8]	97% of the nuclei presented signs suggestive of chromosome 5 trisomy	*P2RY8::CRLF2*
Sample 15	Female	B-ALL	46,XX,del(5)(q33),del(9)(p22),-12,-20,+mar1,+mar2[26] / 46,XX[7]	28% of the nuclei presented 3 copies (2 smaller) of *ETV6*, suggesting a rearrangement. 60% of the nuclei presented 3 copies (2 smaller) of *ABL1*, suggesting a rearrangement.	*ETV6::ABL1*

Supplementary Table 2. Main alterations identified by different conventional methodologies in patients used for the sequencing and the analysis of the pipelines.

Pipeline	Workflow	Aligner	Fusion calling based on	Filters	Reference
Arriba	1- Alignment 2- Detection of chimeric reads 3- Filtering step	STAR-aligner	Split reads Discordant reads	Read-level (single read) event-level (multiple reads)	Uhrig et al., 2021 Genome Res
CICERO	1- Alignment 2- Identification of fusion breakpoint 3- Fusion annotation 4- Ranking of fusion candidates (filtering)	STAR-aligner	Discordant reads Soft clipped reads	signal-to-noise-recognition procedures	Tian et al., 2020 Genome Biol
deFuse	1- Alignment 2- Build a list of candidates 3- AdaBoost classifying (filtering)	Bowtie	Discordant reads Split reads	dynamic programming AdaBoost classifier	McPherson A et al., 2011 PLoS Comput.Biol
Fusion Catcher	1- Alignment (4 methods) 2- Building of a preliminary list of candidates 3- Filtering step	Bowtie, BLAT, STAR-aligner, Bowtie2	Split reads Discordant reads	ChimerDB2, CACG, and ConjoinG BodyMap2 ad-hoc/random fusion	Nicorici et al., 2014 bioRxiv
STAR-fusion	1- Alignment 2- Fusion annotation **3-** Filtering step	STAR-aligner	Split reads Discordant reads	FusionFilter framework	Haas et al., 2019 Genome Biol

Supplementary table 3. Main characteristics of the different algorithms analyzed.