RNA-sequencing for the detection of gene fusions and subtype classification of BCP-ALL based on gene expression signatures

Patient name	Ellen Ripley	Registration date	2023-Mar-14 03:14:15
Patient ID	19790525-1234	Specimen	Blood sample
Sample ID	d5sdfew7f8x	Analysis method	RNA-sequencing (whole transcriptome sequencing, WTS)
RNA-seq BAM file	bcp-all_015_etv6_synt_star_1.0.1.bam	Arriba fusion file	
STAR-Fusion file	bcp-all_heh_notier1or2_star_1.0.0.tsv	FusionCatcher file	bcp-all_etv6_fc_1.0.0.txt

Result summary

Classification of the sample for six subtypes of B-cell precursor acute lymphoblastic leukemia (BCP-ALL): ETV6::RUNX1 or ETV6::RUNX1-like, TCF3::PBX1, BCR::ABL1 or BCR::ABL1-like, KMT2A(MLL)-rearranged, DUX4-rearranged, High hyperdiploidy.

Detected gene fusions of significance:	ETV6::RUNX1
Gene expression-based subtype classification:	ETV6::RUNX1 or ETV6::RUNX1-like

Conclusion

This is an example!

Analysis results

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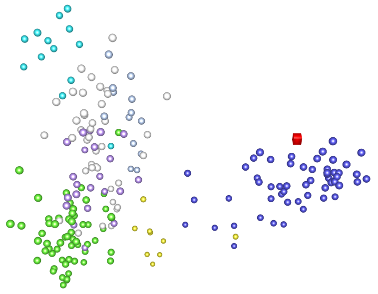
Subtype classification (based on gene expressions)

Subtype	**Probability (%)**
ETV6::RUNX1 or ETV6::RUNX1-like	**100**
High hyperdiploidy	0
BCR::ABL1 or BCR::ABL1-like	0
KMT2A(MLL)-rearranged	0
DUX4-rearranged	0
TCF3::PBX1	0

Sample in relation to training data



The analyzed sample projected on a PCA plot of the samples in the training data using the most important genes for BCP-ALL.

🟥 Sample
🟨 DUX4-rearranged
🟦 ETV6::RUNX1 or ETV6::RUNX1-like
🟩 High hyperdiploidy
🟦 KMT2A(MLL)-rearranged
🟪 BCR::ABL1 or BCR::ABL1-like
🟦 TCF3::PBX1
☐ Unassigned

Gene fusion(s) of significance (Tier 1)

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Gene fusion	**Position A**	**Position B**	**Span. Frgmt.**	**Bkpt. Reads**	**Fusion frameshift**	**Database**	**Fusion caller**
ETV6::RUNX1	12 12022903	21 36265260	5	2	In-frame	Mitelman	FusionCatcher

Gene Fusions related to BCP-ALL (Tier 2)

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Gene fusion	Position A	Position B	Span. Frgmt.	Bkpt. Reads	Fusion frameshift	Database	Fusion caller
RIPOR2::VPS54	6 24843090	2 64120669	35	15	Out-of-frame	Mitelman	FusionCatcher
RIPOR2::VPS54	6 24848253	2 64120669	35	5	Out-of-frame	Mitelman	FusionCatcher
ETV6::RUNX1	12 12022903	21 36340571	5	3	-	Mitelman	FusionCatcher
PTBP1::TMEM259	19 797505	19 1014472	3	3	Out-of-frame	Mitelman	FusionCatcher

Quality metrics (cut-offs)

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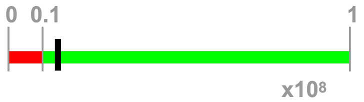
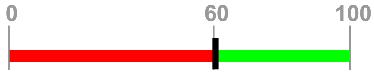
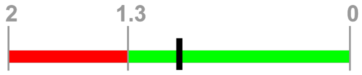
Aligned read pairs	1.45e+07	
Read pairs mapping to features	8.78e+06	
Fraction of read pairs mapping to features (%)	60.6	
Normal cell score	-0.68	
Local outlier factor	1.00	

Quality metrics (informational)

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Paired reads inner distance	$\mu = 5.17, \sigma = 6.60$
Reads fractions (%)	
Ambiguous	0.8
Forward	52.0
Reverse	47.2

Analysis description

Qlucore Diagnostics uses RNA-sequencing (whole-transcriptome sequencing) data generated by next-generation sequencing (NGS) to detect gene fusions and to classify a sample into a BCP-ALL subtype based on gene expression signatures. Following sequencing, reads are aligned to a reference genome (GRCh37, hg19) and gene fusions called using at least two of the tools Arriba, FusionCatcher and/or STAR-Fusion.

Gene expression levels are calculated from the BAM-file and normalized to a reference sample in the training data. Classification based on gene expression levels in the sample is performed using the machine learning-based classifier in the Qlucore Diagnostics BCP-ALL Model. The classifier has been built using a boosted decision tree approach and trained on curated gene expression data from known cases of BCP-ALL. Further details on RNA-extraction, library preparation, sequencing, and bioinformatics using different algorithms are available in the Instructions For Use.

Detected gene fusion(s) are filtered to exclude all gene fusion instances with zero reads over breakpoints or spanning fragments. Thereafter, gene fusion(s) are assigned into three different tiers: Tier 1 includes all in-frame gene fusions listed in the WHO guidelines and in the ICC classification; Tier 2 lists all other gene fusions found in BCP-ALL as reported in the Mitelman database (as of October 12, 2023); Tier 3 (listed in appendix) includes all other gene fusions (might be reported in the Mitelman database, but not found in BCP-ALL).

The classifier in the Qlucore Diagnostics BCP-ALL Model calculates probabilities for the sample to belong to the following six subtypes: ETV6::RUNX1 or ETV6::RUNX1-like, TCF3::PBX1, BCR::ABL1 or BCR::ABL1-like, KMT2A(MLL)-rearranged, DUX4-rearranged, and High hyperdiploidy. If none of the six calculated probabilities reach above the classification threshold, the report will not show any classification probabilities.

The graph on page 1 shows the analyzed sample normalized to training data and projected on a principal component analysis (PCA) plot of all the samples in the training data using the most important genes for BCP-ALL. The analyzed sample is colored in red and the training data samples are colored according to the subtype they belong to.

This case was run with:

REF Qlucore Diagnostics BCP-ALL Model 1.0.17 2025-02-07 UDI (01)07350148190013(8012)1.0.17

REF Qlucore Diagnostics Platform 1.0.4 2025-02-07 UDI (01)07350148190006(240)win(8012)1.0.4

Qlucore AB (publ)
Scheelevägen 17, 223 70 Lund

Appendix: Non-confirmed (unknown) gene fusions (Tier 3)

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Gene fusion	Position A	Position B	Span. Frgmt.	Bkpt. Reads	Fusion frameshift	Database	Fusion caller
GUK1::ARF1	1 228328064	1 228284779	21	15	-	Mitelman	FusionCatcher
CXCR4::GNAS	2 136875616	20 57470667	15	12	In-frame	Mitelman	FusionCatcher
RP11-367G6.3::FAM65B	6 25042079	6 24876045	11	16	-	-	STAR-Fusion
ADGRE2::ADGRE5	19 14885068	19 14499514	34	14	In-frame	-	FusionCatcher
RP11-494M8.4::OVCH2	11 7900554	11 7726221	6	12	-	-	STAR-Fusion
TIMM23::PARGP1	10 51606988	10 51732772	3	11	-	-	STAR-Fusion
NRIP1::AF127936.7	21 16437066	21 16230029	3	10	-	-	STAR-Fusion
SERF1A::SMN1	5 70212875	5 70234666	23	8	Out-of-frame	-	FusionCatcher
CCDC26::LINC00977	8 130587128	8 130229652	1	7	-	-	STAR-Fusion
RIPOR1::GNAS	16 67562817	20 57470667	2	7	-	-	FusionCatcher
TPM4::KLF2	19 16187507	19 16437667	9	7	In-frame	-	FusionCatcher
BRD1::UBB	22 50197852	17 16285216	2	4	-	-	FusionCatcher
AC019118.3::AC019118.2	2 3149318	2 2970447	4	3	-	-	STAR-Fusion
BACH2::PTMA	6 91006199	2 232576058	2	3	-	-	FusionCatcher
CCDC26::LINC00977	8 130365050	8 130229652	1	3	-	-	STAR-Fusion
CCDC26::LINC00977	8 130587128	8 130235615	1	3	-	-	STAR-Fusion
RP11-494M8.4::OVCH2	11 7873820	11 7726221	9	3	-	-	STAR-Fusion
PTMA::LRRFIP1	2 232573461	2 238644232	13	2	-	-	FusionCatcher
RIPOR1::EEF1D	16 67562817	8 144669019	2	2	-	-	FusionCatcher
RIPOR1::EEF1D	16 67562817	8 144669022	2	2	-	-	FusionCatcher
YWHAZ::YPEL5	8 101965497	2 30378716	2	2	-	-	FusionCatcher
C8ORF44::SGK3	8 67592259	8 67705851	1	1	-	-	STAR-Fusion
DNAJC25::UGCG	9 114429226	9 114676885	1	1	-	-	STAR-Fusion
FMR1::RP11-97N5.2	X 147003503	X 135029298	1	1	-	-	STAR-Fusion
NRIP1::AF127936.7	21 16386665	21 16230029	5	1	-	-	STAR-Fusion
SLC5A3::LINC00310	21 35446045	21 35560518	1	1	-	-	STAR-Fusion