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Application Note

Entrogen's Leukemia Translocation Panel v2 enables sensitive quantitative detection of common fusion transcripts in childhood and adult leukemias

- High sensitivity
- Fast and simple workflow
- Clinically actionable results

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Introduction

Leukemia ranks fifth among the most commonly diagnosed cancers globally, with higher prevalence in more developed countries. It is a malignant neoplasm involved in one or more cell-lineages of the hematopoietic system. Based on the origin of the abnormal hematopoietic cells involved, leukemia is categorized into acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL), chronic myeloid leukemia (CML) and chronic lymphocytic leukemia (CLL). The prevalence of leukemia subtypes varies among age groups. While ALL accounts for 75% of pediatric leukemia, AML is more prevalent in adults[1]

Fusion genes are important indicators for leukemia classification and treatment monitoring. Particularly, chromosomal rearrangements (translocations) in MLL-AF4 t(4;11) (q21;q23), TEL-AML1 (12;21)(p13;q22), and E2A-PBX1 t(1;19) (q23;p13) are among the most common cytogenetic subtypes in ALL (Organista-Nava et al., 2016) whereas translocations in AML1-ETO t(8;21) (q22;q22) and CBFb-MYH11 inv(16) (p13q22) are common in AML patients[2] Fusion of PML-RARa .

t(15;17)(q22;q21) is highly prevalent in acute promyelocytic leukemia (APL), a SU btype of AML.

Table 1: Translocation panel coverage by disease

Disease	Translocations	Fusion Transcripts
ALL	t(1;19)	E2A/PBX1 (e13/e2)
	t(12;21)	TEL/AML1 (e5/e2)
	t(4;11)	MLL/AF4 (e9/e5) MLL/AF4 (e9/e4) MLL/AF4 (e10/e4) MLL/AF4 (e10/e5) MLL/AF4 (e11/e4) MLL/AF4 (e11/e5)
APL	t(15;17)	PML/RARα (bcr1) PML/RARα (bcr2) PML/RARα (bcr3)
AML	Inv 16	CBFB/MYH11 (A type) CBFB/MYH11 (D type) CBFB/MYH11 (E type)
	t(8;21)	AML1/ETO (e5/e2)

Moreover, the amounts of fusion transcripts are indicators for measurable residual disease (MRD), which is used for prognostic, predictive, monitoring, and efficacy-response assessments[3] Here, we demonstrate that EntroGen's Leukemia Translocation Panel v2 (cat. no. LEUKMP-RT24) offers a highly sensitive and quantitative method for detecting prevalent fusion genes in acute myeloid and lymphoid leukemias using minimal RNA quantities.

Product Application

The Leukemia Translocation Panel v2 contains all necessary reagents for the detection of 15 common fusion genes in childhood and adult leukemias by real-time quantitative PCR (qPCR). The one-step assay workflow allows for a rapid turnaround time of 2 hours (Figure 1).

Simple and efficient workflows for YOUR lab.

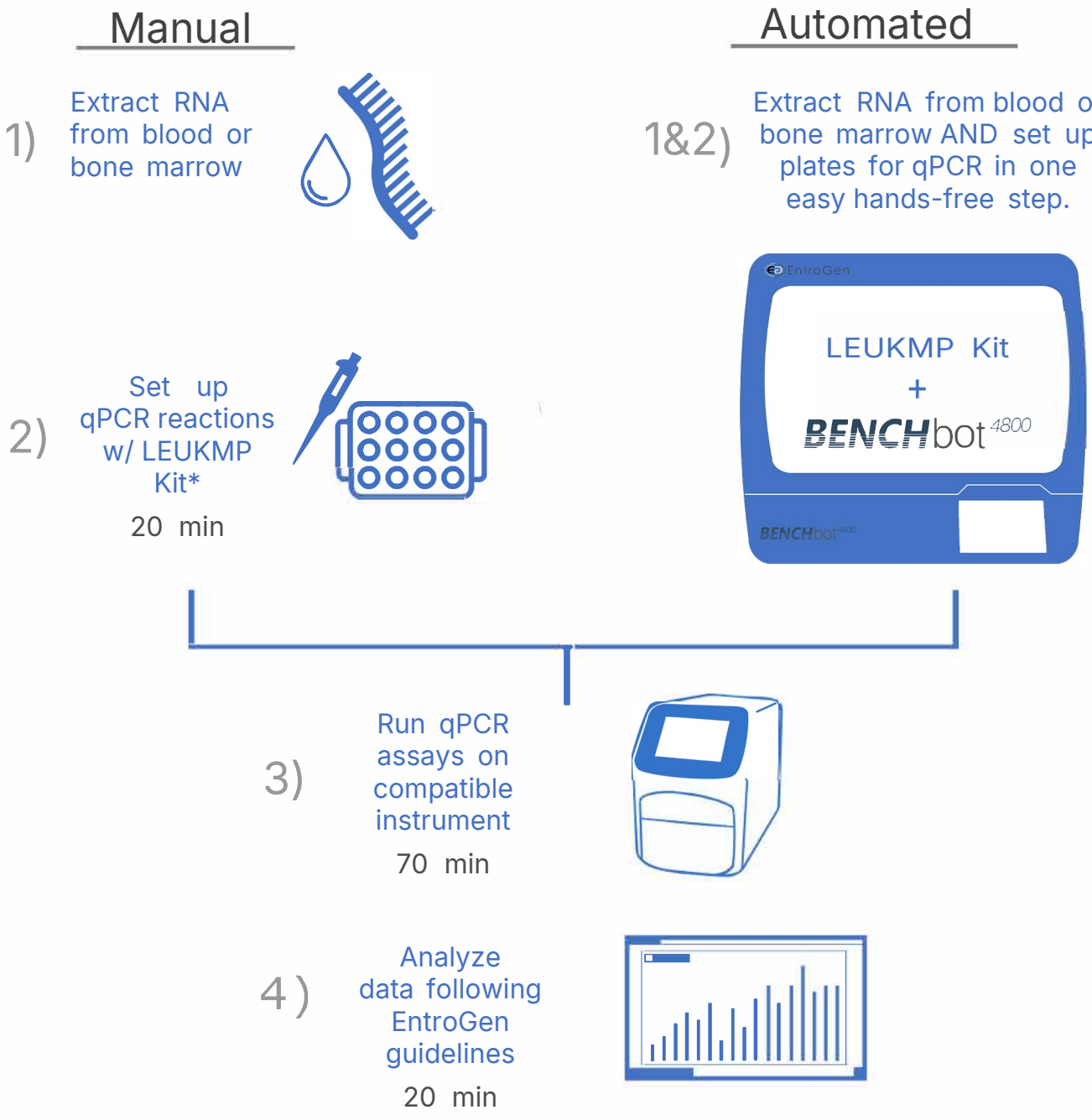

Figure 1. Workflow for the EntroGen's one-step LEUKMP

Robust & Highly Sensitive

In order to model the assay sensitivity, we used contrived samples representing six fusion gene targets to assess Limit of Detection (LoD). These samples were each mixed with a control gene (ABL) at ratios calculated to achieve 10-fold serial dilutions, representing 1 to 5-log reductions. The RNA input tested is equivalent to a Ct value of 20-22 as measured by the control gene. The assay was performed using at minimum 14 replicates per mutant target on a QuantStudio™ 5

As shown in Table 2, five of the six rep rese n tat ive targets achieved an LoD of 0.01% with RNA inputs equivalent to a Ct value of 20 in the control gene. Among these, three targets (namely CBFB-MYH11,
PM L-RARA, and E2A-PBX1) reached a 0.01% LoD with input at a Ct value of 22.

Table 2. Limit of detection for six representative targets in the LEU KM Pv2 assay.

	Target	LoD (%)	% Correct Call
Reaction 1 (AML)	AML1-ETO	0.1	100
	CBFB-MYH11	0.01	100
	PML-RARA	0.01	100
Reaction 2 (ALL)	MLL-AF4	0.01	100
	TEL-AML1	0.01	100
	E2A-PBX1	0.01	100

Simple Data Analysis & Interpretation

For MRD assessment, data analysis is performed using a ratio-based relative quantification calculation:

$$\text{Ratio} = \left(\frac{E_{target}^{\Delta CP_{target}\,(control-sample)}}{E_{ref}^{\Delta CP_{ref}\,(control-sample)}} \right)$$

Where:

E_{target} = Efficiency of target
E_{ref} = Efficiency of reference

ΔCPtarget = Mean target Ct of PC – mean target Ct of the sample
ΔCPref = Mean ABL Ct of PC – mean ABL Ct of the sample

Clinically, measurable/minimal residual disease assessment encompasses the detection of fusion transcripts in a given acute leukemia sample.

MRD is a crucial diagnostic tool that is increasingly used for disease progression prediction, disease status monitoring, and novel treatment evaluation in leukemia. One of the main barriers to MRD assessment for patients is cost[3] Our one-step qPCR assay provides a cost-effective and accurate MRD monitoring solution.

Conclusions

In this study, we demonstrated that EntroGen's Leukemia Translocation Panel v2 can accurately and efficiently detect common fusion transcripts identified in acute child and adult leukemia. Most targets achieved a 0.01% LoD with minimal RNA inputs, providing the sensitivity required for measurable/minimal residual disease detection.

In an independent validation, comparison between LEUKMP and Karyotype results of 300 specimens from newly diagnosed acute myeloid leukemia patients showed 100% concordance (300/300). [4]

Moreover, EntroGen's Leukemia Translocation Panel v2 offers a fast turnaround time of 5 hours, including extraction (two-hours from PCR to results), ease of use and simplified analysis (Figure 1). The Leukemia Translocation Panel v2 combines the high specificity and sensitivity of real-time qPCR with a well-designed multiplex format. Multiplexed qPCR reactions allow for a cost-effective approach with broader coverage than most targeted assays.

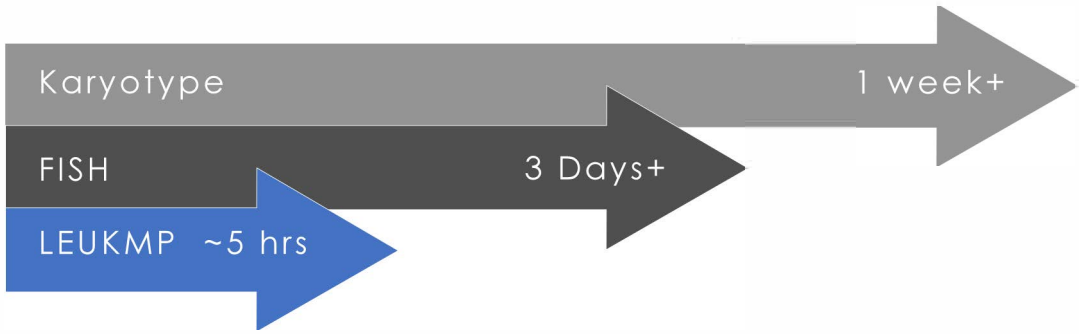

Figure 2. Estimated turnaround time by method

References

1. Tebbi C. Etiology of Acute Leukemia: A Review. Cancers, 2021, 13, 2256.

2. Wang Yet al. Recurrent Fusion Genes in Leukemia: An Attractive Target for Diagnosis and Treatment. Current Genomics, 2017, 18,378-384.

3. Dekker S et al. Using Measurable Residual Disease to Optimize Manag-ing of AML, ALL, and Chronic Myeloid Leukemia. Am Soc Clin Oncol Educ Book, 2023.

4. Butcher DT, et al. (2023, Nov.). Analytical Utility of a One-Step cDNA Synthesis and Real-Time PCR Assay for the Rapid Detection of Common Fusion Genes in Acute Leukemia. AMP Annual Meeting & Expo, USA.

Related Products

BCR-ABL P210 (Mbcr) One-Step Detection Kit
Product Code: BCR210-QRT46

BCR-ABL P190 (mbcr) One-Step Detection Kit
Product Code: BCR190-QRT46

PML-RARA bcrl,2,3 One-Step Detection Kit
Product Code: LEUK4-QRT24

One Step, Many Advantages

- Efficient - No need to perform separate cDNA synthesis and qPCR
- Low contamination risk - No post-PCR product handling
- High-throughput - Up to 46 samples in a single run
- Multiplexed - Reference and target genes assessed in the same reaction
- Fast - Results in under 80 minutes after PCR start

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