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Application Note
#92023006
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Automated JAK2 V617F Mutation Detection on the geneLEAD VIII System

Background

The geneLEAD VIII system is a fully automated, sample-to-result precision instrument. Following the loading of samples, reagents, and consumables, the system automatically performs nucleic acid extraction, qPCR thermal cycling, and result reporting, thereby reducing the user workload. Through optimized threshold and other parameters, the geneLEAD VIII system is adaptable to various qPCR kits for automated detection.

This technical note demonstrates an automated workflow for the GenoQuest™ JAK2 qPCR Assay on the geneLEAD VIII system, including its clinical performance.

Kit: GenoQuest™ JAK2 qPCR Assay (Cat. No.: 92023)

Instrument: geneLEAD VIII (Precision System Science Co., Ltd.) (Cat. No.: A2700)

Sample Type: uncoagulated blood (with anticoagulant)

Input: 200 µL

GenoQuest™ JAK2 qPCR Assay

geneLEAD VIII

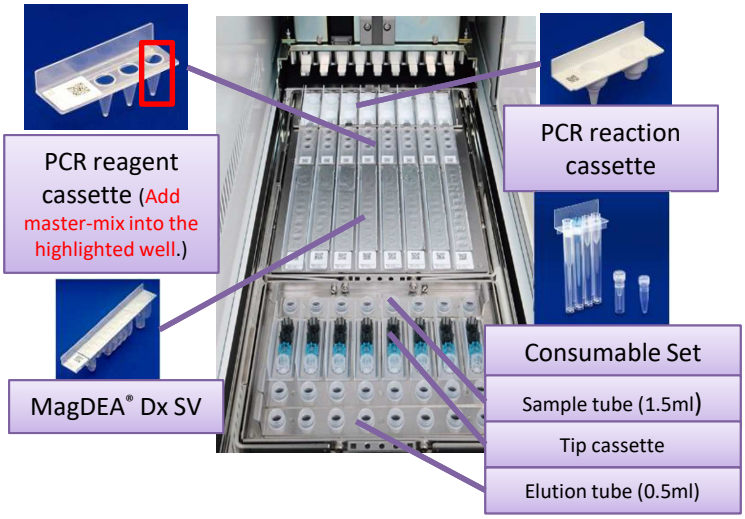

Figure 1: Position of Consumables

Protocol

Note: This Application Note adapts the GenoQuest™ JAK2 qPCR Assay standard protocol (as described in the Instructions for Use) for use on the geneLEAD VIII system.

1. Reagent and Sample Preparation

i. Prepare the real-time PCR master-mix according to the Instruction of use. Pipette 15µL master-mix into PCR reagent cassette (Figure 1 highlights the specific well).

ii. **For blood samples**: Perform a 6-fold pre-extraction dilution of the blood sample using 1x PBS buffer in a sample tube. (33µL blood + 167µL PBS buffer)

2. Instrument Setup

i. On the software home page, click “Perform Run”.

ii. Select “General Mode”.

iii. Enter the following information for each sample in the corresponding lane.
- Sample ID & Elution ID
- Assay: Select “GenoQuest JAK2 qPCR Assay”
- Protocol: Select “Extract + PCR”. (Note: For **extracted DNA**, positive control, and negative control, select “PCR Only”.

iv. Click “Next”.

v. Load the sample and consumables into the system according to the software instructions. (Note: 1. **Remove the caps from the sample and elution tubes.** 2. For blood samples, ensure the elution tube is empty. 3. For **extracted DNA**, positive control, and negative control, add at least 30µL to the elution tube and leave the sample well empty.

vi. Ensure the sample and consumable are loaded correctly (Figure 1), then click “Start”.

Result

1. A HEX channel Ct value **between 23.6 and 26.5** indicates a valid result. (Note: If the HEX Ct value is outside the acceptable range, measure the DNA concentration in the elution tube, adjust it to 10 ng/µL, and repeat the experiment. Suggestion: If Ct < 23.6, dilute the extracted DNA with nuclease-free water and repeat qPCR. If Ct > 26.5, reduce the blood sample dilution factor and repeat the extraction.)
2. For valid results, the FAM channel assay cut-off is **32.4**. A Ct value < 32.4 indicates a positive JAK2 V617F mutation; > 32.4 indicates a negative result (Table 1).

Target(s)		Scientific Control		
JAK2	IAC	PC	NC	Data interpretation
< 32.4	23.6 – 26.5	+	-	$JAK2^{V617F}$ Positive
> 32.4	23.6 – 26.5	+	-	$JAK2^{V617F}$ Negative
-	-	-	-	**Invalid result**
+/-	+/-	+/-	+	Contamination of PCR reaction mix; **Invalid result.**

Table 1: Data Interpretation

Clinical Performance

A clinical study was conducted using 10 human blood samples and 3 spiked samples (containing 1000 copies of JAK2 V617F DNA added to DNA extracted from JAK2 V617F-negative human blood samples). Results show that geneLEAD VIII accurately distinguished positive and negative samples, producing results comparable to ABI ViiA7. (Table 2&3)

Average Ct (HEX)	S.D.	%C.V.	Acceptance Criteria
24.80	0.54	2.19	23.6-26.5

Table 2: Clinical Study Result. Using 50 ng of extracted DNA from blood samples (n=13), geneLEAD VIII produced HEX channel Ct values that fell within the acceptance criteria specified in the instrument's IFU.

		ABI ViiA7	
		Positive	Negative
geneLEAD VIII	Positive	3	0
	Negative	0	10
		Sensitivity = TP/(TP+FN) = 100%	Specificity = TN/(FP+TN) = 100%

Table 3: Result comparison to reference instrument. The sensitivity and specificity evaluated by comparison to ABI ViiA7 were 100%.

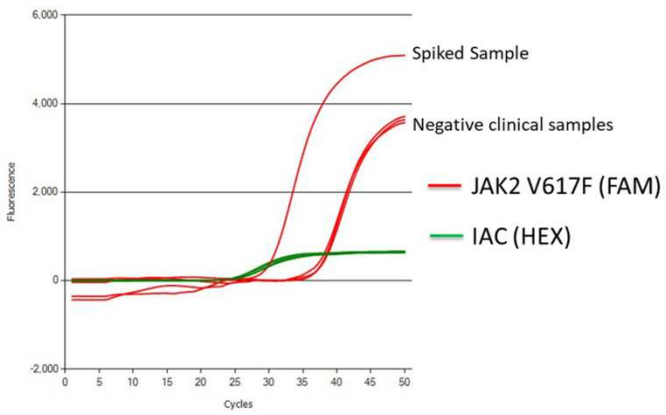

Figure 2: JAK2 Amplification Curve on geneLEAD VIII

Validation of pre-extraction dilution

Undiluted blood samples were extracted using the geneLEAD VIII system. A 4.24- to 7.72-fold dilution (average 6-fold) of the DNA was required to achieve a suitable concentration for the assay (Table 3).

	DNA concentration (ng/µL)	Dilution factors	Average Dilution Factor
Clinical sample 1	42.4	4.24	6.056
Clinical sample 2	71.7	7.17	
Clinical sample 3	52.8	5.28	
Clinical sample 4	44.2	4.42	
Clinical sample 5	61.2	6.12	
Clinical sample 6	60.1	6.01	
Clinical sample 7	71.5	7.15	
Clinical sample 8	57.5	5.75	
Clinical sample 9	77.2	7.72	
Clinical sample 10	67.0	6.70	

Table 4: DNA Concentration Extracted by the Clinical Sample

The 6-fold pre-extraction dilution was validated using blood samples with the highest and lowest DNA concentrations. This protocol yielded results within the acceptable Ct range (23.6–26.5) (Table 4).

	FAM		HEX	
	Trial 1	Trial 2	Trial 1	Trial 2
Clinical sample (Highest DNA concentration)	38.71	37.98	23.68	24.15
Clinical sample (Lowest DNA concentration)	39.03	38.13	24.82	24.39

Table 5: 6-fold Pre-extraction Dilution Validation Result

Reference

Products By Function	Cat. No.
Automated DNA Extraction and Detection	
GenoQuest™ JAK2 qPCR Assay	92023
geneLEAD VIII	A2700
geneLEAD VIII Consumable Set	F8900
geneLEAD VIII PCR Reaction Cassette Set	F8840
geneLEAD VIII PCR Reagent Cassette Set	F8820
MagDEA® Dx SV	E1300
Diluting Agent	
1x PBS Buffer	