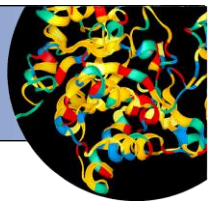
SCARAB GENOMICS

IS Detection Kit Protocol



KIT CONTENTS

Quantities provided are sufficient for analysis of ten samples.

- Positive Control Genomic DNA: 170 µL
- Negative Control Genomic DNA: 170 µL
- IS-specific Forward and Reverse Primers: 80 µL at 5 µM, for each of the following IS elements:

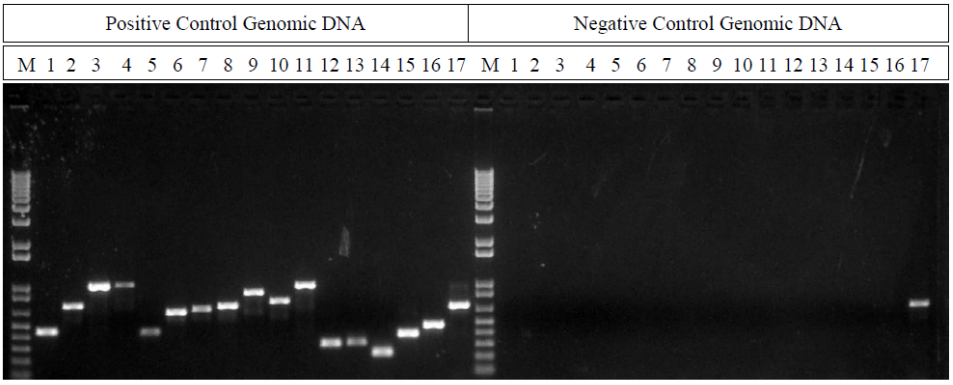
  IS1, IS2, IS3/ISEc17, IS4, IS5, IS10, IS30D, IS150, IS186, IS600/ISsd1, IS609, IS911, ISEc1/3/5, ISEc4, RhsA/B/C, and RhsD/E.

- Positive Control *dnaE* Forward and Reverse Primers: 60 µL of each at 5 µM

Store contents at -20°C. Do not store in a "frost-free" freezer.

PERFORMANCE

IS Detection primers sets are functionally tested using the Positive and Negative Control Genomic DNAs, following the procedure described in this User Protocol. Primers for *dnaE*, a gene present in all *E. coli* strains, serve as a positive control for the quality of the sample genomic DNA. The kit and reaction conditions have been validated with Phusion™ High-Fidelity DNA Polymerase from New England Biolabs. Six microliters of the resulting PCR amplification products are analyzed on 1.0% agarose gel in TAE buffer. No products are visible when water is added in place of template DNA. Expected results are as follows:



Lane	Primer Pair Tested	Expected PCR Product Size (bp)
M = Markers		
1	IS1	433
2	IS2	712
3	IS3/ISEc17	987
4	IS4	982
5	IS5	424
6	IS10	630
7	IS30D	658
8	IS150	696
9	IS186	862
10	IS600/ISsd1	744
11	IS609	966
12	IS911	307
13	ISEc1/3/5	318
14	ISEc4	239
15	RhsA/B/C	389
16	RhsD/E	468
17	dnaE	682

Lane	Primer Pair Tested	Expected PCR Product Size (bp)
M = Markers		
1	IS1	None
2	IS2	None
3	IS3/ISEc17	None
4	IS4	None
5	IS5	None
6	IS10	None
7	IS30D	None
8	IS150	None
9	IS186	None
10	IS600/ISsd1	None
11	IS609	None
12	IS911	None
13	ISEc1/3/5	None
14	ISEc4	None
15	RhsA/B/C	None
16	RhsD/E	None
17	dnaE	682

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PRE-TREATMENT OF PLASMID DNAS

Purified plasmid DNA may be contaminated with chromosomal DNA which could yield a positive PCR result even if the plasmid in question contains no IS elements. Therefore plasmid DNAs should be pretreated with a DNase that degrades linear but not circular DNA. Scarab uses EPICENTRE's Plasmid-Safe™ ATP-Dependent DNase (Cat. #E3101K). Be sure to inactivate the DNAase according to the manufacturer's protocol before using the treated plasmid as a PCR template.

PRE-AMPLIFICATION STEPS

1. Thaw the provided primers and DNA samples for testing on ice
2. Label the required number of 0.2-mL tubes or map out a microplate
3. Dilute the DNA samples:
   - For genomic DNA, dilute to 1-10 ng/μL
   - For plasmid DNA, dilute to 0.3 – 0.5 fmoles/μL (1 ng/μL = 0.30817 fmoles/μL of a 5 kb plasmid)
4. Program the thermal cycler as follows:

Temperature	Time	Cycles
98°C	1 min	1
98°C 61°C 72°C	10 sec 30 sec 1 min	30
72°C	7 min	1
4°C	Hold	--

PCR Procedure

5. For each reaction, mix 2 μL of each of the two primers, 1 μL of the sample DNA and 45 μL PCR master mix

   The master mix will contain buffer, water, dNTPs and thermostable polymerase, per the polymerase manufacturer's recommendations
6. Overlay each reaction mix with 1 drop (~ 20μL) of nuclease-free mineral oil to prevent evaporation. (Mineral oil is not necessary if you are using a thermal cycler with a heated lid)
7. Transfer tubes to thermal cycler and proceed with the PCR.
8. To analyze the results, add 6 μL of each PCR product mix per well of a 1% agarose gel in TAE buffer and electrophorese at 105 V/cm in 1xTAE running buffer then visualize PCR products with your usual method

NOTES

- A complete analysis of a genomic DNA sample requires four reactions for each primer pair: No DNA control, negative control genomic DNA, positive control genomic DNA and sample of interest; and three reactions for the *dnaE* control: No DNA control, negative control genomic DNA, and positive control genomic DNA – for a total of 71 reactions for all primer pairs.
- A complete analysis of plasmid DNA sample requires four reactions for each primer pair: No DNA control, negative control genomic DNA, positive control genomic DNA and sample of interest, but Rhs and dnaE controls are not needed, for a total of 14 primer pairs x 4 reactions each == 56 reactions.