SCARABGENOMICS LLC
CLEAN GENOME® E. coli
www.scarabgenomic.com

White Glove IS Detection Kit
User Protocol, IS-1109-10
FOR RESEARCH ONLY

STORAGE CONDITIONS

–20°C. **Do not store in a frost-free freezer.**

CONTENT

- Positive Control Genomic DNA: 170 µl, sufficient for the analysis of 10 samples.
- Negative Control Genomic DNA: 170 µl, sufficient for the analysis of 10 samples.
- IS-specific Forward (F) and Reverse (R) Primers: 80 µl of each primer at a concentration of 5 µM, sufficient for the analysis of 10 samples.

Forward Primers	Reverse Primer
IS1 Forward Primer	IS1 Reverse Primer
IS2 Forward Primer	IS2 Reverse Primer
IS3/ISEc17 Forward Primer	IS3/ISEc17 Reverse Primer
IS4 Forward Primer	IS4 Reverse Primer
IS5 Forward Primer	IS5 Reverse Primer
IS10 Forward Primer	IS10 Reverse Primer
IS30D Forward Primer	IS30D Reverse Primer
IS150 Forward Primer	IS150 Reverse Primer
IS186 Forward Primer	IS186 Reverse Primer
IS600/ISsd1 Forward Primer	IS600/ISsd1 Reverse Primer
IS609 Forward Primer	IS609 Reverse Primer
IS911 Forward Primer	IS911 Reverse Primer
ISEc1/3/5 Forward Primer	ISEc1/3/5 Reverse Primer
ISEc4 Forward Primer	ISEc4 Reverse Primer
RhsA/B/C Forward Primer	RhsA/B/C Reverse Primer
RhsD/E Forward Primer	RhsD/E Reverse Primer

- Positive Control dnaE Forward Primer and Positive Control dnaE Reverse Primers: 60 µl of each primer at a concentration of 5 µM, sufficient for the analysis of 10 samples.

REAGENT REQUIRED BUT NOT PROVIDED

- Phusion™ High-Fidelity DNA Polymerase (New England Biolabs Product Number F-530)
- Plasmid-Safe™ ATP-Dependent DNase (EPICENTRE Product Number E3101K)

KIT DESCRIPTION

The IS Detection kit is designed to test for the presence of transposable Insertion Sequences (IS) in a DNA of interest. IS elements are naturally present in the genomes of *E. coli* strains commonly used for protein and plasmid production. IS element transposition is known to be stimulated by the cell stress response and can lead to IS element "hopping" into plasmid DNA and or into other regions of the chromosome. Factors such the production of foreign proteins or the burden of carrying a high copy plasmid can induce the cell stress response. To alleviate these undesired transposition events, Scarab Genomics produced the Clean Genome® *E. coli* strains. These strains are devoid of all known IS elements (1-3) thereby creating the ideal hosts for the production of

foreign proteins or plasmid DNA. This kit can be used to detect for the presence of all the specific known IS elements in the genomes of commonly used *E. coli* strains (see table below). It can also be used to determine which elements may have transposed into a plasmid grown in these strains. The kit also detects the presence or absence of known recombination hot spots (Rhs) in the *E. coli* genome.

As demonstrated in the following table, the commonly used *E. coli* lab strains all contain multiple IS elements of many types. Multiple Deletion Strains (MDS) pioneered by Scarab Genomics lack these elements. IS elements detected in the genomic DNA of the indicated strains are highlighted in red, IS elements NOT detected in the genomic DNA of the indicated strains are highlighted in green. The number in each box reflects the number of IS elements of that type present in the indicated strain based on genomic sequence data.

IS Element Frequency in Commonly used *E. coli* Host Strains

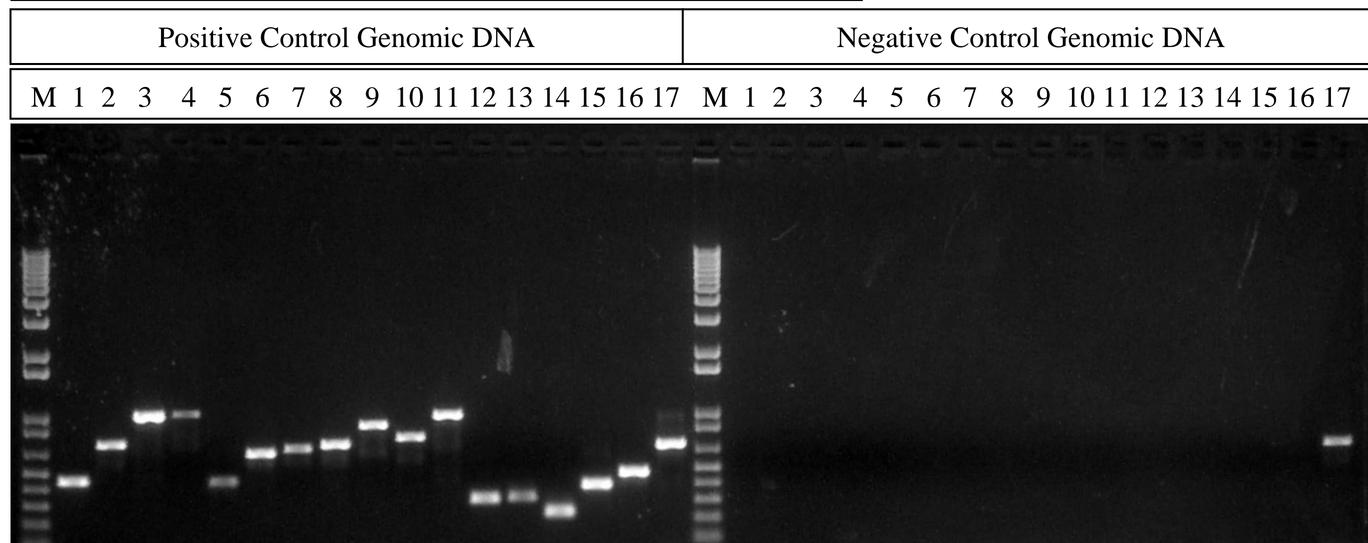
IS Element	BL21(DE3)	DH5alpha	DH10B	W3110	Stabl3	MG1655	MDS42	MDS42 ΔMD64*
IS1A, B, D, F	27	6	11	7	12	7	0	0
IS2	1	6	11	10	10	6	0	0
IS3, ISEc17	3	3	5	5	5	5	0	0
IS4	1	1	1	1	1	1	0	0
IS5	0	12	14	18	12	11	0	0
IS10L, R	0	2	3	0	0	0	0	0
IS186, B	5	3	3	3	3	3	0	0
IS150	4	1	3	1	1	1	0	0
IS30D	1	3	3	3	1	3	0	0
IS600, ISsd1	1	0	0	0	1	0	0	0
IS609*	1	1	1	1	1	1	1*	0
IS911	1	1	1	1	1	1	0	0
ISEc1, 3, 5	3	3	3	3	3	3	0	0
ISEc4	1	1	1	1	1	1	0	0
RhsA, B, C	2	3	3	3	3	3	0	0
RhsD, E	2	2	2	2	1	2	0	0

*See Note in reference section.

PRIMER QUALIFICATION

IS Detection primers sets are functionally tested using the Positive and Negative Control Genomic DNA and by following the procedure described in this User Protocol. Primers for DNA polymerase III, dnaE, are also included to serve as a positive control for the quality of the sample genomic DNA. dnaE is an essential gene and is found in all *E. coli*. The kit and reaction conditions have been validated with Phusion™ High-Fidelity DNA Polymerase from New England Biolabs. The use of other thermostable DNA polymerases may be possible provided that the proper optimization of reaction conditions is performed. Six microliters (6 µl) of the PCR amplification product is analyzed on 1.0% 1X TAE agarose gel. No products are visible when water is added in place of template DNA. The following table lists the expected size of PCR product to be obtained using the Positive and Negative Control Genomic DNA. The associated gel represents the expected electrophoresis pattern.

Primer Validation with Positive and Negative Control Genomic DNA



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

PRETREATMENT OF PLASMID DNA SAMPLES

Purified plasmid DNA samples may contain a small amount of chromosomal DNA contamination. This contamination may be sufficient to yield a false positive PCR result i.e., the PCR test was positive due to amplification of an IS element present in the chromosomal DNA contamination rather than from the presence of an IS element in some portion of the isolated plasmid population. To avoid these false positives, a pretreatment of plasmid DNA samples with EPICENTRE's

Plasmid-Safe™ ATP-Dependent DNase (Product Number E3101K) is recommended. The DNAse in this product degrades linear dsDNA such as contaminating chromosomal DNA but has no activity on nicked or closed-circular dsDNA or supercoiled DNA. Note, the DNAase MUST be inactivated according to the manufactures protocol (incubation at 70C for 30 minutes) prior to using the treated plasmid as a template in PCR reactions.

PRE-AMPLIFICATION STEPS

1. Thaw the provided primer sets on ice. Assemble the DNA samples.

2. Label the necessary number of 0.2 ml tubes.

3. Dilute the DNA samples to be tested in nuclease free water.
   For genomic DNA, dilute to 1-10 ng/µl.
   For plasmid DNA, dilute to 0.3-0.5 pmoles/µl*
   *1 ng/µl = 0.3 pmoles/µl of a 5 kb plasmid

4. Program the thermal cycler using the following cycling parameters:

Temperature	Time	Number of 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	

To perform a complete analysis of a genomic DNA sample of interest, the following reactions will need to be prepared.

<u>Four reactions needed for EACH primer pair to be tested</u>.
No DNA control
Negative Control Genomic DNA
Positive Control Genomic DNA
Sample of interest

<u>Three dnaE Positive Control Gene reactions are needed for each genomic DNA sample.</u>
No DNA control
Negative Control Genomic DNA
Positive Control Genomic DNA

There are 17 primer pairs for the analysis of genomic DNA samples and 14 primers pairs for the analysis of plasmid DNA samples (there is no practical reason to test plasmid DNA samples with the 2 sets of Rhs primer pairs or the dnaE primer pair).
To completely test a genomic DNA sample, a total of 68 reactions (17 primer pairs x 4 reactions) would be needed per sample + 3 control reactions for a total of 71 reactions.

[N# samples X (4 x 17)] + 3

To completely test a plasmid DNA sample, a total of 56 reactions (14 primer pairs x 4 reactions) would be needed.

N# samples X (4 x 14)

PCR PROCEDURE

SAMPLE + PRIMER PREPARATION

Assemble the DNA sample to be tested and the appropriate primer pairs in individual tubes.

Sample + Primer Preparation

Component	Volume per reaction (µl)
5 µM Forward Primer	2
5 µM Reverse Primer	2
DNA Sample	1*

* For genomic DNA use 1 µl at a concentration of 1-10 ng/µl.
For plasmid DNA use 1 µl at a concentration of 0.3-0.5 pmoles/µl.

MASTER MIX (MM) PREPARATION

The following protocol describes the generation of a Master Mix containing everything except the primer pairs and sample to be tested. The Master Mix is then added to the individual primer pair + sample tubes. The protocols are written assuming a 50 µl reaction volume. Volumes can be scaled proportionally if different reaction volumes are used. Assemble the components in the appropriate size tube as dictated by the number of samples to be analyzed. In order to account for pipetting errors an extra reaction is added.

Genomic DNA Sample Master Mix Preparation

Component	Volume per reaction (µl)	# of reactions per sample + 1	Volume to be added (µl)
Nuclease free H_2O	30.5	72	2196
5x Phusion HF Buffer	10	72	720
dNTP mix	4	72	288
Phusion™ High-Fidelity DNA Polymerase	0.5	72	36

Plasmid DNA Sample Master Mix Preparation

Component	Volume per reaction (µl)	# of reactions per sample + 1	Volume to be added (µl)
Nuclease free H_2O	30.5	57	1738.5
5x Phusion HF Buffer	10	57	570
dNTP mix	4	57	228
Phusion™ High-Fidelity DNA Polymerase	0.5	57	28.5

1. Gently vortex the Master Mix tube for 10 seconds and briefly centrifuge.
2. Aliquot 45 µl of the Master Mix to each of the primer pairs + sample or control DNA tubes.
3. Mix briefly and overlay the reaction with 1 drop (~ 20µl) of nuclease-free mineral oil to prevent condensation and evaporation. **Mineral oil addition is not necessary if you are using a thermal cycler with a heated lid.**
4. Place the tubes in a pre-programmed thermal cycler and proceed with the amplification reaction.
5. Analyze 6 µl of the PCR products by agarose gel electrophoresis. Recommended electrophoresis conditions are as follows: constant voltage at 105 V/cm, 1XTAE as running buffer and 1.0% agarose in 1XTAE.
6. Visualize the DNA amplification products using an appropriate technique e.g., UV trans-illumination of the ethidium bromide-stained DNA or other suitable methods.

See the table on page 3 for expected product sizes.

REFERENCE

1. Pósfai, G., Plunkett III, G., Fehér, T., Frisch, D., Keil, G.M., Umenhoffer, K., Kolisnychenko, V., Stahl, B., Sharma, S.S, de Arruda, M., Burland, V., Harcum, S.W., and Blattner, F.R. Emergent Properties of Reduced-Genome *Escherichia coli. Science* **312**, 1044-1046 (2006).
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3. Sharma, S.S., Blattner, F.R., and Harcum, S.W. Recombinant protein production in an Escherichia coli reduced genome strain. *Metabolic Engineering* **9**, 133-141 (2007).
4. Hayashi, T., Makino, K., Ohnishi, M., Kurokawa, K., Ishii, K., Yokoyama, K., Han, C.G., Ohtsubo, E., Nakayama, K., Murata, T., Tanaka, M., Tobe, T., Iida, T., Takami, H., Honda, T., Sasakawa, C., Ogasawara, N., Yasunaga, T., Kuhara, S., Shiba, T., Hattori, M., and Shinagawa, H. Complete genome sequence of enterohemorrhagic Escherichia coli O157:H7 and genomic comparison with a laboratory strain K-12 (Supplement). *DNA Research* **8**, 47-52 (2001).

* Note: Subsequent to the launch of the Clean Genome *E. coli* strains, 2 copies of an atypical IS element named IS609 were recognized in the *E. coli* O157:H7 genome sequence (4). This IS element has not been shown to transpose, although other members of this IS family have been shown to transpose. The ability to transpose requires an intact *orfA*. The single IS609 element found in *E. coli* K-12 and B strains, however, carries a defective *orfA* with a stop codon mutation located near the middle of the ORF. IS609 has been removed in derivatives of the original MDS strains, indicated as “MDS42 ΔMD64” in the table on page 2.

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