InsertFinder™ PCR Quick Screening Kit

(Catalog #K903-500; 500 assays; Store at –20˚C)

I. Introduction:

Identification of clones that contain the desired DNA insert is an essential step in molecular cloning procedure. Traditional methods for identifying the correct DNA inserts require growing up individual colonies, isolating plasmid DNAs, and then digesting with restriction enzymes to analyze each individual clone. Such traditional procedures are very time consuming, laborious, and expensive. The new InsertFinder™ PCR Quick Screening Kit is designed to quickly screen clone candidates using PCR technology. The procedure allows screen of colonies directly from plate, without growing up the cultures. The proprietary Cell Lysis Buffer provided with the kit can efficiently lyse cells (bacteria or yeast) without interfering with PCR reactions. The PCR Enhancer included in the kit allows amplification of hairpin structure or GC-rich region efficiently. PCR products can be directly analyzed on an agarose gel without enzyme digestions. The kit provides a simple, convenient, and economical way to identify correct inserts. It is also suitable for screening large numbers of colonies.

II. Kit Contents:

Component	K903-500	Cap Code	Part Number
Insert Finder Lysis Buffer	0.6 ml	Red	K903-500-1
10X PCR Buffer	1.1 ml	Clear	K903-500-2
dNTP	1.1 ml	Violet	K903-500-3
Taq DNA Polymerase	250 units	Green	K903-500-4
PCR Enhancer	0.6 ml	Blue	K903-500-5

III. InsertFinder™ Assay Procedure:

1. Add 1 µl of InsertFinder Lysis Buffer to the bottom of each labeled PCR tube.
2. Pick a fresh single clone of transformants (bacteria or yeast) with a pipet tip (do not use tooth picks). Carefully transfer a small amount of the colony into the 1 µl of Lysis Buffer prepared in step 1. The buffer will become cloudy.
3. Prepare a reaction Master Mix. For each clone, mix the follows:

2 µl	10X PCR Buffer
2 µl	dNTP
0.05 µg	Primer 1
0.05 µg	Primer 2
0.9 µl	PCR Enhancer
dH_2O	to a total 19 µl
0.2 µl	Taq Polymerase

Notes: a) We suggest including a positive control with a known insert and a negative control without clone added.

b) Primer pairs can both be complementary to the insert, or to the cloning vectors, or in combinations. If using the combination of primers from insert and vector, insert orientation can be selected specifically.

4. Add 19 µl of the Master Mix into each PCR tube containing lysed clones.
5. Run PCR reaction 25 to 35 cycles* as follows:

94˚C	30 sec.
55˚C	120 sec.**
72˚C	60 - 180 sec.***

FOR RESEARCH USE ONLY! Not to be used in humans

Notes:

* We suggest running 25-30 cycles for high copy number plasmid and 30-35 cycles for low copy numbers of plasmid.

**We suggest using an annealing temperature of 55˚C. However, you may use a suggested annealing temperature for your specific primer pairs.

***We recommend using a 60 sec elongation time for an insert < 1 kb, and 180 sec for an insert > 1 kb.

6. Add DNA loading buffer into each sample and load 15 µl of the sample on a standard agarose gel to determine which clones contain the proper DNA insert.

Note: For small inserts (< 300 bp), we suggest using Orange G DNA Loading Buffer (BioVision Cat.# 2110-10) which offers better resolution of DNA fragments on agarose gel.

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