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In vitro DNA Amplification Kit

Store at -20°C

Cat. No.	Product Name	Quantity
E031	*In vitro* DNA Amplification Kit	100 rxns

Product Description

The *In vitro* DNA Amplification Kit offers end users an efficient and fast method for high throughput DNA amplification from minimal amounts of starting material. With a simple set up and short handling time, the following protocol will significantly reduce the cost of high throughput DNA amplifications through eliminating the requirement for miniprep DNA isolation.

Downstream applications of the linear product include (but are not limited to) sequencing and restriction enzyme digestions. This system further serves as a powerful tool for rapid screening of bacterial colonies or even single cells.

Additional applications of this technology include whole genome amplification, DNA library construction, SNP genotyping and *in vitro* cloning of lethal DNA.

Product Components

Part No.	Product Components	Volume
E031-1	Reaction Mix A	1 ml
E031-2	Reaction Mix B	100 µl

Additional Materials Required

PCR tubes or plates.
Finely-pointed instrument for colony picking.

Protocol

Thaw Reaction Mixes on ice before use. Reaction Mixes can be aliquoted into smaller volumes and stored at -20°C to avoid repeated freeze thawing.

1) Pipette 9 µl of Reaction Mix A into each PCR tube or well.
2) If the starting material is a bacterial colony, add it to Reaction Mix A by pinpricking the colony and swirling the tip of the instrument into the PCR tube or well. If the starting material is DNA, add 10 ng of the template into the PCR tube or well.
3) Heat the samples to 95°C for 3 minutes, followed by cooling to 4°C.

4) Add 1 µl of Reaction Mix B to each sample and mix well.
5) Incubate at 30°C for a minimum of 4 hours for restriction enzyme digestion screening, or a minimum of 18 hours for downstream sequencing applications.
6) Incubate at 65°C for 10 minutes to stop the reaction, followed by cooling to 10°C.

The sample is now ready for the following downstream applications:

Restriction Enzyme Screening

a. Cut 2 µl of the reaction product in the restriction enzyme digest.
b. Incubate at optimal digestion temperature for a minimum of 30 minutes.

Isolating DNA Insert Fragment for cloning

a. Cut 5 µl of the reaction product in the restriction enzyme digest.
b. Incubate at the optimal digestion temperature for a minimum of 1 hour.
c. Run the product on an agarose gel, excise the desired band and isolate the DNA fragment using the Column-Pure DNA Gel Recovery Kit (Cat. **D507**) or Direct-Gel-Spin DNA Recovery Kit (Cat. **D210**).

Sequencing Screening

a. Ensure that the 30°C amplification step was incubated for at least 18 hours.
b. Use 1µl of the reaction product as template for the sequencing reaction.

Troubleshooting Guide

Restriction enzyme digestions will naturally produce smeared results on a gel due to the nature of the amplification process. As a result, inserts smaller than 500bp following restriction enzyme digestion may be difficult to detect. Increasing the amount of amplified DNA sample in the restriction digest may help with visualizing smaller bands.

Problem	Explanations	Solution
No DNA or only faint smear visible	- Colony was not picked properly	Ensure only a small amount of the colony is added to the reaction.
	- Reaction Mix has undergone too many freeze thaw cycles	Make aliquots of Reaction Mixes and store at -20°C until use to avoid freeze thawing.
	- Not enough DNA used in the digest	Use more reaction product for the digest.
DNA bands seen, but high amount of smearing	- Too much template	Use a finer tool to pick the colony or use less template DNA.
DNA does not migrate on gel	- Insufficient digestion of product	Increase digestion time or amount of restriction enzyme
Bands are very faint	- Insufficient DNA amplification	Incubate reaction at 30°C overnight.
	- Not enough DNA used in the digest	Use more reaction product for the digest.