

Basic Read Sequence

Solutions for Your Research



Technical detailed list

- DNA and primer should be premixed* in the same tube (1.5ml tube) labelled with the corresponding ID code.

*Primm supplies, free of charge, the most common universal primers (M13for/rev-T7 prom/term-BGHrev-Sp6)

- Customer is kindly requested to provide a copy of the order confirmation received by e-mail.

Basic-PCR

1. DNA Requirements

PCR fragment and primer should be provided premixed in the same tube; lyophilized or in ddH₂O solution in a volume of 6ml according to the following amount:

PCR fragment (<1 Kb)	PCR fragment (≥1 Kb)	OLIGONUCLEOTIDE
5ng/100bp	100ng	2pMol

- Sequencing reactions are performed using standard protocols
- Please verify the samples before sending them to the sequencing facility
- When possible, send double amount of DNA/primer solution to reduce turnaround time when resequencing is necessary

One of the most crucial factors for successful sequencing reactions is to use the right amount of template. We suggest to check the samples concentration by loading them onto an agarose gel rather than measuring UV absorbance.

2. Sample Preparation

It's extremely important to use PCR fragments free from aspecific bands or other contaminants (salts, ethanol, etc.).

Basic-Plasmid

1. DNA Requirements

Plasmidic DNA and primer should be provided premixed in the same tube; lyophilized or in ddH₂O solution in a maximum volume of 6ml according to the following amount:

PLASMID	OLIGONUCLEOTIDE
330ng	3pMol

- Sequencing reactions are performed using standard protocols
- Please verify the suitability of the samples before sending them to the sequencing facility
- When possible, send us a double amount of DNA/primer solution to reduce turnaround time in case of resequencing.

One of the most crucial factors for successful sequencing reactions is to use the right amount of template. Please check the samples concentration by loading them onto an agarose gel rather than measuring UV absorbance.

2. Samples preparation

It's extremely important to use pure samples free of contaminants (salts, ethanol, etc.).

In order to obtain the best quality result, we suggest to prepare your templates according to standardized methods using commercial kits suitable for sequencing analysis.

To resuspend your DNA please use always ddH₂O or 10mM Tris-HCl.

TE or other buffers can cause sequencing failure.

Delivery of results

Primm provides sequencing results as complete electropherograms checked for the absence of technical or electrophoretic problems. On Primm web site customers can download freeware version of the most common tools (PC or MAC-compatible) to view, analyse and align sequencing results.

Be aware: "Single Read" reactions will contain ambiguities and mistakes. Single run sequencing are not publication quality data, therefore we suggest to sequence templates on both strands.

For any other information please contact our Sequencing Staff. (dnaseq@primm.it)