Solutions for Your Research

Smart/Quick Read Sequence

Technical detailed list

•DNA and primers should be sent in a 1.5ml tube labelled with the corresponding ID code.
•Customer is kindly requested to provide a copy of the order confirmation received by e-mail with a gel picture of the samples together with a molecular weight marker.

Smart/Quick-PCR

1. DNA Requirements

PCR fragments can be lyophilized or dissolved in ddH_2O in a maximum volume of 10 µl.
The relative amounts are listed below:

PCR (<1Kb)	PCR (≥ 1Kb)
5ng/100bp	100ng

•Sequencing reactions are performed using standard protocols
•Verify the suitability of the samples before sending them to the sequencing facility
•When possible send double amount of DNA to reduce turnaround time when resequencing is necessary (*failed runs are repeated at no additional charge*)
One of the most crucial factors for successful sequencing is to use the right amount of template. Please check samples concentration by loading them onto an agarose gel rather then measuring UV absorbance.

2. Sample preparation

PCR fragments must be single band. Our service will always perform a free **ExoSAP-IT®** treatment on every PCR product before setting up the sequencing reaction.

PRIMERS Requirements

A list of the most common universal primers is available on Primm web page and are free of charge. Specific oligos can be ordered at Primm or provided by the customer, delivered in water solution at the concentration of 1mM (10µl for each sequencing reaction). Primers that give best results in automated sequencing must have a Tm between 50°-55°C and should not be rich in C or G at the 3' end but can have C or G as the 3' final base. Our staff can help you planning the most suitable oligo to optimize and guarantee the highest quality result.

Smart/Quick-Plasmid

1. **DNA Requirements**

DNA can be lyophilized or dissolved in ddH_2O. The relative amounts are listed below:

PLASMID	BAC
500ng required concentration 150ng/µl	1500 ng required concentration 300ng/µl

•Sequencing reactions are performed using standard protocols
•Verify the suitability of the samples before sending them to the sequencing facility
•When possible send us double amount of DNA to reduce turnaround time when resequencing is necessary *(failed runs are repeated at no additional charge)*
One of the most crucial factors for successful sequencing reactions is to use the right amount of template. Please check samples concentration by loading them onto an agarose gel rather then measuring UV absorbance.

2. Samples preparation
It's extremely important to send pure samples and free of contaminants (salts, ethanol, etc..).
In order to obtain the best quality results we suggest to prepare your templates according to standardized methods using commercial kits suitable for sequencing analysis.
To resuspend your DNA use always ddH_2O or Tris-HCl 10mM.
TE or other buffers can cause sequencing failure.

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DNA Production

1.Samples Requirements
Customer can supply bacteria as:
•O.N. culture in LB or like Medium, with a *minimum* amount of 5ml for each sequencing reaction
•Single colony, on a Petri plate or tube stub
It is mandatory to indicate the antibiotic resistance of the *E.Coli* strain.
Template preparation from the bacterial colony is performed using the commercial kit suitable only for sequencing reaction set-up. (*TempliPhi*)

Delivery of results

Primm provides sequencing results as complete electropherograms checked for the absence of technical or electrophoretic problems. On Primm web site customer can download freeware version of the most common tools (PC or MAC-compatible) to view, analyse and align their 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 your templates on both strands or to require "Safe Read" Service.
For any other information please contact the Sequencing Staff. (dnaseq@primm.it)