Solutions for Your Research

HT Read Sequence

Technical detailed list
•This service is recommended for the customer who sequences great amount of samples.
•DNA should be sent in a 96 well plate format* labelled with the corresponding ID code according to the standard protocols as described below.

At least 48 samples/plate are accepted.

Be aware of keeping empty H12 well for internal positive control (pGem/M13for)
•Customers are kindly requested to provide a copy of the order confirmation received by e-mail.

v **HT READ**
•*for Plasmid and PCR product is available both **"QUICK" and "SAFE"** Service*
•*for bacteria is available both "**QUICK–TempliPhi" and "SAFE-TempliPhi"** Service*

HT-PCR

1. DNA Requirements
PCR fragments can be lyophilized or dissolved in ddH_2O in a maximum volume of 10µl.
The fragments must be rationally normalized for concentration and length.
Please follow the instruction below for the quantity required.

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 in case of resequencing (*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
If the sequencing reactions must be performed with a unique* primer, customer can provide it in a 1.5 ml tube dissolved in ddH_2O at the concentration of 1mM (10µl for each sequencing reaction).
**On the contrary please contact the service.*
Primm can provide the most common universal primers on its web page and they are free of charge.
Specific oligos can be ordered at Primm or provided by the customer.
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.

HT-Plasmid

Solutions for Your Research

1. DNA Requirements

DNA can be lyophilized or dissolved in ddH_2O in a maximum volume of 10mcl.
The fragments must be rationally normalized for concentration.
Please follow the instruction below for the quantity required.

PLASMIDIC DNA
500ng required concentration 150ng/mcl

•Sequencing reactions are performed using standard protocols
•Verify the suitability of the samples before sending them to the sequencing facility.
•When possible send us a double amount of DNA to reduce turnaround time in case of resequencing
One of the most crucial factors for successful runs is to use the right amount of template.
Check your samples concentration by loading them onto an agarose gel rather then measuring UV absorbance.

2. Samples preparation

It's extremely important to send samples pure and 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 use always ddH_2O or Tris-HCl 10mM.
TE or other buffers can cause sequencing failure

PRIMERS Requirements

If the sequencing reactions must be performed with the same primer, customer can provide it in 1.5 ml tube dissolved in ddH_2O at the concentration of 1mM (10mcl for each sequencing reaction).
If you need to perform sequences with different oligonucleotide, please contact the service.
A list of the most common universal primers is available on the web page and they are free of charge.
Specific primers can be ordered to Primm or provided by the customers.
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 contain 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.

DNA PRODUCTION

1.Samples Requirements

Customer can supply bacteria in 96 wpf as:
•O.N. culture in LB or like Medium, with a minimum amount of 50mcl
•Single colony resuspended in 20mcl ddH_2O
It is mandatory to indicate the antibiotic resistance of the *E.Coli* strain.
Template preparation from the bacterial colony is performed with a commercial kit suitable only for sequencing reaction set-up.(*TempliPhy*)

Delivery of results

Primm provides sequencing results as complete electropherograms checked for the absence of technical or electrophoretic problems. On the web page www.primm.it/service/ord/useful.asp 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 you to sequence your templates on both strands or to require "HT Safe Read" Service.
For any other informations please contact the Sequencing Staff.(dnaseq@primm.it)