DNA Sequencing Services

Your Trusted Partner for Sanger Sequencing

Product no.	Product Description
SS1001	**Single Pass DNA Sequencing** Customer to provide purified PCR product or purified plasmid. For Plasmid or long PCR products, we guarantee **minimum 850 bases** from a good quality template in a single reaction.
SS1002	**Single Pass DNA Sequencing, 96-well format** Customer to provide purified PCR product or purified plasmid. Leave well H12 empty, 95 samples with normalised OD. For Plasmid or long PCR products, we guarantee **minimum 850 bases** from a good quality template in a single reaction.
SS1201	**DNA Sequencing Service + Plus** Customer to provide single band un-purified PCR product. For un-purified long PCR product, we guarantee **minimum 1,000 bases** from a good quality template in a single reaction.

Why choose 1st BASE DNA Sequencing?

- Wide range of **FREE universal primers** for dye terminator chemistry
- **100% Quantification** of your DNA templates prior sequencing reaction.
- Stringent quality control: Internal control reaction for each sequencing cycle protocol is provided.
- Best **effort guarantee** for each reaction that you paid.
- **Without the need to ask**, we provide **prompt** technical support and consultation.
- Industry leader in providing **long read results with supreme high quality**
- Industry leader in sequencing difficult templates (high GC, homopolymer, etc)
- Online and / or email notification of results download using password protected web hosted link.

Sample requirement:

Product no.	Type of DNA	Concentration & Volume/ Reaction
SS1001 SS1002	Purified PCR product [90-250bp] Purified PCR product [251-500bp] Purified PCR product [>500bp] Purified Plasmid	[90-250bp] 10 ng/µL, min 10 µL per rxn in dH2O [251-500bp] 20 ng/µL, min 10 µL per rxn in dH2O [>500bp] 40 ng/µL, min 10 µL per rxn in dH2O 100 ng/µL, min 10 µL per rxn in dH2O
SS1201	Single band un-purified PCR product	1 reaction: 100ng/uL, min 20uL in sterilized dH2O 2 reactions: 100ng/uL, min 30uL in sterilized dH2O
	Concentration & Volume/ Reaction	
Primer	10µM or 10pmol/µL, in 5µL per rxn in dH2O	

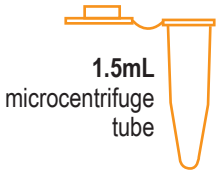
Sample submission:

For tube submission, make sure the label of the matching the order form to avoid delay process of order.

If gel photo is provided, indicate:
(i) lane no. of DNA template and DNA ladder
(ii) name of DNA template and marker (e.g. 1kb.100bp ladder)
(iii) volume of DNA template and ladder loaded per lane

If 1st BASE Sample Collection Card is not available, please wrap plates / tubes with bubble wrap before shipping. For bulk shipment, pack plate in a CLOSED container.

Packing of samples



1.5mL microcentrifuge tube

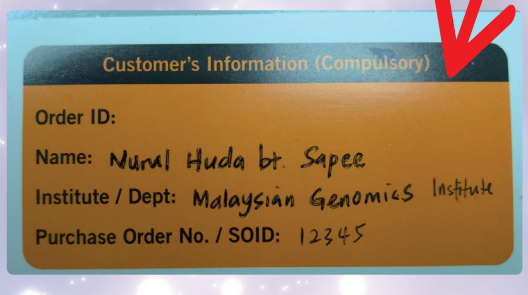
0.5mL microcentrifuge tube

Sample and/or primer tubes

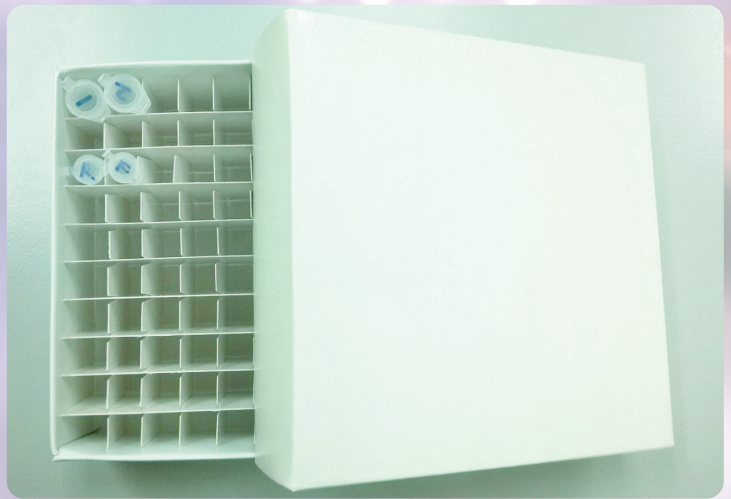
Close lid and seal with parafilm

Label side and lid of the tube with a permanent marker

- **Arrange tubes** on 1st BASE Sample Collection Card, in the same order as indicated on the Order Form
- **Attach print-out of Order Form** to the Card
- **Seal in zip-lock bag and place in fridge for collection**



Sample of Collection Card
(≤ 24 samples)



Sample of Collection Cryobox
(up to 100 tubes)

How to examine the quality of the DNA template before send for Sequencing?

Method 1: Agarose gel electrophoresis (recommended)

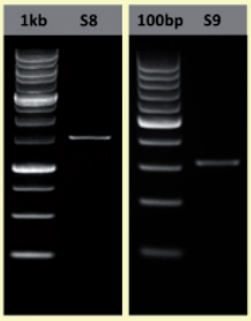
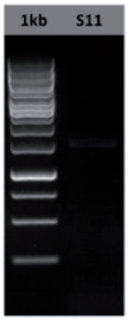
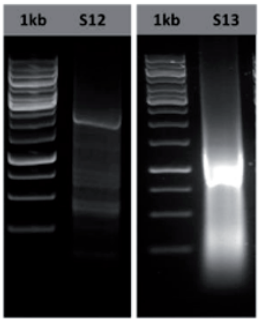
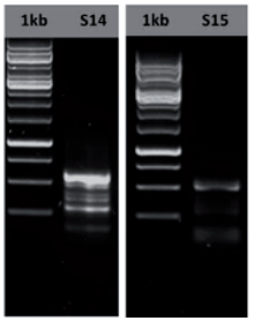
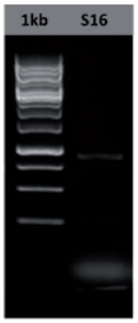
– Purified DNA should run as a single band on an agarose gel. Agarose gels reveal contaminating DNAs and RNAs, but not proteins and salts.

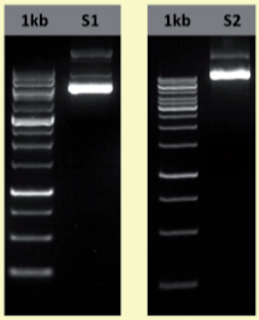
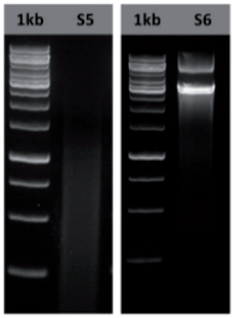
  * uncut plasmid – 2 to 4 bands

Method 2: Spectrophotometry

– The A260/A280 ratio should be 1.8 to 2.0. Smaller ratios usually indicate contamination by protein or organic chemicals. Spectrophotometry can reveal protein contamination, but not DNA, RNA or salts contamination.

Salt contamination does not show up in any of the quantification method – column purification!

Purified PCR Product	Poorly Optimized PCR Reaction / Poorly Purified PCR Product				
✓ Good Quality PCR Product	✗ Absence of Target DNA	✗ Insufficient Template	✗ Smeared Products	✗ Multiple Products	✗ Primer-dimer Contamination
					
Distinct target PCR fragment, free from degradation and contaminants *DNA Concentration within recommended range*	**Suggestion:** Amplify and prepare a new batch of target fragment.	**Suggestion:** Amplify and prepare a new batch of target fragment. Pool replicates if necessary.	**Suggestion:** Amplify and prepare batch of target fragment. Optimize PCR condition to obtain distinct target PCR fragment.	**Suggestion:** Excise the target fragment for gel-DNA extraction. Check by running purified DNA on gel before sending for DNA Sequencing. **For more details, please refer to our FAQ website at http://www.base-asia.com/dna-sequencing-services/support/faqs**	

Purified Plasmid DNA	Poorly Purified Plasmid DNA			
✓ Good Quality Plasmid DNA	✗ Absence of Target DNA	✗ Insufficient Amount of DNA	✗ Degraded Plasmid DNA	✗ RNA Contamination
				
DNA Concentration within recommended range (>100ng/uL) *Free from degradation & contaminants*	**Suggestion:** Extract a new batch of DNA.	**Suggestion:** Extract a new batch of DNA. [>100ng/uL]	**Suggestion:** Extract a new batch of DNA.	**Suggestion:** Treat with RNase-A.

Note: The DNA ladder is not applicable for sizing comparison of non-linear DNA samples (e.g. Plasmid DNA)

More details can be found from our website
http://www.base-asia.com/dna-sequencing-services/support/faqs > Please look for Q8!

Get your Sanger Sequencing done with 1st BASE in just few CLICKS

Step 1: Register for free as a new user with **1st BASE 1oo Online Portal** (**https://order.base-asia.com/**).
Step 2: Upon successful registration, you will be issued a password.
Step 3: Login and start ordering!

Easy ordering step through our Multiple Entry system

1. Download the excel file from our website.
2. Fill in your sample information into the excel file.
3. Copy and paste into our website.
4. That's all!

You may find the complete ordering steps in from the link below.
www.firstbaselab.com/downloads/Steps_to_order_through_1stBASE_1oo_online_portal_multiple_entry_local.pdf

Benefits of *online ordering* for you:

- Order at your convenience: **24hrs a day/ 7 days a week**
- **FREE SMS Notification** for your critical results
- **REWARD POINTS** for every order
- **SECURE** communication ensure your orders and results remain private
- **Easy sharing** of results with other critical lab members
- **High Accuracy** in ordering and processing. No manual inputting in entire processing chain
- **Cloud Storage** Get your results or order info wherever and whenever you want
- **TRACK** your order status as they are processed

1st BASE Online Ordering
https://order.base-asia.com

What will you get after receive the sequencing result?

Two files will be received for each reaction

How do I know whether my sequencing result is good?

We recommend our customer to use Sequence Scanner software, which is free from Applied Biosystems, to view the sequence data.

In each reaction, we emphasize **Quality Value (QV), Contiguous Read Length (CRL) & Trace Score.**

1% PE means 99% Accurate or Probability of error is 1 in 100 bases

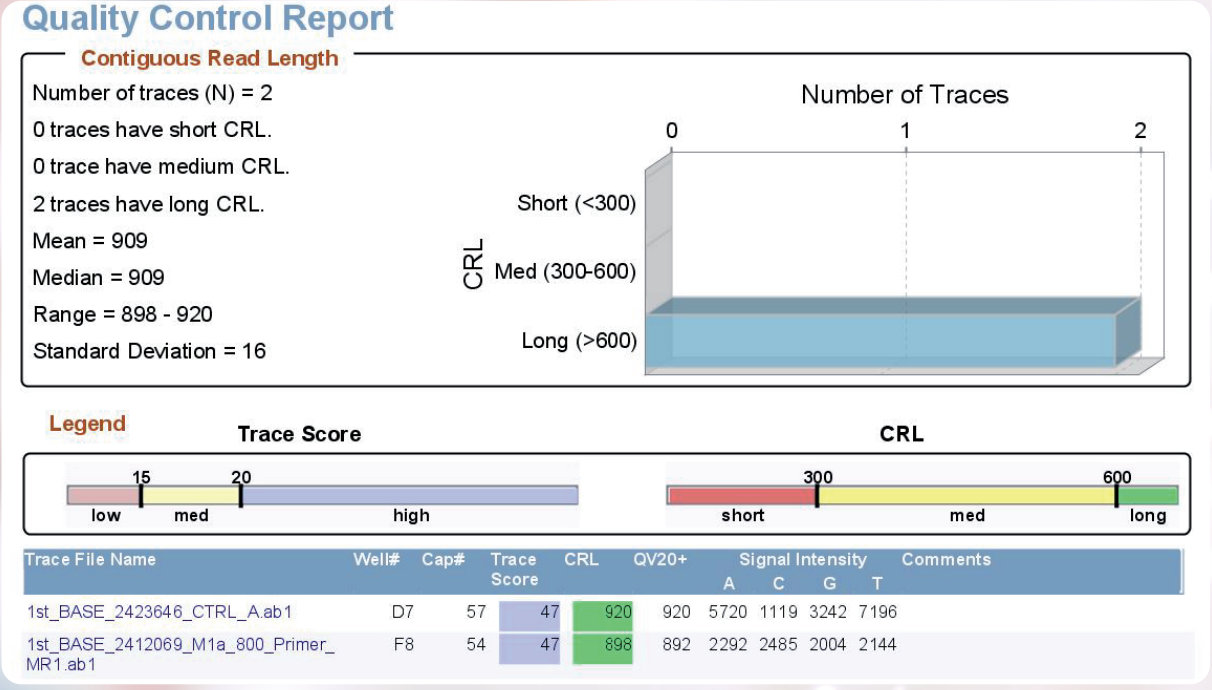
QVn	PE*	QV	PE*	QV	PE*
1	79%	21	0.79%	41	0.01%
5	31%	25	0.31%	45	0.00%
10	10%	30	0.10%	50	0.00%
15	3.20%	35	0.03%	60	0.00%
20	1.00%	40	0.01%	90	0.00%

**PE = The probability that a base was miscalled*

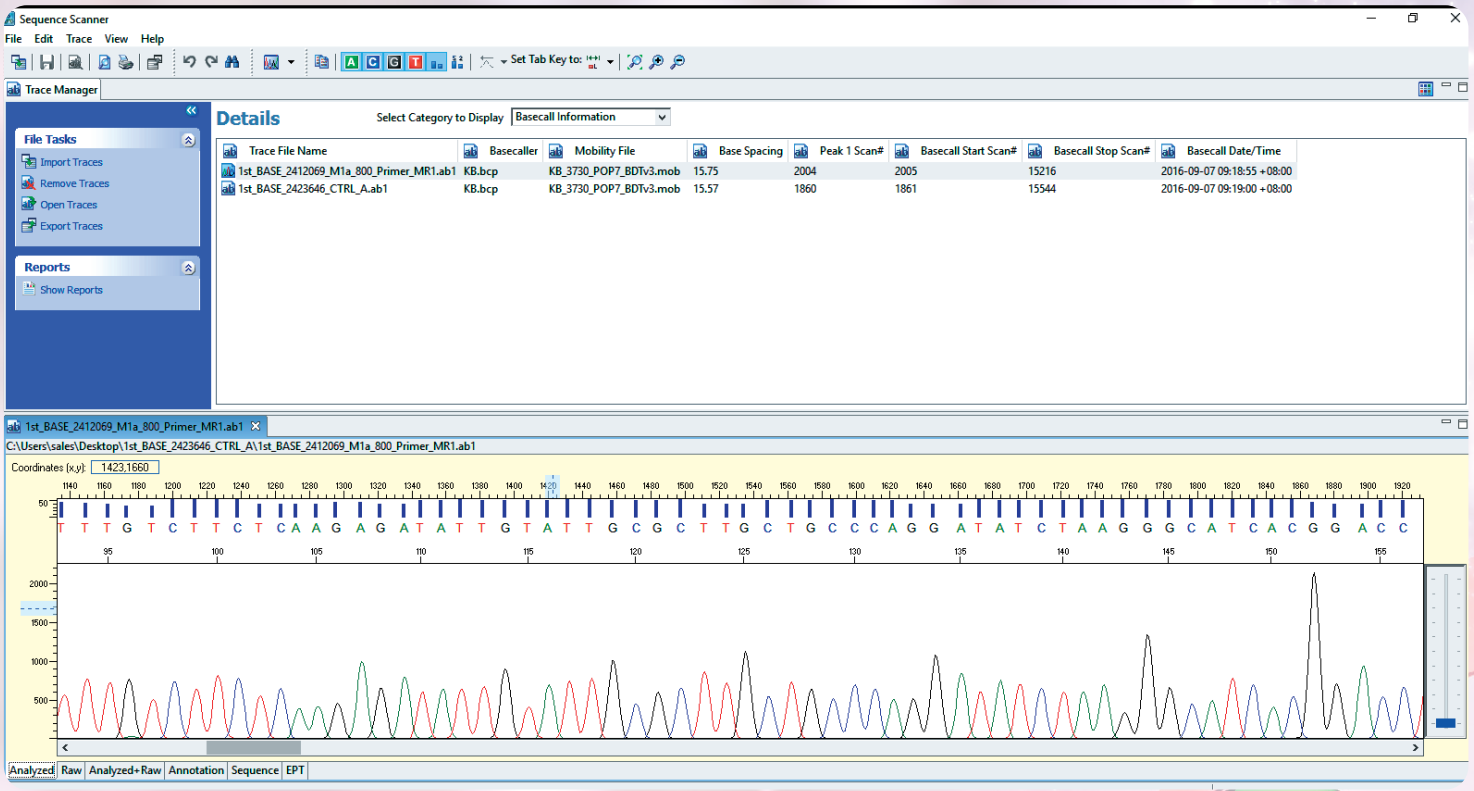
CRL = Longest uninterrupted stretch of high quality base calls

Trace Score = Average QV of a range of bases in this case Contiguous read length or CRL

By using Sequence Scanner, you can view your results in a single click to display the QC report of every sequencing order:



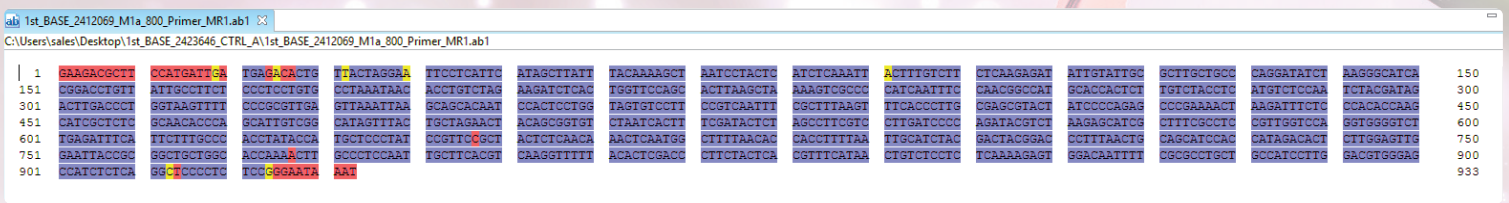
Electropherogram View in Sequence Scanner



Trace View in Sequence Scanner

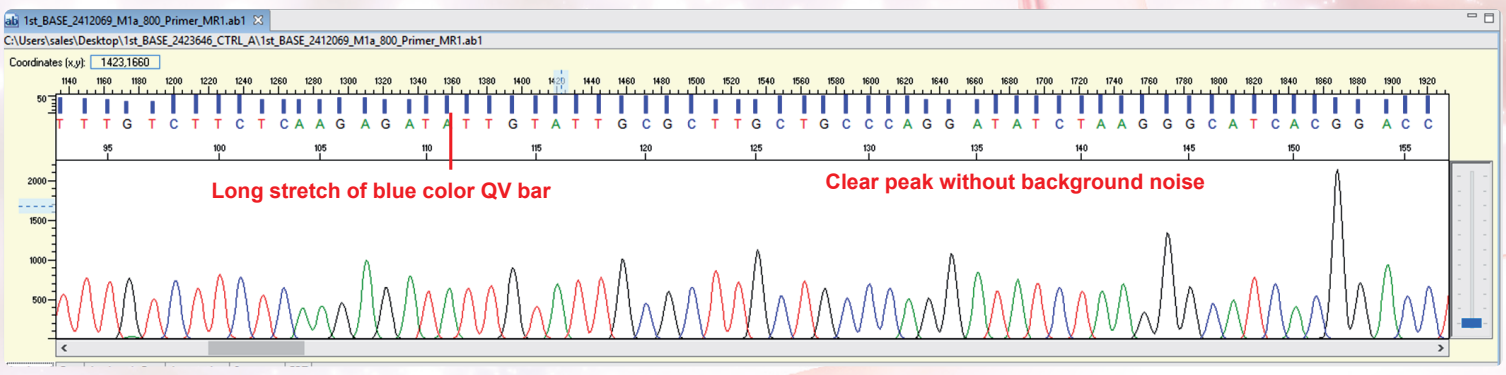
Color represent Quality value

Find/Select > Copy > Paste



Good sequence trace should have 2 characteristics as below:

1. Continuous long stretch or un-interrupted good quality value (QV) of the basecall.
   The QV will be displayed as rectangle bars at the upper part of the electropherograme.
2. The peaks are well defined with almost no or very minimum of background signal.



You may follow the link below to download your Sequence Scanner for FREE:
http://www.base-asia.com/dna-sequencing-services%20/support/design-and-analysis-tools

You may find more information result interpretation in our website as below:
http://www.base-asia.com/dna-sequencing-services/support/technical-support

1st BASE
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www.base-asia.com

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