

Sample Preparation and Requirement

Required Information					DNA Formats Accepted*		Sample Types Accepted		
Type of Service	Sample Name	DNA Type	Sample Type	Primer Name	PCR	Plasmid	Water	HiDi-Formamide	Dried Pellet
<u>Standard</u>	✓	✓		✓	✓	✓	✓		
<u>Ready to run</u>	✓	✓	✓		✓	✓			✓
<u>Ready to run with dye removal</u>	✓	✓	✓		✓	✓	Big Dye 3.1 mixture		
<u>PCR clean up</u>	✓	✓		✓	✓				
<u>Primer walking</u>	✓	✓		✓	✓	✓	✓		

DNA Template Requirements		
Type of DNA	Concentration	Volume/Reaction
PCR products (less than 500bp)	10-20 ng/μl	10 μl
PCR products (500 to 1000bp)	20-30 ng/μl	10 μl
PCR products (over 1000bp)	30-50 ng/μl	10 μl
Plasmid (less than 4kb)	100-200 ng/μl	10 μl
Plasmid (over 4kb)	150-200 ng/μl	10 μl
Genomic DNA for 16s/ITS sequencing*	100-200 ng/μl	20 μl
Primer Walking DNA	150-200 ng/μl	Minimum 40μl

* Genomic DNA is accepted but additional charges for PCR run and PCR clean up applies.

Primer Requirements	
Design:	At least 18 bases long with a Tm > 45°C, G-C content 30-80% (preferred 50-55%)
Purity:	Desalted
Concentration:	10 μM
Volume:	At least 5 μl in distilled water

Packing your samples:

1. Please prepare sample in microcentrifuge tube (preferably in 1.5mL tubes).
2. Label clearly on the side and top of tube with a permanent marker.
3. Ensure that the lid is tightly closed and if necessary, seal the tubes with parafilm.
4. Place samples in Bio Basic Sample collection card and plastic bag and attach the completed DNA Sequencing order form.

For international customer:

5. We'd suggest International customers to prepare a larger volume of sample in case of sample loss due to evaporation during long hour transportation.
6. Protect the samples with bubble wrap, and ensure the lid is tightly closed and sealed with parafilm.
7. Please inform our team regarding the tracking order via admin@biobasic-asia.com and sg-seqencing@biobasic-asia.com .

Please take note of the following:

For plasmids: The success of your sequencing reaction depends on many factors, essentially on the purity and cleanliness of your DNA. We recommend Bio Basic kits for plasmid purification. No matter which prep you use, **do not resuspend in elution buffer**. The EDTA in elution buffer acts as a chelating agent capturing the Mg²⁺ needed as a co-factor for the polymerase in cycle sequencing reaction, which will inhibit your reaction and could cause it to fail completely. **We recommend resuspending your DNA in deionised water.**

For PCR products: The DNA should be purified using a reputable PCR purification method to remove excess primers and dNTP's after your PCR cycling. Please run your PCR products on agarose gel to ensure that there are no multiple products (clear single band would be optimal). **We recommend resuspending your DNA in deionised water.**

For Primers: Please send in at least 10µl of the 10µM primer for 3 sequencing reactions. If you are sending a large quantity of samples for sequencing, the amount of primer to be sent for sequencing should be around **3µl of 10 µM primer per reaction**. This is to prevent the delay of resequencing of samples and result delivery in case of failed sequencing in the first run.