

# SAMPLE SUBMISSION GUIDE

## Primer Walking

Sending samples according to the requirements below helps us to do our job better and provides you with accurate results!

### Sample Submission

- Use **1.5 ml safe-lock tubes** for your templates and primers
- **Do not tape or wrap tubes** with parafilm. Safe-lock tubes offer perfect sealing and evaporation protection
- **Label your template and primer tubes** with our Prepaid Barcode Labels or Free Barcode Labels
- Use a water resistant marker for any additional labelling of template and primer tubes
- Sending us a reference sequence speeds up project time and allows us to define multiple primers right from the start!

### Sample Preparation

Use the following concentrations and volumes below for your samples

Sample Type	Sample Concentration	Sample Volume
Plasmid DNA	Min 100 ng/μl	Min 15 μl
PCR Products	Min 10 ng/μl	Min 15 μl

Service Type	Sample Type	Total Amount
Single strand	Plasmid DNA	Min 1 μg/kb
Single strand	PCR Products	Min 100 μg/kb
Double strand	Plasmid DNA	Min 2 μg/kb
Double strand	PCR products	Min 200 μg/kb

Quantify your template concentration via agarose gel or a photometer to ensure accurate results.

## Sequencing Primers

Send your primers according to the guidelines below to ensure accurate results. View a complete list of our standard primers in our online shop Ecom under [Sequencing Primers](#).

### Optimum Primer Conditions

- Primers must not contain phosphorylation or fluorescent dyes
- The optimum primer length is between 16-25 bases
- The primer melting temperature (T<sub>m</sub>) should be 50 - 62°C
- The GC content of the primer should be 35-60%
- Ideally one G or C should be located at the 3' primer end
- The number of 3' Gs or Cs should not exceed 2 Gs or Cs
- If possible, avoid >3 identical bases in a row in the sequence

### Primer Concentration and Volume

- Exactly **10 pmol/μl** primer concentration is required per sequencing reaction
- Each primer must have a total **volume of 15 μl** (double distilled water or 5mM Tris-HCl); **5 μl of primer volume** is required for every additional sequencing reaction
- Concentration of primers with **wobble bases** must be calculated according to the following formula:  **$n^x \times \text{ConcPrimer}$**

**n** = number of bases within a wobble according to IUPC code, **X** = number of wobbles within the primer sequence. [e.g. 1 V (AGC) =  $3^1 \times 10 \text{ pmol}/\mu\text{l}$ ; 2 V (AGC) (AGC) =  $3^2 \times 10 \text{ pmol}/\mu\text{l}$ ]