

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/ μ l	Min 15 μ l
PCR Products	Min 10 ng/ μ l	Min 15 μ l

Service Type	Sample Type	Total Amount
Single strand	Plasmid DNA	Min 1 μ g/kb
Single strand	PCR Products	Min 100 μ g/kb
Double strand	Plasmid DNA	Min 2 μ g/kb
Double strand	PCR products	Min 200 μ g/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 (Tm) 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: **$n^X \times ConcPrimer$**

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) = $3^2 \times 10 \text{ pmol}/\mu\text{l}$]