

SAMPLE PREPARATION

How to prepare Samples for Custom Sequencing Services at Eurofins MWG Operon

For Value Read Service in Tubes or Plates

- The Value Read process is optimised to send either stab or glycerol cultures with the selected E.coli clone, the purified plasmid DNA or unpurified or purified PCR products
- Use 1,5 ml tubes (no additional sealing with parafilm) or 96well microtiter plates for your samples and primers
Please note that we require **one tube per sequencing reaction**
- For samples in tubes please dissolve the DNA either in doubled distilled water or in 5 mM Tris-HCl (pH 8,0 – 9,0) in a total volume of 15µl **without using parafilm**
- Plates may contain a mixture of plasmids and PCR products as long as no purification is needed
- For optimal results the concentration/amount should be normalised across the plate
- Primers may be arrayed in a separate primer plate according to the sample layout
- Plate position H12 should be kept free for internal quality control (optional)

Concentrations:

- **Purified plasmid DNA:**
50–100 ng/µl in a total volume of 15 µl
- **Purified PCR products:**
 - < 300 bp: 2 ng/µl in a total volume of 15 µl
 - 300–1000 bp: 5 ng/µl in a total volume of 15 µl
 - > 1000 bp: 10 ng/µl in a total volume of 15 µl
- The amount has to be doubled for **unpurified products**
- **Primer concentration:**
2 pmol/µl, minimum volume: 15 µl (corresponds to an amount of 30 pmol)

Premixed Samples in Tubes or Plates:

- **Purified plasmid DNA:**
50–100 ng/µl in a total volume of 15 µl incl. 15 pmol primer
- **Purified PCR products:**
 - < 300 bp: 2 ng/µl in a total of 15 µl incl. 15 pmol primer
 - 300–1000 bp: 5 ng/µl in a total of 15 µl incl. 15 pmol primer
 - > 1000 bp: 10 ng/µl in a total of 15 µl incl. 15 pmol primer

For Ready to Load Sequencing Service in Tubes or Plates

- Use 1,5 ml tubes or 96well microtiter plates
- Use ABI Big Dye Terminator Kit (v3.1)
- Big Dye volume per reaction should not exceed 1 µl
- Total reaction volume of 10 to 20 µl
- Shipment of samples on dry ice is strongly recommended

For Sequencing Service à la Carte

- It is possible to send either a stab or glycerol culture with the selected E.coli clone, the purified DNA, your unpurified or purified PCR product or genomic DNA
- Please use 1,5 ml tubes for your samples and primers
- We recommend to dissolve the DNA either in doubled distilled water or in 5 mM Tris-HCl (pH 8,0 – 9,0) without using Parafilm

Concentrations:

- **Purified plasmid DNA:**
50–100 ng/μl, minimum 1 μg per reaction;
Please send double amount for samples with hairpin- or secondary structures, high GC content or other difficult regions
- **BAC-, PAC-, Cosmid-, Fosmid-DNA:**
200–1000 ng/μl, minimum 4 μg per reaction
- **Purified PCR products:**
 - < 300 bp: 2 ng/μl in a minimum volume of 15 μl
 - 300-1000 bp: 5 ng/μl in a minimum volume of 15 μl
 - > 1000 bp: 10 ng/μl in a minimum volume of 15 μl
- The amount has to be doubled for **unpurified products**
- **Genomic DNA:**
5–50 ng/μl, minimum amount is 200 ng per reaction
- **Primer concentration:**
2 pmol/μl, minimum volume per reaction is 15 μl (corresponds to an amount of 30 pmol)

For Walking Services/GLP-Compliant Sequencing Service

- It is possible to send either a stab or glycerol culture with the selected E.coli clone, the purified plasmid DNA or your unpurified or purified PCR product
- Please use 1,5 ml tubes for your samples
- If available, please send a reference sequence in electronic form

• **Minimum Concentration:**

Plasmid DNA: 100 ng/μl
PCR products: 10 ng/μl

• **Minimum Total Amount:**

– Single Strand Sequencing

Plasmid DNA: 1 μg/1 kb insert
PCR products: 100 ng/1 kb PCR product

– Double Strand Sequencing

Plasmid DNA: 2 μg/1 kb insert
PCR products: 200 ng/1 kb PCR product

– 4fold Coverage Sequencing

Plasmid DNA: 4 μg/1 kb insert
PCR products: 600 ng/1 kb PCR product