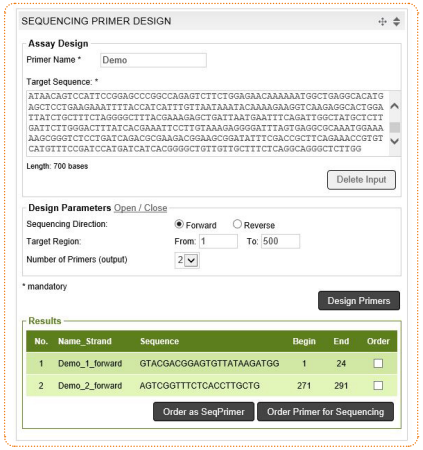
Sequencing Primers

OPTIMUM PRIMER CONDITIONS

- The optimum primer length is between 16-25 bases
- 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
- Primers must not contain phosphorylation or fluorescent dyes



Use our free **Sequencing Primer Design Tool** to design the optimum sequencing primers.

PRIMER CONCENTRATION & VOLUME

- Exactly **10 pmol/µl** primer concentration is required per sequencing reaction
- Each primer must have a **total volume of 20 µl** (double distilled water or 5 mM Tris-HCl)
- **2 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:

$$n^X \times Conc_{Primer}$$

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 x 10 pmol/µl; 2 V (AGC) (AGC) = 3^2 x 10 pmol/µl

Shipping Options & Online Ordering

SHIPPING OPTIONS

- **Eco-friendly paper boxes** for secure shipping of sample plates.
- **DropBox** submission with free pick-up service.
- **Sample Bag** (padded envelope) as an alternative.
- **Address stickers** for institutes/companies with Eurofins postal service available online free of charge.

ONLINE ORDERING

- Place your order via the **PlateSeq Supreme** page at eurofinsgenomics.eu.
- Enter sample and reaction conditions in step two; select the appropriate **PlateSeq Kits and Coupons** per plate.
- **Final step:** review and modify your samples if needed, then add them to your cart.

BOOST YOUR RESEARCH RESULTS

Maximize your research impact – choose PlateSeq Supreme today!

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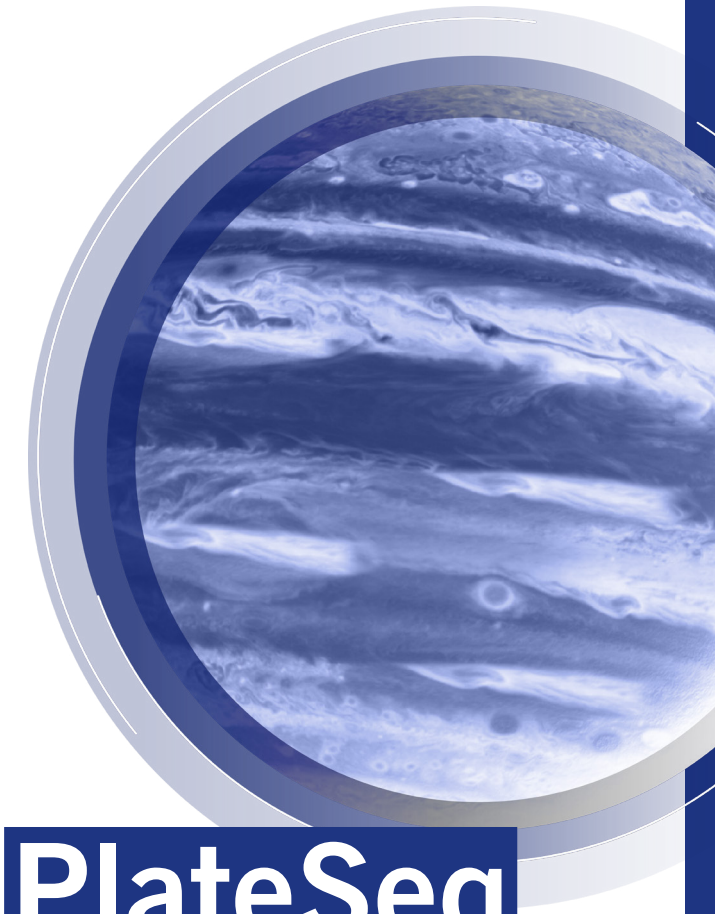

PlateSeq Supreme

Sample Submission & Ordering Guide.

2025_0903

Purified DNA & premixed samples

- Use either our blue **PlateSeq Kit DNA** or the PCR Plate from our sequencing accessories
- Plates may contain plasmid DNA, purified PCR products or premixed (DNA plus primer) samples
- Sample concentration must be **normalised** across the plate
- Well H12 should be kept free for internal quality control.
- **Seal your plates** using **8-cap strips** to prevent material loss
- If you are using your own plates, please use our **PlateSeq Labels** to label your plate on the plate frame
- Samples should be sent at **ambient temperature**
- Premixed samples should consist of **15 µl purified DNA** with either of the concentrations given in below table
- **Add 2 µl of primer** with a **concentration of 10 pmol/µl**
- The **total volume** of your premixed sample **must be 17 µl**

Sample concentration & volume

Sample type	Product length	Sample conc.	Sample vol.
Plasmid DNA	–	50-100 ng/µl	15 µl
Purified PCR Products	150-300 bp 300-1000 bp 1000-3000 bp	1 ng/µl 5 ng/µl 10 ng/µl	15 µl 15 µl 15 µl

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

Unpurified PCR products

- Use either our green **PlateSeq Kit PCR** or the PCR Plate from our sequencing accessories
- Concentration must be **normalised** across the plate
- **Quantify the concentration** via agarose gel or a photometer
- PCR **product size should not vary** by more than a factor of 3
- Well H12 should be kept free for internal quality control
- PCR products should be **sent liquid** in a total volume of 15 µl
- **Seal your plates** using **8-cap strips** to prevent material loss
- If you are using your own plates, please use our **PlateSeq Labels** to label your plate on the plate frame
- Ship samples at ambient temperature to us

Sample concentration & volume

Sample type	Product length	Sample conc.	Sample vol.
Unpurified PCR Products	150-300 bp 300-1000 bp 1000-3000 bp	4 ng/µl 10 ng/µl 20 ng/µl	15 µl 15 µl 15 µl

Plasmid clones as stab culture

- Use either our **PlateSeq Kit Clone** or Agar Plate from our sequencing accessories with appropriate antibiotic
- Use sterile toothpicks to **pick single colonies** from your petri dish and inoculate a single well with one colony
- Cover the plate with a lid and loosely wrap with cellophane
- Incubate plate at **37 °C for 8-12 hours** (overnight)
- If you are using your own plates, please use our **PlateSeq Labels** to label your plate on the plate frame
- Seal the plate with an **adhesive plastic foil**
- Ship your stab cultures at **ambient temperature** to us

PLASMID CLONES AS GLYCEROL CULTURE

- Use transparent 96well plates with 350 µl/well
- Fill each well with **200 µl of liquid medium**
- Include the appropriate antibiotic and add **40 µl glycerol** (final glycerol concentration: 10-20 %)
- Use sterile toothpicks to pick single colonies from your petri dish and inoculate a single well with one colony
- Alternatively transfer already arrayed clones from a storage glycerol plate to a freshly prepared 96well plate
- Cover the plate loosely and **incubate at 37 °C overnight**
- Verify that the **plate surface is dry** before you seal the plate tightly with an adhesive plastic foil
- Use our **PlateSeq Labels** to label your plate
- **Freeze the plate at -80 °C**
- Ship your glycerol cultures on **sufficient dry ice** to us