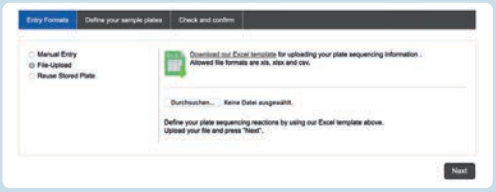
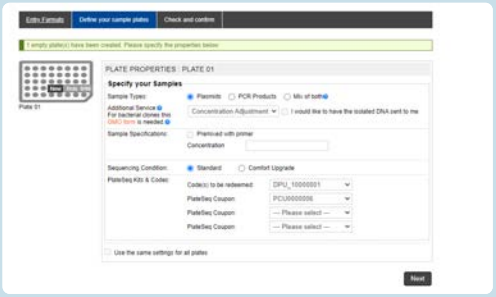
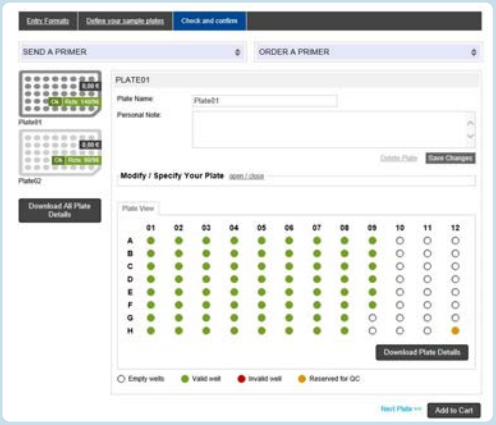
Online Ordering



Go to the **PlateSeq Ultimate** order page on **eurofinsgenomics.eu** and select the entry format.



Specify your samples and reaction conditions on the second step. Based on the sample type, the respective PlateSeq Ultimate Kit and Coupons can be selected per plate.



On the last step you have the possibility to modify and finally check your plates. Confirm your sample plates by adding them to your cart.

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

PRIMER CONCENTRATION & 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 5 mM Tris-HCl)*
- Concentration of **primers with wobble bases** must be calculated according to the following formula:
  $\mathbf{n^X \times Conc_{Primer}}$

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 Y (CT) (CT) = 2^2 x 10 pmol/µl; 1 N (AGCT) = 4^1 x 10 pmol/µl

*Sufficient for up to 200 reactions. 30 µl are needed for up to 350 reactions.
For more reactions, the primer must be entered several times and with a new name (e.g. indexed)

» USE OUR FREE SEQUENCING PRIMER DESIGN TOOL TO DESIGN THE OPTIMUM SEQUENCING PRIMERS

Additional Services

» Shipping options for sending samples to our sequencing lab in Ebersberg can be found here

**» Do you need boxes for safe shipment of your sample tubes?
Visit us here**

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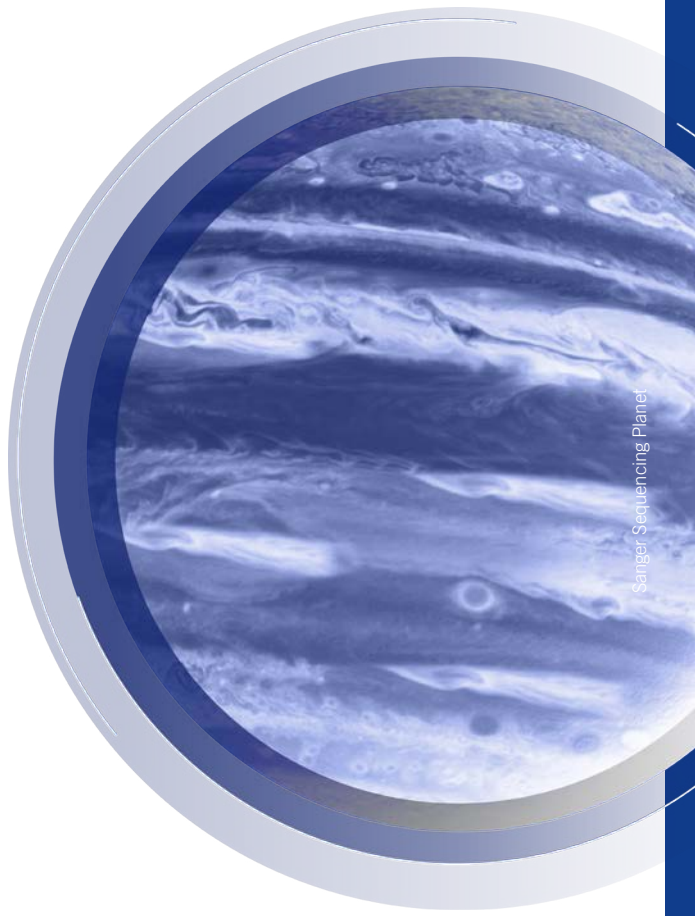

PlateSeq Ultimate

Sample Submission & Ordering Guide.

Purified DNA and premixed samples

PlateSeq Ultimate Kit DNA

PCR Plate

- Use our **PlateSeq Ultimate Kit** for purified DNA and premixed samples
- Alternatively you can use the 96well PCR plate from our accessories or your own 96well plates
- Plates with purified DNA may contain plasmids and PCR products
- Template size should not vary by more than a factor of 3
- Template concentration must be normalised across the plate*
- One well should be kept free for internal quality control
- DNA samples 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 label the plates with our PlateSeq Labels

PREMIXED SAMPLES (MIXTURE OF DNA AND PRIMER):

- Templates should consist of **15 µl purified DNA** with either of the concentrations given in above table
- Add **2 µl** of **primer** with a concentration of **10 pmol/µl**
- Ensure that the **total volume** of your premixed sample is **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

*As an alternative to the information in the table, the optional concentration adjustment allows the DNA concentration to be varied and adjusted across the plate. Nevertheless, the DNA concentration should not deviate by more than a factor of 3. The concentration is determined randomly to provide consistent sequencing conditions for the plate.

Plasmid clones

PlateSeq Ultimate Kit Clone

Agar Plate

PLASMID CLONES AS STAB CULTURE IN SOFT AGAR

- Use either our **PlateSeq Ultimate Kit** for plasmid clones or our agar plates 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 loosely with a lid and incubate at 37 °C for 8-12 hours (over night)
- If you are using your own plates, please label the plates with our PlateSeq Labels
- Seal the plate with an adhesive plastic foil and ship your stab cultures at ambient temperature to us

PLASMID CLONES AS FREEZE GLYCEROL CULTURES

- Only use transparent 96well microtiter plates with a total volume of 350 µl/well
- Fill each well with 200 µl of liquid medium (e.g. LB-medium) including the appropriate antibiotic and add 40 µl glycerol (final glycerol concentration: 10-20%)
- Ensure that liquid cultures are sent only in glycerol!
- Use sterile toothpicks to pick single colonies from your petri dish and inoculate a single well with one colony; or transfer already arrayed clones from a storage glycerol plate to a freshly prepared 96well plate using a multi-channel pipette
- Cover the plate loosely with a lid and incubate at 37°C for 8-12 hours (over night)
- Verify that the plate surface is dry before you manually seal the plate tightly with an adhesive plastic foil to prevent material loss
- Label the plates with our PlateSeq Labels
- Freeze the plate at -80 °C
- Ship your glycerol cultures on sufficient dry ice to prevent sample decay

Unpurified PCR products

PlateSeq Ultimate Kit PCR

PCR Plate

- Use our **PlateSeq Ultimate Kit** for unpurified PCR products
- Alternatively you can use the 96well PCR plate from our accessories or your own 96well plates
- The DNA concentration should not vary by more than a factor of 3; The values in the following table can serve as a guide. The concentration is then randomly measured after cleaning to determine consistent sequencing conditions for the plate
- PCR product size should not vary by more than a factor of 3
- PCR products should be **sent liquid** in a total volume of **15 µl**
- One well 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 label the plates with our PlateSeq Labels

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

SHIP YOUR SAMPLES AT AMBIENT TEMPERATURE TO OUR SEQUENCING LAB IN EBERSBERG

EUROFINS GENOMICS
Anzinger Str. 7a / D-85560 Ebersberg, Germany