Last updated: 29-Dec-11

Sample Requirements for "*Cycle, Clean & Run*"

1. DNA Template

The amount of DNA template is dependent on template length:

100-200 bp	5-20 ng
200-500 bp	10-40 ng
500-1000 bp	20-50 ng
1000-2000 bp	40-100 ng
> 2000 bp	50-150 ng
plasmids	150-300 ng
cosmids or BACs	300-1000 ng

2. Sequencing Primer

The amount of primer should be between 2-10 pmol. The optimal amount depends on the specific primer sequence and is not always predictable. **3.2 pmol primer** is a good starting point and should work in the majority of cases. The primer should be 18-25 bases long with a T_m of 52-60°C.

3. Template/Primer MIx

The template/primer mix should be provided in 10 mM Tris/Cl, pH 8.5 (**no EDTA**, since it interferes with the sequencing reaction) in a total volume of **7 µl**. Please use 200 µl PCR tubes (or 8-strips/plates) if possible.

4. Submission of samples

Sign up your samples for "*Cycle, Clean & Run*" with either BigDye v1.1 or v3.1 on the sequencing homepage (http://www.genetik.biologie.uni-muenchen.de/sequencing). **Clearly labeled** samples that are deposited in the fridge in room G03.031 (Sequencing Service im LMU Biozentrum, Großhaderner Str. 2-4, 82152 Martinsried) by 10:00 AM will be processed on the same day. Usually, sequence data can be downloaded from the sequencing homepage on the following working day.

5. Troubleshooting

The majority of sequencing problems are due to either incorrect template or primer concentration or contaminants in the template. Contaminants known to interfere with the sequencing reaction are: salts (NaCl, NaAc, KAc, KCl), chelators (EDTA, EGTA), proteins, detergents (SDS, Triton X-100), RNA, chromosomal DNA, organic chemicals (ethanol, chloroform, phenol), divalent cations (Mg^{2+}, Ca^{2+}, Mn^{2+}), and excess PCR primers, dNTPs, enzyme, and buffer components from PCR. Be sure to clean your DNA template and check the quantity and quality before submitting for sequencing.

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