Last updated: 29-Dec-11

Short BigDye Sequencing Protocol for ABI 3730 (for *Clean & Run*)

1. Setting up the Sequencing reaction

Choose the lowest amount of BigDye Sequencing Mix that still provides satisfactory results ($^1/_8$x reactions are a good starting point):

	$^1/_1$x	$^1/_2$x	$^1/_4$x	**$^1/_8$x**	$^1/_{16}$x	
BigDye Terminator Sequencing Mix (2.5x)* (v1.1 or v3.1)	4.00	2.00	1.00	**0.50**	0.25	µl
5x Sequencing Buffer* (to compensate for lower BigDye conc.)	0.00	1.00	1.50	**1.75**	1.88	µl
DNA Template (in ddH_2O or buffer without EDTA)	----------		**5-1000**	----------		**ng**
Sequencing Primer (**3.2 pmol** is a good starting point)	----------		**2-10**	----------		**pmol**
ddH_2O	----------		**ad 10**	----------		**µl**

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. Cycling conditions

96°C/1min – [96°C/10sec – 50°C/15sec – 60°C/4min] x 35 – 4°C/∞

3. Preparation and submission of samples

Sign up your samples for "*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 within the same day.

4. 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.

* Big Dye Terminator v1.1 or v3.1 Sequencing Mix, as well as 5x Sequencing Buffer can be obtained from the freezer in room G03.031. Please sign on the sign-up sheet for the aliquots you take.

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