Recommendations for the 3730xl DNA Analyser (96 capillaries)

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Big Dye Terminator Reaction Mix **version 1.1 or 3.1**

NB A HOT START IS PERFORMED FOR ALL CYCLE SEQUENCING REACTIONS.

For PCR products we now use a 1/8 reaction (1µl Big Dye Terminator Reaction Mix)

1-2 ng DNA per 100 bps of product
1 pMol of Primer
2 µl Big Dye Dilution Buffer (2.5x)
dH_2O up to 8 µl

Add the following after a 5 minute pre denaturation
1 µl Big Dye Terminator Mix
1 µl Buffer 2.5x

For Plasmids we now use a 1/16 reaction (0.5 µl Big Dye Terminator Reaction Mix)

100 ng Plasmid DNA
0.8 pMol Primer
2.0 µl Big Dye Dilution Buffer (2.5x)
dH_2O up to 8 µl

Add the following after a 5 minute pre denaturation
0.5 µl Big Dye Terminator Mix
1.5 µl Buffer 2.5x

NB To ensure accurate pipetting of Big Dye, prepare a master mix of Big Dye / Buffer Master Mix (eg 1 µl Big Dye + 1 µl 2.5x Buffer OR 0.5 µl Big Dye + 1.5 µl 2.5x Buffer). Prepare enough master mix for the number of samples to be sequenced.

Cycling Parameters for Applied Biosystems/Perkin-Elmer Thermal Cyclers

In the Sequencing Facility we always do a Hot Start WITHOUT the Big Dye Terminator Reaction Mix for 5 minutes at 96˚C.

The samples are then transferred to ice and the Big Dye added and mixed by pipetting up and down 2 or 3 times.

The samples are returned to the Thermal Cycler for the usual 25 cycles

96˚C	10 sec
50˚C	5 sec
60˚C	4 min

Cool to 4˚C

Ethanol Precipitation of Sequencing Reactions.

The following volumes are for a 10 µl sequencing reaction:

47.5 µl	Ethanol (99.7 – 100 %)
1 µl	3 M Sodium Acetate pH 5.2
11.5 µl	ddH_2O
60.0 µl	Total

Prepare a master mix of the Sequencing Precipitation Mix fresh each time for the required number of samples. 80 % Ethanol can be prepared and stored.

Precipitation protocol in 1.5 µl microcentrifuge tubes

1. Add 60 µl of precipitation mix to a 1.5 ml microcentrifuge tube.
2. Transfer sequencing reaction to tube.
3. Mix and place on ice for 15 minutes.
4. Spin at 12,500 rpm for 20 minutes at 15˚C.
5. Carefully remove supernatant and discard.
6. Add 60 µl of 80 % Ethanol.
7. Spin at 12,500 rpm for 10 minutes at 15˚C.
8. Carefully remove supernatant and discard.
9. Repeat wash steps (6 – 8).
10. Transfer tubes to a heating block.
11. Dry samples by heating in block at 90˚C for 2 minutes.

Precipitation protocol in 96-well plates

1. Add 60 µl of precipitation mix to each well.
2. Place on ice for 15 minutes.
3. Spin at 3940 rpm (2499 g) for 30 minutes at 15˚C.
4. Invert plate over sink and discard liquid.
5. Blot onto tissue.
6. Place plate onto fresh tissue and transfer inverted plate to centrifuge.
7. Spin inverted plated at 2085 rpm (700 g) for 1 minute.
8. Add 60 µl of 80 % Ethanol.
9. Spin at 3940 rpm (2499 g) for 10 minutes at 15˚C.
10. Discard liquid as before (Steps 4 – 7).
11. Repeat wash (steps 8 – 9).
12. Discard liquid as before (Steps 4 – 7).
13. Transfer plate to a thermal cycler.
14. Dry samples by heating in cycler at 90˚C for 2 minutes.