

Sequencing Reaction Set-up

Purpose: To set-up the 10 μL sequencing reaction in preparation to use the Applied Biosystems 3730 DNA Analyzer to sequence plasmid DNA and PCR products.

Materials:

- 8-strip PCR tubes (ISC Bioexpress, cat # T-3113-1)
- 30 μL Matrix pipette tips (Matrix, cat # 7631)
- 250 μL Matrix pipette tips (Matrix, cat # 7151)
- Matrix 12.5 μL pipettor (Matrix, cat # 2003)
- Matrix 125 μL pipettor (Matrix, cat # 2001)
- Autoclaved MilliQ water
- Big Dye Terminator 5x Sequencing Buffer (Applied Biosystems, Part # 4336699)
- Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Part # 4336921)
- DNA Template (provided by customer; PCR products should be brought in at 3.0 ng/ μL /100 bp; Plasmid DNA should be brought in at 0.2 $\mu\text{g}/\mu\text{L}$.)
- Primers (obtained from Oligonucleotide Facility, <http://www.daf.jhmi.edu/synthesis.index.html>)
- pGEM standard solution (see Preparation of pGEM standard solution protocol)
- MJ Research Tetrad PCR Machine (MJ Research, cat # PTC-0225)

Procedure:

- 1- Place 8-strip PCR tubes in a 96-well base. This is the reaction plate that will later be placed in the Thermal Cycler.
- 2- Distribute 8 μL of master mix solution to each well of the reaction plate (see Preparation of Master Mix Solution protocol) using a multi-channel pipette. One way to do this is to draw 250 μL of master mix into an 8-channel pipette using 250 μL tips and dispensing 8 μL into each of the twelve rows.
- 3- Vortex and spin down all template tubes. Dispense 2 μL of template from the 96-well flat to each corresponding well in the 96-well base using a multi-channel pipette. Do this by drawing 2 μL of template into a 12.5 μL multi-channel pipette and dispensing 2 μL into the corresponding reaction plate of 8-strip PCR tubes.
- 4- Add 2 μL pGEM standard solution (see preparation of pGEM standard solution protocol) into well H12.
- 5- Cover plate with the septa seal and spin in the centrifuge for 10 seconds at 200 RCF.
- 6- Place lid onto 96-well flat containing the individual tubes from each investigator. Label this flat, and place in -20°C freezer. You may dispose of these plates after one week, after having ensured that the data is accurate and that no re-runs of the samples are necessary.
- 7- Place Reaction plate into Thermal Cycler using the DAF-SEQ program. See Table #1 for cycling program.

Table #1: Thermal Cycle Conditions for a 10 μ L Sequencing Reaction

Stage #	Temperature	Time
1 (repeat for 24 cycles)	96 °C	10 seconds
	50 °C	5 seconds
	60 °C	4 minutes
2	4 °C	Hold

Contact:

Breon Beech
Sikha Singh
Joe Stolkovich

410-614-6548
410-614-6548
410-614-6548

bbeech@jhmi.edu
singhs@jhu.edu
jstolkov1@yahoo.com

For frequently asked questions go to the following address:

<http://www.daf.jhmi.edu>