

Take out box of reagents and allow to warm up to room temperature before using (~45minutes)

Gel-Dye mix is good for 1 month

Preparing Gel-Dye Mix: Add 25 $\mu$ L DNA dye concentrate (blue) to a DNA gel matrix vial (red)

Vortex well, transfer to spin filter tube

Spin at ~2240g (5000 rpm) for 15 minutes

Discard filter and label tube with initials and date made

Loading a Chip: Add 9 $\mu$ L Gel-Dye Mix into the well marked "G" with a dark circle

Place chip onto priming station

Make sure plunger is at 1mL

Close priming station, silver clip on base will click shut

Press down on plunger until syringe clip holds plunger in place

Wait 1 minute

Release syringe clip and wait for the syringe to rise up on its own

Slowly pull plunger back to 1mL position

Release silver clip on base of station

Remove the chip from the station

Add 9 $\mu$ L Gel-Dye Mix (blue) into the 2 wells labeled "G"

Add 1 $\mu$ L Ladder (yellow) into the well marked with a ladder symbol

Add 5 $\mu$ L Marker (green) into the ladder well

Add 5 $\mu$ L marker (green) in all wells that will contain sample

Add 6 $\mu$ L marker (green) in all wells that will not contain sample

Add 1 $\mu$ L sample to appropriate sample wells

Remove bubbles: Place chip onto vortexer (place back end in first)

Vortex for 1 minute at the indicated setting

**Note: Run chip within 5 minutes of preparing it**

Run chip: Open the lid of the Bioanalyzer

Place chip into slot

Close the lid of the Bioanalyzer

The chip should appear as an icon

Select/verify Assay→Electrophoresis→dsDNA→DNA 1000 Series II

Name your chip to include case # and item #

Choose the number of wells to be run

## Click Start\*

Name your samples in the sample information table to include case # and item #

Fill in chip and kit lot #'s

Information is automatically saved to the chip

\*Alternatively, naming your samples and filling in lot # information can be done prior to hitting Start

End of Run: When all wells have been analyzed, the machine will beep  
Remove your chip from the Bioanalyzer and place into biohazard bucket  
Fill the cleaning chip with 350 $\mu$ L of dH<sub>2</sub>O to clean the electrodes  
Place cleaning chip into the Bioanalyzer and close the lid for 10 seconds  
Lift the lid, remove the cleaner, and wait 10 seconds  
Close the lid

Analyzing Results: The concentration of NC's and RB's must be less than or equal to 10% of your sample to proceed to cycle sequencing. Refer to mtDNA SOP-1 when NC's and RB's are greater than 10%.

**Note: With Knowns processed in a batch using the EZ1 Advance XL and amplified in a set, The NC and RB of the set, must be less than or equal to 10% of the individual sample to proceed to cycle sequencing. For example, some samples in the set may qualify for cycle sequencing while others may not.**

Your Positive control and Sample must be greater than or equal to 1ng/ $\mu$ L to proceed to cycle sequencing

Printing out Data Sheets: Under 'Data View', go to File→Print  
You will be presented with options, click on/verify the following:  
Print Item Tab: Electropherograms, Results Table  
Wells Tab: Wells 1-8 (or the number of wells run)  
Options Tab: 4 per page  
Alternatively, quant data can be saved to a thumb drive.

**Note: After sequencing on the 3130- your NC's/RB's cannot be the same sequence as your sample.**