

*Approved by Director: Dr. Guy Vallaro*

Take out pre-aliquoted Formamide and allow to warm up to room temperature (250µL aliquots)

Check the Maintenance of the Instrument: Buffer and water are changed weekly or as necessary  
POP-6 must be changed monthly or as necessary  
(Refer to mtDNA SOP-08 for Maintenance Instructions)

Name a plate: Initials \_ Case # \_ Item # \_ Date of Sequencing

Re-suspend samples: Add 10µL of Formamide to the dried samples  
Either pipette up/down or vortex/spin the re-suspended tubes  
Transfer the samples to the individually labeled wells on the plate  
\*Two columns= One Run  
Make sure the run is complete by adding 10µL of Formamide to the wells that do not contain any sample  
Cover the wells with septa  
Place covered plate into centrifuge and spin briefly

Assemble plate: Assemble the sample plate into the black plate base  
Snap on white cover plate

Place assembled plate onto autosampler: Press TRAY button on outside of instrument  
When tray has completely moved forward, open doors and place assembled plate onto tray (notched end of plate base away from you)  
Make sure plate fits flat into the autosampler  
Under Plate View, the gray plate indicator will turn to yellow

Create Plate Record: Under Plate Manager, click 'New'  
Name the plate as above  
Select the sequencing application  
Select 96 well plate  
For owner, type in analysts initials  
For operator, type in 3130-5  
When done, click 'Finish'  
The Plate Editor Spreadsheet will open

Label Plate Editor Sheet: Name the samples: Sample \_ Case# \_ Item# \_ Primer \_ Date of Seq\*  
Under Results Group, click 'mtDNA' and fill down  
Under Instrument Protocol, click 'mtDNA\_seq' and fill down  
Under Analysis Protocol, click 'BC\_3130POP6\_BDv1.1' and fill down

*Approved by Director: Dr. Guy Vallaro*

When finished, click 'OK'

Your plate will appear under Plate View tab

Link your Plate: Under Plate View, select the plate name you want to link

Choose the plate position that you are linking and click on it

The yellow indicator box will change to green

(Two plates can be linked at a time)

Start the Run(s): Under Instrument Status, click on the 'Green Arrow'

The instrument will indicate 'you are about to begin processing a plate', click 'OK'

Each Run takes ~1 hr and 15 minutes

End of Run: When the run has finished, remove the plate from the tray and store in -20°C up to 2 weeks

Transfer the data from 3130 computer to desk computer for analysis

\*Naming Samples:

Ex2 2<sup>nd</sup> Extraction

Amp2 2<sup>nd</sup> Amplification

Seq2 2<sup>nd</sup> Cycle Sequencing

Inj2 2<sup>nd</sup> Injection

Place these additional events after the primer and before the date sequenced

Ex. NC\_Case#\_Item#\_A1\_Ex2\_Amp3\_Seq2\_010203