TROUBLE-SHOOTING YOUR ASSAY

1) Are you using only the **2 µl and 10 µl Pipettes?**

2) Did you remember to check for **air bubbles?**

3) Did you set up the **priming station** correctly**?**

- **a.** clean / unclog the **gasket** with a wet KimWipe (di-H_2O) and dry KimWipe.
- **b.** **Silver Clip** is in the correct position (Nano/Pico RNA top, HS DNA bottom)
- **c.** **Syringe securely attached** to plate, verified by attempting to give a ¼ turn.
- **d.** Syringe shows **immediate rebound** between 0.7 ml and 1 ml for Nano/Pico RNA; above 0.3 ml for DNA.

4) Are you using the correct **assay from the pull-down menu?**

5) Are your **reagents freshly prepared** and/or within the recommended using dates**?**

Filtered Gel: RNA (1 month),
Gel-dye Mix: RNA Nano (1 week), RNA Pico (24 hrs), DNA (4 to 6 weeks)

6) Did you **spin down for 10 mins Max Speed prior to** loading your Gel-Dye Mix**?**

7) Was there **sample splash** during vortexing? Check volumes under the light & adjust with a few µl's of 🟢 marker as needed.

8) Do all sample **wells have 5 µl volume + 1 µl** of sample or H_2O**?**

9) Do any well **volumes appear low?** If so, add "🟢 Marker" to make up the volume**.**

10) Is the electrode CLEAN & DRY? Refer to the sign-out sheet for previous assay.

a. Clean electrodes may be found in the sonicator bath or in the box marked "Clean Electrodes". Electrode & bayonet must be completely dry prior to use.

b. To dry, spray with 70% ethanol, pat dry with a KimWipe, and use N_2 gun on both sides (15s-30s per side, twice) to completely dry all crevices. If bayonet is wet, dry it, too. Turn off N_2 gun valve when you are done to avoid running tank dry.

c. An electrode may be re-used by rinsing between assays. You need 3 H_2O electrode cleaning chips to rinse the electrode. Fill each one with 350 µl of Nuclease-Free Water. Rinse 30s-60s per chip. Allow to air dry 30s-60s.

d. For DNA assay, please rinse as (c) between assays.

e. For RNA Nano assay, you may use the same clean electrode for multiple assays. Decontaminate with RNAse Zap, rinse with H_2O as (c), and allow to air dry per protocol.

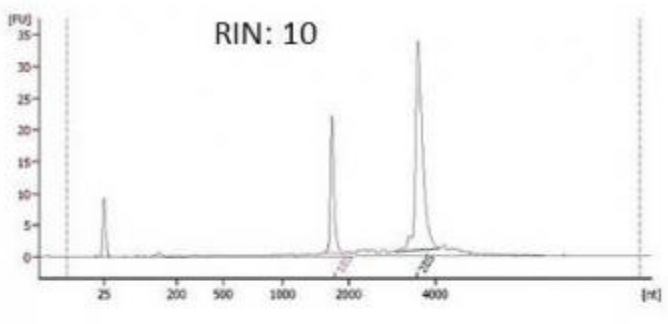
f. For RNA Pico assay, a clean electrode is recommended after 2 assays. Decontaminate with RNAse Zap,
g. rinse with H_2O as (c), and allow to air dry per protocol.

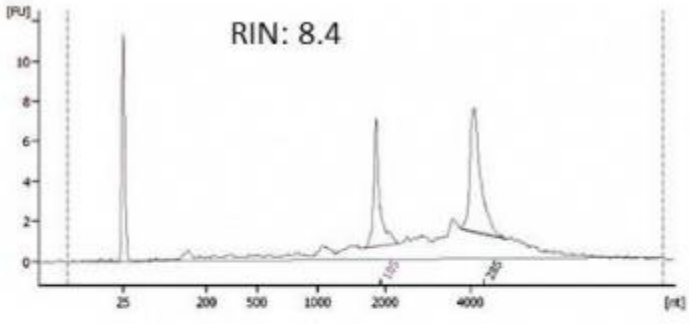
More troubleshooting help can be found here:
http://www.chem.agilent.com/Library/usermanuals/Public/G2946-90003_MandTguide.pdf

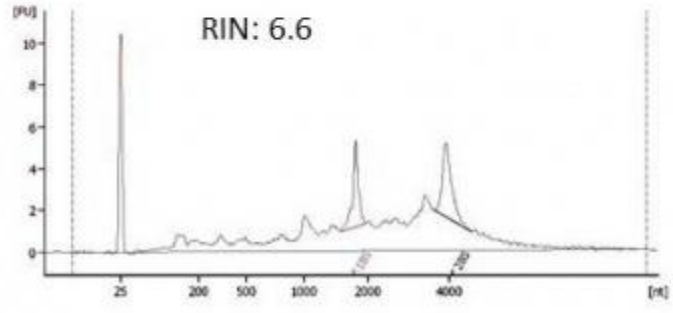
Interpreting RNA Bioanalyzer results

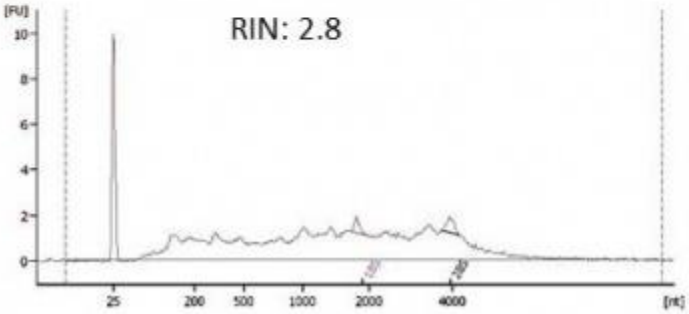
Initial QC

Submitted RNA samples are run on the Bioanalyzer to determine the RNA Integrity Score or RIN score. This number ranges from 2-10 where 10 is intact RNA and 2 is highly degraded RNA. RNA library protocols suggest starting with samples with a RIN score of 8 or above for best results.



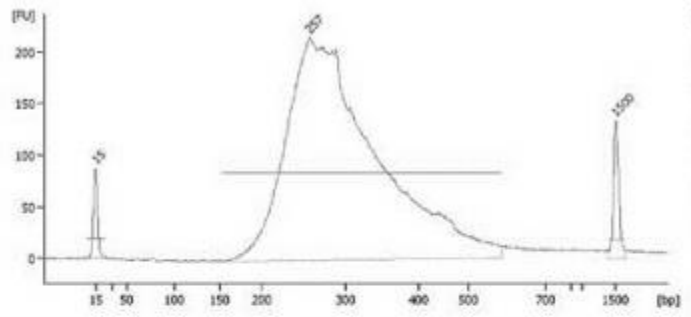




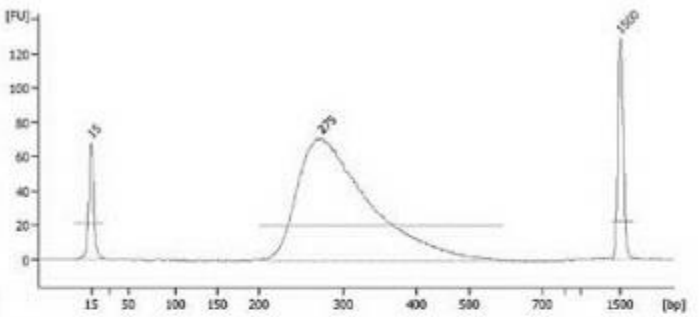


Final QC

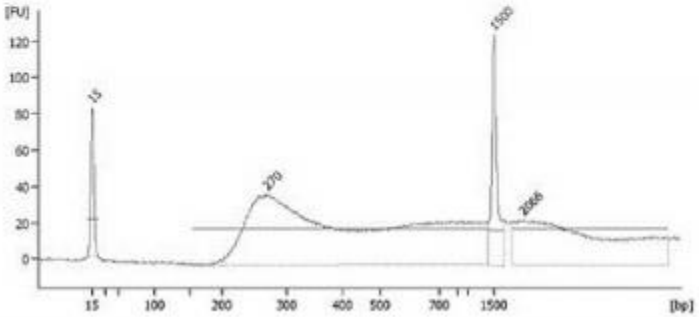
Completed mRNA seq libraries are run on the Bioanalyzer to visualize the size and purity of the library. Libraries should have a single peak between 200-300 bp.



NuGen mRNA library



TruSeq mRNA library

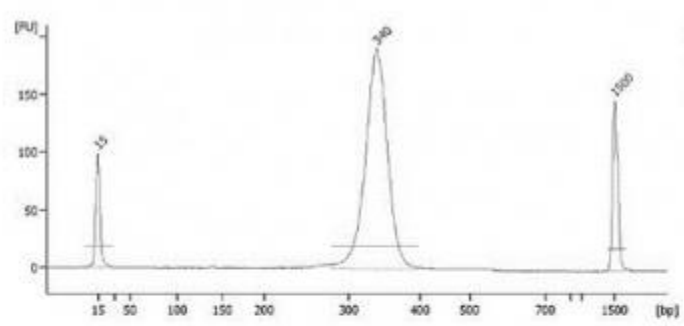


RNA library with Genomic DNA contamination

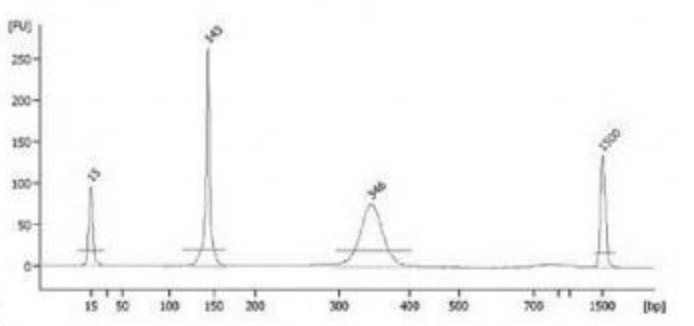
Interpreting DNA Bioanalyzer results

Completed QC

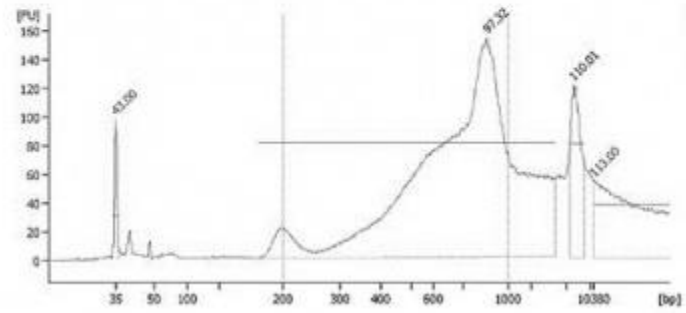
Completed DNA libraries are run on the Bioanalyzer to visualize the size and purity of the library. Libraries should have a single peak between 200-500 bp depending on the project specifications. A spike is sometimes seen in the Bioanalyzer trace. This can be due to dye blobs in the gel/dye mix or the instrument getting bumped during the run. It is not a real sample peak.



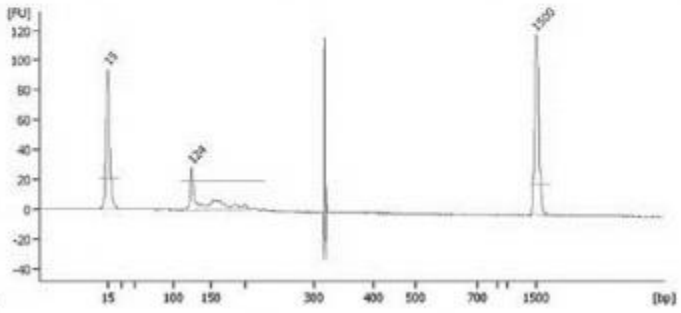
DNA library



DNA library with adapter diamer peak (143bp)



DNA library – insert size too large



Spike (320bp)

High Sensitivity DNA Ladder Well Results

To check the results of your run, select the Gel or Electropherogram tab in the **Data** context. The electropherogram of the ladder well window should resemble to those shown below.

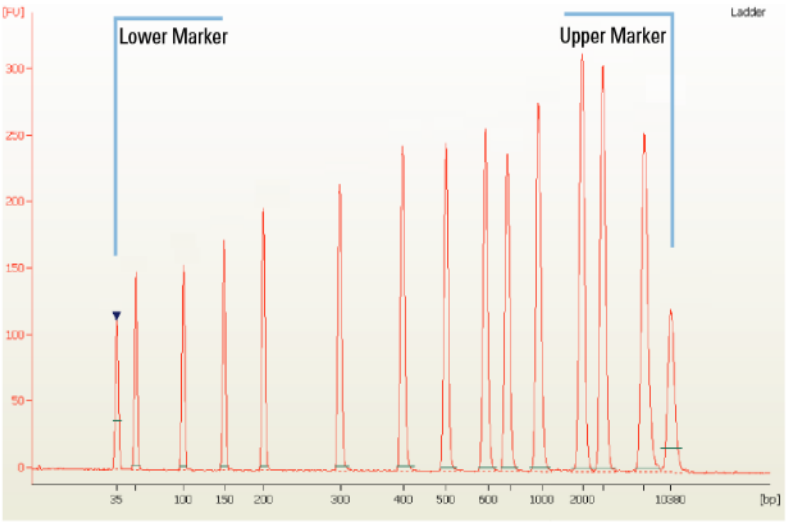
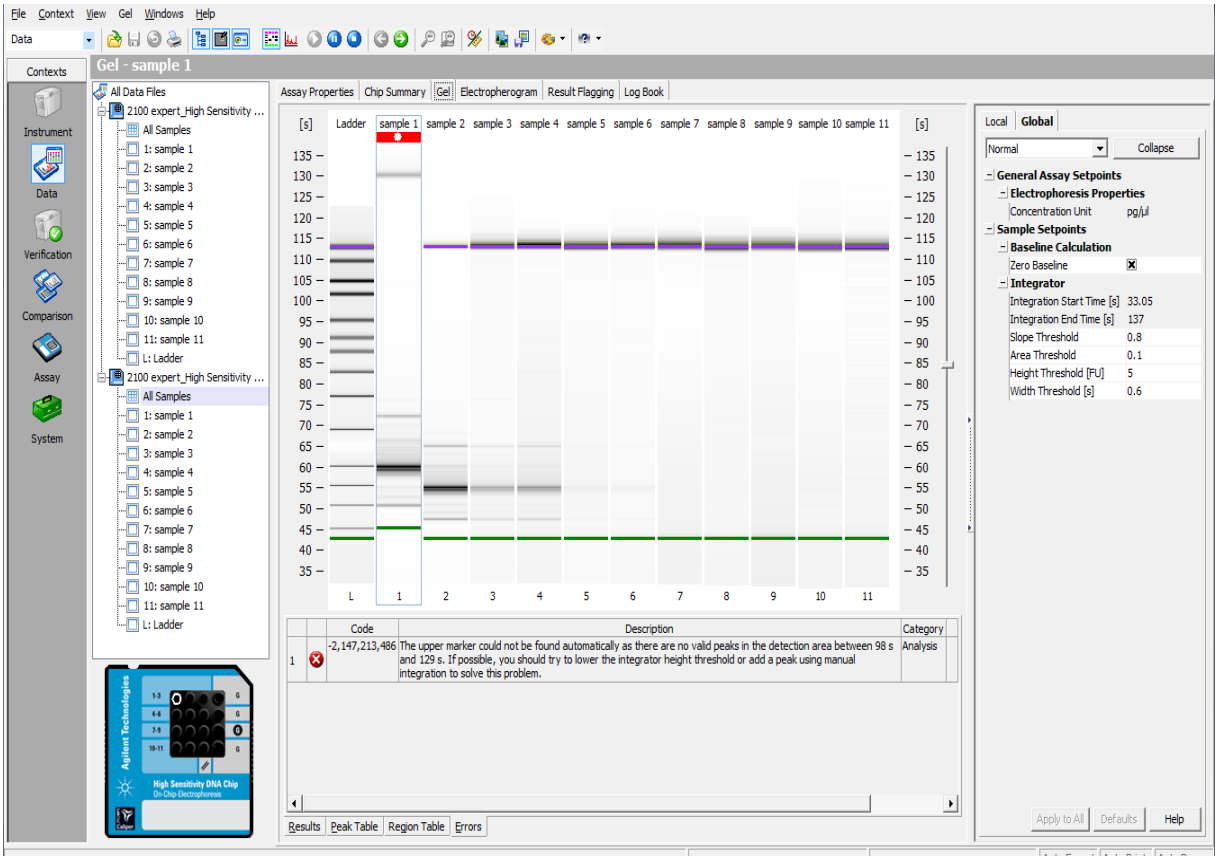
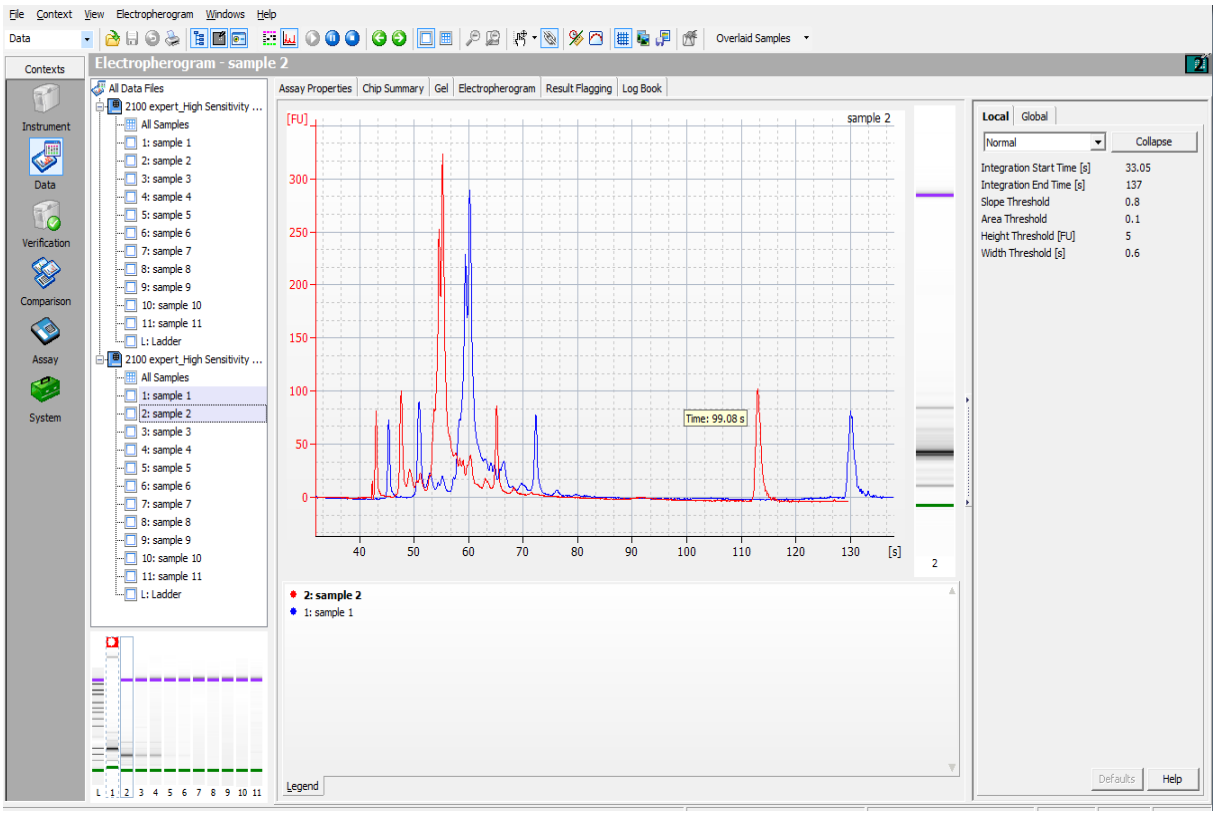


Figure 1 High Sensitivity DNA ladder

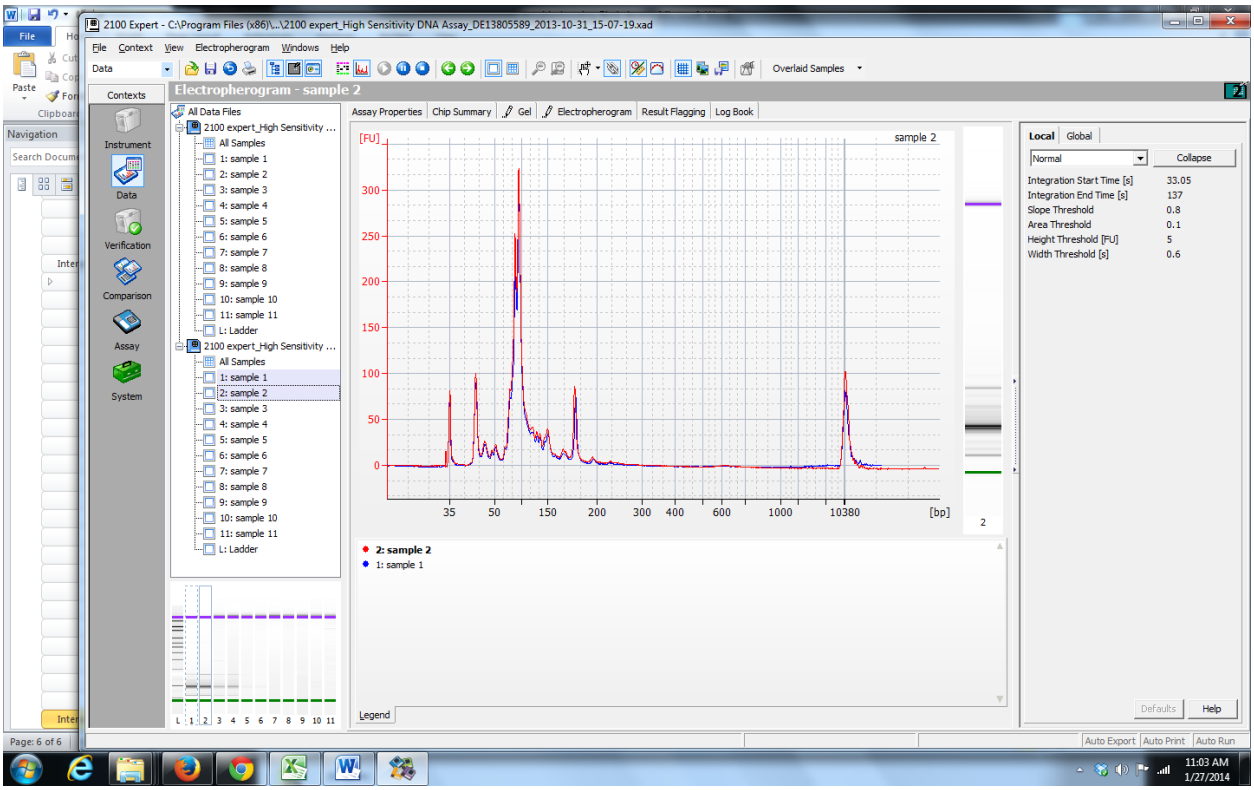
Examples of problem data:
Marker doesn't run uniformly. Notice the markers in lane 1 of the run do not match all the proceeding lanes. The lane appears to have similar peaks to sample 2 lane (see red & blue comparison peaks on electropherogram) and does not appear to have degradation.





If this occurs you can manually set the markers in lane 1 to coincide with the rest of the run (the BioA may suggest using the ‘Manual Integrator’ tool.

1. Using the tabs closest to the top of the sample data, click on Electropherogram. Make sure to click on the sample you need to adjust so that it is displayed.
2. Click on ‘peak table’ near the bottom of the sample display
3. Right click in the ‘Observations’ column in the marker lane you need to adjust (you need to know how big your marker lanes need to be). It will give you the option to ‘Manually Set Marker’.
4. Choose this option (based on which marker you need to adjust; i.e. lower/upper). Once both markers have been manually adjusted, you will see an adjustment in the gel/electropherogram.
5. Be sure to save your adjusted data.



If your data looks like this, you probably have the silver clip on syringe holder in the wrong place for your type of assay. See page (1) for proper clip positioning.