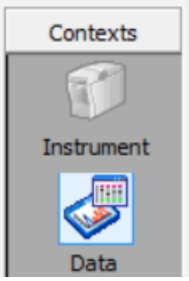
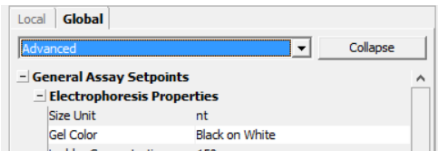
# Smear Analysis of FFPE Samples for NanoString

1. Run the FFPE sample RNA on the Agilent Bioanalyzer using the RNA Nano Chip or RNA Pico Chip
2. Open the Agilent 2100 Expert Software
3. Select the Data context on the left hand side of the program

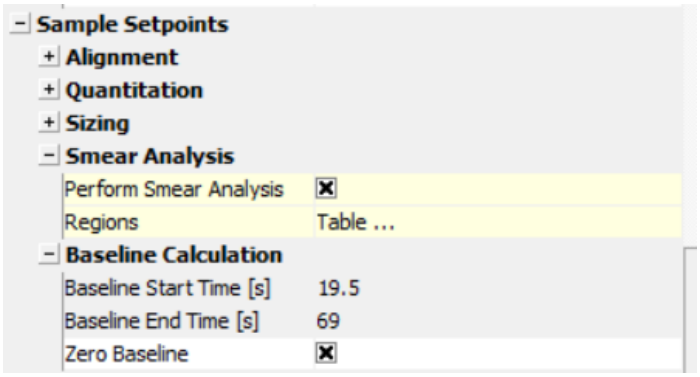



4. Open ".xad" file that contains your samples in Agilent 2100 Expert Software by going to File →Open… or select the  button.
5. Select Electropherogram → Show Sizes in the menu to change the x-axis from seconds (s) the nucleotides bases (nt)
6. Select the "Assay Properties" in the top of sample tab

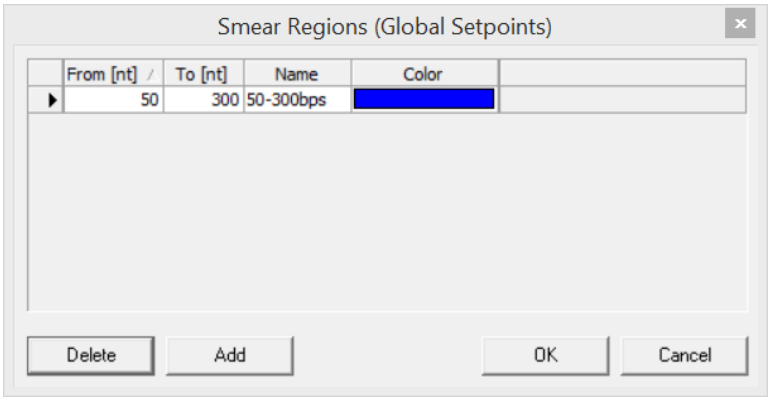

7. Select the Global tab and change the menu from Normal to Advances on the right hand of the window




8. Scroll down then reveal the **Smear Analysis** options by clicking the "+" on the **Sample Setpoints**.
9. Make sure the **Baseline** Calculation options are set as shown below




10. Select the region of interest by the small box with "…" to the right of "Regions Table…" options




11. Click Add and add a region starting at 50 to 300

12. Name the region and click OK
13. Select Apply to All in the bottom right of the option window
14. For each sample
    a. Select each sample in the lane on the left
    b. Select the Region Table tab below the electropherogram
    c. Copy the % of Total for the 50-300 nt region to an excel spreadsheet

## Calculating the input amount

The target input amount for degraded RNA depends on the fragments below 300 bp and the desired input amount of RNA fragments. The formula is below

$$Adjusted\ Input = \frac{Target\ Input}{100 - [\%between\ 50 - 300nt]} * 100$$

Enter the percentage between 50-300 nt, sample concentration and target input into an Excel spreadsheet. The adjusted input can be calculated in Excel using the formulas below.