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 \rightarrow Open... or select the $\stackrel{\frown}{=}$ 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

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Assay Properties Chip Summary Gel Electropherogram Result Flagging Log Book
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7. Select the Global tab and change the menu from Normal to Advances on the right hand of the window

Local Global				
Advanced	-	Collapse		
- General Assay Set	points	^		
 Electrophoresis Properties 				
Size Unit	nt			
Gel Color	Black on White			

- 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

Sample Setpoints	
+ Alignment	
+ Quantitation	
+ Sizing	
 Smear Analysis 	
Perform Smear Analysis	×
Regions	Table
 Baseline Calculation 	
Baseline Start Time [s]	19.5
Baseline End Time [s]	69
Zero Baseline	×

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

	Smear Regions (Global Setpoints)						
	From [nt] /	To [nt] Name 300 50-300bps	Color				
options	Delete	Add	OK	Cancel			

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.

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	Α	В	С	D	E	F	G	H 🔺
1		% between 50-300nt	Concentration	Target Input (ng)	Adjusted Input (ng)	Adjusted Input Amount (ul) water to 5ul	L
2 Sa	mple 1	22.5	45.3	100	129.03	2.85	2.15	
3						Δ	h	
4								
5					=(D2/(100-B2))*100	=F2/C2	=5-F2	
6								