Analysis of 6x mRNA on BioAnalyzer

Email from Kyle (PAN, Stanford University):
Hi Do Soon,

Please find the requested data files attached to this email. Please remember that for an accurate analysis, the ladder sizing polynomial fit equation should be redone for every run.

Since 10-12-16-12 did not have a ladder, you can use the sizing distribution from 16-05 to estimate the sizes. Please also remember that 10-12-17-13 was diluted 1:1 since I did not have enough remaining sample for that run.

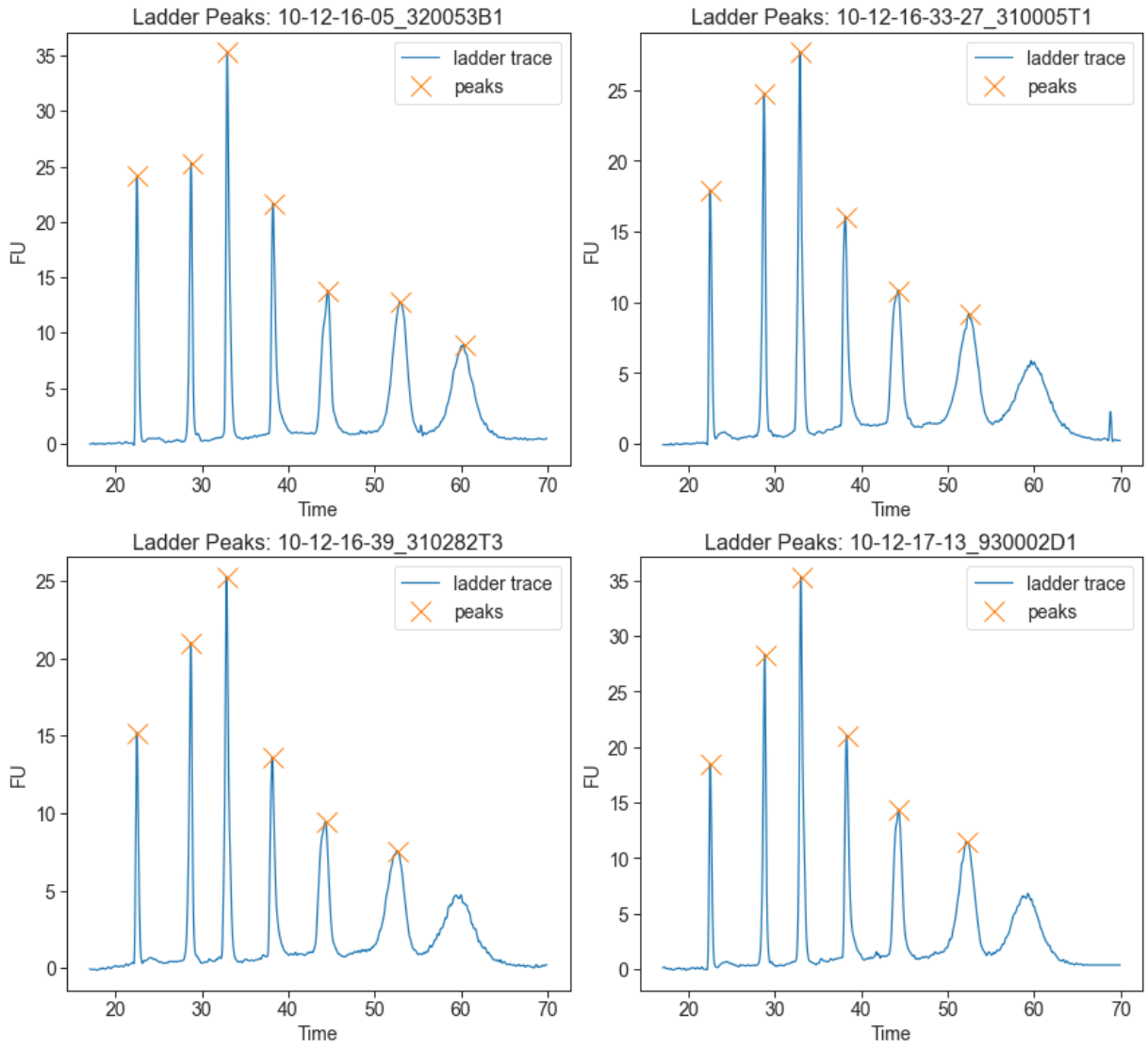
Please let me know if you have any questions.

Best,
Kyle

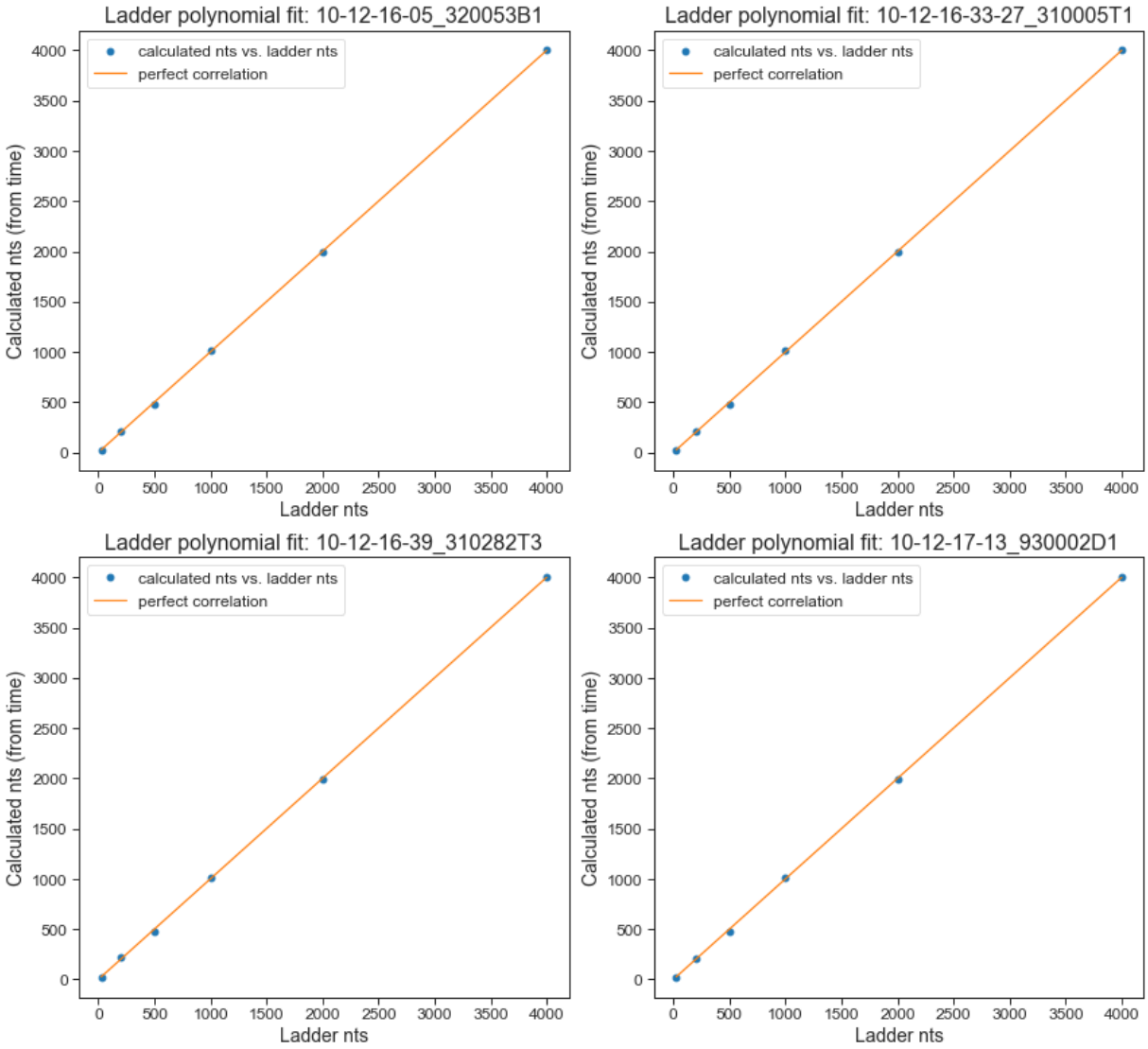
17-13-16: RNA4
16-39-16: RNA8
16-33-27: RNA7
16-05-51: RNA3

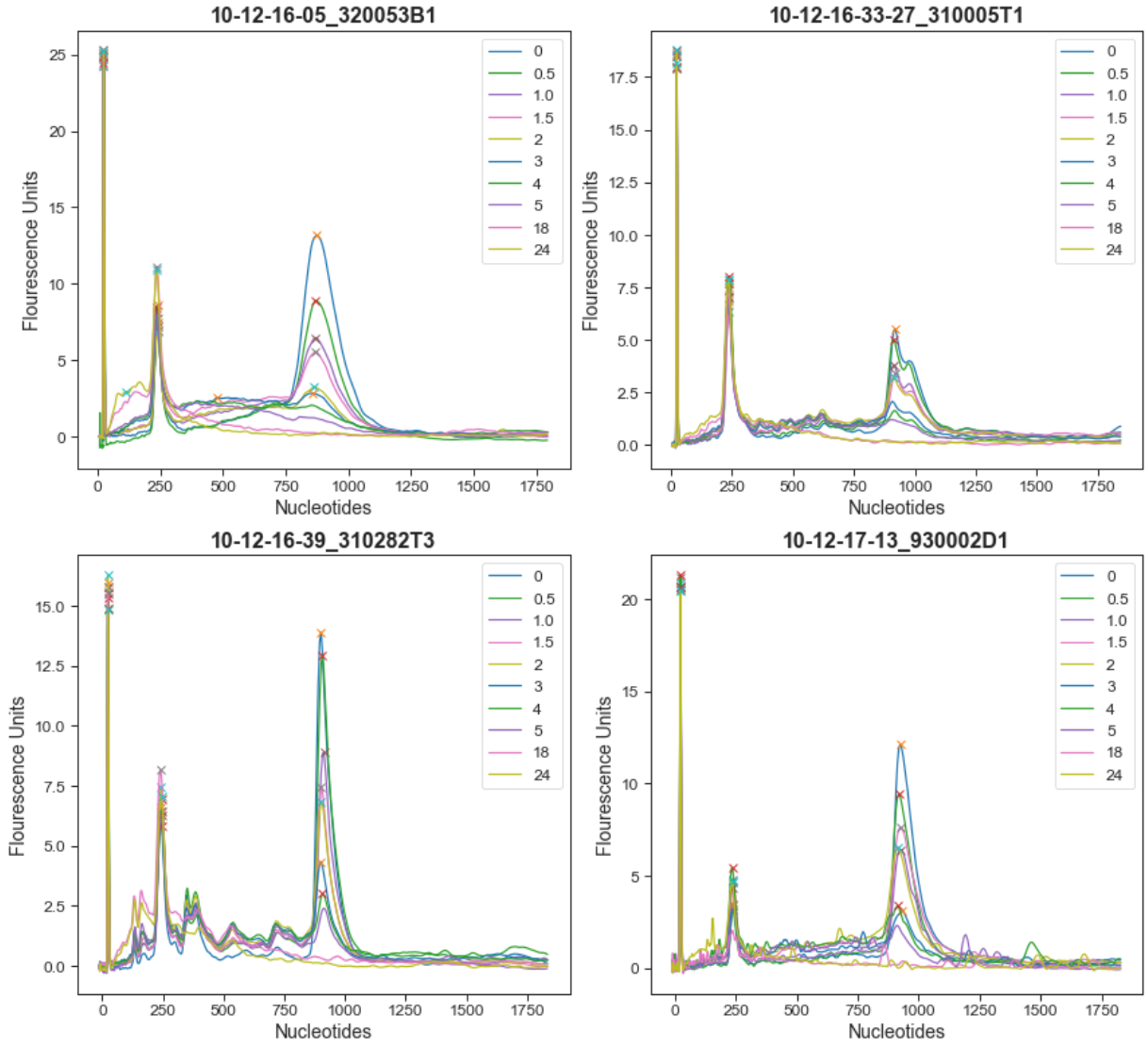
RNA1: 310002T1, Eterna_hHBB+3XHANluc+JEV+hHBB+F30Pepper_EiFisker_Yellowstone
RNA2: 310003T1, Eterna_hHBB+3XHANluc+JEV+hHBB+F30Pepper_jiabei618_LinearDesign-1
RNA3: 320053B1, hHBB_Nluc_hHBB
RNA4:930002D1, Eterna_hHBB+3XHANluc+JEV+hHBB+F30Pepper_GC-rich_2
RNA7: 310005T1, Eterna_hHBB+3XHANluc+JEV+hHBB+F30Pepper_John2000_LinearDesign-LocalDesign_w50 ()
RNA8: 310282T3, Eterna_hHBB+3XHANluc+JEV+hHBB+F30Pepper_-423.7 kCal

First fitting ladders to nucleotides:

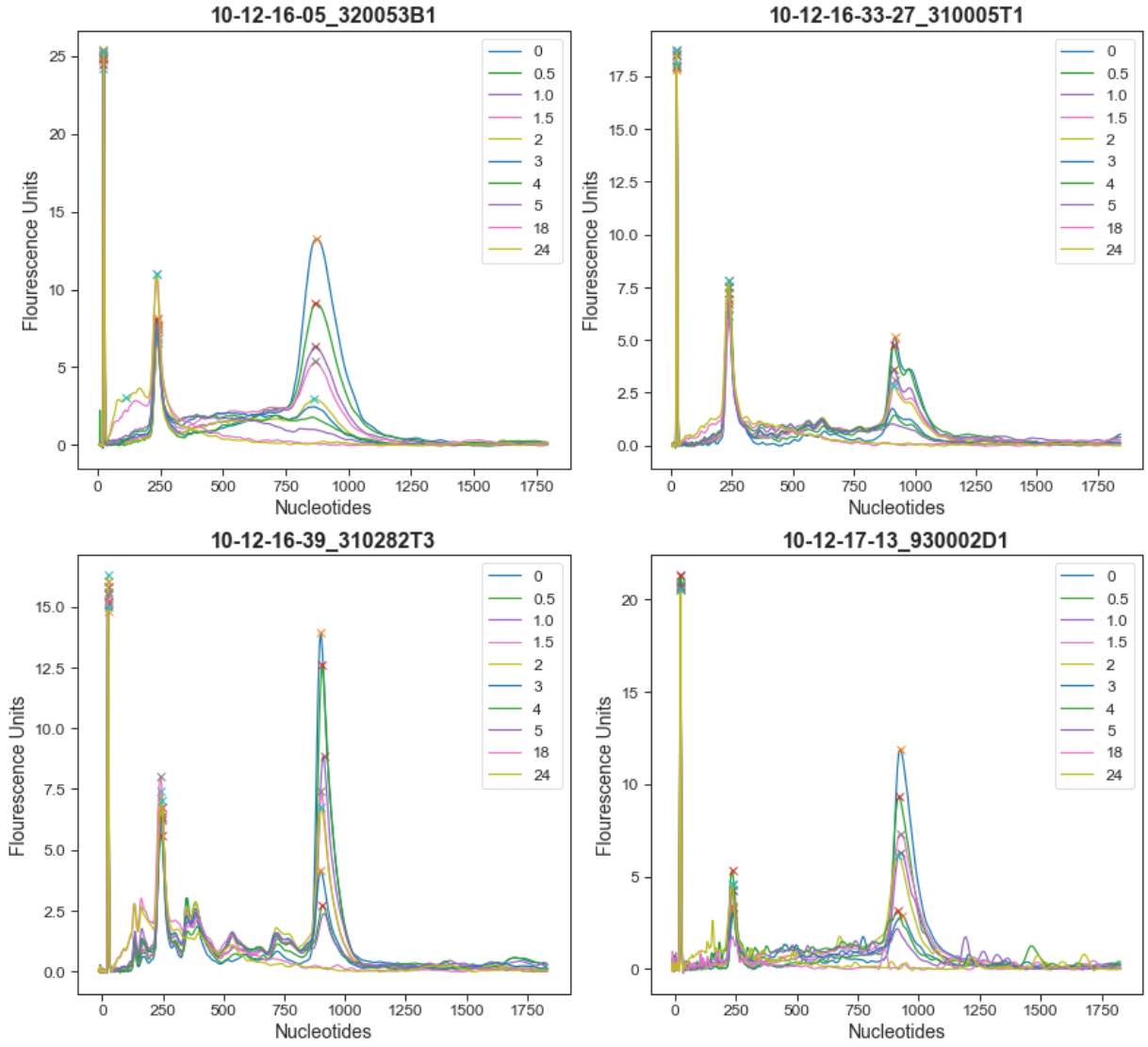


And fitting polynomials to convert to nucleotides:


Ladder polynomial fit: 10-12-16-05_320053B1
calculated nts vs. ladder nts
perfect correlation
Calculated nts (from time)
Ladder nts
Ladder polynomial fit: 10-12-16-33-27_310005T1
calculated nts vs. ladder nts
perfect correlation
Calculated nts (from time)
Ladder nts
Ladder polynomial fit: 10-12-16-39_310282T3
calculated nts vs. ladder nts
perfect correlation
Calculated nts (from time)
Ladder nts
Ladder polynomial fit: 10-12-17-13_930002D1
calculated nts vs. ladder nts
perfect correlation
Calculated nts (from time)
Ladder nts

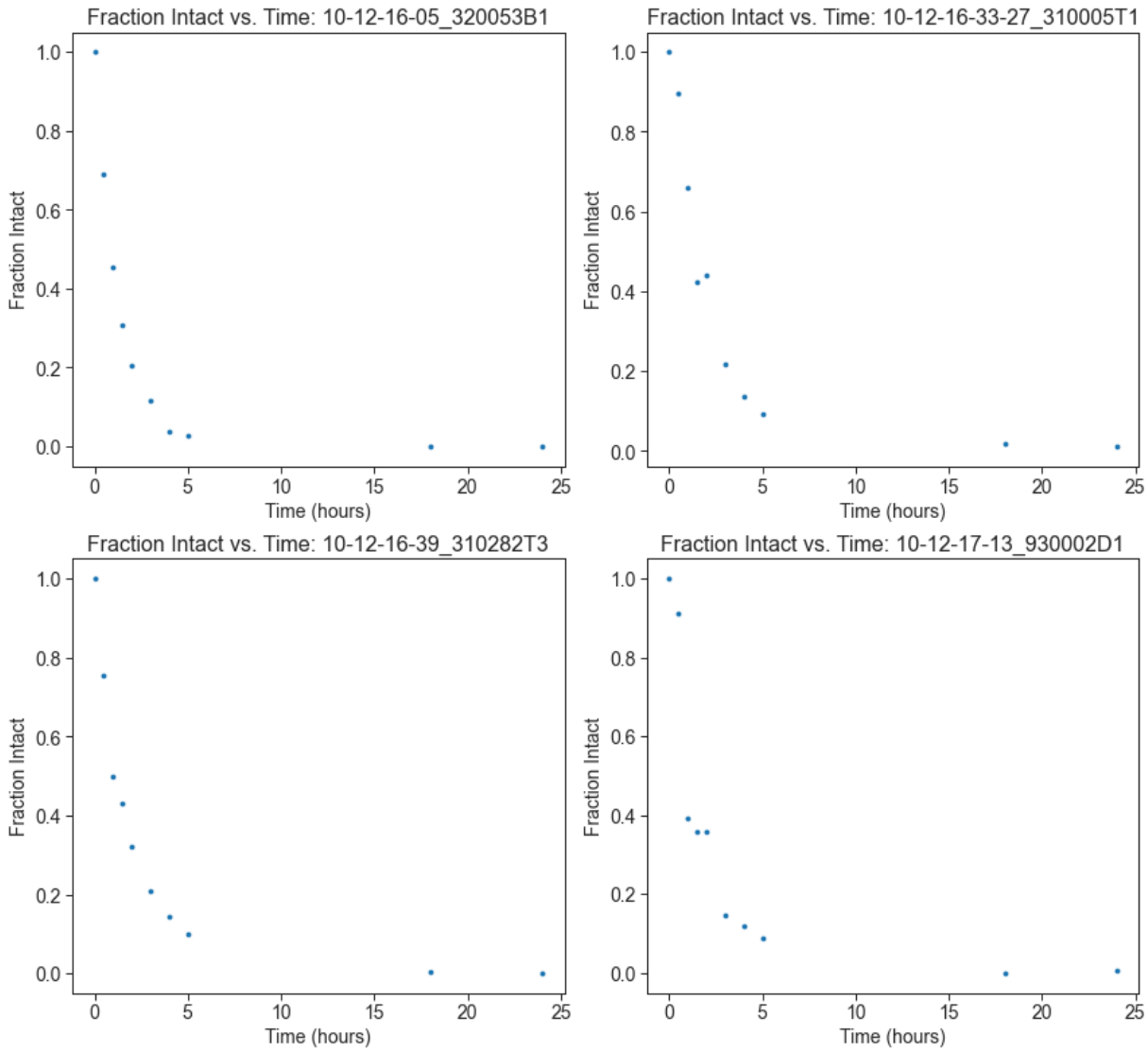


Looks messy. Need to apply baseline subtraction.

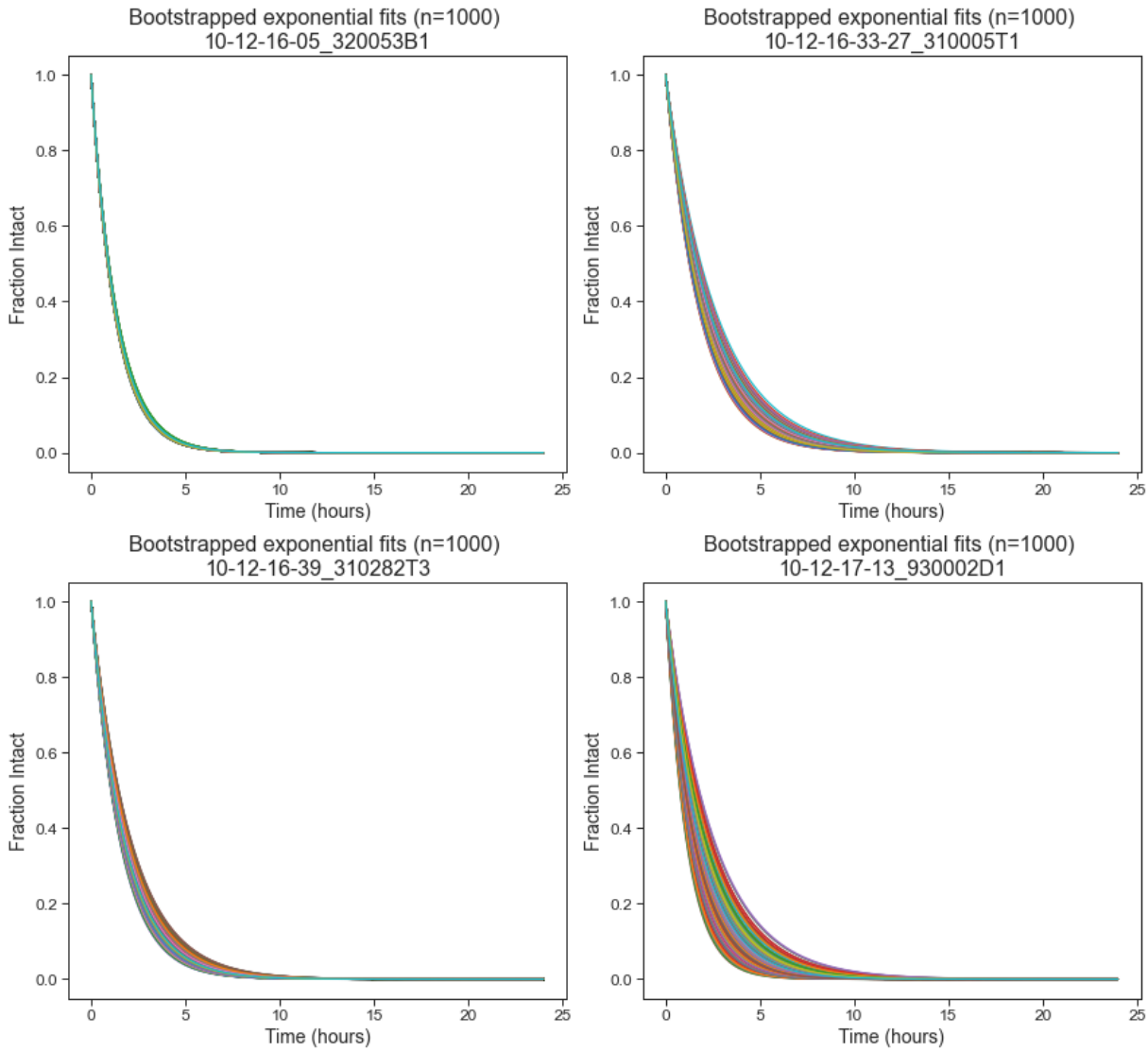


Better, although I see much room for improvement with the little peaks coming up. Much dirtier than previous samples (RNA1-Yellowstone & RNA2-LinearDesign). Especially in 930002D1 and 310282T3. I think this means the RNA samples are not as clean, and likely not an artifact of degradation (see contaminating peaks at higher nts).

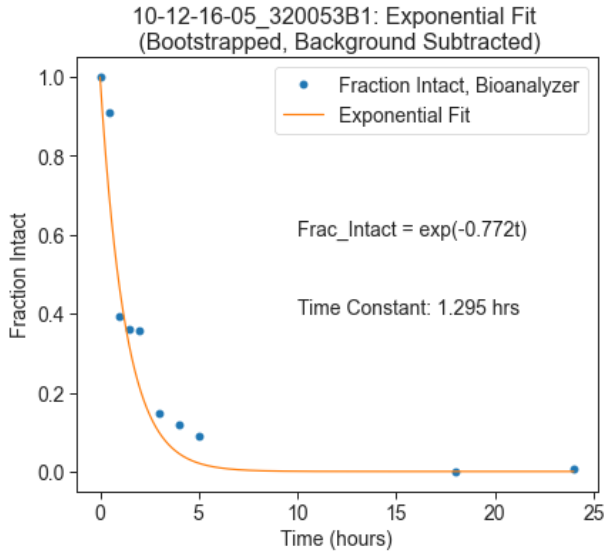
One thing to watch out for: notice how 320053B1 is shifted lower in nts (this is expected given the shorter RNA), so make sure to adjust nucleotides accordingly!

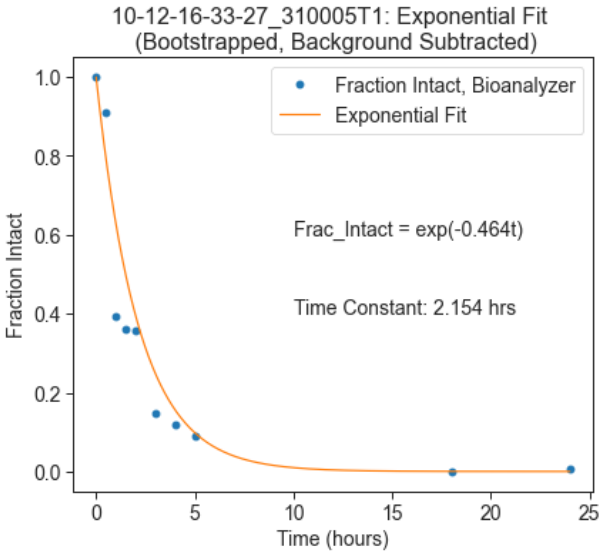


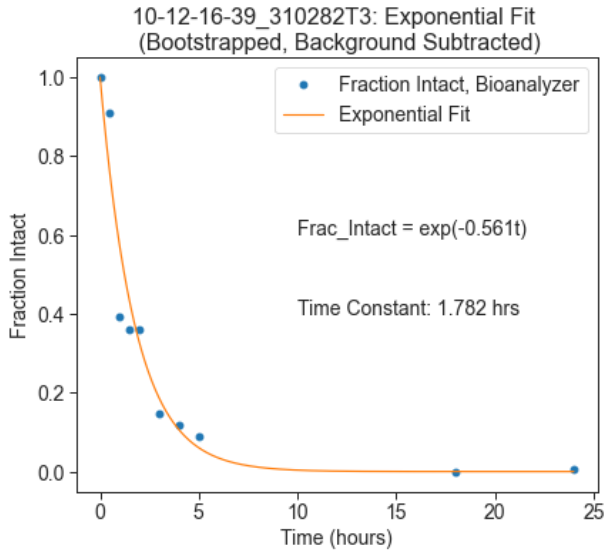
Looks pretty good!

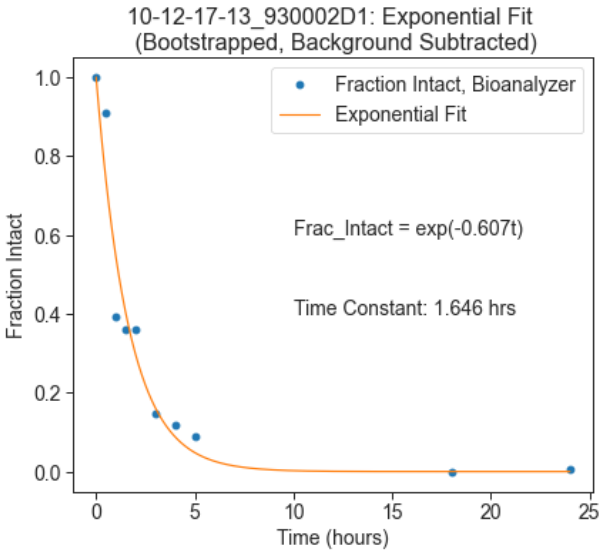


Now plotting the exponential decays:


10-12-16-05_320053B1: Exponential Fit
(Bootstrapped, Background Subtracted)
Fraction Intact, Bioanalyzer
Exponential Fit
Frac_Intact = exp(-0.772t)
Time Constant: 1.295 hrs
Fraction Intact
Time (hours)


10-12-16-33-27_310005T1: Exponential Fit
(Bootstrapped, Background Subtracted)
Fraction Intact, Bioanalyzer
Exponential Fit
Frac_Intact = exp(-0.464t)
Time Constant: 2.154 hrs
Fraction Intact
Time (hours)


10-12-16-39_310282T3: Exponential Fit
(Bootstrapped, Background Subtracted)
Fraction Intact, Bioanalyzer
Exponential Fit
Frac_Intact = exp(-0.561t)
Time Constant: 1.782 hrs
Fraction Intact
Time (hours)


10-12-17-13_930002D1: Exponential Fit
(Bootstrapped, Background Subtracted)
Fraction Intact, Bioanalyzer
Exponential Fit
Frac_Intact = exp(-0.607t)
Time Constant: 1.646 hrs
Fraction Intact
Time (hours)