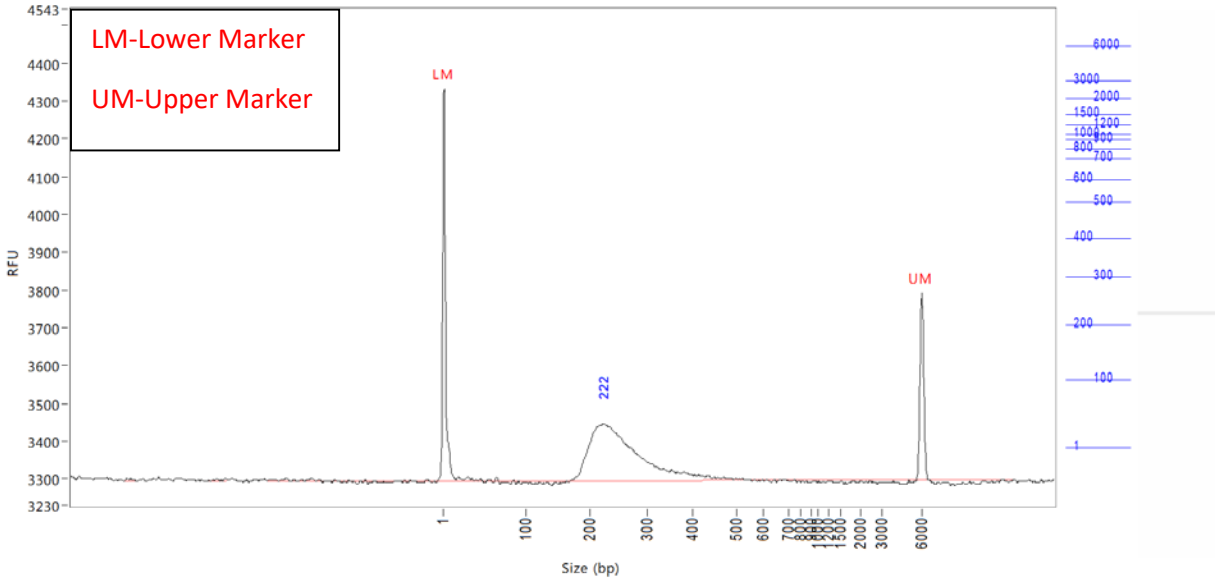
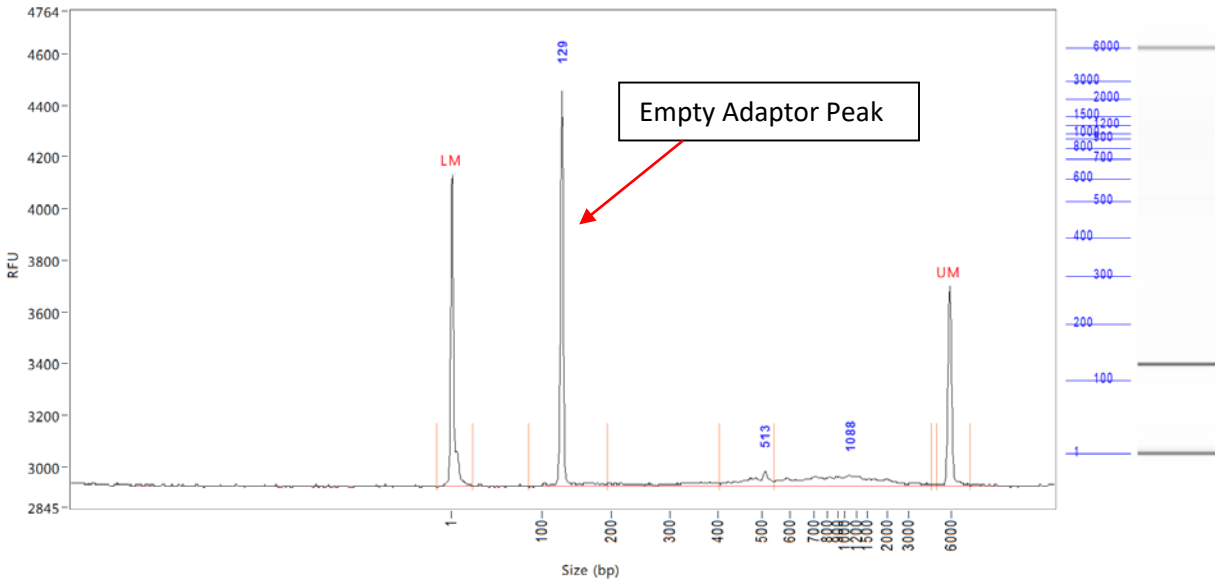
Fragment Analyzer QC: Electropherogram Reading and interpretation

Typical Illumina Sequencing Library: Library size 200-400bp



The LM at 1bp and UM at 6000bp are internal markers that are added to ensure proper sizing of your sample. They are added by the Core Facility during the loading process and are not part of you sample.

Empty Adaptor Illumina Sequencing Library:



Illumina's standard primers are ~65bp in length with empty adaptors (primer dimers) appearing at ~130bp. We recommend empty adaptor contamination make up 20% or less of the molarity of the sample to ensure quality sequencing results. Empty adaptors will bind to the flowcell and sequence, resulting in a decrease in usable reads from your run.

Free floating primers are ~65bp in length and may also cause problems when sequencing if they are prominent in your sample. They can bind to the flowcell and prevent proper cluster generation resulting in a decrease in the total yield for a sample

In both cases, if possible, we recommend doing an additional clean-up on the sample in order to increase the quality of your sequencing results.

Fragment Analyzer QC: Peak Table Analysis

Clean Illumina Library:

Peak	Size (bp)	Conc. (ng/uL)	From (bp)	To (bp)	RFU	Rel. Conc. % (ng/uL)	Molarity (nmole/L)
1	1 (LM)	0.0122	0	15	1039		15.8950
2	222	0.2493	120	568	151	100.0	1.5894
3	6000 (UM)	0.0034	5312	7021	494		0.0009
	TIC:	0.2493	ng/uL				
	TIM:	1.5894	nmole/L				
	Total Conc.:	0.2550	ng/uL				

Empty Adaptor Library:

Peak	Size (bp)	Conc. (ng/uL)	From (bp)	To (bp)	RFU	Rel. Conc. % (ng/uL)	Molarity (nmole/L)
1	1 (LM)	0.3108	0	24	1204		406.0831
2	129	3.5210	86	194	1530	62.8	44.8461
3	513	0.5659	404	546	57	10.1	1.9398
4	1088	1.5166	546	4646	42	27.1	2.0995
5	6000 (UM)	0.1064	5033	7485	773		0.0292
	TIC:	5.6034	ng/uL				
	TIM:	48.8853	nmole/L				
	Total Conc.:	6.1117	ng/uL				

High Empty Adaptor peak Molarity, we would NOT recommend sequencing this library without a clean-up

Definition of Terms

Peak number: numbers the peaks in the electropherogram from left to right, including the LM and UM

Size: tallest point of that peak, **not** the average size of the peak

Conc.: concentration in ng/ul for that peak number

"From" and "To": start and end of that peak number

RFU: Relative **F**luorescence **U**nit for this run. RFU's change from run to run and should not be used as a comparison across multiple samples

Rel. Conc.: relative concentration represented as a percent of the whole sample

Molarity: molarity of that peak number

TIC: Total **I**ntegrated **C**oncentration in ng/µL. Concentration of all detected peaks, does **not** include LM and UM.

TIM: Total **I**ntegrated **M**olarity in nmole/L. Molarity of all detected peaks, does **not** include LM and UM.

Total conc.: total concentration in ng/µL of sample, does **not** include LM and UM

The Genomics Facility recommends you use this QC file as a **qualitative** measure only. The concentrations given by this instrument are often inaccurate. It is recommended that a qubit or some other intercalating dye is used to determine quantification.