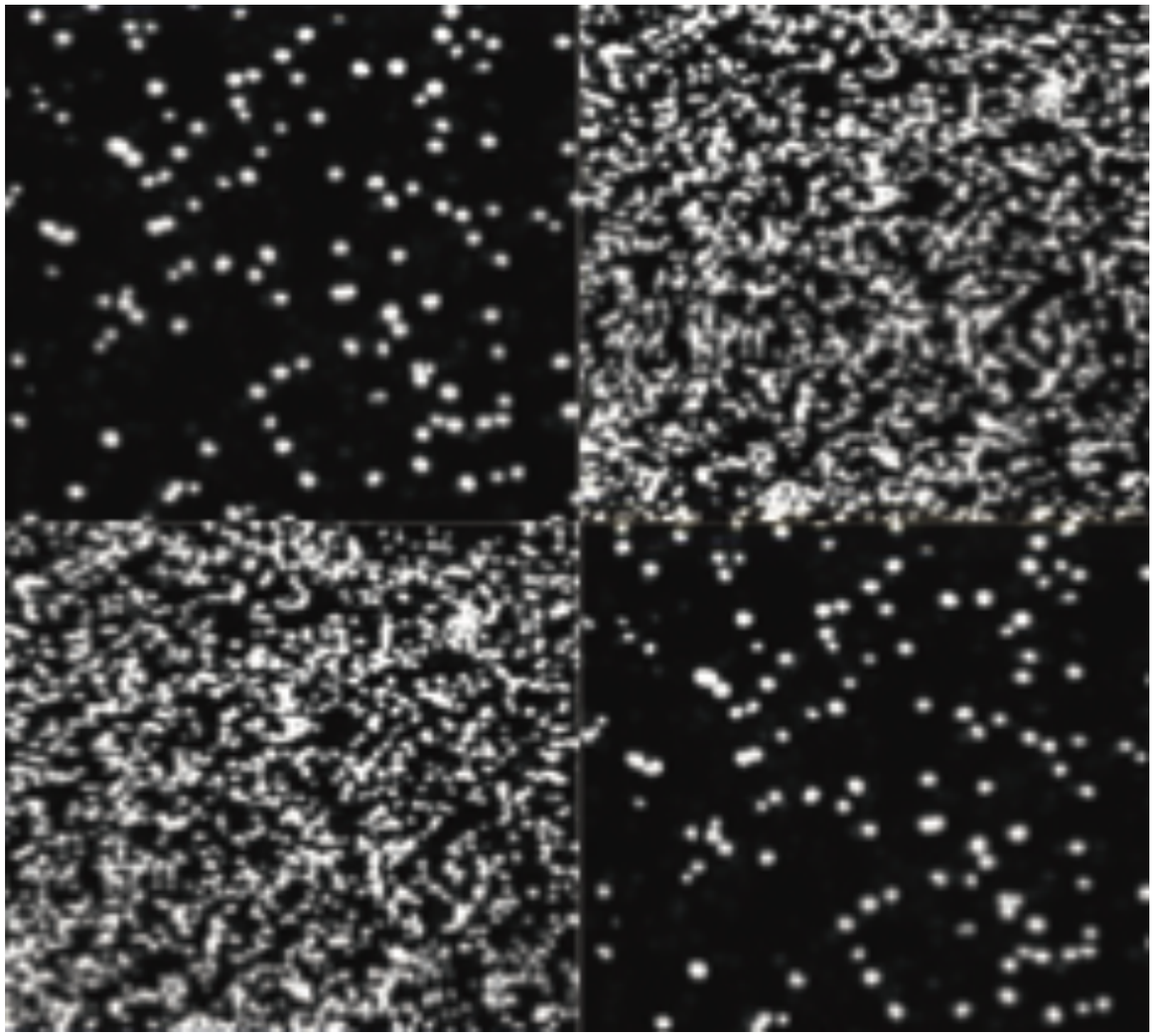
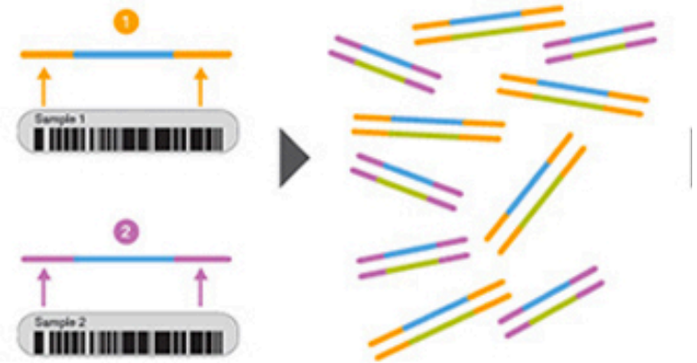
Library Quantification



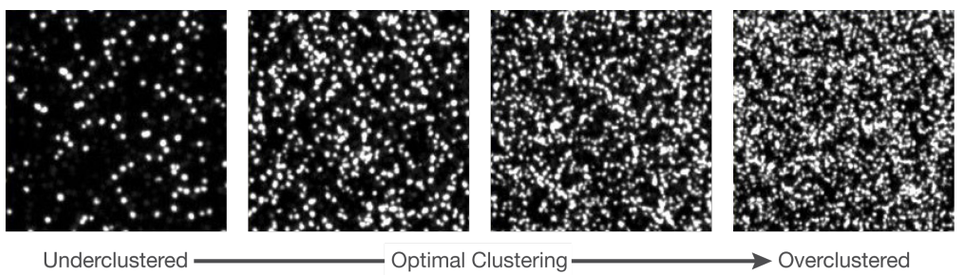
Library Quantification

Why you need to quantify libraries prior to sequencing…

1. Pool multiplexed libraries in desired ratios



2. Load correct amount of library on the sequencer

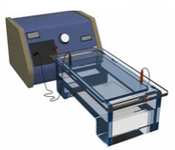


Is your library at a high enough concentration?

- Protocols for loading a library onto a sequencer often require a starting concentration of at least 2 nanomolar (nM).
- You have generated an amplicon library for sequencing with an expected fragment size (including adapters) of **400 bp**. According to a qubit analysis, the dsDNA concentration of your library is 1.3 ng/µl.
- Note that the molecular weight of DNA is ~650 daltons per bp.
- What is the molarity of your library? Do you have enough to proceed with loading the sequencer?

Measuring the Library Molarity

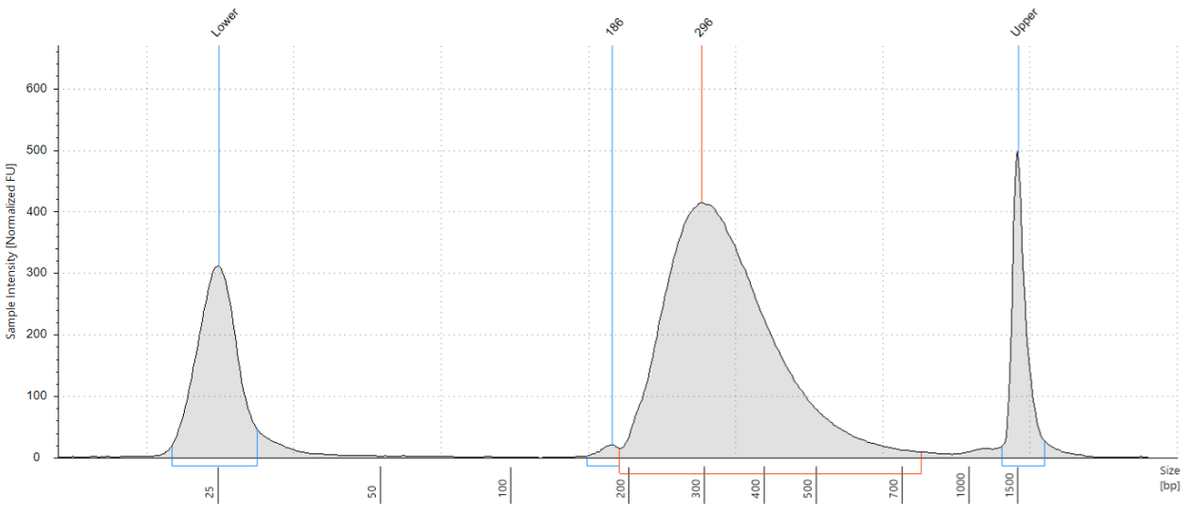
A meaningful library quantification is based on the concentration of *sequenceable* molecules (molarity). It therefore combines information on DNA mass, average molecular weight, and presence of ligated adapters.

		DNA Mass/Concentration	Size Distribution	Presence of Adapters
NanoDrop		Yes	No	No
Qubit		Yes	No	No
Gel Electrophoresis		No	Yes*	No
Bioanalyzer/TapeStation		Yes	Yes	No
qPCR		Yes*	No	Yes

Gold standard is do TapeStation AND qPCR

Library QC with TapeStation

TapeStation High-Sensitivity D1000 reagents can effectively measure mass, size distribution, and molarity for typical libraries, but they cannot assess whether molecules have adapters.



Sample Table

Well	Conc. [pg/µl]	Sample Description	Alert	Observations
A1	1600	Duplex Lib Pool		

Peak Table

Size [bp]	Calibrated Conc. [pg/µl]	Assigned Conc. [pg/µl]	Peak Molarity [pmol/l]	% Integrated Area	Peak Comment	Observations
25	456	-	28100	-		Lower Marker
186	12.9	-	107	0.81		
296	1580	-	8230	99.19		
1500	250	250	256	-		Upper Marker

Library QC with qPCR

The KAPA Library Quantification kit provides a series of library standards of known molarity to estimate molarity of amplifiable (adapter-containing) molecules in your library. It is highly precise, but final estimates require a correction factor based on average molecule length.