Primer Dimer Sequencing Requirements

What is Primer Dimer?

At the HTSF, primer dimer and adapter dimer are used interchangeably for convenience. QAQC results will likely refer to any unexpected small peak in the 120-170bp region as a primer dimer. Both adapter dimer and primer dimer refer to any partially formed or full-length index sequence that is able to bind and cluster on the flowcell. This becomes an issue as smaller size fragments cluster more efficiently on the flowcell. As a result, even small amounts of primer dimer can take up a large portion of the data and skew the results.

Primer Dimer Causes

Primer dimer can occur if there is insufficient starting material during library prep or if the starting material is poor quality. That is why it is important to clean-up samples with primer dimer greater than the threshold for the future sequencing platform.

Primer Dimer Threshold for MiSeq and HiSeq

Sequencing Platform			
Primer Dimer Threshold	MiSeq and MiSeq Nano	HiSeq 2500 RR and HO	HiSeq 4000
	Up to 5% maximum	Up to 2.5% maximum	Up to 0.5% maximum

Primer Dimer Threshold for NovaSeq

Per Illumina's experience, 0.5% primer dimer maximum is recommended for the NovaSeq platform. Please note, this is based on experience and has not been validated by Illumina. Based on the chart below and the linked experimental data, the HTSF has decided to recommend a **0.3% primer dimer maximum** for the NovaSeq platform. However, any amount of primer dimer present will affect the data output even if only to a small degree as seen in the

chart below. More information can be found at: https://support.illumina.com/bulletins/2020/12/how-short-inserts-affect-sequencing-performance.html.

Primer Dimer Reduction in Output by Percentage					
% PD	10%	5%	1%	0.5%	0.1%
% Adapters Identified in Downstream Analysis	84.25%	60.44%	6.64%	2.71%	0.81%

Clean Up Recommendations for Primer Dimer

It is always the HTSF's recommendation to perform a beadwash on libraries, not pools, when possible. Beadwashing libraries allows us to preserve pool balance. Balancing pools' high dimers is hard as they throw off the relationship between the usable fragments and the observed molarity of the pool. This is especially important if samples are intended to run on the NovaSeq as the NovaSeq is extremely sensitive to pool balance.

Beadwashes will automatically be performed on any HTSF made libraries at no charge to the study if needed. For study made libraries and pools, the HTSF will notify the project if any of their samples exceeds the primer dimer threshold for the selected sequencing platform. Projects will need to sign off on proceeding with or without a beadwash and acknowledge any risks to data quality and quantity.