Appendix C: SMART Adapter in Illumina Primer 2 Read

Blocked PCR primers are especially useful when preparing cDNA for library construction on next-generation sequencing platforms. The primer used for amplification of the double-stranded cDNA is blocked (Figure 2), which prevents ligation of the sequencing adapter to the 5' ends of double-stranded cDNA fragments containing the SMART sequence.

In many library preparation methods for Illumina sequencing, double-stranded adapters are added to cDNA fragments through ligation. Unfortunately, in these reactions, ligation may also take place between the bottom strand of the cDNA fragment and the Illumina adapter containing Read Primer 2, at a low and somewhat variable rate. If ligation is also successful on the other, unblocked side of the same cDNA fragment, this bottom strand can be amplified by the subsequent PCR and can ultimately form clusters for sequencing on Illumina machines.

When these clusters are sequenced, the SMART adapter will be present in the first 30 cycles in Read 2. In addition, the dT30 sequence from the 3' SMART-Seq CDS Primer II A will also be present after the adapter in a subset of these clusters. The presence of the SMART adapter in Read 2 occurs at a high enough rate to be observed in the base distribution by cycle graph generated by the run analysis (Figure 5, cycles 77–106), as does the dT30 sequence (Figure 5, cycles 107–136).

If you wish to avoid sequencing the SMART adapter, there are three options:

1. Use the Low Input Library Prep Kit (Cat. No. 634947). This unique adapter addition method does not permit erroneous ligation.
2. Use the Nextera XT DNA Library Preparation Kit from Illumina to prepare your library. We recommend using an input amount of 100–150 pg amplified cDNA.
3. Sequence only from Read Primer 1.

If you have already sequenced with Read Primer 2, the SMART adapter sequence can be trimmed from reads prior to mapping to your transcriptome.

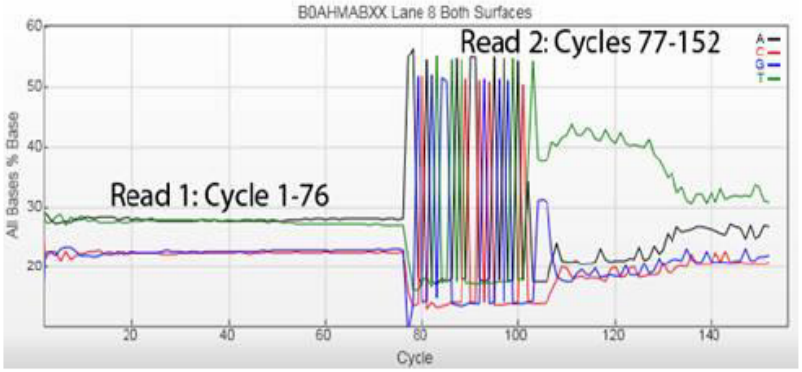

Figure 5. SMART adapter in Primer 2 Read. The presence of the SMART adapter in Read 2 commonly occurs at a high enough rate to be observed in the base distribution by cycle graph generated by the run analysis (cycles 77–106), as does the dT30 sequence (cycles 107–136).