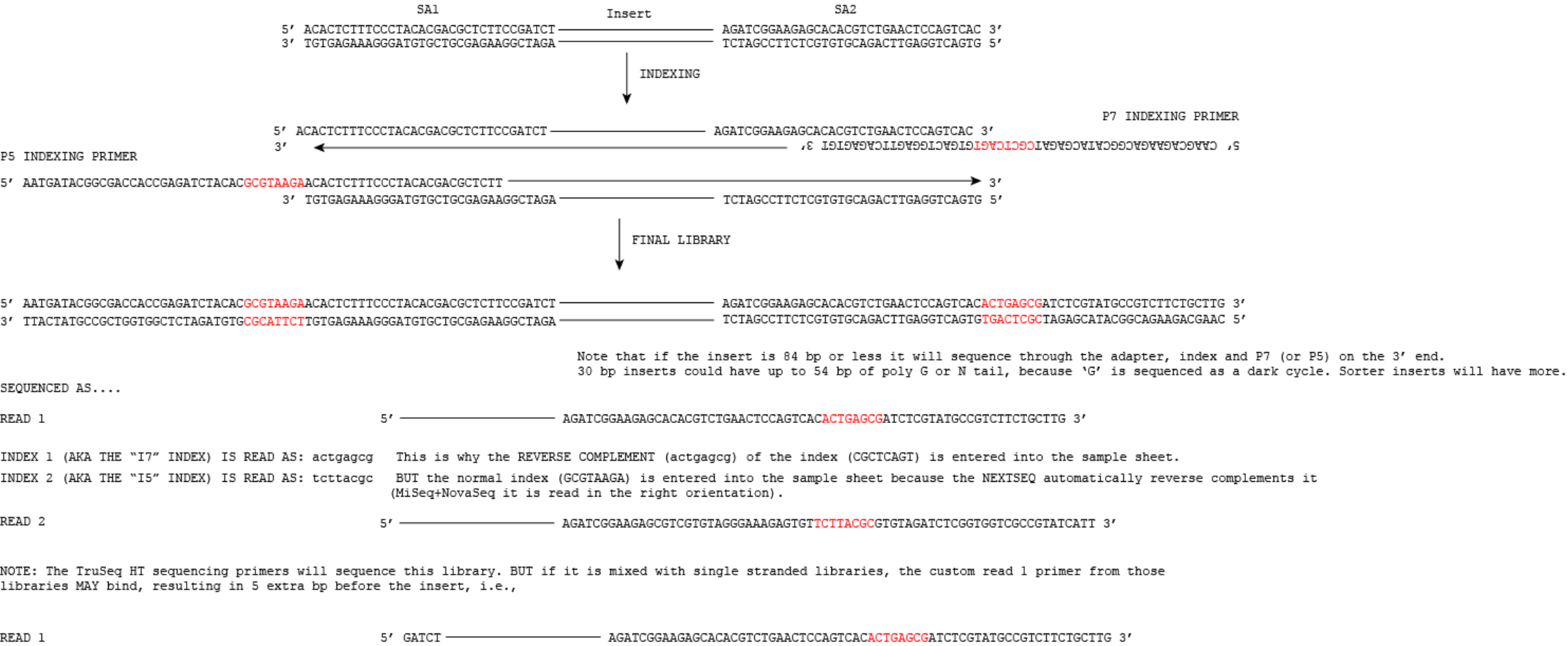

Note that if the insert is 84 bp or less it will sequence through the adapter, index and P7 (or P5) on the 3′ end.
30 bp inserts could have up to 54 bp of poly G or N tail, because ‘G’ is sequenced as a dark cycle. Sorter inserts will have more.

SEQUENCED AS....

READ 1 5′ AGATCGGAAGAGCACACGTCTGAACTCCAGTCACACTGAGCGATCTCGTATGCCGTCTTCTGCTTG 3′

INDEX 1 (AKA THE “I7” INDEX) IS READ AS: actgagcg This is why the REVERSE COMPLEMENT (actgagcg) of the index (CGCTCAGT) is entered into the sample sheet.

INDEX 2 (AKA THE “I5” INDEX) IS READ AS: tcttacgc BUT the normal index (GCGTAAGA) is entered into the sample sheet because the NEXTSEQ automatically reverse complements it (MiSeq+NovaSeq it is read in the right orientation).

READ 2 5′ AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTTCTTACGCGTGTAGATCTCGGTGGTCGCCGTATCATT 3′

NOTE: The TruSeq HT sequencing primers will sequence this library. BUT if it is mixed with single stranded libraries, the custom read 1 primer from those libraries MAY bind, resulting in 5 extra bp before the insert, i.e.,

READ 1 5′ GATCT AGATCGGAAGAGCACACGTCTGAACTCCAGTCACACTGAGCGATCTCGTATGCCGTCTTCTGCTTG 3′