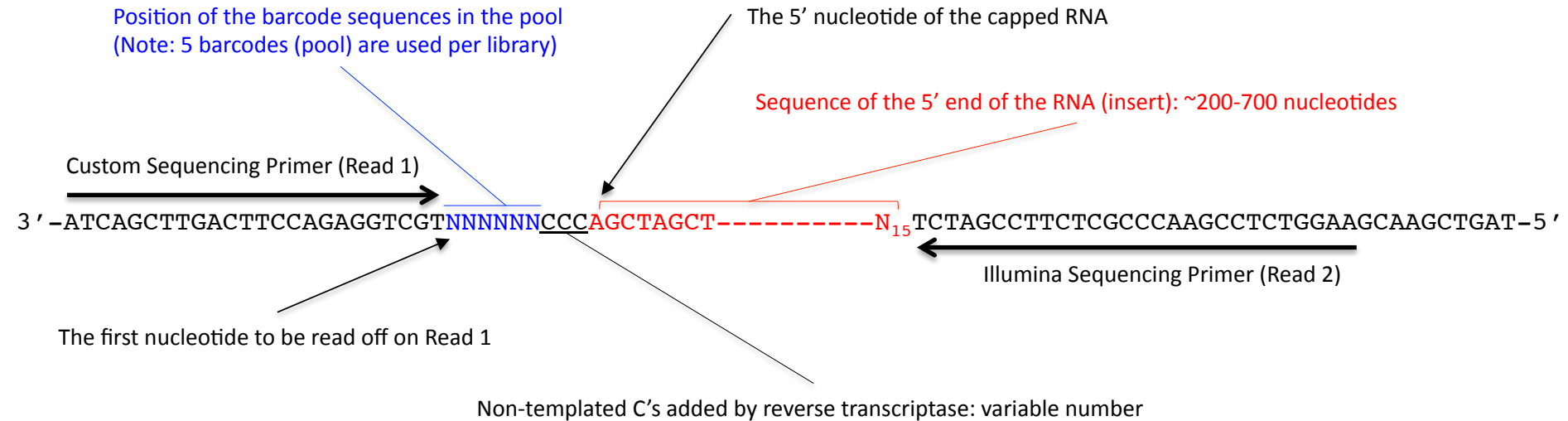


RAMPAGE Library Structure

(This is a partial sequence showing the structure of the insert, barcodes and non-templated C's relative to the sequencing primers)



- Reverse Transcription Primer: 5'- TAGTCGAACGAAGGTCTCCGAACCCGCTCTTCCGATCTN₁₅
- Template-Switching Primer: 5'- TAGTCGAAGTCTCCAGCANNNNNNrGrGrG – the last 3 G's must be riboguanosines
- Forward PCR Primer: 5'- AATGATACGGCGACCACCGAGATCTACACTAGTCGAACTGAAGG
- Reverse PCR Primer: 5'- CAAGCAGAAGACGGCATAACGAGATCGGTCTCGGCATTCTGCTGAACCGC
- Read 1 Sequencing Primer (custom): 5'- TAGTCGAAGTCTCCAGCA
- Read 2 Sequencing Primer (Illumina): 5'- CGGTCTCGGCATTCTGCTGAACCGCTCTTCCGATCT

Annealing the Reverse Transcription Primer

5' ***-TCGATCGA_{~200-700 n'tds} NNNNNNNNNNNNNNNNN_x to the 3' end
 <-NNNNNNNNNNNNNNNNNTCTAGCCTTCTCGCCCAAGCCTCTGGAAGCAAGCTGAT-5'

After Reverse Transcription: addition of non-templated C's

5' ***-TCGATCGA_{~200-700 n'tds} NNNNNNNNNNNNNNNNN_x to the 3' end
 CCCAGCTAGCT-----NNNNNNNNNNNNNNNNNTCTAGCCTTCTCGCCCAAGCCTCTGGAAGCAAGCTGAT-5'

Annealing of the Template-switching oligo

5' -TAGTCGAACTGAAGGTCTCCAGCANNNNNNNGGG
 CCCAGCTAGCT-----N₁₅TCTAGCCTTCTCGCCCAAGCCTCTGGAAGCAAGCTGAT-5'

After template-switching

5' -TAGTCGAACTGAAGGTCTCCAGCANNNNNNNGGG
 ATCAGCTTGACTTCCAGAGGTCGTNNNNNNCCAGCTAGCT-----N₁₅TCTAGCCTTCTCGCCCAAGCCTCTGGAAGCAAGCTGAT-5'

PCR

Position of the sequencing primers: (Note the blue sequence below is a partial sequence and does not include the PCR primers, so that it fits on the slide)

3' -ATCAGCTTGACTTCCAGAGGTCGTNNNNNNCCAGCTAGCT-----N₁₅TCTAGCCTTCTCGCCCAAGCCTCTGGAAGCAAGCTGAT-5'

- Reverse Transcription Primer: 5'- TAGTCGAACGAAGGTCTCCGAACCCGCTCTTCCGATCTN₁₅
- Template-Switching Primer: 5'- TAGTCGAACTGAAGGTCTCCAGCANNNNNNrGrGrG
- Forward PCR Primer: 5'- AATGATACGGCGACCACCGAGATCTACACTAGTCGAACTGAAGG
- Reverse PCR Primer: 5'- CAAGCAGAAGACGGCATAACGAGATCGGTCTCGGCATTCTGCTGAACCGC
- Read 1 Sequencing Primer (custom): 5'- TAGTCGAACTGAAGGTCTCCAGCA
- Read 2 Sequencing Primer (Illumina): 5'- CGGTCTCGGCATTCTGCTGAACCGCTCTTCCGATCT

Red: RNA
 Blue: Products after extension
 Black : oligos