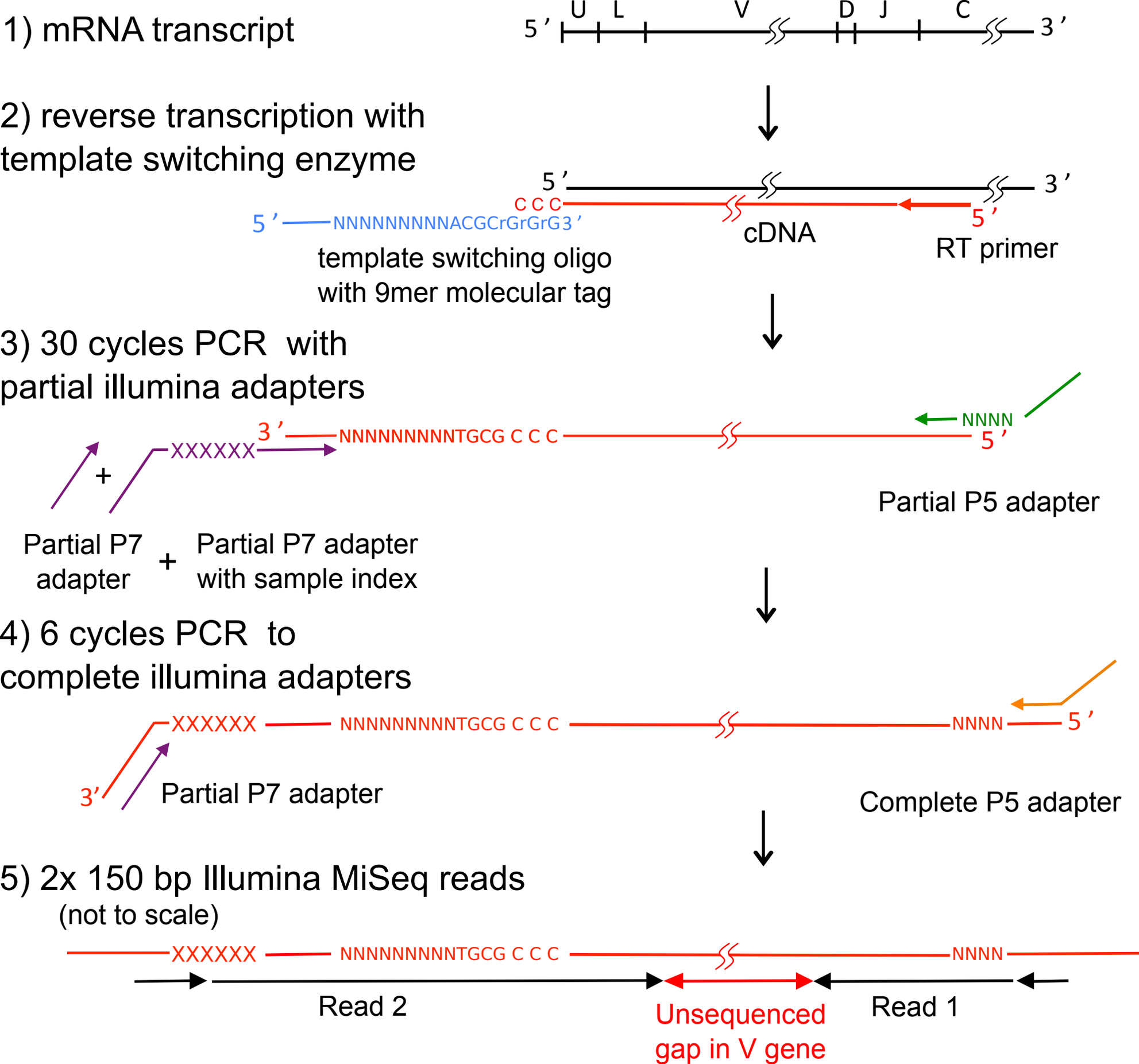
1) mRNA transcript
5 ’
U
L
V
D
J
C
3 ’
2) reverse transcription with template switching enzyme
5 ’
3 ’
C C C
5 ’ NNNNNNNNNACGCrGrGrG 3 ’
cDNA
5 ’
RT primer
template switching oligo with 9mer molecular tag
3) 30 cycles PCR with partial illumina adapters
3 ’
NNNNNNNNNTGCG C C C
NNNN
5 ’
XXXXXX
+
Partial P5 adapter
Partial P7 adapter
+
Partial P7 adapter with sample index
4) 6 cycles PCR to complete illumina adapters
XXXXXX
NNNNNNNNNTGCG C C C
NNNN
5 ’
3’
Partial P7 adapter
Complete P5 adapter
5) 2x 150 bp Illumina MiSeq reads
(not to scale)
XXXXXX
NNNNNNNNNTGCG C C C
NNNN
Read 2
Unsequenced gap in V gene
Read 1