

**A****STEP 1: DIGEST W/ MmeI**

5' 5PHOS NNNNNNNNNNNNNNNNNNNNNACAGGTTGGA TRANSPOSON TCCAACCTGTNNNNNNNNNNNNNNNNNN 3'  
 3' NNNNNNNNNNNNNNNNNNNNNTGTCCAACCT NOSOPSNART AGGTTGGACTNNNNNNNNNNNNNNNNNN 5PHOS 5'

**STEP 2: PHOSPHOTASE DNA**

5' NNNNNNNNNNNNNNNNNNNNNACAGGTTGGA TRANSPOSON TCCAACCTGTNNNNNNNNNNNNNNNNNN 3'  
 3' NNNNNNNNNNNNNNNNNNNNNTGTCCAACCT NOSOPSNART AGGTTGGACTNNNNNNNNNNNNNNNNNN 5'

**STEP 3: LIGATE ADAPTOR****Adapter:**

5' ACACTCTTTCCCTACACGACGCTCTTCCGATCTNN  
 3' 3Phos TGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA 5Phos

**Final Product:**

5' ACACTCTTTCCCTACACGACGCTCTTCCGATCTNN NNNNNNNNNNNNNNNNNNNACAGGTTGGA TRANSPOSON AGGTTGGACTNNNNNNNNNNNNNNNNNN AGATCGGAAGAGCGTCTGTAGGGAAGAGTGT 3'  
 3' TGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA NNNNNNNNNNNNNNNNNNNTGTCCAACCT NOSOPSNART TCCAACCTGTNNNNNNNNNNNNNNNNNN NNTCTAGCCTTCTCGCAGCACATCCCTTTCTCACA 5'

**STEP 4: PCR AMPLIFY TRANSPOSON ENDS**

PCR.1 5' CAAGCAGAAGACGGCATACGAGATCGTGATGTGACTGGAGTTCAGACGCTGTGCTCTTCCGATCTAGACCGGGACTTATCATCCAACCTGT 3'  
 U. 5' AATGATACGGCGACCACCGAGATCTACACTTTCCCTACACGACGCTCTTCCGATCT 3'

**P7 FLOW CELL BINDING** **BARCODE** **INDEXING PRIMING SITE** **TRANSPOSON** **GENOMIC DNA** **PRIMER READ 1 SITE** **P5 FLOW CELL BINDING**

5' CAAGCAGAAGACGGCATACGAGATCGTGATGTGACTGGAGTTCAGACGCTGTGCTCTTCCGATCTAGACCGGGACTTATCATCCAACCTGT  
 TRANSPOSON TCCAACCTGTNNNNNNNNNNNNNNNNNN AGATCGGAAGAGCGTCTGTAGGGAAGAGTGT  
 NOSOPSNART AGGTTGGACTNNNNNNNNNNNNNNNNNN NNTCTAGCCTTCTCGCAGCACATCCCTTTCTCACA  
TCTAGCCTTCTCGCAGCACATCCCTTTCTCACACTAGAGCCACCAGCGCATAGTAA 3'

**Final Product:**

**P7 FLOW CELL BINDING** **BARCODE** **INDEXING PRIMING SITE** **TRANSPOSON (MmeI site)** **GENOMIC DNA** **PRIMER READ 1 SITE** **P5 FLOW CELL BINDING**

5' CAAGCAGAAGACGGCATACGAGAT CGTGAT GTGACTGGAGTTCAGACGCTGTGCTCTTCCGATCT AGACCGGGACTTATCATCCAACCTGT NNNNNNNNNNNNNNNNNNN AGATCGGAAGAGCGTCTGTAGGGAAGAGTGT AGATCTCGTGGTCCGCGTATCAT 3'

**B**