Sample Preparation:

(only one strand is shown, in the 5’ to 3’ direction)

Tripartite adapter is ligated to the end of the target molecule:
Ligated target – NNN CCTACACGACGCTCTTCCGATCT NNNNNNNNNNNNNNNN CC AGGAATAGTTATGTGCATTAATGAATGG CGCC

↓

Target molecules with adapters at both ends are amplified and the PCR primer annealing region is removed:
Ligated target – NNN CCTACACGACGCTCTTCCGATCT NNNNNNNNNNNNNNNN CC

↓

Amplified target molecules are fragmented and circularized:
Ligated target end – NNN CCTACACGACGCTCTTCCGATCT NNNNNNNNNNNNNNNN CC – ligated region of interest

↓

Circularized DNA is fragmented and fragments containing adapter sequences are prepared for sequencing:
Illumina adapter 1 – CCTACACGACGCTCTTCCGATCT NNNNNNNNNNNNNNNN CC – ligated region of interest – Illumina adapter 2

↓

Resulting sequencing read:
NNNNNNNNNNNNNNNN CC – ligated region of interest

↓

In computational pipeline, the sequences at the start of the read are used to determine the sample and target molecule of origin:
NNNNNNNNNNNNNNNN CC – ligated region of interest

↓ Determines target molecule of origin

↓ Confirms upstream sequence is a barcode

↓ Contains sequence information