Transposome Assembly Using Diagenode pA-Tn5 Transposase

pA-Tn5 transposase (Cat. No. C01070002) is a fusion protein of hyperactive Tn5 transposase and protein A developed for the **CUT&Tag** assay. For flexibility of use, the fusion protein is not pre-loaded with sequencing adapters. The fusion protein should be loaded with appropriate oligonucleotides prior to use. Oligonucleotides should contain 19-mer Tn5 mosaic ends (underlined) recognized by the transposase and the sequences (bold) allowing the PCR amplification with Illumina-compatible barcoded i7/i5 primers. These sequences have to be adapted to a particular experimental design and take into account the sequencing platform requirements.

Mosaic end_reverse: [PHO]CTGTCTCTTATACACATCT

Mosaic end_Adapter A: **NNNNNNNNNNNNNNN**AGATGTGTATAAGAGACAG

Mosaic end_Adapter B: **NNNNNNNNNNNNNNN**AGATGTGTATAAGAGACAG

Protocol

1. Design and order the lyophilized oligonucleotides that you would like to use to load the pA-Tn5 transposase. You will need 3 oligonucleotides that we can call A, B and Rev.
2. Prepare the following Annealing Buffer: 40mM Tris-HCl (pH8.0), 50mM NaCl.
3. Resuspend the oligos in Annealing Buffer to stock concentration of 100 µM.
4. In a PCR tube, mix 10 µL of oligo Rev with 10 µL of oligo A.
5. In a separate PCR tube, mix 10 µL of oligo Rev with 10 µL of oligo B.
6. Vortex and place PCR tubes in a thermocycler.
7. Run the following program:

Temperature	Time
95°C	5 minutes
Cool to 65°C	-0.1°C/second
65°C	5 minutes
Cool to 4°C	-0.1°C/second

Note: *Annealed linker oligos can be stored at -20°C.*

8. In a chilled PCR tube, mix 6.25 µL of the annealed oligo A/oligo Rev with 6.25 µL the annealed oligo B/oligo Rev.
9. Add 10 µL of pA-TN5 transposase (unloaded) (Cat. No. C01070002).
10. Pipet gently and incubate at 23°C for 30 minutes in a thermocycler.

    CAUTION: Do not exceed 60 minutes incubation time, or the pA-TN5 transposase will lose activity
11. Add 12.5 µL of glycerol and store at -20°C.

Reference

Picelli S, Björklund AK, Reinius B, Sagasser S, Winberg G, Sandberg R. Tn5 transposase and tagmentation procedures for massively scaled sequencing projects. Genome Res. 2014;24(12):2033–2040. doi:10.1101/gr.177881.114

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