Transposome assembly using Diagenode Tagmentase

Diagenode Tagmentase is a hyperactive Tn5 transposase. Its ability to cut DNA and insert sequencing adapters in one step makes it the perfect companion for Next-Generation Sequencing experiments using powerful technologies such as ATAC-seq or ChIPmentation.

For flexibility of use, the protein is not pre-loaded with sequencing adapters and 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 e.g., 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. Order the oligos that you would like to use to load the tagmentase. You will need 3 oligos that we can call A, B and Rev. They should be lyophilized.
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.*
You can therefore prepare a bigger volume and freeze it.

8. In a chilled PCR tube, mix 5 µL of the annealed oligo A/oligo Rev with 5 µL the annealed oligo B/oligo Rev.
9. Add 10 µL of Tagmentase (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 Tagmentase will lose activity.*

11. Add 10 µl of glycerol and store at -20°C.

    Note: *If dilution of the transposome is needed we recommend using the Tagmentase Dilution Buffer which is available separately (Cat. No. C01070011). This buffer contains 50% glycerol*

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