Transposome Assembly Using Hologic Diagenode Tagmentase Tn5 Transposase

Cat. No. C01070010

Hologic Diagenode Tagmentase (unloaded, Cat. No. C01070010) is a hyperactive Tn5 transposase. Its ability to simultaneously cut DNA and insert sequencing adapters makes it an ideal tool for Next-Generation Sequencing.

For flexibility, the protein is not pre-loaded with sequencing adapters and must be loaded with appropriate oligonucleotides before use. These oligonucleotides contain 19-mer Tn5 mosaic ends (underlined) recognized by the transposase, as well as any sequences needed for PCR amplification (e.g., Illumina-compatible barcoded i7/i5 primers). The exact sequences should be tailored to the specific experimental design and must account for the requirements of the chosen sequencing platform.

Mosaic End Sequences:

- **Mosaic end_reverse:** [PHO]CTGTCTCTTATACACATCT
- **Mosaic end_Adapter A:** $(N)_n$* _AGATGTGTATAAGAGACAG
- **Mosaic end_Adapter B:** $(N)_n$* _AGATGTGTATAAGAGACAG

$(N)_n$ = custom sequence

Protocol

The following protocol describes Tagmentase loading at an enzyme-to-adapters molar ratio of 1:1.3, which is recommended for optimal performance.

1. Oligonucleotides Preparation

- Order the three lyophilized oligonucleotides (A, B, and Rev) you plan to use for loading the Tagmentase.
- Prepare the **Annealing Buffer**:
  - **40 mM Tris-HCl (pH 8.0)**
  - **50 mM NaCl**
- Resuspend each of the lyophilized oligonucleotides (A, B, and Rev) in Annealing Buffer to a stock concentration of **200 µM**.

2. Set Up Oligo Mixes

- In one PCR tube, mix equal volumes of oligonucleotide A and oligonucleotide Rev (e.g., 100 µl each).
- In a second PCR tube, mix equal volumes of oligonucleotide B and oligonucleotide Rev (e.g., 100 µl each).
- **Vortex** both tubes and **place them in a thermocycler**.

3. Perform Annealing

- Run the following program on the thermocycler:

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

- This annealing step generates a **100 µM Annealed Adapter Mix (A/Rev and B/Rev)**.

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4. Prepare Annealed Adapters Mix

- In a new 1.5 mL tube, mix **equal volumes** of the annealed **oligos A/Rev** (e.g., 100 µl) with the annealed **oligos B/Rev** (e.g., 100 µl).
- This final mixture is your **100 µM Annealed Adapter Mix**.
- Make aliquots and store at –20°C until use.

5. Load Tagmentase

- Mix an **equal volume** of **unloaded Tagmentase** (Cat. No. C01070010) with an equal volume of the **100 µM Annealed Adapter Mix** (e.g., 10 µl of each).
- Pipette gently and incubate at **23°C** for 30 minutes in a thermocycler.

  CAUTION: *Do not exceed the recommended incubation time to avoid activity loss.*
- Add a volume of **glycerol** equal to the volume of Tagmentase used in the loading step (e.g., 10 µl).
- The loaded enzyme is now ready to use. **Store at –20°C**.

Note: *Each new lot or specific application may require enzyme titration to ensure optimal activity. If further dilution of the transposome is required, use the Tagmentase Dilution Buffer (Hologic Diagenode, Cat. No C01070011).*

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