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Loading Tn5 Enzyme with sci-protocol Oligonucleotides

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1 Works for me dx.doi.org/10.17504/protocols.io.6zahf2e

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ABSTRACT

Loading of Tn5 enzyme for sci-ATAC and s3 protocols.

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PROTOCOL CITATION

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PROTOCOL INTEGER ID

27394

PARENT PROTOCOLS

In steps of
s3-ATAC
s3-WGS
s3-GCC

Prepare Reagents

1 Prepare **50 mL 2.125X Tn5 Dilution buffer** for protein dilution.

Reagent	Stock Concentration	Final Concentration	Amount of Stock
HEPES-KOH (pH 7.2)	1M	100mM	5mL
NaCl	5M	200mM	2mL
Glycerol	100%	25%	12.5mL
Triton-X100	100%	0.2%	100uL
ddH2O			30.4mL (to 50mL)
DTT	Dry	2mM	15.4 mg

Tn5 Dilution buffer can be stored at 4C for up to 2 months.

2 Prepare Mosaic End reverse compliment (ME'), i7, i5 oligonucleotides at [M]**100 Micromolar (µM) Tris-HCl buffer (pH 8.0)**

See attached spreadsheet for oligonucleotide sequences.

Three sets of oligonucleotides are listed for both i5 and i7 Tn5 loading.
This yields (3 i5 sets) x (3 i7 sets)=9 uniquely identifiable 96 well plates or 864 unique well barcode combinations.

Mosaic End oligonucleotide sequence used for Tn5 loading is also listed within the spreadsheet.

Example_sciTn5_Oligos.xlsx

Synthesis quality of these oligonucleotides is critical. HPLC purification is essential. We find that Eurofins oligos outperform IDT by roughly 10 fold in library complexity.

All indexes are designed to be 2 or greater Hamming distance from all others to allow for sequencing errors.

Anneal Indexed Oligoes to Mosaic End Reverse Compliment

3 **Preparation of dsDNA through annealing.**
Volumes are adjusted for a single 96-well plate loading.

1. For each i5 barcoded oligo prepare the following reaction (8 total):

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12.5 uL	100 uM i5 Tn5 Indexed oligo
12.5 uL	100 uM Mosaic End Reverse Compliment oligo
53.125 uL	2.125x Tn5 Dilution Buffer

Henceforth refered to as **i5/ME'**

2. For each i7 barcoded oligo prepare the following reaction (12 total):

8.5 uL	100 uM i7 Tn5 Indexed oligo
8.5 uL	100 uM Mosaic End Reverse Compliment oligo
36.125 uL	2.125x Tn5 Dilution Buffer

Henceforth refered to as **i7/ME'**

4 Anneal Oligo mixtures within a Thermocycler with the following reaction.

- **95 °C** **00:05:00**
- Slow ramp down to **20 °C at a rate of -2.5C/min**
- **20 °C hold**

This results in **16 Micromolar (µM)** annealed oligo species per reaction (**i7/ME'** and i5/ME').

Oligoes should be freshly annealed prior to loading Tn5 transposome

Plate Annealed Oligos

5 Prepare a 96-well plate with the following loading schema.

1. Add **5 µl** of i5/ME' (**16 Micromolar (µM)**) to each respective wells in a row-wise fashion.

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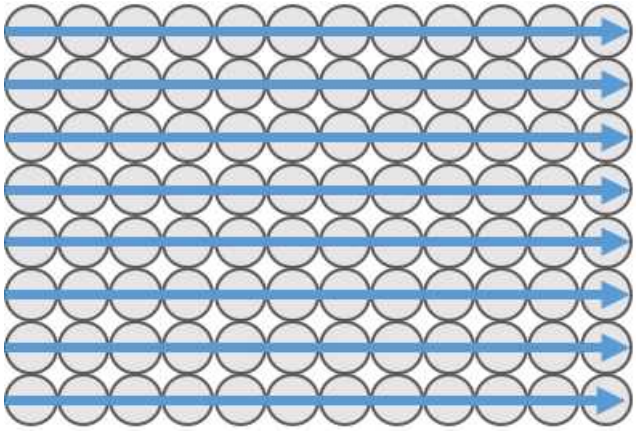
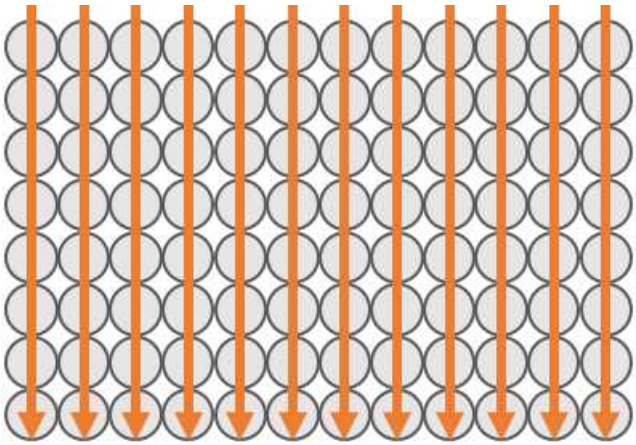


2. Add **5 µl** of i7/ME' (**[M]16 Micromolar (µM)**) to each respective wells in a column-wise fasion.



This results in **10 µl i5/ME' and i7/ME' Indexed Oligos** at **[M]8 Micromolar (µM)** /well

Adjust Salt Concentration on Tn5 and Load

6 Prepare Tn5 protein as described in *"Generation and Purification of pTXB1.Tn5"* protocol.

Prior to loading Tn5 protein adjust NaCl concentration.
Combine:

1152 uL	Prepared Tn5
144 uL	5M NaCl

This adjusts salt to a final concentration of **[M]555.55 Milimolar (mM)** NaCl

7 Add **12 µl** of salt-corrected Tn5 to each well of the 96 well plate.

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Assemble the Tn5/oligo mixture via incubation at **25 °C** for **01:00:00** .

Store at -20C for no more than 8 months.

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