MAY 07, 2021

Loading Tn5 Enzyme with sci-protocol Oligonucleotides

Ryan Mulqueen¹, Andy Fields¹, Andrew Adey¹¹Oregon Health & Science University

Andrew Adey

Oregon Health & Science University

ABSTRACT

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

OPEN ACCESS

DOI:

dx.doi.org/10.17504/protocols.io.6zahf2e

Protocol Citation: Ryan Mulqueen, Andy Fields, Andrew Adey 2021. Loading Tn5 Enzyme with sci-protocol Oligonucleotides. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.6zahf2e>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
We use this protocol and it's working

Created: Aug 30, 2019

Last Modified: May 07, 2021

PROTOCOL integer ID:
27394

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%	12.5 mL
Triton-X100	100%	0.2%	100uL
ddH2O			30.4 mL (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 complement (ME'), i7, i5 oligonucleotides at 100 micromolar (μ M) 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

Note

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.

Note

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 Complement

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):

12.5 uL	100 uM i5 Tn5 Index ed oligo
12.5 uL	100 uM Mosai c End Rever se Comp liment oligo
53.12 5 uL	2.125 x Tn5 Dilutio n Buffer

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

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

8.5 uL	100 uM i7 Tn5 Index ed oligo
--------	---

8.5 uL	100 uM Mosai c End Reverse Complement oligo
36.125 uL	2.125 x Tn5 Dilution Buffer

Henceforth referred 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 **[IM] 16 micromolar (μM)** annealed oligo species per reaction (**i7/ME'** and **i5/ME'**).

Note

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' (**[IM] 16 micromolar (μM)**) to each respective wells in a row-wise fashion.

2. Add 5 μL of i7/ME' ($16 \mu\text{M}$) to each respective wells in a column-wise fashion.

This results in 10 μL i5/ME' and i7/ME' Indexed Oligos at $8 \mu\text{M}$ /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 555.55 millimolar (mM) NaCl

7 Add 12 μ L of salt-corrected Tn5 to each well of the 96 well plate.

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

Note

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