TagMap
Tn5 Tagmentation-based pBac mapping
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Protocol - Preliminaries

1 – Anneal the oligonucleotides

Combine:

Rx1 (adapter 1 10 uM)

10uL Tn5ME-A (100 uM)
10uL Tn5MErev-InT (100 uM)
80uL Reassociation Buffer

Mix well.

Anneal primers in a thermal cycler with the following Reassociation Program

Step	Temp	Time
1	95°C	10 min
2	90°C	1 min
3	Reduce temp by 1°C/cycle 60 times	
4	4°C	Hold

2 – Dilute Tn5 to 20ng/uL

e.g.
1 uL Tn5 protein (200 ng / uL)
9 uL Reassociation buffer

Mix well by pipetting 10 times.

3 – Dilute the adaptors.

1 uL Adaptor 1 (10 uM)
9 uL H20

Mix by pipetting 10 times.

4 – Pre-charge the Tn5

Combine, in the following order, mix after each addition:

21 uL Tn5 (20 ng / uL)
10 uL Glycerol
10 uL Diluted Adaptors (1uM)

Pre-charge at 37°C for 30 minutes

5 - Tagment

Combine:

1 uL Precharged Tn5
1 uL DNA (10 ng / uL *or less!*)
2 uL 5 X TAPS
6 uL H_20

Mix well by pipetting up and down 10 X.

Incubate at 55°C for 7 minutes.

6 – Kill the Tn5

Add to each reaction:

2.5 uL 0.2% SDS

Incubate at 55° 7 min

7 – PCR 1

1uL	Tagmentation reaction
1uL	5uM Tn5ME-B-pBac-142R
1uL	5uM Tn5ME-B-pBac-3287F
1uL	5uM A_idx_i5 primer
1uL	4 mM dNTP
4uL	5X Phusion Reaction Buffer
0.5uL	Phusion Polymerase
10.5uL	H_20

Thermocycler settings

Step	Temp	Time
1	95°C	5 min
1	95°C	15 sec
2	61°C	15 sec
3	72°C	1 min
4	Cycle to step 3 17 times	
5	72°C	2 min
6	4°C	Hold

8 – PCR 2

1 uL	PCR1 Reaction
1 uL	10 uM Primer 1 – Tn5-Illumina-Primer1 (FC2)
1 uL	10 uM Primer 2 – i7_idx-primer
1 uL	dNTPs
4 uL	Phusion 5X Buffer
0.5uL	Phusion Polymerase
11.5uL	H_20

Cycle 12 times with same thermocycler settings as PCR 1

12 – Clean up product - Ampure

Pool 10uL of each reaction

0.5 X Ampure (e.g. 50ul / 100uL library)
Incubate 1 min
Separate on magnet
Keep supernatant, discard beads

Add 0.35 X Ampure (e.g. 35ul/ 100uL original library. Total volume 185uL)
Separate on Magnet
Wash 2X 80% Ethanol
Dry beads 5 min on magnet
Resuspend in water
Separate on magnet, keep 95% of liquid

13 – Quantify final library

Reassociation Buffer – *store at R.T.*
10 mM Tris pH 8.0
50 mM NaCl
1 mM EDTA

5x TAPS-DMF buffer from Picelli paper
50 mM TAPS-NaOH,
25 mM MgCl2,
50% v/v DMF (pH 8.5) at 25°C

-100 g TAPS in 500 ml H2O, pH = 9.9 -Add 5-10 ml concentrate HCl to get to pH 8.5
-complete to 754 ml for 500mM (Paper says TAPS-NaOH but I added HCl instead)