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Generation and Purification of pTXB1.Tn5

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

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ABSTRACT

Generation of Tn5 transposome, protein purification and loading for the sci- family of protocols.

Protein purification of pTXB1.Tn5 transformed into T7 Express LysY (NEB C3031) cells;
reference is PTXB1-Tn5 Addgene #60240 from Picelli et al. Genome Res. 2014."

This protocol is built largely off of work described in
Picelli, Simone, et al. "Tn5 transposase and tagmentation procedures for massively scaled sequencing projects." Genome research 24.12 (2014): 2033-2040.

DOI

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26995

PARENT PROTOCOLS

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

MATERIALS TEXT

MATERIALS

Carbenicillin **Contributed by users**

HEPES **Sigma Aldrich Catalog #H6147**

Potassium hydroxide **Sigma Aldrich Catalog #1050121000**

Poly(ethyleneimine) solution **Sigma Aldrich Catalog #03880-100ML**

Chitin

Resin **NEB Catalog #S6651L**

Prior to day 1

1 Generate LB agarose plates with [M]**100 ug/mL Carbenicillin** following addgene suggested protocol. https://www.addgene.org/mol-bio-reference/#antibiotics

2 Streak out E. coli stab with **pTXB1-Tn5** plasmid received from Addgene (https://www.addgene.org/60240/).

3 Select single colony with a sterile pipette tip and grow out colony in liquid LB with [M]**100 ug/mL Carbenicillin**

4 Incubate with shaking (**250 rpm**) at **30 °C** overnight.

5 Extract plasmid using Qiagen Miniprep spin column following manufacturer's instructions.

6 Transform T7 Express LysY(NEB C3031) cells with extracted plasmid.

7 Grow out E. coli to generate long term storage stock in [M]**25 % volume glycerol** in a cryovial tube as described in https://www.addgene.org/recipient-instructions/myplasmid/#long.
Store at **-80 °C** indefinitely.

Day 1: Grow fresh colonies

8 Freshly streak out a LB agarose plates with [M]**100 ug/mL Carbenicillin** from the glycerol stock. Allow to grow overnight at **30 °C** in an incubator.

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Day 2: Expand single colony

9 Pick a single colony to inoculate **5 mL LB/carb** and incubate with shaking (**250 rpm**) at **30 °C** overnight.

Day 3: Induce Expression

10 Add 2mL inoculum (expanded liquid colony) to **1 L LB/carb** in a 2 L capacity Erlenmeyer flask.

11 Grow to O.D.600 = .400-500 (**04:00:00 roughly**) at **30 °C** with shaking (**250 rpm**) , then let cool to **Room temperature** .

12 Add IPTG to **0.1 Milimolar (mM)** final concentration (**1 mL** of **100 Milimolar (mM)** stock) for induction at **18 °C** - **22 °C** on shaker with mild agitation (**20 rpm**) overnight.

We found no difference in temperature ranges from 18C to 22C during induction.

Day 4: Cell Pelleting and Protein Extraction

13 Spun down cells with a JA-10 rotor (Beckman Coulter) at **6000 x g** for **00:25:00** in 500 ml bottles (make sure bottles are balanced).

14 Decant supernatant and keep pellets on ice.

Safe stopping point: Pellets can be frozen in **-80 °C** and stored for a week.

15 Prepare **1 L** HEGX Buffer.

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Reagent	Stock Concentration	Final Concentration	Amount of Stock
HEPES-KOH (pH 7.2)	1M	100mM	20mL
NaCl	Dry	800mM	46.8g
EDTA (pH 8.0)	0.5M	1mM	2mL
Glycerol	100%	10%	100mL
Triton-X100	100%	0.2%	2mL

16 **Perform steps 16 onwards in a cold room.**

Resuspend pellet in **75 mL** **4 °C (ice chilled)** HEGX Buffer and **3 tablets** of EDTA-free protease inhibitor tablets in 100 ml beaker. Keep everything at **4 °C** .

17 Add a sterile magnetic stir bar and break up pellet on a stir plate in a cold room. Keep everything at **4 °C** .

18 Sonicate for **00:15:00** total:

Perform **00:00:15** **14 Watt pulses** pulses with a **00:00:15** break between each pulse. (30 pulses total).

Remove stir bar from beaker before sonicating. Keep everything on ice during sonication. Avoid foaming solution during sonication

19 Spin down lysate in 2 x 50 mL oak ridge tubes in JA-16 or JA-20 rotor.
Make sure tubes are balanced.

15000 rpm for **00:30:00** , **4 °C**

20 **Aliquot** **30 µl** **supernatant for future protein gel to check induction and purity.**

Pour remaining supernatant into clean 100 ml beaker with stir bar.

21 **Precipitate DNA:**

To supernatant in beaker, add **2 mL** dropwise of **5 % volume** Poly(ethyleneimine) Solution as it stirs on stir plate to precipitate DNA.

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Reagent	Stock Concentration	Final Concentration	Amount of Stock
Poly(ethyleneimine) Solution	50%	5%	1mL
HEPES-KOH (pH 7.2)	1M	20mM	200 uL

Add PEI solution very slowly, dropwise down sides of beaker by a transfer pipette.
Solution will become cloudy.
Do not precipitate DNA too quickly.

22 Spin down supernatant in 2 x 50mL oak ridge tubes in JA-16 or JA-20 rotor.
Make sure tubes are balanced.

12000 rpm for **00:10:00** , **4 °C**

23 **Aliquot 30 µl supernatant for future protein gel to check induction and purity.**

Pour remaining supernatant (henceforth called Tn5 lysate) into clean 500 ml beaker on ice.
Bring volume up to **150 mL** ice cold HEGX Buffer.

24 **Chitin Column preparation and loading Tn5 lysate:**

Clamp column on a stand in cold room for gravity flow.

Column type: Kimble-Chase Flex-Column Economy Columns with two 3-way valve stop-cocks

We've been using a 30cm (1 cm ID) column which enables you to add 24 mls volume during elution.

25 Add **10 mL** of Chitin resin using 10 ml serological pipet to column and allow resin to settle and pack on bottom of column

26 Equilibrate resin with **200 mL** of HEGX by gravity flow. Discard flow through.

27 Load **150 mL** of Tn5 Lysate by gravity flow over column.

SAVE this flow through for future protein gel to check induction and purity.

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28 Once all protein solution is loaded on column, wash column with **200 mL** HEGX by gravity flow. Discard any flow through.

29 **Elution:**

Allow all wash buffer to drain through column and close bottom stopcock.

29.1 Add **24 mL** of HEGX with **100 Milimolar (mM)** DTT directly to top of column material (Chitin) by a 25 ml serological pipette.

Reagent	Stock Concentration	Final Concentration	Amount of Stock
HEGX Buffer	1X	1X	24mL
DTT	Dry	100mM	0.37g

29.2 Open bottom stopcock and let **11 mL** of HEGX with **100 Milimolar (mM)** DTT to flow through column. Measure volume by allowing volume to flow into 15mL falcon tube.

29.3 Then close stop-cock and cap stop-cock to prevent any liquid from leaking from column. Cap stopcock on top of column too.

29.4 Leave HEGX/DTT solution on column in cold room for **48:00:00** to **72:00:00**

Day 5: Elution and Dialysis

30 **Elution continued:**

Collect **9 mL** of flow through from column.

31 Dialyze against **1 L** of Dialysis buffer for **02:00:00** to **03:00:00** in a cold room.4

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Reagent	Stock Concentration	Final Concentration	Amount of Stock
HEPES-KOH (pH 7.2)	1M	100mM	100mL
NaCl	5M	200mM	40mL
EDTA (pH 8.0)	0.5M	0.2mM	400uL
Glycerol	100%	20%	200mL
Triton-X100	100%	0.2%	2mL
Prior to adding DTT, Dialysis buffer can be stored at 4C			
DTT	Dry	2mM	0.308g

We use a Slide-a-Lyzer G2 cassettes with 10 KDa cutoff (ThermoFisher, No. 87731).

32 Exchange buffer with **1 L** of fresh dialysis buffer and dialyze overnight.

Day 6: Size check and storage

33 Measure O.D.280. It should be about 4.0.

34 **Aliquot 30 µl supernatant for future protein gel to check induction and purity.**

Run an SDS-PAGE gel following manufacturer's instruction on aliquots from steps 20, 23, 27 and 34 to check purity, and induction.

35 After dialysis, add approximately **6 mL** of sterile 100% glycerol to bring final concentration of glycerol to 55% (for approx. 15 ml final volume).

Aliquot in cryotubes and freeze at **-20 °C** . Protein is good for at least 1 year with proper storage.

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