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Homemade Tn5 transposome assembly and activity evaluation V.1

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Wei Xu[1]

[1]GMU-GIBH Joint School of Life Sciences, The Guangdong-Hong Kong-Macao Joint Laboratory for Cell Fate Regulation and Diseases,Guangzhou Medical University

Nianchao NGS

Wei Xu
GMU

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

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Abstract

Tn5 Transposome Assembly and Activity Assessment.

Guidelines

This protocol is suitable for the assembly of purified Tn5 protein with a concentration between 0.5~1 μg/μl.

Materials

	A	B	C
	Reagent	Source	Cat.No.
	BSA protein standards	Abcam	ab270701
	2% (wt/vol) digitonin	Promega	G9441
	DNA Clean and Concentrator-5 kit	zymo	Cat#D4014
	SYBR Green	Thermo	S7563
	NEBNext® High-Fidelity 2X PCR Master Mix	NEB	M0541L

Before start

This protocol is basically for homemade Tn5 assembly and activity evaluation in ISSAAC-seq. Tn5 activity is determined by bulk ATAC-seq experiments, which mimics the *in situ* tagmentation condition in ISSAAC-seq experiments.

Unloaded Tn5 is available from vendors such as Diagenode (C01070010-10) or Lucigen (Cat. No. TNP92110). We obtain Tn5 from a local vendor, supplied at approximately 0.5 μg/μl. Also, there are many standard protocols for Tn5 purification, and you can produce your own Tn5 protein if needed. Here are some references for Tn5 purification:

1) **Generation and Purification of pTXB1.Tn5 (protocols.io)**

2) Picelli, Simone, et al. "Tn5 transposase and tagmentation procedures for massively scaled sequencing projects." Genome research 24.12 (2014): 2033-2040.

3) Soroczynski,J., et al., OpenTn5 Project: Open-source resource for robust and scalable Tn5 transposase purification and characterization. Molecular Biology of the Cell, 2023. 34(2): p. 1026-1026.

Tn5 protein concentration determination by Western blot.

1d

1 The concentration and purity of in-house purified Tn5 protein could be further confirmed by Western blot before you start assembly. Tn5 protein is about 53 kDa, and its target band is between 55 kDa and 70 kDa markers on the gel.

1.1 Prepare BSA protein concentration standards: dilute 3 µL of 2 mg/ml BSA standard with 21 µL of water to a final concentration of 0.25 mg/ml.

1.2 Prepare BSA standards for WB : 5m

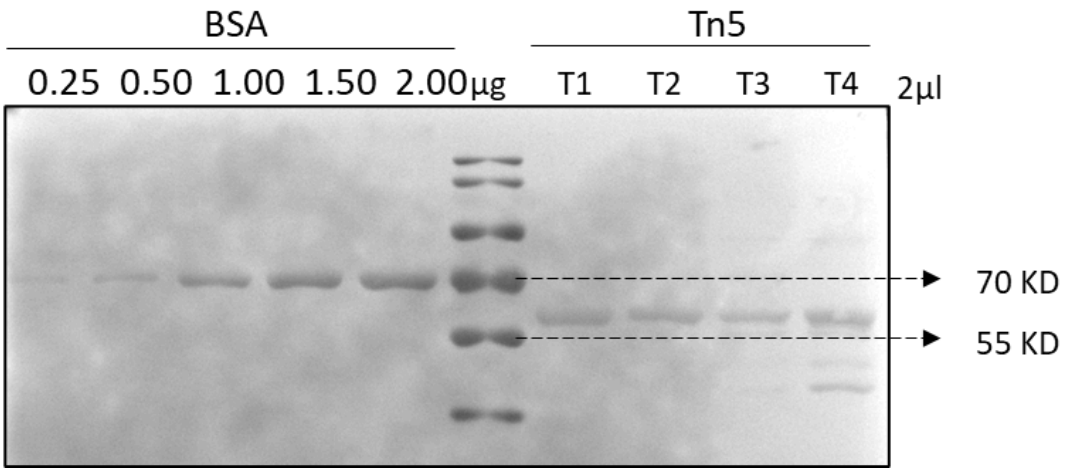
	A	B	C	D	E
	Standard No.	0.25 mg/ml BSA (µl)	Water (µl)	5x loading (µl)	Amount (µg)
	1	1	15	4	0.25
	2	2	14	4	0.50
	3	4	12	4	1.00
	4	6	10	4	1.50
	5	8	8	4	2.00

Incubate at 95 °C for 00:05:00 , centrifuge briefly .

1.3 For each batch, mix 2 µL Tn5, 4 µL 5x loading buffer, and 14 µL water. 5m
Incubate at 95 °C for 00:05:00 , centrifuge briefly .

1.4 Load the samples on a SDS-PAGE gel. After electrophoresis, the protein was transferred to NC membrane and stained with Ponceau S. Compare Tn5 with BSA standards to determine protein concentration.
Expected result 1

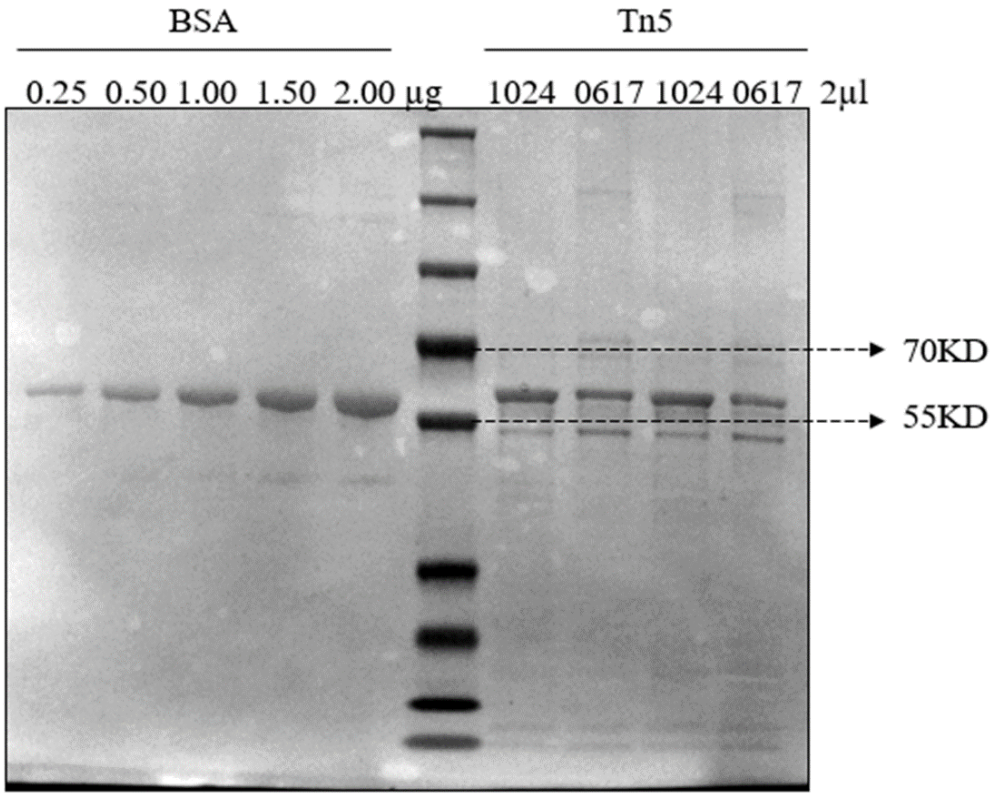
Expected result



Tn5 concentration detremination by WB. (Sample T1-T4 are four different batches of purified Tn5 protein.The concentrations of samples T1 to T4 are all between 0.5-0.75 µg/µl, and can be used for the next step of assembly. Sample T4 has a relatively high concentration.Therefore, assembled T4-Tn5 may require a reduced amount to achieve similar cleavage efficiency compared to other groups.)

Expected result 2

Expected result



Tn5 concentration detremination by WB. (1024 and 0617 are two different batches of purified Tn5. The concentration of batch1024 is about 0.5 µg/µl, and can be used for the next step of assembly. Batch 0607's concentration is about 0.25 µg/µl, which is not suitable for this protocol and needs to be further concentrated.)

Reagent Setup

30m

2 Reagent Setup

2.1 Prepare Annealing Buffer:

	A	B	C	D
	Component	Stock concentration	Final concentration	For 1 ml
	Tris-HCl, pH=8.0	1 M	10 mM	10 µl

	A	B	C	D
	NaCl	5 M	50 mM	10 µl
	EDTA, pH=8.0	0.5 M	1 mM	2 µl
	Nuclease-free water	--	--	978 µl

Store at -20 °C for up to 6 months.

2.2 Prepare Coupling Buffer:

	A	B	C	D
	Component	Stock concentration	Final concentration	For 1 ml
	Tris-HCl, pH=7.5	1 M	50 mM	50 µl
	NaCl	5 M	100 mM	20 µl
	EDTA, pH=8.0	0.5 M	0.1 mM	0.2 µl
	Triton X-100	10%	0.1%	10 µl
	DTT	1 M	1 mM	1 µl
	Glycerol	100%	50%	500 µl
	Nuclease-free water	-	-	412 µl

Store at -20 °C for up to 6 months.

2.3 Prepare oligonucleotides :

	A	B	C
	#name	seq (5'-3')	Purification
	ME_S5	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG	HPLC
	ME_S7	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG	HPLC
	ME_bottom	Phos-C*T*G*T*C*T*C*T*T*A*T*A*C*A*C*A*T*C*/iInvdT/	HPLC
	N7 primer	CAAGCAGAAGACGGCATACGAGATTCGCCTTAGTCTCGTGGGCTCGG	HAP
	S5 primer	AATGATACGGCGACCACCGAGATCTACACCTCTCTATTCGTCGGCAGCGTC	HAP

Dissolve the oligos in annealing buffer from above, to a final concentration of 100 µM.
Store at -20 °C for up to 6 months.

2.4 Prepare 4x THS TD Buffer:

	A	B	C
	Reagent	Final concentration	For 1 ml
	1 M Tris-HCl (pH 8.0)	132 mM	132 µl
	5 M potassium acetate	264 mM	52.8 µl
	1 M Magnesium acetate	40 mM	40 µl
	N,N-dimethylformamide	64%	640 µl
	Nuclease-free water	-	135.2 µl

2.5 Prepare 0.1% (wt/vol) digitonin: Mix 0.5 µL 2% (wt/vol) digitonin stock with 9.5 µL nuclease-free water.
CRITICAL Make fresh. The Promega 2% (wt/vol) digitonin stock is dissolved in DMSO. Thaw at RT prior to use.

Tn5 transposome Assembly

1h 3m

3 Adapter annealing.

3.1 Mix 25 µL ME_S5 oligo (100 µM) with 25 µL ME_Bottom oligo (100 µM) in a PCR tube, and label as "S5_adapter".

3.2 Mix 25 µL ME_S7 oligo (100 µM) with 25 µL ME_Bottom oligo (100 µM) in a PCR tube, and label as "S7_adapter".

3.3 Anneal adaptor oligo mixtures in a Thermocycler with the following reaction: 3m

- 98 °C 00:03:00
- slowly cooled to 16 °C with a temperature ramp of −0.1 °C/s.
- 16 °C hold.

3.4 Dilute the S5_adaptor ([M] 50 micromolar (µM)) and S7_adaptor([M] 50 micromolar (µM)) with nuclease-free water to([M] 20 micromolar (µM)).
Continue to Step 8 or store at -20 °C for up to 6 months.

3.5 Assemble the Tn5 enzyme and annealed oligos: 1h

	A	B
	Component	Amount (µl)
	Tn5	48

	A	B
	Annealed S5_adapter (20 µM)	12
	Annealed S7_adapter (20 µM)	12
	Coupling buffer	69
	Total	141

Incubate at room temperature for 01:00:00. Store at -20 °C for up to 1 year.

Tn5 activity evaluation

1d

4 Perform a fast ATAC-seq experiment to determine the activity of assembled Tn5.

4.1 Pellet 50,000 cells for each sample (#1 illumina Tn5 , #2 Tn5_batch1, #3 Tn5_batch2...) at 1,000g, 4 °C for 3 min in a fixed-angle centrifuge.

4.2 Aspirate and discard all supernatants, resuspend cell pellets in 1 mL ice-cold 1× DPBS–0.5% (wt/vol) BSA and centrifuge at 1,000g, 4 °C for 3 min.

4.3 Aspirate all supernatants using a P1000 pipette, and briefly centrifuge again to collect leftover buffer from the bottom of the tube. Remove traces of the buffer using a P200 pipette with a 10 µL tip fitted on top of a 200 µL tip.

4.4 Prepare 50 µL tagmentation mix for each sample by combining and mixing the reagents listed in the table below:

	A	B	C
	Reagent	Amount (µL)	Final concentration
	4× THS TD buffer	12.5	1×
	0.1% (wt/vol) Digitonin	5	0.01% (wt/vol)
	Tn5	2.5	-
	Nuclease-free water	30	-
	Total	50	-

4.5 Resuspend the cell pellet in 50 µL tagmentation mix by pipetting up and down 25 times.

4.6 Put the 50 µL reaction on a thermomixer, and incubate at 37 °C, 800 rpm for 30 min.

4.7 Cleanup reaction with a Zymo DNA Clean and Concentrator-5 kit, elute DNA in 21 µL of elution buffer.

4.8 Set up the following PCR reaction:

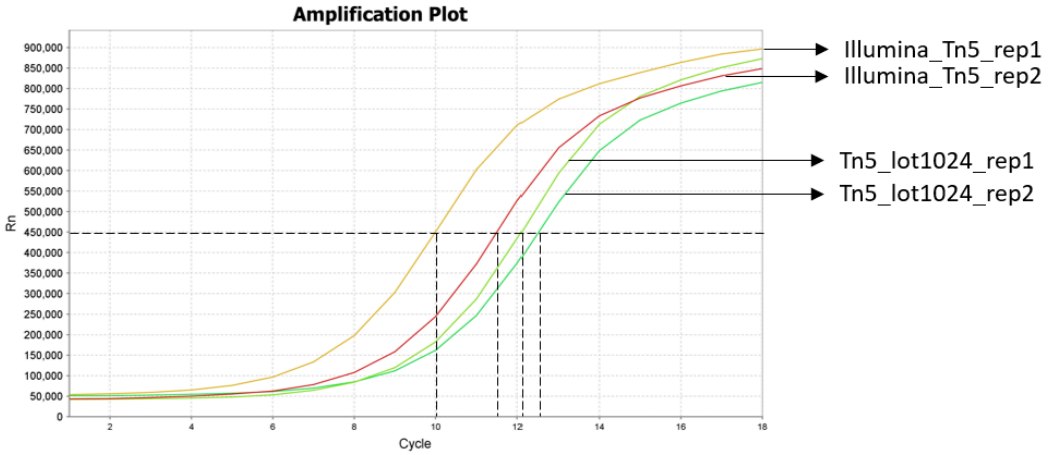
	A	B
	Reagent	Amount (µL)
	Purified sample	8
	N7 primer (10 µM)	1
	S5 primer (10 µM)	1
	NEBNext High-Fidelity 2× PCR Master Mix	10
	Total	20

4.9 Take out 9 µL of each reaction, and mix with 1 µL 10X SYBR Green and perform a qPCR analysis to decide the cycle number.

4.10 Use the following cycling condition to perform a qPCR analysis, and monitor the amplification curve in linear scale.

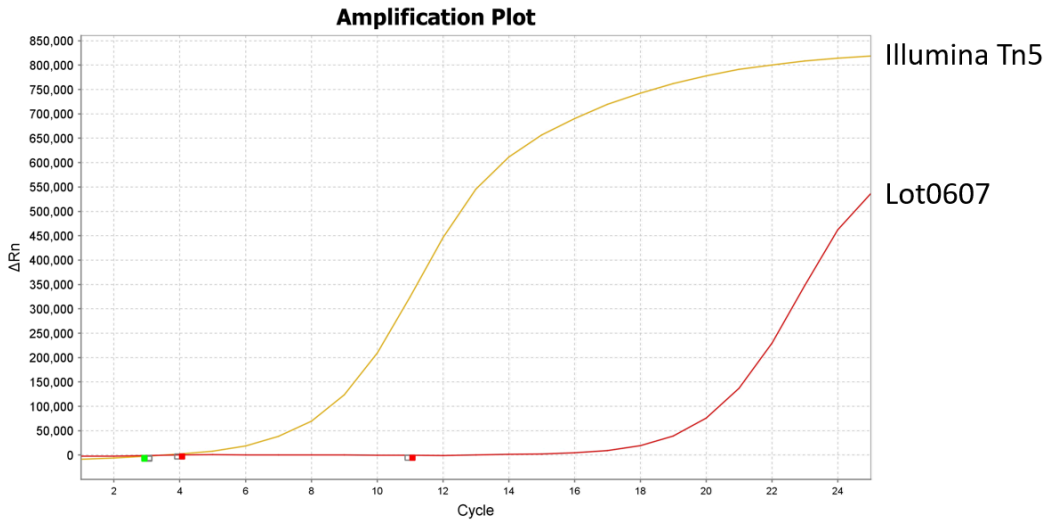
	A	B	C	D
	Steps	Temperature(℃)	Time	Cycles
	Gap fill-in	72	5 min	1
	Initial Denaturation	98	1 min	1
	Denaturation	98	10 s	30
	Annealing	63	30 s	
	Extension	72	20 s	

Expected result



Amplification Plot of qPCR. The Ct values of Tn5_lot1024 are comparable to illumina_Tn5, thus can be used in subsequent experiments.

Expected result

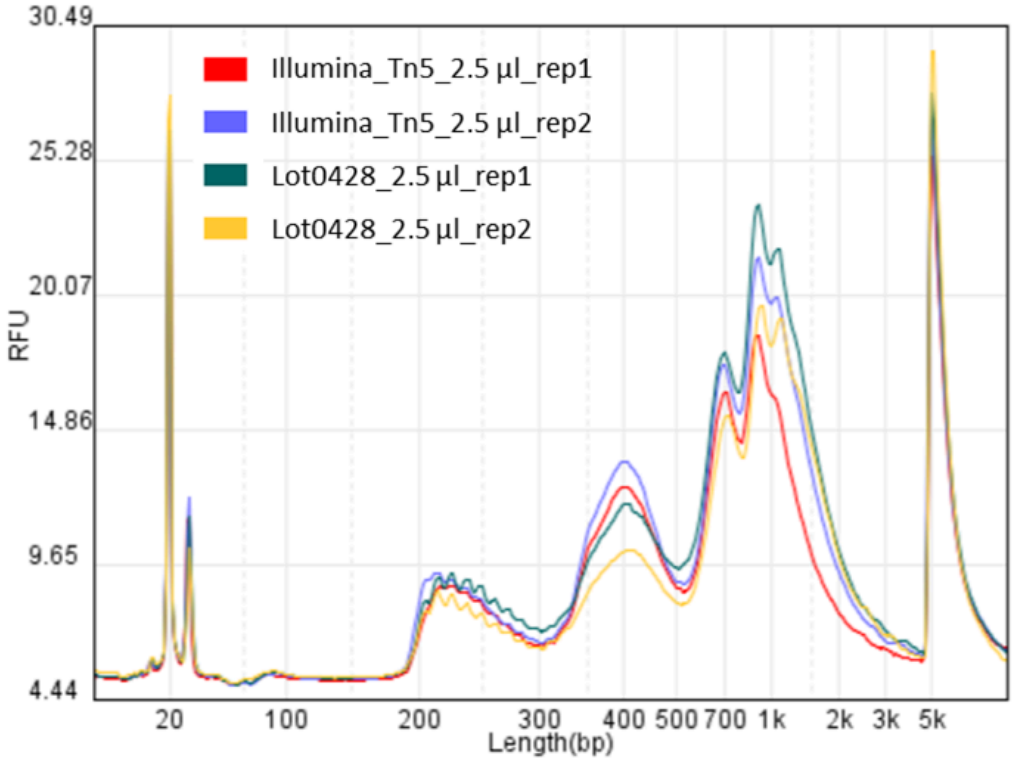


Amplification Plot of qPCR. The Ct value of lot0607 was significantly different from that of the illumina control, indicating that the purification may fail, and this batch of Tn5 cannot be used in subsequent experiments.

4.11 Validation of ATAC-seq library fragment length distribution.

Amplify the rest 11 µL PCR reaction for 12 cycles, purify the PCR product using 1.2x VAHTS DNA Clean Beads, elute in 20 µL water and check the size distribution using a bioanalyzer.

Expected result



Distribution of ATAC-seq libraries. (Successful experiments with a prominent nucleosomal ladder pattern.)