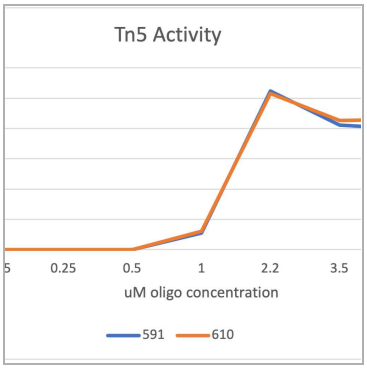

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We use this protocol and it's working

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Assay for determination of functional concentration of Tn5 transposase

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ABSTRACT

Tn5 is used by multiple labs world-wide for its ability to introduce DNA oligos and barcode sequences into libraries and for genomic assays. In many instances, labs produce their own Tn5 enzyme in-house rather than from commercial sources. Our method provides a means of determining the functional concentration of Tn5 in preparations by qPCR to standardize amounts of Tn5 used in assays and identify batch/lot variability. The current standard for assaying in-house produced Tn5 is a plasmid smear. Purified Tn5 is loaded with oligonucleotides and mixed with a plasmid substrate. The plasmid is then run on an agarose gel and checked for smearing, indicating the DNA was cut by the Tn5. The amount of Tn5 is standardized by measuring protein concentration using a protein assay or absorbance at 280nM which provides an approximation based on total protein present, not the functional concentration.This method is modified from Rykalina et al. to evaluate and empirically determine the functional concentration of Tn5 transposomes in homemade enzyme preps by qPCR. The principle is based on decreased Cp(or Cq) values correlating with increased fragmentation caused by the transposome.The change in Cp values functions as a measurement of the activity of the Tn5 at that concentration of oligonucleotide (the greater the number of cycles required to produce a product correlates with the increased cleavage of the plasmid substrate by Tn5) as plasmids with transposomes insertions (cleaved regions) will not amplify. The change in Cp values is then plotted against the oligonucleotide concentration in a line graph and a plateau will appear at the concentration at which the Tn5 is saturated by oligo. The first point in the plateau is the functional concentration.

This method may also be applied to testing the efficiency of changing DNA sequences in oligos used to assemble transposomes as the activity of the transposase is dependent upon the binding sequences contained within the oligo as well as secondary structure formed by the oligo. This property enables testing of oligo variations and barcode efficiencies in our assay. For instance, oligos containing different lengths or different barcodes sequences can be assembled in transposomes and tested in comparison to standard oligos

MATERIALS

HEPES, pH 7.2 (1M)	FisherScientific	AAJ16924K2
NaCl (5M)	Invitrogen	AM9760G
EDTA (0.5M)	Invitrogen	AM9260G
Triton X-100 (10%)	VWR	97063-864
DTT (1M)	Krackeler	45-43816-50ML
Glycerol (100%)	VWR	MK509202
Nuclease-free water	Invitrogen	AM9932
Tn5 or TDE1	homemade or Illumina	20034197
SDS (10%)	Invitrogen	15553027
pUC19	NEB	N3041S
EcoRI	NEB	R3101S
Zymo Research Clean and Concentrate-5	Zymo	D4014
LUNA 2x qPCR master mix	NEB	M3003S
Qubit 1X dsDNA HS Assay Kit	Invitrogen	Q33231

Equipment:

Eppendorf Thermomixer

qPCR thermocycler

Qubit

Primers:

Transposome

A	B
Tn5ME_Rev	/5Phos/CTGTCTCTTATACACATCT
Tn5ME-A (Illumina FC-121-1030)	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG
Tn5ME-B (Illumina FC-121-1031)	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG

qPCR primers

A	B	C
Name	**Sequence**	pUC19 location(bp)
591bp_pUC19_Tn5_F	GCTCACTCAAAGGCGGTAAT	748-1319
591bp_pUC19_Tn5_R	CTTCAGCAGAGCGCAGATAC	
602bp_pUC19_Tn5_F	CTTTCACCAGCGTTTCTGGG	2396-248
602bp_pUC19_Tn5_R	GCTGGCGTAATAGCGAAGAG	
610bp_pUC19_Tn5_F	CCTATCTCAGCGATCTGTCTATTTC	1651-2241
610bp_pUC19_Tn5_R	GCGCGGTATTATCCCGTATT	

Transposome preparation and assembly

1h 29m

1 Prepare ME A/Rev or ME-B/rev oligos by annealing equal concentrations of primers. 22m
We typically target 40uM stocks.
Example: 40uL 100uM Tn5-ME-A +40uL 100uM Tn5-REV +20uL nuclease-free water or 0.1x TE (can be scaled as needed)
Thermocycler program: 95°C for 2min, slow cool to 25deg at 0.1°C/sec, total time ~22 minutes.
00:22:00

1.1 Prepare working stocks of annealed oligos by serial dilution(1:1): 1.25uM, 2.5uM, 5uM, 10uM, 20uM, 40uM

2 Prepare Tn5 transposomes by combining 9uL of the Tn5 to be tested with 1uL annealed oligos (equates to final concentration of: 0.125uM,0.25uM, 0.5uM, 1uM, 2uM, 4uM)
a. If the estimated concentration of Tn5 is high, prepare serial dilutions first in Tn5 exchange buffer
b. Volumes may be titrated down if Tn5 to be tested is in limited quantities(i.e 0.5uL oligo in 4.5uL Tn5)

2.1

	A	B	C	D	E	F	G
	Oligo Concentration(uM)	1.25	2.5	5	10	20	40
	Oligo(uL)	1	1	1	1	1	1
	Tn5(uL)	9	9	9	9	9	9
	Final vol	10	10	10	10	10	10
	Final Conc.(uM)	0.125	0.25	0.5	1	2	4

2.2 Incubate for 21 hours at 25°C w/shaking(600rpm) on Eppendorf Thermomixer
Overnight 21 h
Shorter incubations (1 hour) are possible, but we find the longer incubations provides better, more consistent, results.

Template preparation

1h 29m

3 01:00:00 — 1h

Digest pUC19 with EcoRI to generate a linear template.

a. Column purify the plasmid DNA and adjust concentration to 25ng/uL

b. The digest reaction may be scaled to provide a stock of linearized plasmid for future use

Tagmentation

1h 29m

4 In duplicate, prepare tagmentation master mix and test each concentration of transposome on 25ng linearized pUC19

a. Include a no Tn5 control for reference (0uM)

b. For no Tn5 control, increase water to 11.5uL/reaction

c. 10X Tango restriction enzyme buffer (ThermoFisher) or 10X Cutsmart buffer(NEB) are used to provide the magnesium Tn5 requires for tagmentation. A recipe for an alternative 2X buffer is listed below.

	A	B
	Stock	***Vol per 25uL rxn (uL)***
	10X Tango RE Buffer	2.5
	Nuclease-free water	18
	DMF	2.5
	Linearized pUC19(25ng/uL)	1
	Tn5	1

Tagmentation Master Mix

2X Tagmentation Buffer

	Stock	Vol for 10mL	Final Conc.	Supplier
	1M Tris-HCl pH7-8	200uL	20mM	Invitrogen #AM9850G, #AM9855G
	1M MgCl2	100uL	10mM	Invitrogen #AM9530G
	Nuclease-free water	9.7mL		Invitrogen AM9932

5 Incubate at 37°C for 1hr at 600rpm

6 Stop the reaction by adding 1uL of 2%SDS to each reaction(final conc. 0.08%) 7m
a. 55°C for 7 minutes to remove transposomes 00:07:00

7 Quench SDS by adding 3ul 10% Triton X-100 to reactions

8 Final volume should be 29uL

9 Qubit or nanodrop DNA for normalization (~0.86ng/uL)
a. Quantifying the DNA first enables the assay to be more quantitative

qPCR 1h 29m

10 Dilute transposed DNA 1:10 in nuclease free water for qPCR
a. using too much DNA may lead to difficulty in determining Cp and lead to higher data variability
b. Controls: linearized pUC19 without Tn5 and a NTC control

11 Setup qPCR reactions using primer pairs (recommend running at least 2 pairs)
a. Pairs 591, 602, and 610 may be run on the same program

12 Run qPCR using following settings:
a. 95°C for 1 min, followed by 35(minimum) to 45 cycles of 95°C for 15 sec, 60°C for 20 sec, 72°C for 30sec, then a melt curve
Two-step protocols may also be used alternating 95°C for 15 sec, 60°C for 20 sec

13 Analyze and graph delta Cp versus concentration.
a. The no Tn5 control provides the reference value as the plasmid should be intact (i.e Subtract the Geomean of the no Tn5 replicates from the Cp of the test samples).
b. The higher the delta Cp, the more cleavage of the plasmid, indicating higher Tn5 tagmentation activity
c. The concentration at which the graph plateaus or peaks at, is the functional concentration of

the Tn5 prep

Data Analysis Example

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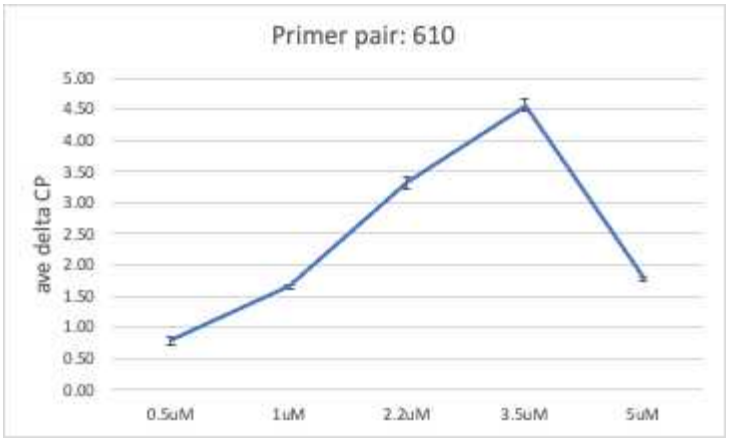
	Primer pair:610					
	Sample	Cp	Ave Cp	delta Cp		
	0.5	8.89	8.78	8.98	8.88	0.74
	0.5	9.12	8.81	8.97	8.97	0.83
	1	9.74	9.8	9.74	9.76	1.62
	1	9.67	9.99	9.79	9.82	1.68
	2.2	11.36	11.34	11.49	11.40	3.26
	2.2	11.37	11.67	11.57	11.54	3.40
	3.5	12.52	12.63	12.73	12.63	4.49
	3.5	12.7	12.74	12.86	12.77	4.63
	5	9.83	9.94	9.92	9.90	1.76
	5	9.79	10.05	9.97	9.94	1.80
	noTn5	8.2	8.02	8.18	8.13	-0.01
	noTn5	8.25	7.97	8.21	8.14	0.00
	NTC	24.04	23.92	23.9	23.95	15.81
	NTC	24.27	24.25	24.26	24.26	16.12

Example results from qPCR testing of a Tn5 prep

	Primer pair:610		
	Conc.(uM)	ave delta CP	stdev
	0.5uM	0.79	0.06
	1uM	1.65	0.04
	2.2uM	3.33	0.10
	3.5uM	4.56	0.10
	5uM	1.78	0.03


Primer pair: 610
ave delta CP
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
0.5uM
1uM
2.2uM
3.5uM
5uM