Diluted Transposome 96-well Tagmentation and Amplification

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This protocol uses the following purchased reagents:

Company	Kit	Catalog Number
Illumina	Nextera DNA Sample Preparation Kit	FC-121-1030
IDT	Indexed primers	custom order
Clonetech	Ex Taq	RR001A
Qiagen	QIAquick Gel Extraction Kit	28704 or 28706

Tagmentation

1. Dilute DNA samples first to 1.66 ng/μl.
   - If you used a column-based assay (e.g. Omega BioTek prep) and lysed enough worms, you can saturate the column such that each column elutes approximately the same amount of material.
   - On average, we get 35 ng/μl and we dilute each sample as if it has a concentration equal to that of the average: 9.5 μl of DNA sample with 190.5 μl of dH_2O.
   - M**ake sure that the plates are in the same orientation and change tips between rows!**

2. Dilute the DNA samples a second time to 0.2 ng/μl by combining 12 μl of the 1.66 ng/μl DNA with 88 μl of dH_2O.
   - **Make sure that the plates are in the same orientation and change tips between rows!**

3. Dilute Illumina-purchased Tagmentation Enzyme 1/35 in 1X Tris buffer (see recipe below).
   - For tagmenting a single 96-well plate, you can add 7.5 μl of the Tagmentation Enzyme to 255 μl of 1X Tris Buffer for a total of 262.5 μl.

4. Prepare a master mix for the tagmentation reaction:
   - Final tagmentation volume is 10 μl.
   - You might have to alter the DNA and the dH2O volumes depending on the amount of DNA you want to add. We used 0.75 ng of DNA prepped from the Omega Bio-tek kit in a 10 μl tagmentation reaction.
   - *Prepare reactions on ice.*

Reagent	1 Reaction (μl)	100 Reactions (μl)
10X Tris Buffer	1	100
dH2O	2.75	275
0.2 ng/μl DNA	3.75	-
1/35 Transposome	2.5	250

5. Add 6.25 μl of the master mix to each well of your 96-well PCR tagmentation plate.

6. Use a multi-channel micropipette to add 3.75 μl of the 0.2 ng/μl DNA samples to each well. **Make sure the plates are in the same orientation and change tips between each addition!**

7. Incubate the reactions in a thermocycler with the following program:

Number of Cycles	Temperature	Time
1	55ºC	5 minutes
1	10ºC	hold

8. Immediately proceed to amplification step.

Amplification

9. Prepare an amplification master mix according to the recipe listed below.
   - Use a 10 μM mix of primers that contains both the Index 1 and Index 2 primers, with *each* primer at a concentration of 10 μM.
   - Final volume of PCR is 25 μl.

Reagent	1 Reaction (μl)	100 Reactions (μl)
10X Ex Taq Buffer	2.5	250
2.5 mM dNTPs	2	200
Ex Taq Polymerase	0.1875	18.75
dH2O	14.8125	1481.25
10 μM Primer Mix (indices)	0.5	-
Template (Tagmentation Reaction)	5	-

10. Add 19.5 μl of the master mix to each well of a 96-well PCR amplification plate.

11. Add 0.5 μl from each well of the 10 μM Primer Mix plate to each corresponding well of the amplification plate.

- Use a multi-channel micropipette to add the primers. **Make sure that the plates are in the same orientation and change tips between rows!**

12. Add 5 μl from each well of the tagmentation reaction plate to each corresponding well of the amplification plate.
    - Use a multi-channel micropipette to add the DNA. **Make sure that the plates are in the same orientation and change tips between rows!**
    - Freeze remaining tagmentation samples at -20ºC.

13. Incubate in a thermocycler with the following program:

Number of Cycles	Temperature	Time
1	72ºC	3 minutes
1	95ºC	30 seconds
20	95ºC	10 seconds
	62ºC	30 seconds
	72ºC	3 minutes
1	10ºC	hold

14. In a microcentrifuge tube, combine 8 μl from each library to make a 96-sample pooled library.
    - You can use a 12-channel pipettor to pipette 8 μl from each row into a sterile trough. **Change tips between each row!** Then, combine all the material in the trough into an eppendorf tube.
    - You can also electrophorese 5 μl of each of the amplification samples to determine the state of tagmentation/amplification. After you have conducted this protocol a few times, this should not be necessary.

15. Remove 170 μl of the pooled material and put in a new microfuge tube. Freeze the remaining pooled material. To the 170 μl, add 30 μl of 6X loading dye.

16. Electrophorese the 170 μl of pooled library on a 2% agarose gel.
    - If you have large-volume combs, use these to make your gel.
    - If you don't have large-volume combs, tape together wells on your normal gel comb to create a large well that can hold 200 μl of sample.

17. Excise the DNA that corresponds to 450-600 bp in size.

18. Weigh the gel slice in a clear microcentrifuge tube. Add 3 volumes of Buffer QG to 1 volume of gel (e.g. - add 300 µl of Buffer QG to each 100 mg of gel).
    - If the gel slices weigh more than 400 mg, you can either:
      - proceed as if the gel weighed 400 mg or

- add the appropriate Buffer QG and isopropanol (see below) based on the weight of the gel slice. If you do this, you will have to do multiple spins to bind all the sample to one column.

19. Incubate at 50°C for 10 minutes or until the gel slice has completely dissolved.

20. Add 1 gel volume of isopropanol to the sample and mix by inverting the tube several times.

21. Place a labeled QiaQuick column in a provided 2 ml collection tube in a suitable rack.

22. To bind DNA, apply the sample to the labeled QiaQuick column and centrifuge for 1 min.

23. Discard flow-through. Place the QiaQuick column back into the same tube.

24. Add 500 µl of Buffer QG to the spin column and centrifuge for 1 min.

25. Discard flow-through. Place the QiaQuick column back into the same tube.

26. To wash, add 750 µl Buffer PE to the QiaQuick column, incubate at room temperature for 2 min and centrifuge for 1 min.

27. Discard flow-through and place the QiaQuick column back in the same tube. Centrifuge the column for an additional 1 min at maximum speed.
    - Note: Residual ethanol from Buffer PE will not be completely removed unless the flow-through is discarded before this additional centrifugation.

28. Place the QiaQuick column in a clean, labeled 1.5 ml microcentrifuge tube.

29. To elute DNA, add **30 µl** Buffer EB (10 mM Tris·Cl, pH 8.5) to the center of the membrane, let the column stand for 1 min, and then centrifuge for 1 min.
    - Note: Ensure that the elution buffer is dispensed directly onto the center of the membrane for complete elution of bound DNA.

30. Quantitate your pooled libraries using the Qubit HS kit.
    - Label enough Assay Tubes for your samples, standard 1 and standard 2.
    - Prepare the Qubit Working Solution by diluting the Qubit HS reagent 1:200 in Qubit buffer. Prepare 200 µl of Working Solution for each standard and sample.
    - For each standard, add 190 µl of of Working Solution to the appropriate tubes.
    - For each sample, add 199 µl of Working Solution to the appropriate tubes.
    - Add 10 µl of each standard to the appropriate tube.
    - Add 1 µl of each sample to the appropriate tube.
    - Briefly vortex all tubes.
    - Incubate the tubes for 2 minutes at room temperature.
    - Insert tubes in the Qubit Fluorometer to take readings.

31. Determine molarity on this site: http://molbiol.edu.ru/eng/scripts/01_07.html.

Pooling more than 96 samples?

- If you are pooling multiple plates of samples, you can make a pool of pools:

- For each plate, combine the samples as suggested above (8 μl of each sample).
- Electrophorese 170 μl of each plate pool separately and excise the appropriate DNA (400-600 bp) band for each plate pool.
- Gel purify each plate pool independently.
- Determine the DNA concentration for each purified sample.
- Convert to nM and dilute each of the plate pools to 10 nM (in either a 10 μl or 20 μl volume - use the 20 μl volume if the 10 μl volume would require pipetting to low of a volume of sample).
- Combine 5 μl of each of the 10 nM plate pools to make a pool of pools!

10X Tris Buffer Recipe
100 mM Tris-HCl pH 8.0
50 mM MgCl2