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

TaME-seq2

Forked from a private protocol

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ABSTRACT

Tagmentation-assisted multiplex PCR enrichment sequencing for viral genomic profiling (TaME-seq2). Viral genome and integration enrichment library preparation protocol.

Manuscript:
TaME-seq2: Tagmentation-assisted multiplex PCR enrichment sequencing for viral genomic profiling.
Alexander Hesselberg Løvestad, Milan S. Stosic, Jean-Marc Costanzi, Irene Kraus Christiansen, Hege Vangstein Aamot, Ole Herman Ambur, Trine B. Rounge medRxiv 2022.12.22.22283851
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Before starting:
Prepare samples consisting of extracted DNA or reverse transcribed cDNA.

The method consists of three main parts:

Part 1:
Tagmentation of samples using the Illumina DNA Prep kit.

Part 2:
Multiplex PCR reaction of tagmented DNA/cDNA, using virus-specific primers and i5/i7 indexes.

Part 3:
Clean-up and size selection using purification beads/Ampure XP beads.

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Keywords: integration, library preparation, illumina, virus, viral integrations, snp, snps, variants, whole genome sequencing, genome, genomics, enrichment, multiplex PCR

MATERIALS

	A	B	C
	Component	Supplier	Catalog number
	Illumina® DNA Prep, (M) Tagmentation (96 Samples)	Illumina	20018705
	QIAGEN Multiplex PCR Kit (100)	Quiagen	206143
	AMPure XP 60 mL	Beckman Coulter	A63881

Sample preparation

1 Prepare and normalize DNA/cDNA samples by measuring sample concentration and diluting in nuclease-free water if necessary.

Sample volume should be 15 µL

50 ng input is recommended, but the protocol works with less and performance is more dependent on viral load.

Tagmentation

2 Prepare a master mix for the tagmentation reaction and add sample DNA and master mix to individual wells.

	A	B	C
	Reagent	1x	10x
	Bead linked transposoms (BLT)	5 µL	50 µL
	Tagmentation buffer 1 (TB1)	5 µL	50 µL
	Sample DNA/cDNA	15 µL	
	Total	25 µL	10x10 µL

Tagmentation reagent mix table

Note

You should prepare 10% extra to account for loss during pipetting

3 Incubate the samples as follows: 15m

00:15:00 at 55 °C

Hold at 4 °C

Note

Let it cool down to 4 °C before taking it out of the thermocycler.

4 Add 5 µL of Tagmentation stop buffer (TSB) to each sample.

Note

The Tagmentation stop buffer needs to be vortexed well before use.

5 Incubate sample for 00:15:00 at 37 °C 15m

Note

The samples can be stored in the fridge for a few hours after this step.

6 Wash the samples 9m

1. Place tubes on the magnetic rack for 00:03:00 (or until solution is clear)
2. Discard supernatant
3. Remove tube from magnetic rack and add 50 µL Tagment wash buffer (TWB) and mix to resuspend beads

4. Place tubes on magnetic rack for 00:03:00 and remove supernatant
5. Repeat step 3 and 4 for a total of 2 washes
6. Add 50 µL TWB to samples and mix
7. Close cap on tubes and place on magnet. Allow to incubate for at least 00:03:00 and continue the protocol. The samples will be used later in the PCR

Note

The samples are submerged in TWB to stop the beads from overdrying while working on the next steps.

The samples can also be eluted in 14 µL nuclease-free water and stored in the freezer, if so do not add H2O/elution buffer to the PCR master mix in the next step.

Amplification of tagmented DNA

7 20m

1. Make the PCR master mix

	A	B	C
	Reagent	1x	10x
	2x PCR master mix	12,5 µl	125 µl
	Q-solution x5	2,5 µl	25 µl
	Primer pool (15 µM)	1 µl	
	i5/i7 indexes (10 µM)	2 µl	
	Nuclease-free water	7 µl	70 µl

	A	B	C
	Total	25 µl	22 µL x 10 µL + 3 µL primers per sample

Note

One master mix need to be prepared for each of the forward and reverse primer pools.

Nuclease-free water, primer pool and i5/i7 indexes will be dispensed in each individual well in the downstream PCR 96-well plate.

If samples were eluted in nuclease-free water after step 6, add 7 µL of the eluted sample to the individual wells instead of nuclease-free water.

2. Remove supernatant from samples prepared in step 6.7 and remove from magnetic rack
3. Add 30 µL of PCR MM to the samples, mix well and resuspend the beads (the beads can be difficult to resuspend, but they don't need to be completely respuspended)
4. Pipette 15 µL out of the sample to a new well containing the nuclease-free water, primer pool and i5/i7 indexes, for seperate F and R reactions
5. Run the touchdown PCR reaction using the following program on the thermal cycler:

Step	Temperature	Time	Cycles
Initial denaturation	95 °C	00:05:00	1
Touchdown PCR:			
Denaturation	95 °C	00:00:30	10
Annealing	68 °C – 58 °C	00:01:30	10 (decrease 1 °C per cycle)
Extension	72 °C	00:00:30	10
PCR:			
Denaturation	95 °C	00:00:30	26
Annealing	58 °C	00:01:30	26
Extension	72 °C	00:00:30	26
Final extension	68 °C	00:10:00	26
Hold	4 °C		

Note

We use a touchdown PCR as this gives the best amplification yield for us. This, and other PCR parameters, might differ between primer pools.

If amplification yield is low, consider changing the ramping temperature.

Clean up and size selection, pooling and quality control

5m

8

38m

1) After the PCR reaction is finished place the plate on the magnet for 00:05:00

2) Transfer 20 µL of the supernatant to a fresh well (transfer less than the total volume to compensate for differences in volume during pipetting).

3) Pool 10 µL of each sample in an eppendorf tubes.

4) Vortex and invert purification beads (PB) to fully resuspend.

5) Prepare at master mix of diluted PB (Note: The volumes used in the master mix is for each sample that has been pooled).

	A	B
	Reagent	Volume per reaction
	PB	10 µL
	Nuclease-free water	8,85 µL

6) Vortex and mix the diluted master mix and add 18.75 µL (per reaction) master mix to the pool and **mix well**

Note

The mixing is essential for a good size selection and increasing yield

7) Seal the tubes and incubate at room temperature for 00:05:00

8) Place on magnet for 00:05:00 5 min or until clear

9) Add 3.7 µL (per reaction) of PB to a new eppendorf tube

10) Transfer 27 µL (per reaction) of the supernatant to the eppendorf tube containing PB and **mix well**

Note

The mixing is essential for a good size selection and increasing yield

11) Seal the tube and incubate at room temperature for 00:05:00
12) Place the tube on a magnet for 00:05:00 or until clear
13) Remove and discard the supernatant without disrupting the beads
14) With the tube on the magnet, add fresh 80% ethanol to cover the beads without mixing and incubate 00:01:00
15) Remove the ethanol
16) Repeat steps 14 and 15 for a total of 2 washes
17) Remove any excess liquid from the tube
18) Air-dry on the magnet for 00:05:00 or until dry. The bead should not dry so long that it cracks while ethanol residues should have evaporated.
19) Remove the tube from the magnet and add 205 µL (more or less depending on desired reaction volume to elute in) resuspension buffer/water-free nuclease to the beads and mix
20) Incubate at room temperature for 00:05:00
21) Place tube on magnet for 00:02:00 or until clear
22) Transfer 200 µL of the supernatant into a fresh tube
23) Clean up two more times (start from step 12) using 0,65x ratio Ampure beads, elute in 42 µL and transfer 40 µL to a new tube before assessing size distribution again.

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Assess the quality of the pools using a Bioanalyzer or an equivalent instrument to see the fragment size distribution.

If there is an excess of small fragments in the library, clean up one or more times (start from step 11) using 0,65x ratio Ampure beads, elute in 42 µL and transfer 40 µL to a new tube before assessing size distribution again.