Rapid, high throughput library preparation of SARS-CoV2 using Illumina's DNA Prep Library Preparation Kit V.3

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ABSTRACT

This procedure provides instructions on how to prepare DNA libraries for whole genome sequencing on an Illumina MiSeq or NextSeq using Illumina's DNA Prep Library Preparation Kit scaled to half reaction volumes with modifications to the post-PCR procedures; tagmentation stop buffer and associated washes are removed and libraries are pooled post PCR then a single size selection is performed.

This protocol is used to sequence SARS-CoV-2 using the cDNA/PCR protocol: https://dx.doi.org/10.17504/protocols.io.b3viqn4e

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

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MATERIALS

A	B	C
Reagents	**Equipment**	**Supplies**
Illumina DNA Prep, (M) Tagmentation, 96 samples (Cat: 20018705)	Post-PCR thermal cyclers	Applied Biosystems Standard 96 well plates
Nextera UD Indexes A-D or custom IDT adapters	Single and multichannel pipettes	Adhesive plate seals and applicator
Nuclease-free water	Vortex	Reagent boats
	Plate spinner	Pipet tips; various sizes. Filter plugged and nuclease free
	SPRI 96-well Magnetic Plate	Disposable Powder-free Gloves and Gowns
	Magnetic tube rack for 1.5ml tubes	Discard pail and waste bags
		1.5ml Lo-bind snap-top tubes

Tagment Genomic DNA

10s

1 Add 15 µL of DNA to each well of 96 well PCR plate. If the DNA volume is less than 15 µl, add nuclease-free water to the DNA samples to bring the total volume to 15 µl.

Note: it is assumed that the samples from your PCR plate (labeled " amplicon") have at least 3.3ng/µl.

2 Vortex the BLT and TB1 tubes vigorously for 00:00:10 to resuspend. Spin down the tubes, but only briefly to not sediment the beads. 10s

3 Combine the following volumes to prepare the tagmentation master mix: Reagent overage is included in the 96 sample volume to ensure accurate pipetting.

A	B	C

	A	B	C
	Reagent	Volume per sample	Volume (96 samples)
	Bead-Linked Transposome (BLT)	5 µl	565 µl
	Tagmentation Buffer (TB1)	5 µl	565 µl
	Final volume	10 µl	1130 µl

4 Vortex the tagmentation master mix thoroughly to resuspend. Divide the tagmentation master mix volume equally by pipetting 141.25 µL into each well of an 8-tube strip or alternatively add the master mix to a low dead volume reservoir.

5 Using a multichannel pipette, transfer 10 µL of the tagmentation master mix to each well of the plate, pipetting each sample 10 times to resuspend. Use fresh tips for each sample column.

6 Seal the plate with adhesive seal, spin down briefly, and run the TAG program on the thermal cycler as follows:

	A	B
	Temperature	Time
	55 C	15 minutes
	10 C	Hold

Amplify Tagmented DNA

7 Thaw the Enhanced PCR Mix (EPM) on ice, vortex, and briefly centrifuge. Thaw your plate of index adapters to room temperature.

8 Combine the following volumes to prepare the PCR master mix:

	A	B	C
	Reagent	Volume per sample	Volume (96 samples)
	Enhanced PCR Mix (EPM)	10 µl	1130 µl
	Nuclease-free water	10 µl	1130 µl
	Final volume	20 µl	2260 µl

Reagent overage is included in the volume to ensure accurate pipetting.

9 Vortex and centrifuge the PCR master mix.

10 Remove the BLT plate from the thermal cycler and place on a magnet. Once the liquid is clear, use a multichannel pipette to remove and discard the supernatant.

11 Remove the plate from the magnet.

12 Using a low dead volume reservoir and a multichannel pipette, immediately add 20 µL of the PCR master mix directly onto the beads in each sample well. Pipette the mix until the beads are fully re-suspended.

13 Spin down your adapter plate before use. Using a multichannel pipette, puncture the foil seal of the adapter plate and add 5 µL of pre-pared i7 and i5 index adapters to each sample. Pipette 10 times to mix.

14 Seal the plate and spin down to remove as many bubbles as possible.

15 Place on the thermal cycler and run the BLT PCR program as follows:

	A	B	C
	Temperature	Time	Cycles
	68 C	3 minutes	1
	98 C	3 minutes	1
	98 C	45 seconds	8*
	62C	30 seconds	
	68C	2 minutes	
	68 C	1 minute	1

*Depending on the total DNA input at the beginning of the experiment, the number of PCR cycles must be altered accordingly. For specimens >100ng, 5 PCR cycles must be performed. It was found with this rapid protocol that 8 PCR cycles produced the best results. After the BLT PCR program, libraries can be stored at 2-8C for up to 3 days.

16 Proceed to procedure "Clean Up Pooled Libraries"

Clean Up Pooled Libraries

18m 30s

17 Warm the Sample Purification Beads (SPB) to room temperature before use. Thaw the Resuspension Buffer (RSB) to room temperature before use.

18 Make 500 µL of 70-80% ethanol for washes.

19 After the BLT PCR program has completed, remove the plate from the thermal cycler and quickly spin down to collect the contents to the bottom of the well.

20 Put the plate on the magnetic stand until the liquid is clear (~ 00:01:00) 1m

21 Multi-channel 2 µl from each column of the BLT PCR plate into an 8-tube strip avoiding bead carry over, then combine all 8 of the tubes into a 1.5mL Lo-bind Eppendorf tube labelled with the run name followed by “BLT Pool” for long term storage. The BLT PCR plate can be discarded.

22 Add 40 µL of nuclease free water to a new 1.5mL Lo-bind tube labelled “1”.

23 Vortex and spin down the BLT Pool and then transfer 45 µL to tube 1, the Lo-bind tube containing the water.

24 Vortex and invert the SPB tube to resuspend. Add 45 µL of SPB directly into tube “1” containing the BLT Pool and water. Mix by pipetting slowly 10 times, avoiding bubbles.

25 Incubate at room temperature for 00:05:00 . 5m

26 During this incubation, add 15 µL of SPB into a brand new 1.5mL tube labelled “2”

27 Once the 5 minute incubation is complete, place tube “1” on the magnet and wait until the liquid is clear (~1 minute).

28 Once the liquid is clear, transfer 125 µL of supernatant from tube “1” into tube “2” containing the 15 µL SPB. Pipette 10 times to mix, again slowly to avoid bubbles. Discard the original tube “1”.

29 Incubate tube “2” for another 00:05:00. 5m

30 Place tube “2” on the magnetic stand until the liquid is clear (~1 minute).

31 Without disturbing the beads, remove and discard the supernatant out of tube “2”.

32 Perform two washes as follows: 30s

With tube “2” remaining on the magnetic stand, add 200 µL of the previously made 70-80% ethanol to each well without mixing

Incubate for 00:00:30 Without disturbing the beads, remove and discard the supernatant. Repeat the wash a second time.

33 Use a 20 µl pipette to remove and discard residual ethanol from each well.

34 Air-dry on the magnetic stand for 00:05:00. 5m

35 Remove from the magnetic stand.

36 Using a single channel pipet, add 35 µL of RSB to the beads, pipetting up and down to resuspend, slowly.

37 Incubate at room temperature for 00:02:00. 2m

38 Place tube “2” on the magnetic stand and wait until the liquid is clear (~2 minutes).

39 Transfer 30 µL of the supernatant to a new 1.5mL Lo-bind Eppendorf tube labelled with the Run ID and “Libraries” for long term storage. Dispose of tube “2”.

40 If you are stopping, store your libraries in -20 °C freezer for up to a month.

41 If you are not stopping, proceed to the appropriate protocol for quantification, diluting, and denaturing as per your sample type.