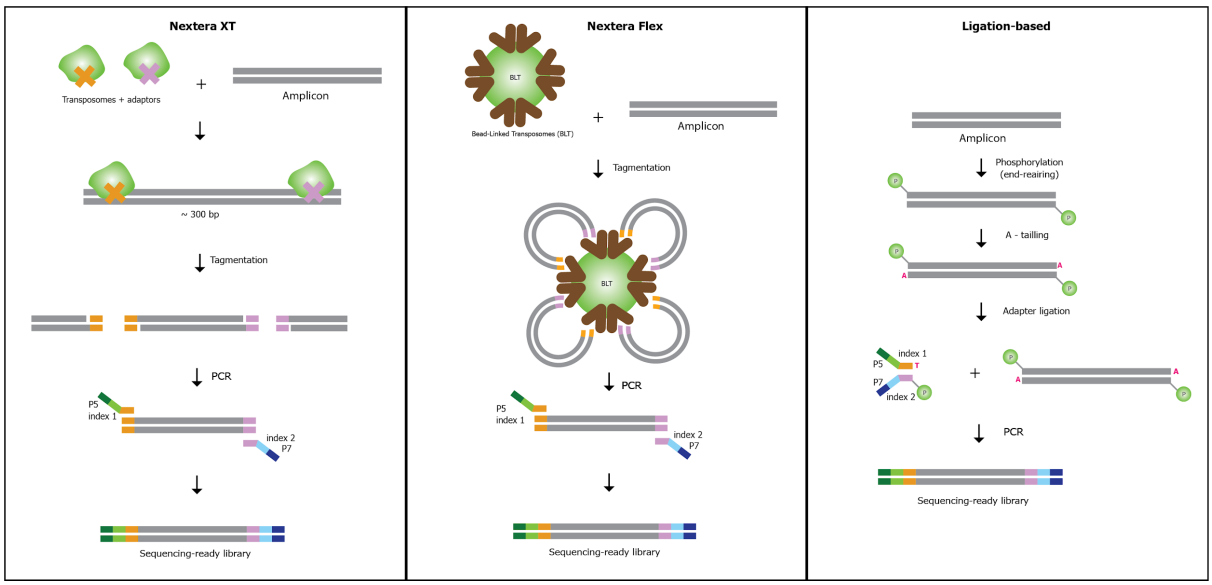
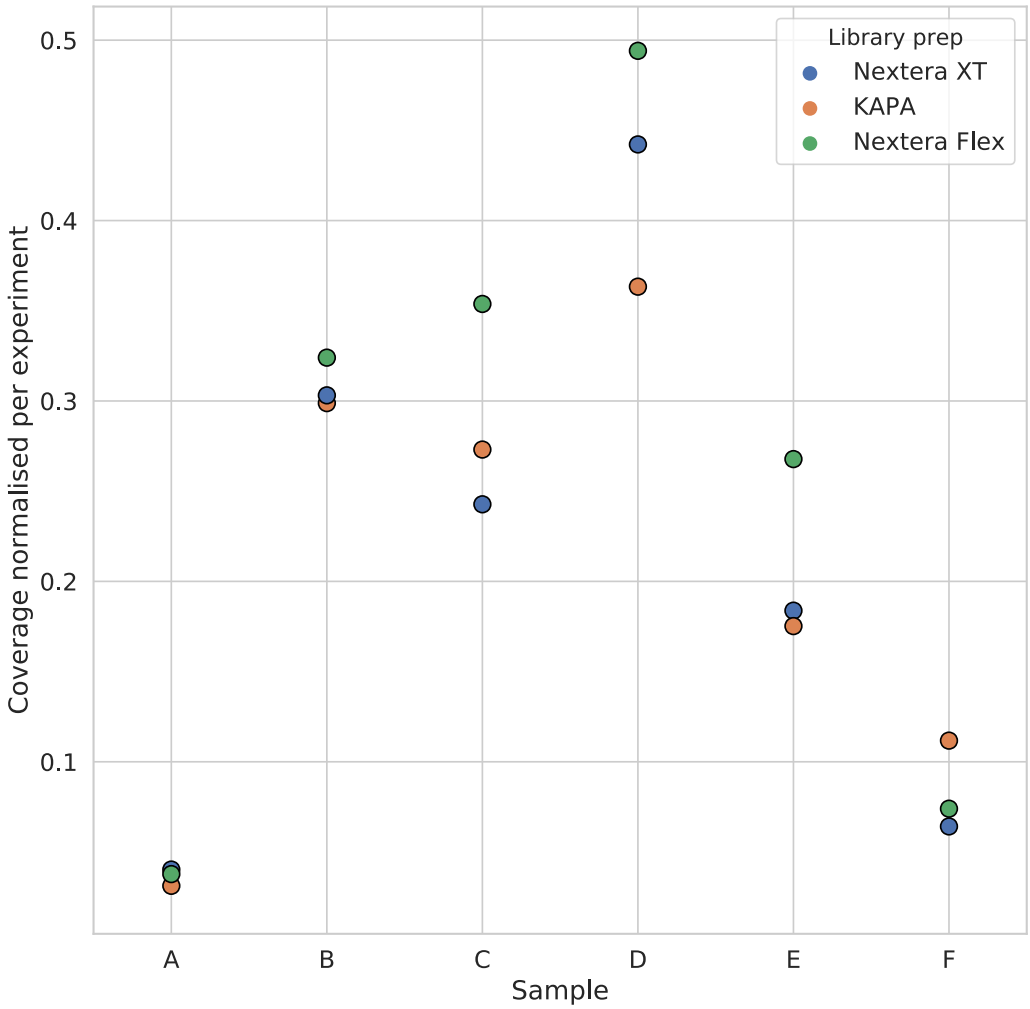
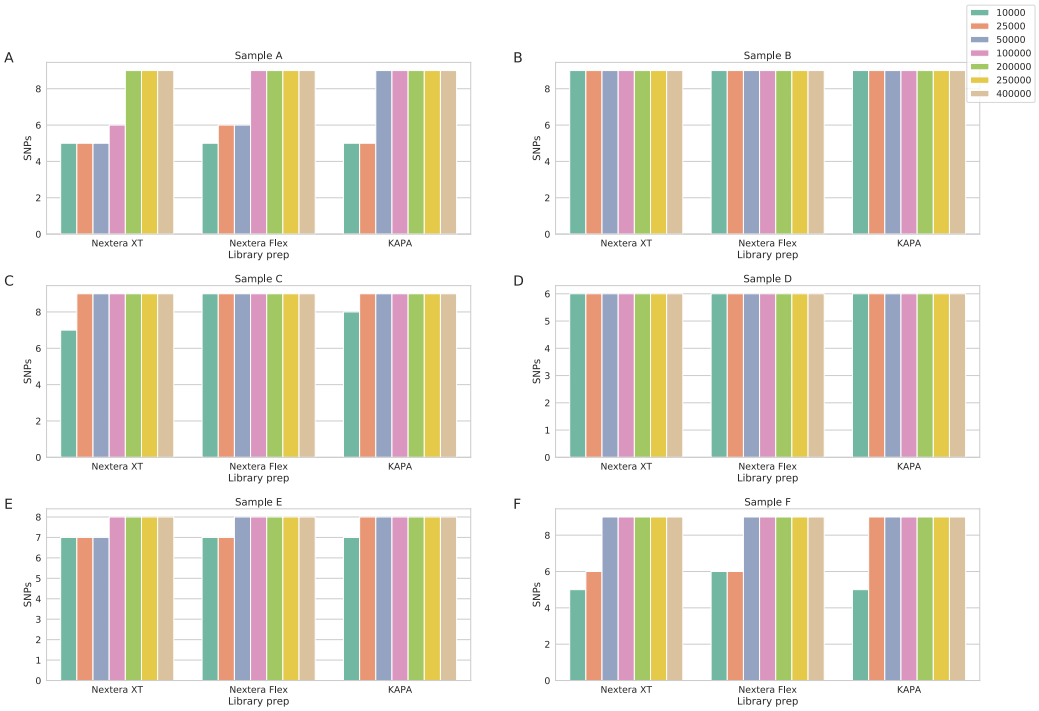
Supplementary Figures



Supplementary Figure 1. Schematic workflow for each library preparation method.

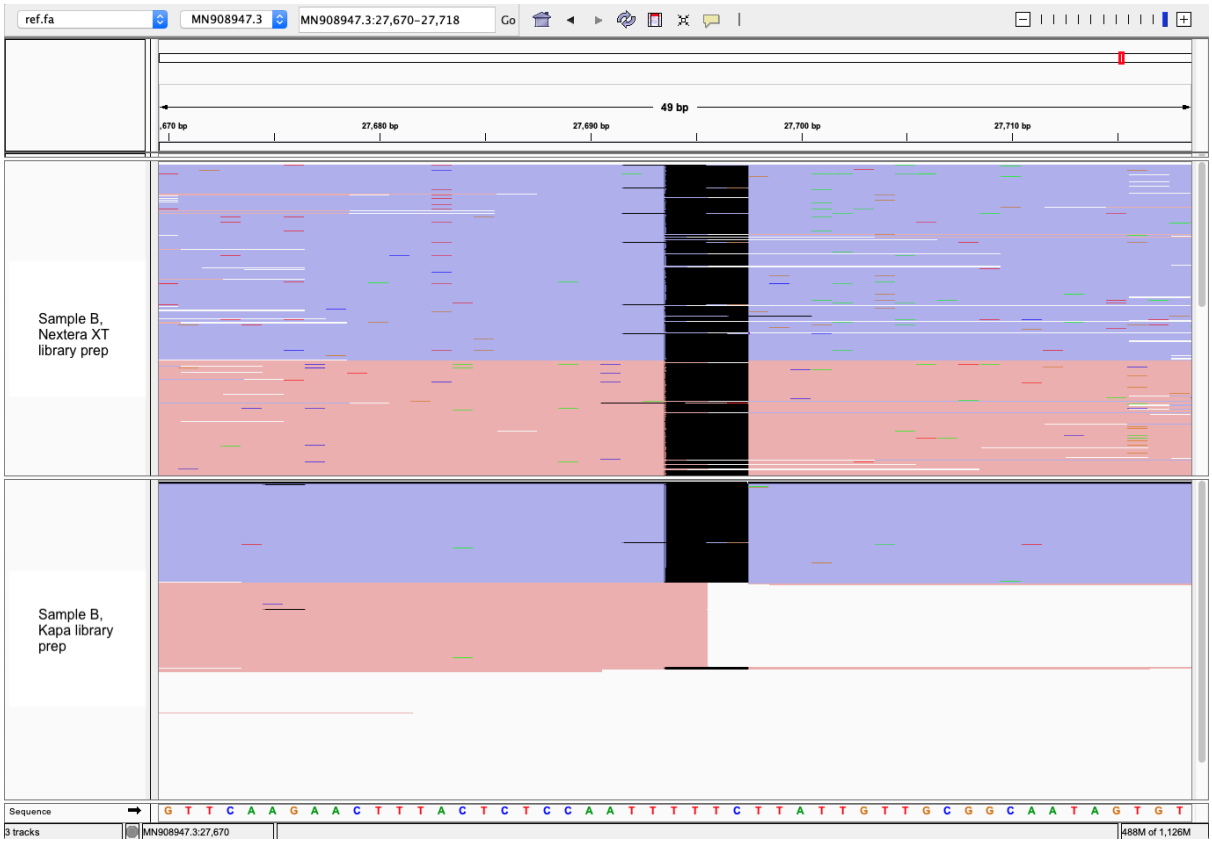


Supplementary Figure 2. Median coverage across amplicon 97 for each library. Coverage is normalised within each experiment. Samples A and F have a SNP in the amplicon 97 primer region.



Supplementary Figure 3. Number of SNPs called in each sample by library prep method and number of read pairs used.

Supplementary Figure 4. Screenshot of IGV alignment around position 15168 in the Nextera XT library prep sample, showing the variant position in a red box. The alternative base call is seen only in negative strand reads.



Supplementary Figure 5. Screenshot of IGV alignment around position 27693 in the Nextera XT and KAPA library prep for the same sample. The black bar shows the deletion, which is not seen in the positive strand reads in the KAPA library prep (pink bars), as the read alignment is soft-clipped.

Supplementary Tables

Sample	C_t (Orf1a)	C_t (RdRp)
A	22	22.4
B	13.5	13.6
C	27.3	undetermined
D	24.1	22.1
E	20.6	20.6
F	18.59	19

Supplementary Table 1. C_t values of RNA samples used in this study. The qRT-PCR was carried out previously by Ramathibodi Hospital for clinical diagnostic test.

Sample	Amplicon yield (ng)		Library concentration (ng/µl)			Library yield (ng)			Library fragment size (bp)		
	Pool 1	Pool2	Nextera XT	Nextera Flex	Ligation-based	Nextera XT	Nextera Flex	Ligation-based	Nextera XT	Nextera Flex	Ligation-based
A	1137	1296	5.08	2.15	30.2	254	64.5	906	308	465	512
B	594	867	5.41	2.06	58.2	270.5	61.8	1746	259	467	538
C	477	651	5.61	2.11	27	280.5	63.3	810	260	467	512
D	762	1071	5	2.32	42.8	250	69.6	1284	269	466	518
E	221.7	312	6.51	2.57	36.4	325.5	77.1	1092	272	469	530
F	519	477	6.27	2.77	81.1	313.5	83.1	2433	262	470	556
NTC1	18	17.28	7.2	0.987	0.216	360	29.61	6.48	290	459	512
NTC2	20.73	17.64	6.28	0.662	1.87	314	19.86	56.1	320	440	512

Supplementary Table 2. RT-PCR and library preparation QC. Concentrations were measured using Qubit fluorometer. Library fragment sizes were based on Fragment Analyzer System. NTC, negative control.

Sample	Library prep	Reads mapped to SARS-CoV-2	Reads remaining after Ivar quality filtering and primer trimming
NTC1	Nextera XT	3165	464
NTC2	Nextera XT	2251	310
NTC1	Nextera Flex	7602	3787
NTC2	Nextera Flex	4873	2341
NTC1	KAPA	20358	268
NTC2	KAPA	5907	779

Supplementary Table 3. The number of reads which were mapped to SARS-CoV-2 after the initial filtering step of our pipeline, and the number which remained after final quality filtering and trimming. NTC, negative control.