**Supplementary method**

**ViReMa (v0.25)**

**With filtration and standardization:**

java -jar Trimmomatic-0.39/trimmomatic-0.39.jar PE ${name}\_R1.fastq.gz ${name}\_R2.fastq.gz output\_${name}\_paired\_R1.fastq output\_${name}\_unpaired\_R1.fastq output\_${name}\_paired\_R2.fastq output\_${name}\_unpaired\_R2.fastq ILLUMINACLIP:TruSeq3-PE.fa:2:30:10:2:True LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:75

python3 interleave\_paired\_end\_fastq.py output\_${name}\_paired\_R1.fastq output\_${name}\_paired\_R2.fastq > interleaved\_${name}.fastq

python3 ViReMa.py SARS-COV2\_Reference\_MN908947.3\_padded.fasta interleaved\_${name}.fastq ViReMa25\_SARS2\_${name}\_recombinations.sam --Output\_Dir ViReMa25\_SARS2\_${name} --Output\_Tag ViReMa25\_SARS2\_${name} --Seed 20 -BED --MicroInDel\_Length 5

python3 standardize\_alignments.py SARS-COV2\_Reference\_MN908947.3\_padded.fasta ./ViReMa25\_SARS2\_${name}/ViReMa25\_SARS2\_${name}\_recombinations.sam > ./ViReMa25\_SARS2\_${name}/ViReMa25\_SARS2\_standardized\_${name}\_recombinations.bam

python3 filter\_aligned\_reads.py SARs-CoV-2\_v5.3.2\_400.primer.bed ./ViReMa25\_SARS2\_${name}/ViReMa25\_SARS2\_standardized\_${name}\_recombinations.bam --min-deletion-length 6 --max-overhang-primer-frac 1 --min-aligned-length 75 --virema > ./ViReMa25\_SARS2\_${name}/filtered\_ViReMa25\_SARS2\_standardized\_${name}\_recombinations.bam

python3 extract\_deletions.py ./ViReMa25\_SARS2\_${name}/filtered\_ViReMa25\_SARS2\_standardized\_${name}\_recombinations.sorted.bam --primer-bed SARs-CoV-2\_v5.3.2\_400.primer.bed --min-deletion-length 6 --virema > ./ViReMa25\_SARS2\_${name}/filtered\_ViReMa25\_SARS2\_standardized\_${name}\_recombinations.sorted.txt

samtools view -S -b ViReMa25\_SARS2\_${name}\_recombinations.sam > ViReMa25\_SARS2\_${name}\_recombinations.bam

samtools sort ViReMa25\_SARS2\_${name}\_recombinations.bam -o ViReMa25\_SARS2\_${name}\_recombinations.sorted.bam

samtools depth -a -m 0 ViReMa25\_SARS2\_${name}\_recombinations.sorted.bam > ViReMa25\_SARS2\_${name}\_recombinations.coverage

**Without filtration and standardization:**

java -jar Trimmomatic-0.39/trimmomatic-0.39.jar PE ${name}\_R1.fastq.gz ${name}\_R2.fastq.gz output\_${name}\_paired\_R1.fastq output\_${name}\_unpaired\_R1.fastq output\_${name}\_paired\_R2.fastq output\_${name}\_unpaired\_R2.fastq ILLUMINACLIP:TruSeq3-PE.fa:2:30:10:2:True LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:65

python3 interleave\_paired\_end\_fastq.py output\_${name}\_paired\_R1.fastq output\_${name}\_paired\_R2.fastq > interleaved\_${name}.fastq

python3 ViReMa.py SARS-COV2\_Reference\_MN908947.3\_padded.fasta interleaved\_${name}.fastq ViReMa25\_SARS2\_${name}\_recombinations.sam --Output\_Dir ViReMa25\_SARS2\_${name} --Output\_Tag ViReMa25\_SARS2\_${name} --Seed 20 -BED --MicroInDel\_Length 5

samtools view -S -b ViReMa25\_SARS2\_${name}\_recombinations.sam > ViReMa25\_SARS2\_${name}\_recombinations.bam

samtools sort ViReMa25\_SARS2\_${name}\_recombinations.bam -o ViReMa25\_SARS2\_${name}\_recombinations.sorted.bam

samtools depth -a -m 0 ViReMa25\_SARS2\_${name}\_recombinations.sorted.bam > ViReMa25\_SARS2\_${name}\_recombinations.coverage

python3 extract\_deletions.py ./ViReMa25\_SARS2\_${name}/ViReMa25\_SARS2\_${name}\_recombinations.sorted.bam --min-deletion-length 6 --virema > ./ViReMa25\_SARS2\_${name}/annotated\_ViReMa25\_SARS2\_${name}\_recombinations.sorted.txt

**STAR (v2.7.3a)**

**With filtration and standardization:**

~/TrimGalore-0.4.3/trim\_galore --stringency 3 -q 30 -e .10 --length 15 --paired ./${SRA}/${SRA}\_1.fastq ./${SRA}/${SRA}\_2.fastq

STAR --readFilesIn ./${SRA}\_1\_val\_1.fq ./${SRA}\_2\_val\_2.fq --outFileNamePrefix ${name} --genomeDir ./Genome\_Dir --outFilterType BySJout --outFilterMultimapNmax 20 --alignSJoverhangMin 8 --alignSJDBoverhangMin 1 --outSJfilterOverhangMin 12 12 12 12 --outSJfilterCountUniqueMin 1 1 1 1 --outSJfilterCountTotalMin 1 1 1 1 --outSJfilterDistToOtherSJmin 0 0 0 0 --outFilterMismatchNmax 999 --outFilterMismatchNoverReadLmax 0.04 --scoreGapNoncan -4 --scoreGapATAC -4 --chimScoreJunctionNonGTAG 0 --chimOutType Junctions WithinBAM HardClip --alignSJstitchMismatchNmax -1 -1 -1 -1 --alignIntronMin 20 --alignIntronMax 1000000 --alignMatesGapMax 1000000

python3 standardize\_alignments.py NC\_045512.2.fasta ${name}Aligned.out.sam > ${name}\_standardizationAligned.out.bam

python3 annotate\_alignment\_with\_primers.py ARTIC\_primers\_v3.bed ${name}\_standardizationAligned.out.bam > ${name}\_annotated\_standardization\_Aligned.out.bam

python3 filter\_aligned\_reads.py ARTIC\_primers\_v3.bed ${name}\_annotated\_standardization\_Aligned.out.bam --min-deletion-length 20 --max-overhang-primer-frac 1 --min-aligned-length 75 --primer-pool-matching --max-primer-dist 1 > filtered\_${name}\_annotated\_standardizationAligned.out.bam

samtools sort filtered\_${name}\_annotated\_standardizationAligned.out.bam -o filtered\_${name}\_annotated\_standardizationAligned.out.sorted.bam

samtools depth -a -m 0 filtered\_${name}\_annotated\_standardizationAligned.out.sorted.bam > filtered\_${name}\_annotated\_standardizationAligned.out.sorted.coverage

python3 extract\_deletions.py filtered\_${name}\_annotated\_standardizationAligned.out.sorted.bam --primer-bed ARTIC\_primers\_v3.bed --min-deletion-length 20 --ignore-secondary > filtered\_${name}\_annotated\_standardizationAligned.deletion.sorted.txt

**Without filtration and standardization:**

~/TrimGalore-0.4.3/trim\_galore --stringency 3 -q 30 -e .10 --length 15 --paired ./${SRA}/${SRA}\_1.fastq ./${SRA}/${SRA}\_2.fastq

STAR --readFilesIn ./${SRA}\_1\_val\_1.fq ./${SRA}\_2\_val\_2.fq --outFileNamePrefix ${name} --genomeDir ./Genome\_Dir --outFilterType BySJout --outFilterMultimapNmax 20 --alignSJoverhangMin 8 --alignSJDBoverhangMin 1 --outSJfilterOverhangMin 12 12 12 12 --outSJfilterCountUniqueMin 1 1 1 1 --outSJfilterCountTotalMin 1 1 1 1 --outSJfilterDistToOtherSJmin 0 0 0 0 --outFilterMismatchNmax 999 --outFilterMismatchNoverReadLmax 0.04 --scoreGapNoncan -4 --scoreGapATAC -4 --chimScoreJunctionNonGTAG 0 --chimOutType Junctions WithinBAM HardClip --alignSJstitchMismatchNmax -1 -1 -1 -1 --alignIntronMin 20 --alignIntronMax 1000000 --alignMatesGapMax 1000000

python3 annotate\_alignment\_with\_primers.py ARTIC\_primers\_v3.bed ${name}Aligned.out.sam > ${name}\_annotated\_Aligned.out.bam

python3 filter\_aligned\_reads.py ARTIC\_primers\_v3.bed ${name}\_annotated\_Aligned.out.bam --min-deletion-length 20 --max-overhang-primer-frac 1 --primer-pool-matching --max-primer-dist 1 > ${name}\_annotated\_primer\_Aligned.out.bam

samtools sort ${name}\_annotated\_primer\_Aligned.out.bam -o ${name}\_annotated\_primer\_Aligned.out.sorted.bam

samtools depth -a -m 0 ${name}\_annotated\_primer\_Aligned.out.sorted.bam > ${name}\_annotated\_primer\_Aligned.out.sorted.coverage

python3 extract\_deletions.py ${name}\_annotated\_primer\_Aligned.out.sorted.bam --primer-bed ARTIC\_primers\_v3.bed --min-deletion-length 20 --ignore-secondary > ${name}\_before\_filteration\_and\_standardization\_deletion.sorted.txt

**Identification of TRS-related and TRS-independent deletions**

tail -n +2 all\_groups\_all\_deletions.txt | cut -f 1-2 | perl translate.pl 1> all\_groups\_all\_deletions\_translated.xls 2> all\_groups\_all\_deletions\_translated.txt

(the deletion coordinates in the input of *translate.pl* are the first and last nucleotides deleted (donor site+1, acceptor site -1))