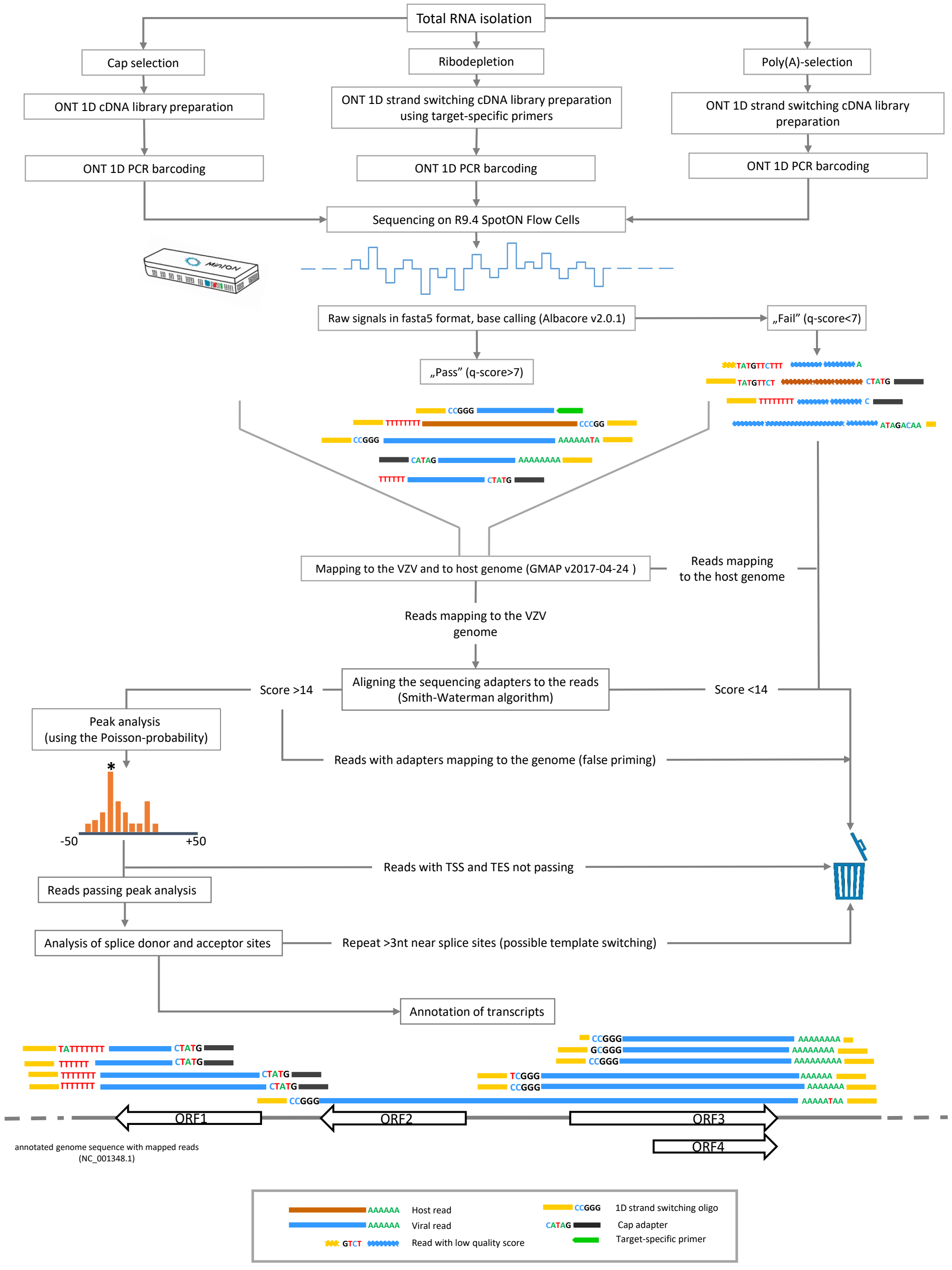
Total RNA isolation
Cap selection
Ribodepletion
Poly(A)-selection
ONT 1D cDNA library preparation
ONT 1D strand switching cDNA library preparation using target-specific primers
ONT 1D strand switching cDNA library preparation
ONT 1D PCR barcoding
ONT 1D PCR barcoding
ONT 1D PCR barcoding
Sequencing on R9.4 SpotON Flow Cells
MinION
Raw signals in fasta5 format, base calling (Albacore v2.0.1)
„Fail" (q-score<7)
„Pass" (q-score>7)
Mapping to the VZV and to host genome (GMAP v2017-04-24)
Reads mapping to the host genome
Reads mapping to the VZV genome
Score >14
Aligning the sequencing adapters to the reads (Smith-Waterman algorithm)
Score <14
Peak analysis (using the Poisson-probability)
Reads with adapters mapping to the genome (false priming)
-50
+50
Reads with TSS and TES not passing
Reads passing peak analysis
Analysis of splice donor and acceptor sites
Repeat >3nt near splice sites (possible template switching)
Annotation of transcripts
ORF1
ORF2
ORF3
ORF4
annotated genome sequence with mapped reads (NC_001348.1)
Host read
Viral read
Read with low quality score
1D strand switching oligo
Cap adapter
Target-specific primer