

A) 3' SMART CDS Primer II A + SMARTer II A Oligonucleotide (X = undisclosed base):

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AAGCAGTGGTATCAACGCAGAGTAC-XXXXX-RNA_MOLECULE-AAAAAAAAAAAAAAAA-GTACTCTGCGTTGATACCACTGCTT  
TTCGTCACCATAGTTGCGTCTCATG-XXXXX-----TTTTTTTTTTTTTTT-CATGAGACGCAACTATGGTGACGAA
```

SMART CDS Primer II A contains T₃₀ and thus is complementary to the poly-A tail.

Flanks are symmetric (except for T₃₀ vs X₅), thus it is not possible to distinguish between original and RC strands of the insert.

Smart-seq2 (Picelli 2013 - PMID 24056875) has identical oligo system for cDNA synthesis. However, the ds cDNA is then tagged by Nextera DNA Sample preparation kit, thus the reads are expected to have Nextera flanks rather than the SMARTer flanks.

B) Modification in Jia et al., 2020 (PMID 32541953):

SMART CDS Primer II A was replaced by NEB Universal miRNA Cloning Linker (ligates to 3' end of RNA molecule) and a complementary oligo:

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AAGCAGTGGTATCAACGCAGAGTAC-XXXXX-RNA_MOLECULE-AAAAAAAAAAAAAAAA-CTGTAGGCACCATCAATGTACTCTGCGTTGATACCACTGCTT  
TTCGTCACCATAGTTGCGTCTCATG-XXXXX-----TTTTTTTTTTTTTTT-GACATCCGTGGTAGTTACATGAGACGCAACTATGGTGACGAA
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The sequence of miRNA Cloning Linker (CTGTAGGCACCATCAAT) is unique for 3' end of the RNA molecule.

Detection of strandedness in R: see Jia_2020_(PMID_32541953).R