Hoque 2014 (PMID 24590784):

mRNA -> poly(A)-enrichment by chimeric U_5T_{45} oligos -> RNaseH treatment of A-T duplexes (A-U part remains untreated) -> PASS (PAS supporting) reads are expected to begin with unmapped T bases.

"A short poly(A) sequence unalignable to the genome is used as evidence for the poly(A) tail, which is important for identification of genuine pAs. Since RT is primed at the 3′ adapter region, internal priming at A-rich sequences is avoided"

Also it has 4bp UMI!

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    5' adapter                                  3' adapter

CCUUGGCACCCGAGAAUUCC-NNNN-mRNA-AAAAA-NNNN-GATCGTCGGACTGTAGAACTCTGAAC

                                  <------CTAGCAGCCTGACATCTTGAGACTTG     RT primer



PCR primer 2

CAAGCAGAAGACGGCATACGAGAT[INDEX]GTGACTGGAGTTCCTTGGCACCCGAGAATTCC----->

             FS-cDNA                      GGAATTCTCGGGTGCCAAGG-NNNN-mRNA-TTTTT-NNNN-CTAGCAGCCTGACATCTTGAGACTTG



CAAGCAGAAGACGGCATACGAGAT[INDEX]GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA-NNNN-mRNA-AAAAA-NNNN-GATCGTCGGACTGTAGAACTCTGAAC              PCR primer 1

                                                                                        <----AGCCTGACATCTTGAGACTTGCACATCTAGAGCCACCAGCGGCATAGTAA


Final library:

CAAGCAGAAGACGGCATACGAGAT[INDEX]GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA-NNNN-mRNA-AAAAA-NNNN-GATCGTCGGACTGTAGAACTCTGAACGTGTAGATCTCGGTGGTCGCCGTATCATT

GTTCGTCTTCTGCCGTATGCTCTA[INDEX]CACTGACCTCAAGGAACCGTGGGCTCTTAAGGT-NNNN-mRNA-TTTTT-NNNN-CTAGCAGCCTGACATCTTGAGACTTGCACATCTAGAGCCACCAGCGGCATAGTAA


CAAGCAGAAGACGGCATACGAGAT[INDEX]GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA-NNNN-mRNA-AAAAA-NNNN-GATCGTCGGACTGTAGAACTCTGAACGTGTAGATCTCGGTGGTCGCCGTATCATT

                                                                    <--------CTAGCAGCCTGACATCTTGAGACTTGCACATC  Insert sequencing primer
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Zheng 2016 - PMID 27512124:

"pAs located in a long stretch of A's cannot be effectively identified by 3'READS because the short poly(A) tail left after RNase H digestion can be completely aligned to the A-stretch sequence, leaving no additional A's as evidence of the poly(A) tail".

Key advantage of 3'READS+: "generation of reads with an optimal number of T's (~13) for accurate identification of genuine pAs located in A-stretch regions".