5P CITE-seq assay scheme – 10x Genomics single cell 5’ chemistry

cDNA production from 10x single cell 5’ chemistry (From 10x Genomics)

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Gel bead oligo primer annealed to antibody-conjugated oligo – partial small RNA read 2 handle

5P 10x gel bead oligo

5’-CTACACGACGCTCTTCCGATCT-NNNNNNNNNNNNNNNN-NNNNNNNNN-TTTCTTATATrGrGrG-3’

3’ AAAGAATATA-C-C-Cxxxxxxxxxxxx**ACCTTAAGAGCCCACGGTTCC**

antibody-conjugated 5P CITE-seq oligo

double stranded cDNA (after cDNA PCR with additive) – 94 nt:

ADT cDNA additive primer: ***5’-CCTTGGCACCCGAGAATT*C*C-3’***

5’-CTACACGACGCTCTTCCGATCT-NNNNNNNNNNNNNNNN-NNNNNNNNN-TTTCTTATATGGGxxxxxxxxxxxxTGGAATTCTCGGGTGCCAAGG-3’

3’-GATGTGCTGCGAGAAGGCTAGA-NNNNNNNNNNNNNNNN-NNNNNNNNN-AAAGAATATACCCxxxxxxxxxxxx**ACCTTAAGAGCCCACGGTTCC**-5’

Library PCR – addition of P5 and P7 ends. SI-PCR (P5) and RPI-x (P7) – shown with 6 nt i7 index

3’-ACCTTAAGAGCCCACGGTTCCTTGAGGTCAGTGxxxxxx**TAGAGCATACGGCAGAAGACGAAC**

5’-CTACACGACGCTCTTCCGATCT-NNNNNNNNNNNNNNNN-NNNNNNNNN-TTTCTTATATGGGxxxxxxxxxxxxTGGAATTCTCGGGTGCCAAGG-3’

3’-GATGTGCTGCGAGAAGGCTAGA-NNNNNNNNNNNNNNNN-NNNNNNNNN-AAAGAATATACCCxxxxxxxxxxxxACCTTAAGAGCCCACGGTTCC-5’

AATGATACGGCGACCACCGA-GATCTACACTCTTTCCCTACACGACGCTC-3’

5P CITE-seq ADT final library – 173 nt (175 nt with 8 nt i7 index)

Cell barcode (16) UMI (10)

READ 1 --> ••••••••••••••••-••••••••••

i7 index read --> ••••••••

AATGATACGGCGACCACCGA-GATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-NNNNNNNNNNNNNNNN-NNNNNNNNN-TTTCTTATATGGGxxxxxxxxxxxxTGGAATTCTCGGGTGCCAAGGAACTCCAGTCACxxxxxx**ATCTCGTATGCCGTCTTCTGCTTG**

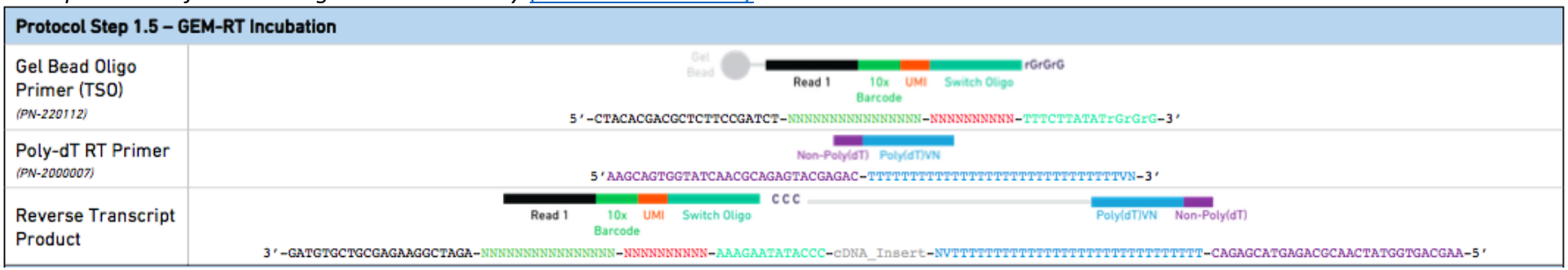
TTACTATGCCGCTGGTGGCT-CTAGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA-NNNNNNNNNNNNNNNN-NNNNNNNNN-AAAGAATATACCCxxxxxxxxxxxxACCTTAAGAGCCCACGGTTCCTTGAGGTCAGTGxxxxxx**TAGAGCATACGGCAGAAGACGAAC**

.....•••••••••••• <-- READ 2

Ab barcode (e.g. 12 nt)

5P Cell Hashing assay scheme – 10x Genomics single cell 5’ chemistry

cDNA production from 10x single cell 5’ chemistry [(From 10x Genomics)]



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Gel bead oligo primer annealed to antibody-conjugated oligo – partial TruSeq read 2 handle

5P 10x gel bead oligo

```
5’-CTACACGACGCTCTTCCGATCT-NNNNNNNNNNNNNNNN-NNNNNNNNN-TTTCTTATATrGrGrG-3’
                                                3’ AAAGAATATA-C-C-CxxxxxxxxxxxxTCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTG
```

antibody-conjugated 5P Hashtag oligo

double stranded cDNA (after cDNA PCR with additive) – 107 nt:

HTO cDNA additive primer: ***5’-GTGACTGGAGTTCAGACGTGTGC*T*C-3’***

```
5’-CTACACGACGCTCTTCCGATCT-NNNNNNNNNNNNNNNN-NNNNNNNNN-TTTCTTATATGGGxxxxxxxxxxxxAGATCGGAAGAGCACACGTCTGAACTCCAGTCAC-3’
3’-GATGTGCTGCGAGAAGGCTAGA-NNNNNNNNNNNNNNNN-NNNNNNNNN-AAAGAATATACCCxxxxxxxxxxxxTCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTG-5’
```

Library PCR – addition of P5 and P7 ends. SI-PCR (P5) and D7xx_s (P7)

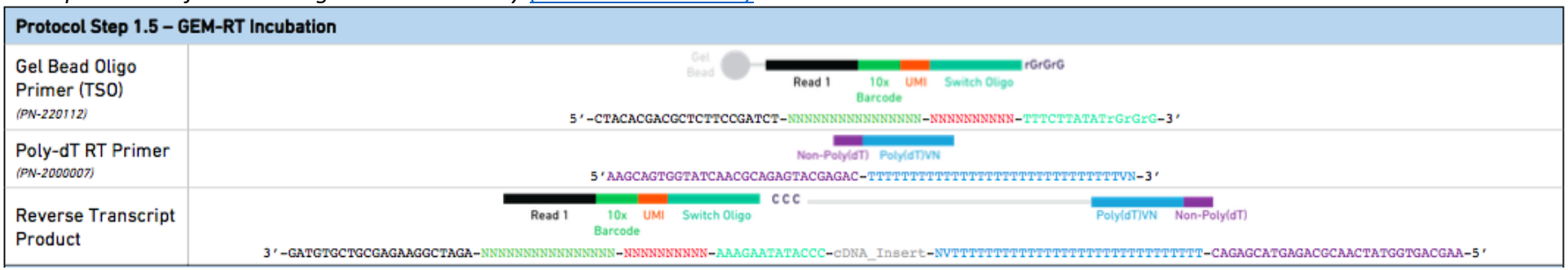
```
                                                                                                3’-CGTGTGCAGACTTGAGGTCAGTGxxxxxxxxTAGAGCATACGGCAGAAGACGAAC
                                  5’-CTACACGACGCTCTTCCGATCT-NNNNNNNNNNNNNNNN-NNNNNNNNN-TTTCTTATATGGGxxxxxxxxxxxxAGATCGGAAGAGCACACGTCTGAACTCCAGTCAC-3’
                                  3’-GATGTGCTGCGAGAAGGCTAGA-NNNNNNNNNNNNNNNN-NNNNNNNNN-AAAGAATATACCCxxxxxxxxxxxxTCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTG-5’
AATGATACGGCGACCACCGA-GATCTACACTCTTTCCCTACACGACGCTC-3’
```

5P Cell Hashing HTO final library – 176 nt

```
                                        Cell barcode (16) UMI (10)
                                READ 1  -->  ••••••••••••••••-••••••••••                                    i7 index read  -->  ••••••••
AATGATACGGCGACCACCGA-GATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-NNNNNNNNNNNNNNNN-NNNNNNNNN-TTTCTTATATGGGxxxxxxxxxxxxAGATCGGAAGAGCACACGTCTGAACTCCAGTCACxxxxxxxxATCTCGTATGCCGTCTTCTGCTTG
TTACTATGCCGCTGGTGGCT-CTAGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA-NNNNNNNNNNNNNNNN-NNNNNNNNN-AAAGAATATACCCxxxxxxxxxxxxTCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTGxxxxxxxxTAGAGCATACGGCAGAAGACGAAC
                                                                                  .......••••••••••••  <--  READ 2
                                                                                  Ab barcode (e.g. 12 nt)
```

5P Direct Guide Capture assay scheme – 10x Genomics single cell 5’ chemistry

cDNA production from 10x single cell 5’ chemistry [(From 10x Genomics)]



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Spiked in SpCas9 guide RT primer: gd_RT_v4: AGCAAGTGAGAAGCATCGTGTCAAAGCACCGACTCGGTGCCAC

Anneal

SpCas9 guide-RNA

xxxxxxxxxxxxxxxxxxxxGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTT-3’

3’-CACCGTGGCTCAGCCACGAAAACTGTGCTACGAAGAGTGAACGA

Guide RT primer

Extend and Template switch

5P 10x gel bead oligo

5’-CTACACGACGCTCTTCCGATCT-NNNNNNNNNNNNNNNN-NNNNNNNNN-TTTCTTATATrGrGrG-3’

C-C-CxxxxxxxxxxxxxxxxxxxxCAAAATCTCGATCTTTATCGTT/--/CTGTGCTACGAAGAGTGAACGA

guide-derived first-strand cDNA

double stranded cDNA (after cDNA PCR with additive) – 183 nt:

Guide cDNA additive primer: ***5’-AGCAAGTGAGAAGCATCGTG*T*C-3’***

5’-CTACACGACGCTCTTCCGATCT-NNNNNNNNNNNNNNNN-NNNNNNNNN-TTTCTTATATGGGxxxxxxxxxxxxxxxxxxxxGTTTTAGAGCTAGAAATAGCAA/--/GACACGATGCTTCTCACTTGCT-3’

3’-GATGTGCTGCGAGAAGGCTAGA-NNNNNNNNNNNNNNNN-NNNNNNNNN-AAAGAATATACCCxxxxxxxxxxxxxxxxxxxxCAAAATCTCGATCTTTATCGTT/--/CTGTGCTACGAAGAGTGAACGA-5’

Library PCR – addition of P5 and P7 ends. SI-PCR (P5) and internal nested primer Next_nst_[index] (P7)

Next_nst_[index] CAAGCAGAAGACGGCATACGAGATxxxxxxxxGTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTATTTCTAGCTCTAAAAC

GACAGAGAATATGTGTAGAGGCTCGGGTGCTCTGxxxxxxxxTAGAGCATACGGCAGAAGACGAAC

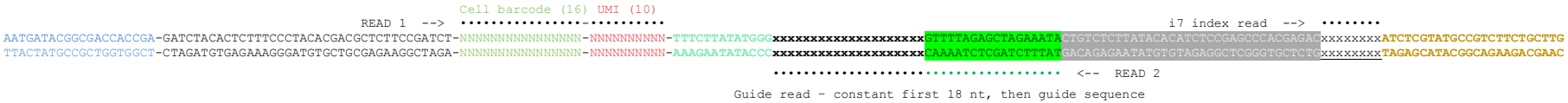
CAAAATCTCGATCTTTAT

5’-CTACACGACGCTCTTCCGATCT-NNNNNNNNNNNNNNNN-NNNNNNNNN-TTTCTTATATGGGxxxxxxxxxxxxxxxxxxxxGTTTTAGAGCTAGAAATAGCAA/--/GACACGATGCTTCTCACTTGCT-3’

3’-GATGTGCTGCGAGAAGGCTAGA-NNNNNNNNNNNNNNNN-NNNNNNNNN-AAAGAATATACCCxxxxxxxxxxxxxxxxxxxxCAAAATCTCGATCTTTATCGTT/--/CTGTGCTACGAAGAGTGAACGA-5’

AATGATACGGCGACCACCGA-GATCTACACTCTTTCCCTACACGACGCTC-3’

5P Direct Guide Capture final library v4 – 201 nt



Note: shown as read by paired end sequencing. Alternative sequencing run is to do a long (~60 nt) read 1 and sequence over switch region into variable guide sequence.

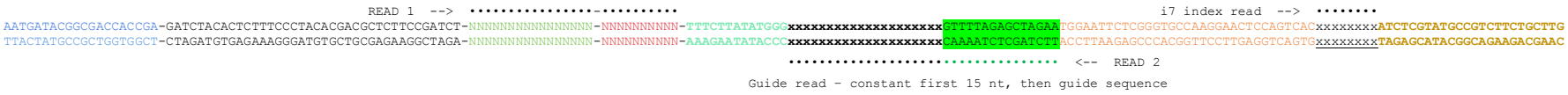
Comparison of v3 and v4 schemes

These schemes have both been used successfully. They differ only in the identity of the P7 primer in the final PCR

- **v3** - Small RNA read 2 sequencing primer, 15 nt region of complementarity to guide-adjacent sequence

smRNA_nst_[index] **CAAGCAGAAGACGGCATACGAGAT**xxxxxxxx GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA TTCTAGCTCTAAAAC

Final library (197 nt)



- **v4** - Nextera read 2 sequencing primer, 18 nt region of complementarity to guide-adjacent sequence

next_nst_[index] **CAAGCAGAAGACGGCATACGAGAT**xxxxxxxx GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TATTTCTAGCTCTAAAAC

Final library (201 nt)