CITE-seq assay scheme – 10x Genomics single cell 3’ v2 chemistry

cDNA production from 10x single cell 3’ v2 chemistry [(From 10x Genomics)]

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

10x gel bead oligo

```
5’-CTACACGACGCTCTTCCGATCT-NNNNNNNNNNNNNNNN-NNNNNNNNN-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTVN-3’
                                                3’ AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAB xxxxxxxxxxxxx ACCTTAAGAGCCCACGGTTCC
```

antibody-conjugated CITE-seq oligo

double stranded cDNA after RT (and cDNA PCR with additive) – 112 nt:

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

```
5’-CTACACGACGCTCTTCCGATCT-NNNNNNNNNNNNNNNN-NNNNNNNNN-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTV xxxxxxxxxxxxx TGGAATTCTCGGGTGCCAAGG-3’
3’-GATGTGCTGCGAGAAGGCTAGA-NNNNNNNNNNNNNNNN-NNNNNNNNN-AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAB xxxxxxxxxxxxx ACCTTAAGAGCCCACGGTTCC-5’
```

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

```
                                                                                                                                3’-ACCTTAAGAGCCCACGGTTCCTTGAGGTCAGTG xxxxxx TAGAGCATACGGCAGAAGACGAAC
                                5’-CTACACGACGCTCTTCCGATCT-NNNNNNNNNNNNNNNN-NNNNNNNNN-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTV xxxxxxxxxxxxx TGGAATTCTCGGGTGCCAAGG-3’
                                3’-GATGTGCTGCGAGAAGGCTAGA-NNNNNNNNNNNNNNNN-NNNNNNNNN-AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAB xxxxxxxxxxxxx ACCTTAAGAGCCCACGGTTCC-5’
AATGATACGGCGACCACCGA-GATCTACACTCTTTCCCTACACGACGCTC-3’
```

CITE-seq ADT final library – 190 nt (192 nt with 8 nt i7 index)

```
                                                Cell barcode (16) UMI (10)
                                    READ 1  -->  ••••••••••••••••-••••••••••                                                  i7 index read  -->  ••••••••
AATGATACGGCGACCACCGA-GATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-NNNNNNNNNNNNNNNN-NNNNNNNNN-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTV xxxxxxxxxxxxx TGGAATTCTCGGGTGCCAAGGAACTCCAGTCAC xxxxxx ATCTCGTATGCCGTCTTCTGCTTG
TTACTATGCCGCTGGTGGCT-CTAGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA-NNNNNNNNNNNNNNNN-NNNNNNNNN-AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAB xxxxxxxxxxxxx ACCTTAAGAGCCCACGGTTCCTTGAGGTCAGTG xxxxxx TAGAGCATACGGCAGAAGACGAAC
                                                                                                  .......••••••••••••  <--  READ 2
                                                                                                    Ab barcode (e.g. 12 nt)
```

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