

# ASAP-seq assay scheme – 10x Genomics single cell ATAC NextGEM v1.1 chemistry

## Version H: Using TotalSeq-A Hashing reagents (BioLegend)

ATAC fragment barcoding from 10x single cell ATAC NextGEM v1.1 chemistry ([From 10x Genomics](#))

### Protocol Step 2.5 – GEM Incubation

Gel Bead Oligo  
Primer  
PN-2000210



5'-AATGATACGGCGACCACCGAGATCTACAC-NNNNNNNNNNNNNNNNNN-TCGTCCGCGAGCGTC-3'

Linear Amplification  
DNA Product



5'-AATGATACGGCGACCACCGAGATCTACAC-NNNNNNNNNNNNNNNNNN-TCGTCCGCGAGCGTCAGATGTGTATAAGAGACAG----insert----CTGTCTCTTATACACATCTCCGAGCCCACGAGAC-3'

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### Bridging / extension and linear amplification reactions

#### 1. Bridging

##### 10x ATAC gel bead oligo

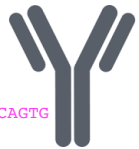
5' AATGATACGGCGACCACCGAGATCTACACNNNNNNNNNNNNNNNNNNTCGTCCGCGAGCGTC 3'

**Bridge Oligo A (BOA)**

5' TCGTCCGCGAGCGTCAGATGTGTATAAGAGACAGNNNNNNNNNNVTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT 3' [blocked]

3' <-- AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAABxxxxxxxxxxxxxxxxTCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTG

**TotalSeq™-A Hashing reagent**



#### 2. Round 1 extension in droplets

Bridge Oligo A (BOA)

5' TCGTCCGCGAGCGTCAGATGTGTATAAGAGACAGNNNNNNNNNNVTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT 3' [blocked]

3' AGCAGCCGTCGCAGTCTACACATATTCTCTGTCNNNNNNNNNNBAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAABxxxxxxxxxxxxxxxxTCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTG

**TotalSeq™-A Hashing reagent (extended)**

### 3. Subsequent rounds of linear extension in droplets

#### 10x ATAC gel bead oligo

5' AATGATACGGCGACCACCGAGATCTACACNNNNNNNNNNNNNNNNNNNNTCGTCGGCAGCGTC --> 3'  
 3' <-- AGCAGCCGTCGCAGTCTACACATATTCTCTGTCNNNNNNNNNBAAAAAAAAAAAAAAAAAAAAAAAAAAAAAABXXXXXXXXXXXXXXXXXCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTG  
**TotalSeq™-A Hashing reagent (extended)**

#### Linear amplification product (bottom strand also produced, not shown)

5' AATGATACGGCGACCACCGAGATCTACACNNNNNNNNNNNNNNNNNNNNTCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNNNNNNNVTTTTTTTTTTTTTTTTTTTTTTTTTTTTTVXXXXXXXXXXXXXXXXXAGATCGGAAGAGCACACGTCTGAACTCCAGTCAC

#### Library PCR – addition of P5 and P7 ends. Partial\_P5 and D7xx\_s

5' AATGATACGGCGACCACCGAGA  
 5' AATGATACGGCGACCACCGAGATCTACACNNNNNNNNNNNNNNNNNNNNTCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNNNNNNNVTTTTTTTTTTTTTTTTTTTTTTTTTTTTTVXXXXXXXXXXXXXXXXXAGATCGGAAGAGCACACGTCTGAACTCCAGTCAC 3'  
 3' -CGTGTGCAGACTTGAGGTCAGTGXXXXXXXXXTAGAGCATACGGCAGAAGACGAAC 5'

#### ASAP-Seq ADT (TotalSeq™-A) final library

5' AATGATACGGCGACCACCGAGATCTACACNNNNNNNNNNNNNNNNNNNNTCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNNNNNNNVTTTTTTTTTTTTTTTTTTTTTTTTTTTTTVXXXXXXXXXXXXXXXXXAGATCGGAAGAGCACACGTCTGAACTCCAGTCACXXXXXXXXXATCTCGTATGCCGCTTCTGCTTG 3'  
 3' TTAGTATGCCGCTGGTGGCTCTAGATGTGNNNNNNNNNNNNNNNNNNNNAGCAGCCGTCGCAGTCTACACATATTCTCTGTCNNNNNNNNNBAAAAAAAAAAAAAAAAAAAAAAAAAAAAAABXXXXXXXXXXXXXXXXXCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTGXXXXXXXXXTAGAGCATACGGCAGAAGACGAAC 5'  
 i5 \*\*\*\*\*  
 Cell barcode (16)  
 UBI  
 READ 1 --> \*\*\*\*\*  
 i7 index read --> \*\*\*\*\*  
 \*\*\*\*\* <-- read 2  
 antibody barcode

#### Sequencing for ASAP-seq with TotalSeq-A Hashtag detection (spiked into ATAC run)

| Read    | Length | ATAC             | Protein Tag    |
|---------|--------|------------------|----------------|
| Read 1: | 50     | Genomic fragment | 1-10 = UBI     |
| i7:     | 8      | sample index     | sample index   |
| i5:     | 16     | cell barcode     | cell barcode   |
| Read 2: | 50     | Genomic fragment | 1-15 = hashtag |