

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

## Version A: Using TotalSeq-A 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-TCGTCGGCAGCGTC-3'

Linear Amplification  
DNA Product



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

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### Bridging / extension and linear amplification reactions

#### 1. Bridging

##### 10x ATAC gel bead oligo

5' AATGATACGGCGACCACCGAGATCTACACNNNNNNNNNNNNNNNNNNTCGTCGGCAGCGTC 3'

##### Bridge Oligo A (BOA)

5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNNNNNNNNVTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT 3' [blocked]

3' <-- AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAABxxxxxxxxxxxxxxxxxxACCTTAAGAGCCACGGTTCC

##### TotalSeq™-A reagent



#### 2. Round 1 extension in droplets

Bridge Oligo A (BOA)

5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNNNNNNNNVTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT 3' [blocked]

3' AGCAGCCGTCGCAGTCTACACATATTCTCTGTCNNNNNNNNNBAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAABxxxxxxxxxxxxxxxxxxACCTTAAGAGCCACGGTTCC

##### TotalSeq™-A reagent (extended)

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

#### 10x ATAC gel bead oligo

```
5' AATGATACGGCGACCACCGAGATCTACACNNNNNNNNNNNNNNNNNNNNTCGTCGGCAGCGTC --> 3'
3' <-- AGCAGCCGTCGCAGTCTACACATATTCTCTGTCNNNNNNNNNNBAAAAAAAAAAAAAAAAAAAAAAAAAAAAAxxxxxxACCTTAAGAGCCACGGTTCC
TotalSeq™-A reagent (extended)
```

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

```
5' AATGATACGGCGACCACCGAGATCTACACNNNNNNNNNNNNNNNNNNNNTCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNNNNNNNVTTTTTTTTTTTTTTTTTTTTTTTTTTTTVxxxxxxxxxxxxxxTGGAATCTCGGGTGCCAAGG
```

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

```
5' AATGATACGGCGACCACCGAGA
5' AATGATACGGCGACCACCGAGATCTACACNNNNNNNNNNNNNNNNNNNNTCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNNNNNNNVTTTTTTTTTTTTTTTTTTTTTTTTTTTTVxxxxxxxxxxxxxxTGGAATCTCGGGTGCCAAGG
3' -ACCTTAAGAGCCACGGTTCCTTGAGGTCAGTxxxxxxxxxTAGAGCATACGGCAGAGACGAAC
```

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

```

          UBI
          READ 1 --> .....
5' AATGATACGGCGACCACCGAGATCTACACNNNNNNNNNNNNNNNNNNNNTCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNNNNNNNVTTTTTTTTTTTTTTTTTTTTTTTTTTTTVxxxxxxxxxxxxxxTGGAATCTCGGGTGCCAAGCACTCCAGTCACxxxxxxxxATCTCGTATGCCGTCTTCTGCTTG 3'
3' TTACTATGCCGCTGGTGGCTCTAGATGTGNNNNNNNNNNNNNNNNNNNNAGCAGCCGTCGCAGTCTACACATATTCTCTGTCNNNNNNNNNBAAAAAAAAAAAAAAAAAAAAAAAAAAAAAxxxxxxxxxxxxACCTTAAGAGCCACGGTTCCTTGAGGTCAGTxxxxxxxxxTAGAGCATACGGCAGAGACGAAC 5'
          i5 .....
          Cell barcode (16)
          antibody barcode <-- read 2
          i7 index read --> .....
```

#### Sequencing for ASAP-seq with TotalSeq-A protein 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 = antibody tag