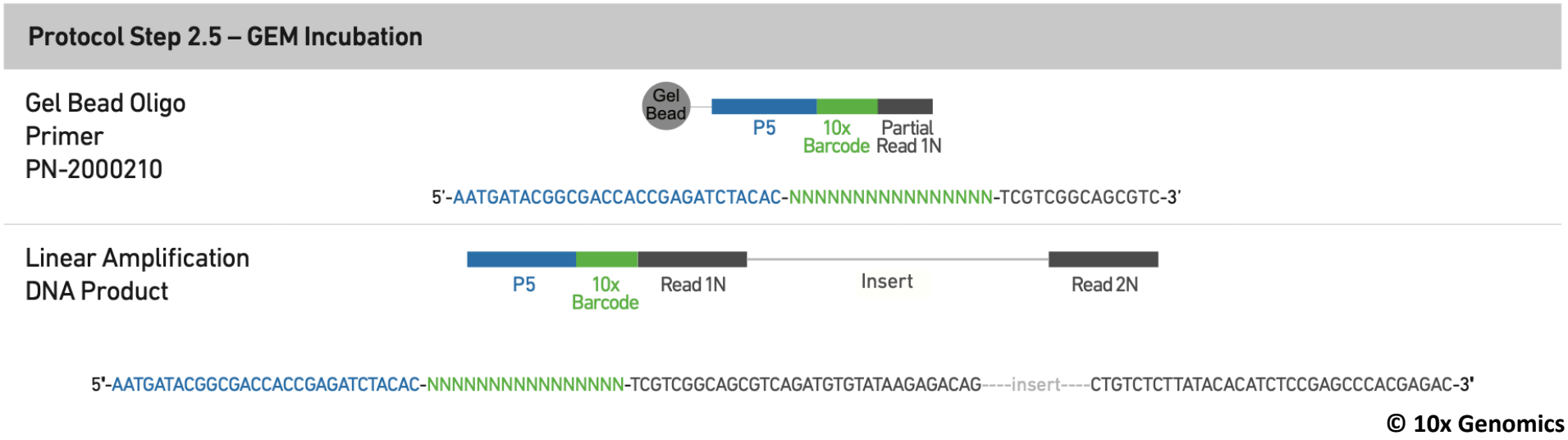
ASAP-seq assay scheme – 10x Genomics single cell ATAC NextGEM v1.1 chemistry

Version B: Using TotalSeq-B reagents (BioLegend)

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



Bridging / extension and linear amplification reactions

1. Bridging

10x ATAC gel bead oligo

5′ AATGATACGGCGACCACCGAGATCTACACNNNNNNNNNNNNNNNNTCGTCGGCAGCGTC 3′

Bridge Oligo B (BOB)

5′ TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTTGCTAGGACCGGCCTTAAAGC 3′ [blocked]

3′ <-- AACGATCCTGGCCGGAATTTCGNNNNNNNNNxxxxxxxxxxxxxxxNNNNNNNNNNTCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTG

TotalSeq™-B reagent

2. Round 1 extension in droplets

Bridge Oligo B (BOB)

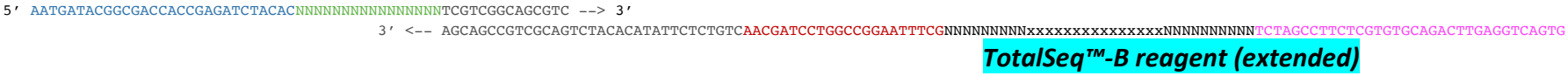
5′ TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTTGCTAGGACCGGCCTTAAAGC 3′ [blocked]

3′ AGCAGCCGTCGCAGTCTACACATATTCTCTGTCAACGATCCTGGCCGGAATTTCGNNNNNNNNNxxxxxxxxxxxxxxxNNNNNNNNNNTCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTG

TotalSeq™-B reagent (extended)

3. Subsequent rounds of linear extension in droplets

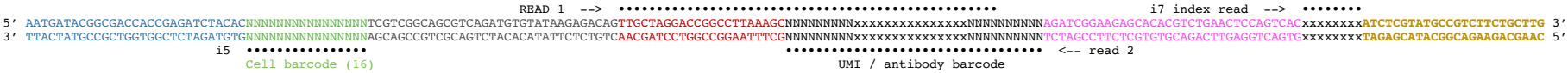
10x ATAC gel bead oligo



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

Library PCR – addition of P5 and P7 ends. Partial_P5 and D7xx_s

ASAP-Seq ADT (TotalSeq™-B) final library



Sequencing for ASAP-seq with TotalSeq-B protein detection (spiked into ATAC run)

Read	Length	ATAC	Protein Tag
Read 1:	50	Genomic fragment	(discard)
i7:	8	sample index	sample index
i5:	16	cell barcode	cell barcode
Read 2:	50	Genomic fragment	1-10 = UMI1, 11-25 = Antibody tag, 26-34 = UMI2

Sequencing this library alone will cause problems due to lack of sequence diversity in read 1. We highly recommend spiking this into the ATAC libraries generated together in the same assay.

UMI and tag barcode can be recovered from either read 1 or read 2.