

Split-seq GEX Molecular Biology

- Round 1 RT Barcodes: 5'-/5Phos/ACTGTGG-N8-TTTTTTTTTTTTTT-3' or 5'-/5Phos/ACTGTGG-N8-TNNNNNN-3'
- Round 2 Barcodes: 5'-/5Phos/CATCGCGTACGACT-N8-ATCAGCTGCTGTGAG-3'
- Round 3 Barcodes: 5'-/5Biosg/CAGACGTGTGCTTCCGACTNNNNNNNN-N8-GTGGCCGATGTTTCG-3'
- Round2 Linker: 5'-CCACAGTCTCAAGCAGC-3'
- Round2 Blocking: 5'-CGTGTGAGACTGTGG-3'
- Round3 Linker: 5'-TACGCCGATGCGGAAACATCG-3'
- Round3 Blocking: 5'-GTGGCCGATGTTTCGACTGGCGTACGACT-3'

Round 1 RT:

5' - NNNNNNA15-3'
<- 3'-CCC-cDNA-NVT15-N8-GGTGTCA\5Phos\ -5'

Round 2 Ligation:

Round 2 linker: 5'-CCACAGT CTCAAGCAGC-3'
<- 3'-CCC-cDNA-NVT15-N8-GGTGTCA\5AGTTCGTGCACCTA-N8-TCAGCATGCGGCTAC-5'
Round 2 blocking: 3'-GGTGTCA GAGTTCGTGC-5'

Round 3 Ligation:

Round 3 linker: 5'-TACGCCGATG CGAAACATCG-3'
<- 3'-CCC-cDNA-NVT15-N8-GGTGTCA-GAGTTCGTGCACCTA-N8-TCAGCATGCGGCTAC\GCTTTGTAGCCGGTG-N8-NNNNNNNNNTCTAGCCTTCTCGTGTGCAGAC-5'
Round 3 blocking: 3'-TCAGCATGCGGCTAC GCTTTGTAGCCGGTG-5'

- Split_TSO: 5'-/5dSp/AAGCAGTGGTATCAACGACAGTGAATrGrGrG-3'

Reverse Transcription: TSO-GGG-cDNA-A15-R1 N8-linker-R2 N8-linker-R3 N8-UMI- Partial_TruSeqRead2

5'-AAGCAGTGGTATCAACGACAGTGAATGGG-cDNA-NB A15-N8-CCACAGT-CTCAAGCAGCTGGAT-N8-AGTCGTACGCCGATG-CGAAACATCGGCCAC-N8-N10-AGATCGGAAGAGCACACGCTCTG-3'
3'-TTGTCACCATAGTTCGCTCTCACTTACCC-cDNA-NVT15-N8-GGTGTCA-GAGTTCGTGCACCTA-N8-TCAGCATGCGGCTAC-GCTTTGTAGCCGGTG-N8-N10-TCTAGCCTTCTCGTGTGCAGAC-5'

- Split_Partial-TSO: 5'-AAGCAGTGGTATCAACGACAGT-3'

- Split_TruSeq-Read2: 5'-CAGACGTGTGCTTCCGATCT-3'

cDNA Amplification: TSO-GGG-cDNA-A15-R1 N8-linker-R2 N8-linker-R3 N8-UMI- Partial_TruSeqRead2

5'-AAGCAGTGGTATCAACGACAGTGAATGGG-cDNA-NB A15-N8-CCACAGT-CTCAAGCAGCTGGAT-N8-AGTCGTACGCCGATG-CGAAACATCGGCCAC-N8-N10-AGATCGGAAGAGCACACGCTCTG-3'
3'-TTGTCACCATAGTTCGCTCTCACTTACCC-cDNA-NVT15-N8-GGTGTCA-GAGTTCGTGCACCTA-N8-TCAGCATGCGGCTAC-GCTTTGTAGCCGGTG-N8-N10-TCTAGCCTTCTCGTGTGCAGAC-5'

- Split_Adaptor_Top: 5'-ACACTCTTCCCTACACGACTCTCCGATCT-3' (*=phosphorothioated)

- Split_Adaptor_Bottom: 5'-GATCGGAAGAGCGCTGTGAGGAAAGAGTGT-3'

o 5'-ACACTCTTCCCTACACGACTCTCCGATCT-3'

o 3'-TGTGAGAAAGGATGTGCTGCGAAGGCTAG-5'

Adaptor Ligation: TruSeqRead1-cDNA-A15-R1 N8-linker-R2 N8-linker-R3 N8-UMI-Partial_TruSeqRead2

5'-ACACTCTTCCCTACACGACTCTCCGATCT-cDNA-BA15-N8-CCACAGT-CTCAAGCAGCTGGAT-N8-AGTCGTACGCCGATG-CGAAACATCGGCCAC-N8-N10-AGATCGGAAGAGCACACGCTCTG-3'
3'-TGTGAGAAAGGATGTGCTGCGAAGGCTAGA-cDNA-VT15-N8-GGTGTCA-GAGTTCGTGCACCTA-N8-TCAGCATGCGGCTAC-GCTTTGTAGCCGGTG-N8-N10-TCTAGCCTTCTCGTGTGCAGAC-5'

- Split_P5: P5-Sample_Index(i5)-Partial_TruSeqRead1

o 5'-AATGATACGGCACCAGGATCTACAC-N6-ACACTCTTCCCTACACGACGC-3'

- Split_P7: P7-Sample_Index(i7)-TruSeqRead2

o 5'-CAAGCAGAAGACGGCATACGAGAT-N6-GTGACTGGAATTCAGACGTGTGCTCTCCGATCT-3'

GEX Index PCR: P5-Sample_Index(i5)-TruSeqRead1-cDNA-A15-R1 N8-linker-R2 N8-linker-R3 N8-UMI-TruSeqRead2-Sample_Index(i7)-

5'-AATGATACGGCACCAGGATCTACAC-N6-ACACTCTTCCCTACACGACTCTCCGATCT-cDNA-A15-N8-CCACAGTCTCAAGCAGCTGGAT-N8-AGTCGTACGCCGATGCGAAACATCGGCCAC-N8-N10-AGATCGGAAGAGCACACGCTGAACTCCAGTAC-N6-ATCTCGTATGCCGCTCTTCTGCTTG-3'
3'-TTACTATGCCGCTGTGGCTCTAGATGTG-N6-TGTGAGAAAGGATGTGCTGCGAAGGCTAGA-cDNA-T15-N8-GGTGTCAAGTTCGTGCACCTA-N8-TCAGCATGCGGCTACGCTTTGTAGCCGGTG-N8-N10-TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGT-N6-TAGAGCATACGGCAGAAGACGAAC-5'

Sequencing:

ACACTCTTCCCTACACGACTCTCCGATCT→R1(cDNA):74 I1 (i7):6
5'-AATGATACGGCACCAGGATCTACAC-N6-ACACTCTTCCCTACACGACTCTCCGATCT-cDNA-A15-N8-CCACAGTCTCAAGCAGCTGGAT-N8-AGTCGTACGCCGATGCGAAACATCGGCCAC-N8-N10-AGATCGGAAGAGCACACGCTGAACTCCAGTAC-N6-ATCTCGTATGCCGCTCTTCTGCTTG-3'
3'-TTACTATGCCGCTGTGGCTCTAGATGTG-N6-TGTGAGAAAGGATGTGCTGCGAAGGCTAGA-cDNA-T15-N8-GGTGTCAAGTTCGTGCACCTA-N8-TCAGCATGCGGCTACGCTTTGTAGCCGGTG-N8-N10-TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGT-N6-TAGAGCATACGGCAGAAGACGAAC-5'
I2 (i5):6 R2(UMI+R3_N8+R2_N8+R1_N8):86←TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGT

Split-seq gRNA Molecular Biology

- Split_TSO: 5'-/5dSp/AAGCAGTGGTATCAACGACAGTGAATrGrGrG-3'

Reverse Transcription: TSO-GGG-mCherry-U6-gRNA-scaffold-A15-R1 N8-linker-R2 N8-linker-R3 N8-UMI- Partial_TruSeqRead2

5'-AAGCAGTGGTATCAACGACAGTGAATGGG-mCherry-U6-gRNA-scaffold-NB A15-N8-CCACAGT-CTCAAGCAGCTGGAT-N8-AGTCGTACGCCGATG-CGAAACATCGGCCAC-N8-N10-AGATCGGAAGAGCACACGCTCTG-3'
3'-TTGTCACCATAGTTCGCTCTCACTTACCC-mCherry-U6-gRNA-scaffold-NVT15-N8-GGTGTCA-GAGTTCGTGCACCTA-N8-TCAGCATGCGGCTAC-GCTTTGTAGCCGGTG-N8-N10-TCTAGCCTTCTCGTGTGCAGAC-5'

- Split_U6_outer: 5'-GGGCTATTTCCTGATTCCTTC-3'

- Split_TruSeq-Read2: 5'-CAGACGTGTGCTTCCGATCT-3'

cDNA Amplification: U6-gRNA-scaffold-A15-R1 N8-linker-R2 N8-linker-R3 N8-UMI- Partial_TruSeqRead2

5'-U6-gRNA-scaffold-NB A15-N8-CCACAGT-CTCAAGCAGCTGGAT-N8-AGTCGTACGCCGATG-CGAAACATCGGCCAC-N8-N10-AGATCGGAAGAGCACACGCTCTG-3'
3'-U6-gRNA-scaffold-NVT15-N8-GGTGTCA-GAGTTCGTGCACCTA-N8-TCAGCATGCGGCTAC-GCTTTGTAGCCGGTG-N8-N10-TCTAGCCTTCTCGTGTGCAGAC-5'

- Split_U6_inner: 5'-ACACTCTTCCCTACACGACTCTCCGATCT-CGATTCTTGGCTTATATATCTTGTGGAAGGACG-3'

- Split_TruSeq-Read2: 5'-CAGACGTGTGCTTCCGATCT-3'

CRISPR Feature PCR: TruSeqRead1-U6-gRNA-scaffold-A15-R1 N8-linker-R2 N8-linker-R3 N8-UMI-Partial_TruSeqRead2

5'-ACACTCTTCCCTACACGACTCTCCGATCT-U6-gRNA-scaffold-BA15-N8-CCACAGT-CTCAAGCAGCTGGAT-N8-AGTCGTACGCCGATG-CGAAACATCGGCCAC-N8-N10-AGATCGGAAGAGCACACGCTCTG-3'
3'-TGTGAGAAAGGATGTGCTGCGAAGGCTAGA-U6-gRNA-scaffold-VT15-N8-GGTGTCA-GAGTTCGTGCACCTA-N8-TCAGCATGCGGCTAC-GCTTTGTAGCCGGTG-N8-N10-TCTAGCCTTCTCGTGTGCAGAC-5'

- Split_P5: P5-Sample_Index(i5)-Partial_TruSeqRead1

o 5'-AATGATACGGCACCAGGATCTACAC-N6-ACACTCTTCCCTACACGACGC-3'

- Split_P7: P7-Sample_Index(i7)-TruSeqRead2

o 5'-CAAGCAGAAGACGGCATACGAGAT-N6-GTGACTGGAATTCAGACGTGTGCTCTCCGATCT-3'

CRISPR Index PCR: P5-Sample_Index(i5)-TruSeqRead1-U6-gRNA-scaffold-A15-R1 N8-linker-R2 N8-linker-R3 N8-UMI-TruSeqRead2-Sample_Index(i7)-

5'-AATGATACGGCACCAGGATCTACAC-N6-ACACTCTTCCCTACACGACTCTCCGATCT-U6-gRNA-scaffold-A15-N8-CCACAGTCTCAAGCAGCTGGAT-N8-AGTCGTACGCCGATGCGAAACATCGGCCAC-N8-N10-AGATCGGAAGAGCACACGCTGAACTCCAGTAC-N6-ATCTCGTATGCCGCTCTTCTGCTTG-3'
3'-TTACTATGCCGCTGTGGCTCTAGATGTG-N6-TGTGAGAAAGGATGTGCTGCGAAGGCTAGA-U6-gRNA-scaffold-T15-N8-GGTGTCAAGTTCGTGCACCTA-N8-TCAGCATGCGGCTACGCTTTGTAGCCGGTG-N8-N10-TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGT-N6-TAGAGCATACGGCAGAAGACGAAC-5'

Sequencing:

ACACTCTTCCCTACACGACTCTCCGATCT→R1(U6+gRNA):74 I1 (i7):6
5'-AATGATACGGCACCAGGATCTACAC-N6-ACACTCTTCCCTACACGACTCTCCGATCT-U6-gRNA-scaffold-A15-N8-CCACAGTCTCAAGCAGCTGGAT-N8-AGTCGTACGCCGATGCGAAACATCGGCCAC-N8-N10-AGATCGGAAGAGCACACGCTGAACTCCAGTAC-N6-ATCTCGTATGCCGCTCTTCTGCTTG-3'
3'-TTACTATGCCGCTGTGGCTCTAGATGTG-N6-TGTGAGAAAGGATGTGCTGCGAAGGCTAGA-U6-gRNA-scaffold-T15-N8-GGTGTCAAGTTCGTGCACCTA-N8-TCAGCATGCGGCTACGCTTTGTAGCCGGTG-N8-N10-TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGT-N6-TAGAGCATACGGCAGAAGACGAAC-5'
I2 (i5):6 R2(UMI+R3_N8+R2_N8+R1_N8):86←TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGT