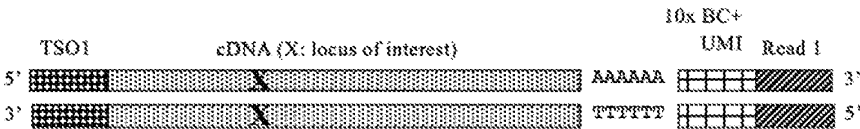
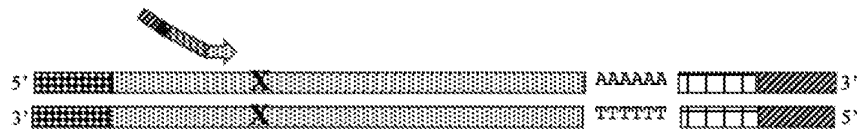


1. Amplified cDNA from 10x Genomics Chromium single-cell 3' libraries



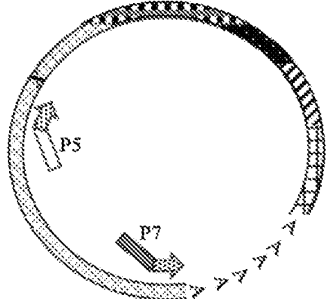
2. Hemi-nested gene-specific PCR (PCR no. 1: gene-specific primers)



3. Amplified targeted single-stranded amplicons



4. Circularization of single-stranded amplicons with splint ligation and gene-specific PCR (PCR no. 2: P5-GSP + P7-GSP primers **OR** P7-polyA)



Legends

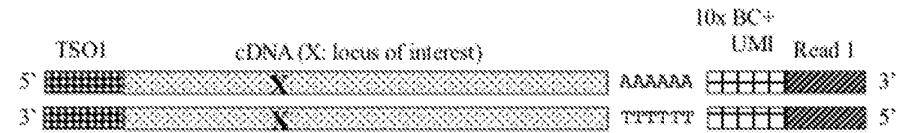
- Template-switch oligo (TSO1)
- cDNA
- 10x barcode (BC) + UMI
- Illumina Read 1
- Illumina Read 2
- Illumina index 1, i7
- Illumina P5
- Illumina P7

5. Final sequencing library structure

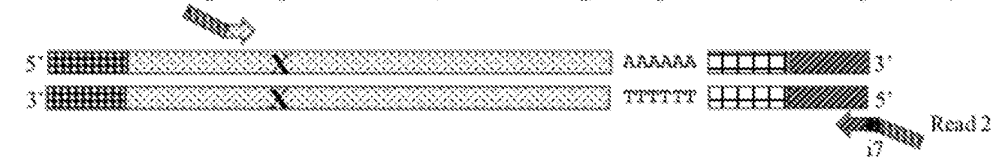


FIG. 1A

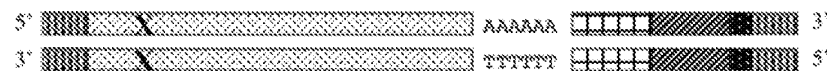
1. Amplified cDNA from 10x Genomics Chromium single-cell 3' libraries



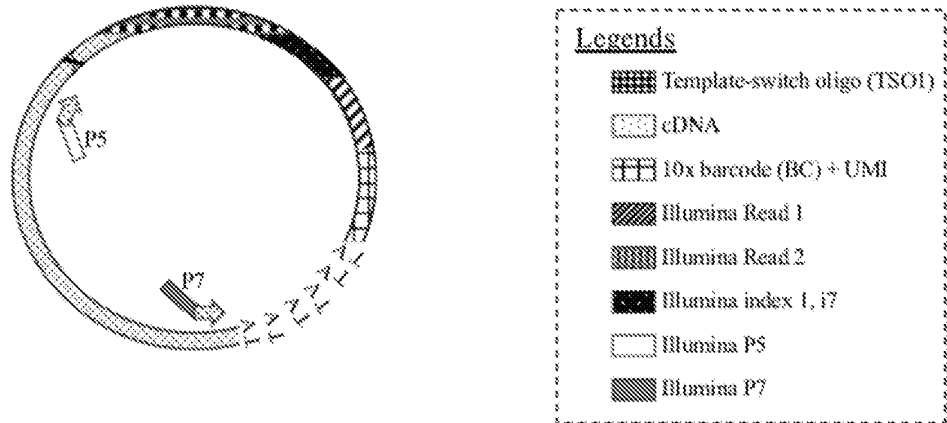
2. Hemi-nested gene-specific PCR (PCR no. 1: gene-specific + Read 1 primers)



3. Amplified targeted amplicons with Read 2 homology arms



4. Circularization of amplicons with Gibson assembly and gene-specific PCR (PCR no. 2: P5-GSP + P7-GSP primers OR P7-polyA)



5. Final sequencing library structure



FIG. 1B

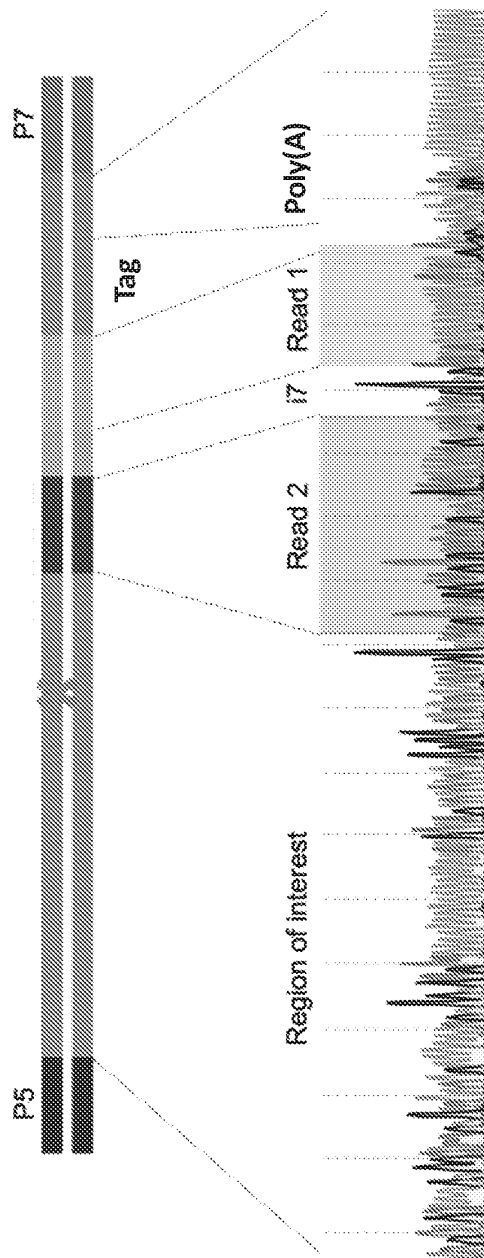
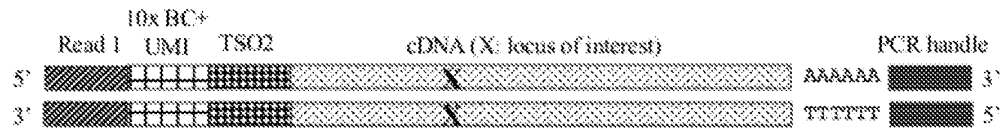
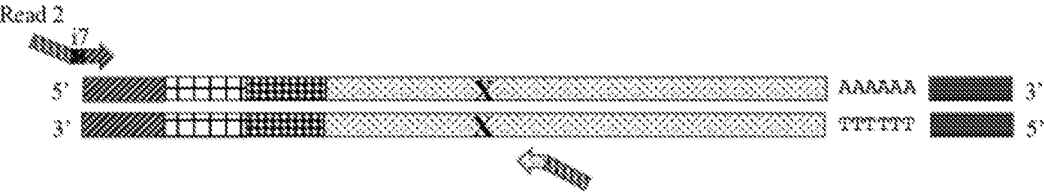


FIG. 1C

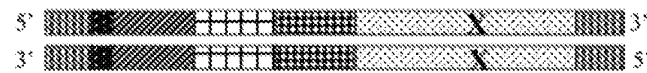
1. Amplified cDNA from 10x Genomics Chromium single-cell 5' libraries



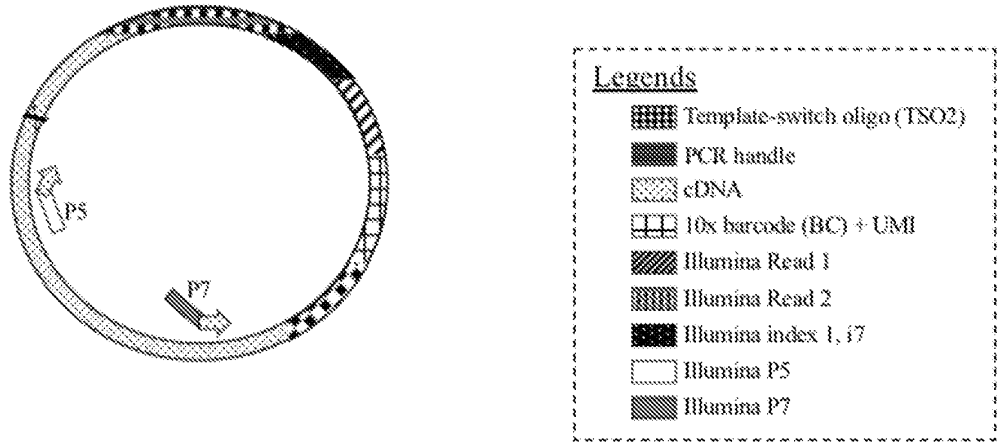
2. Hemi-nested gene-specific PCR (PCR no. 1: gene-specific + Read 1 primers)



3. Amplified targeted amplicons with Read 2 homology arms



4. Circularization of amplicons with Gibson assembly and gene-specific PCR (PCR no. 2: P5-GSP + P7-GSP primers OR P7-TSO)



5. Final sequencing library structure



FIG. 2

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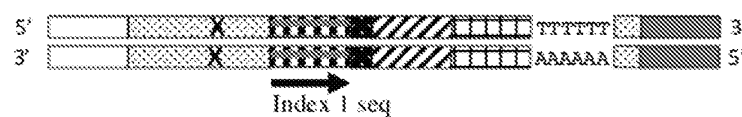
1. Final SIT-seq sequencing library structure



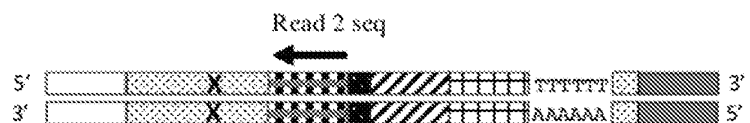
2. Read 1 sequencing



3. Index 1, i7 sequencing



4. Read 2 sequencing

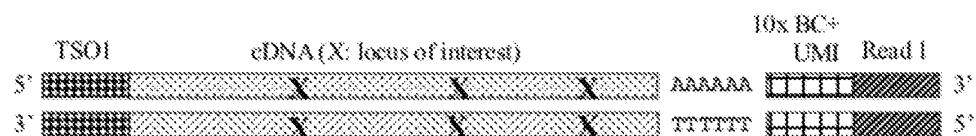
**Legends**

- cDNA
- 10x barcode (BC) + UMI
- Illumina Read 1
- Illumina Read 2
- Illumina index 1, i7
- Illumina P5
- Illumina P7

FIG. 3

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1. Amplified cDNA from 10x Genomics Chromium single-cell 3' libraries

**Alternative A**

2. Gene-specific probe with PCR handle targeting 5' most upstream locus



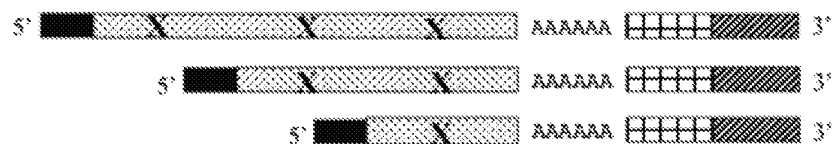
3. Elongated probe with gene-specific probe targeting 5' most upstream locus

**Alternative B**

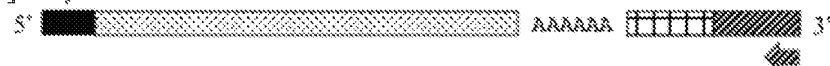
2. Tiling gene-specific probes with PCR handle targeting each locus



3. Elongated probes with tiling gene-specific probes



4. Minimal PCR amplification and use amplicons for hemi-nested gene-specific PCR [FIG. 1B, Step 2]

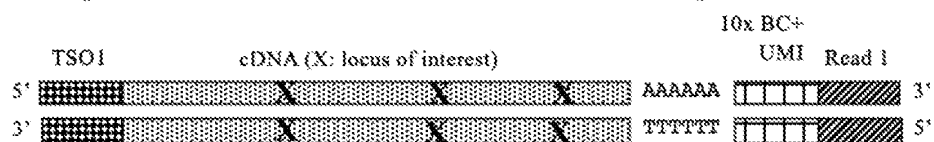
**Legends**

Template-switch oligo (TSO1)
cDNA

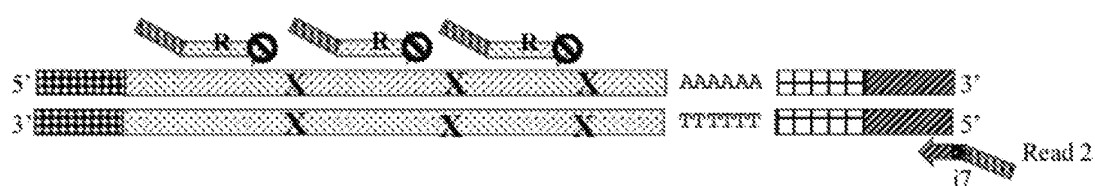
10x barcode (BC) + UMI
Illumina Read 1
PCR handle

FIG. 4

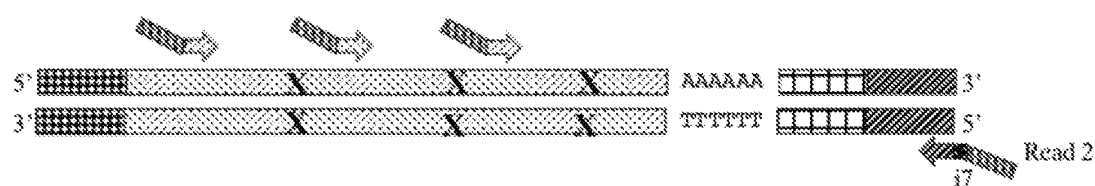
1. Amplified cDNA from 10x Genomics Chromium single-cell 3' libraries



2. Hemi-nested gene-specific PCR with rhPCR primers (PCR no. 1: gene-specific rhPCR + Read 1 primers)



3. RNaseH2-dependent PCR



4. Use amplicons for circularization of amplicons with Gibson assembly [FIG. 1B, Step 4]

FIG. 5