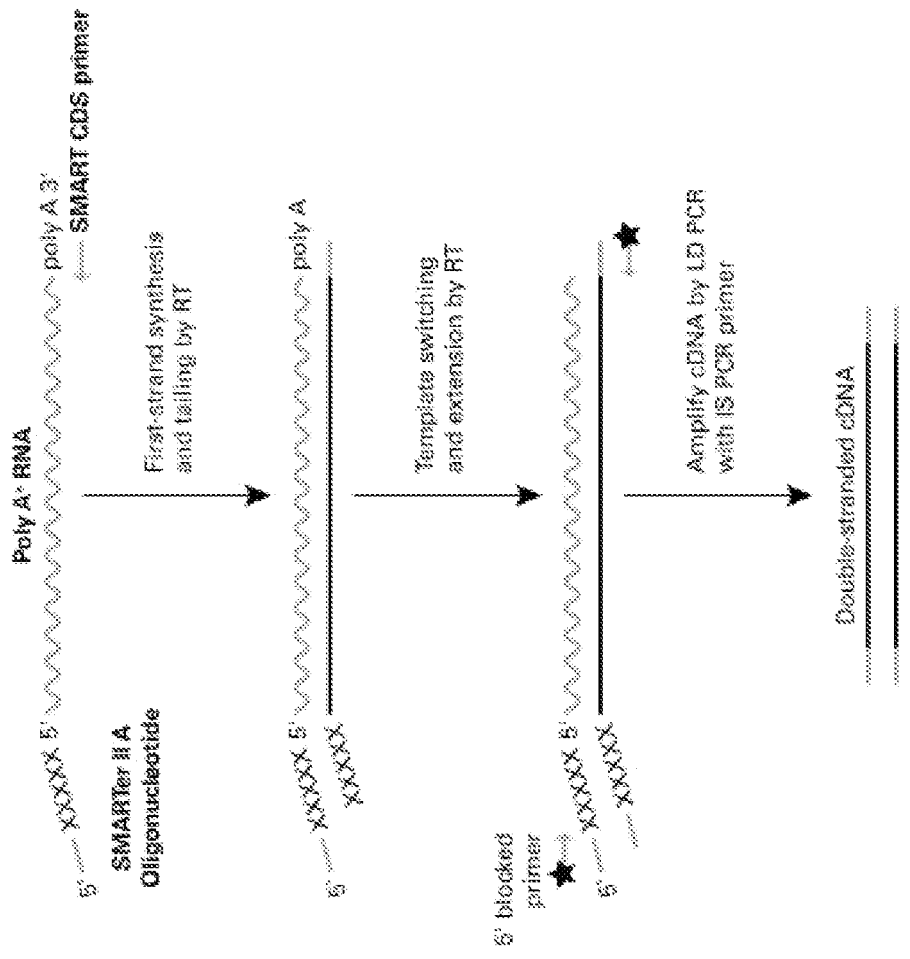

Poly A+ RNA
poly A 3'
SMART CDS primer
SMARTer II A Oligonucleotide
First-strand synthesis and tailing by RT
poly A
Template switching and extension by RT
5' blocked primer
Amplify cDNA by LD PCR with IS PCR primer
Double-stranded cDNA

Fig. 1

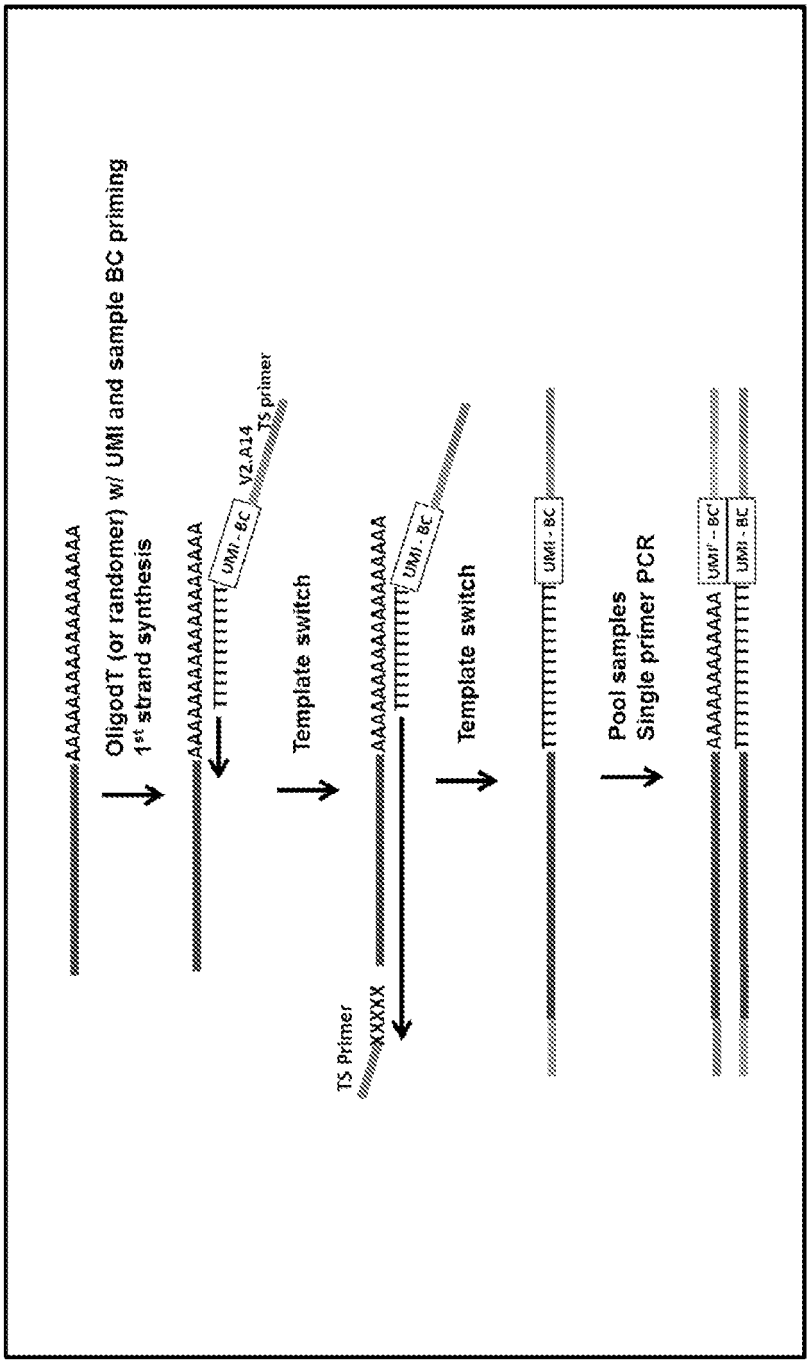

AAAAAAAAAAAAAAAAA
OligodT (or randomer) w/ UMI and sample BC priming
1st strand synthesis
AAAAAAAAAAAAAAAAA
TTTTTTTTTTTTTTTT
UMI - BC
V2.A14
TS primer
Template switch
AAAAAAAAAAAAAAAAA
TTTTTTTTTTTTTTTT
UMI - BC
TS Primer
XXXXX
Template switch
TTTTTTTTTTTTTTTT
UMI - BC
Pool samples
Single primer PCR
AAAAAAAAAAAAAAAAA
UMI - BC
TTTTTTTTTTTTTTTT
UMI - BC

Fig. 2A

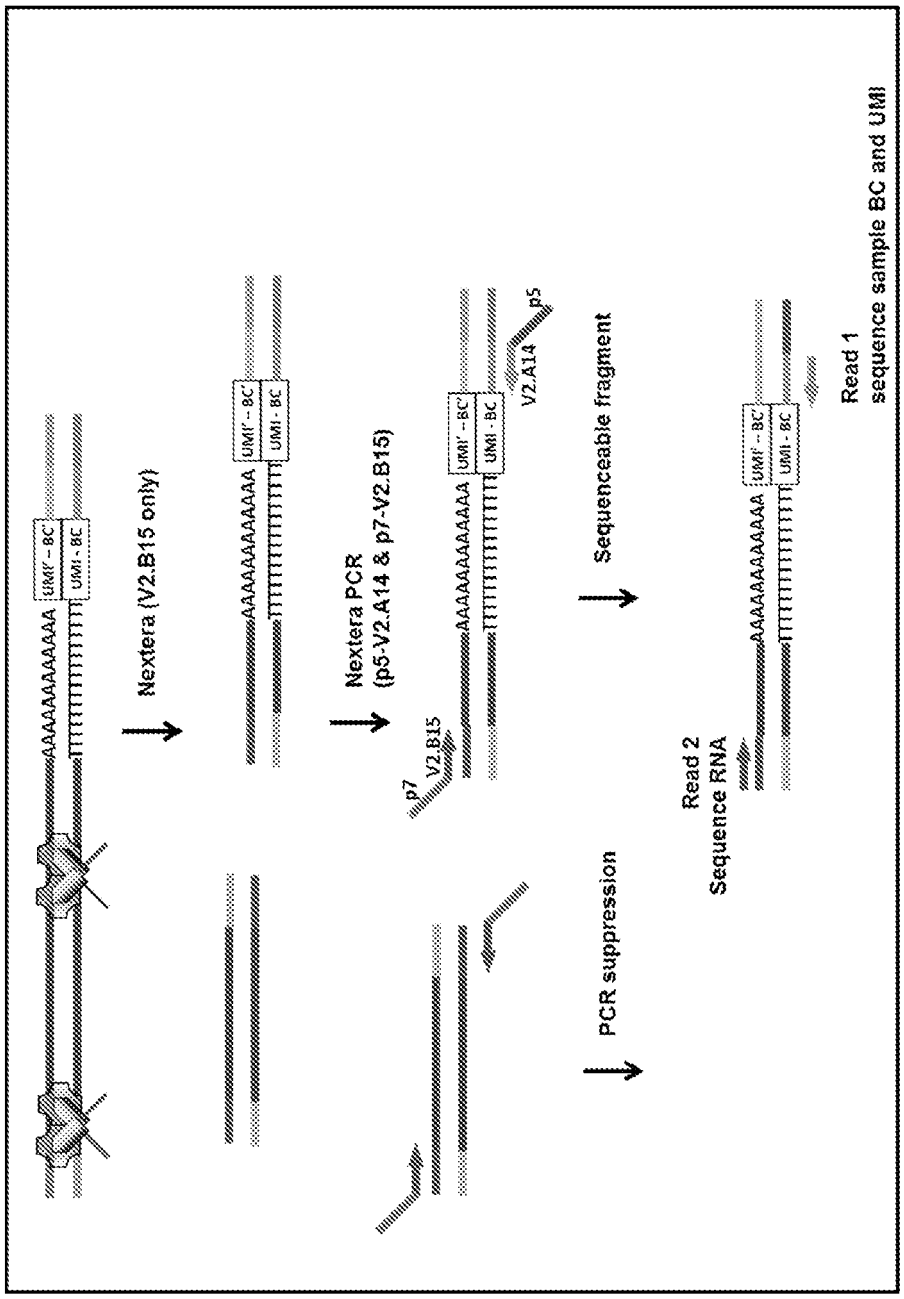

AAAAAAAAAAAA
UMI' - BC'
UMI - BC
TTTTTTTTTTTTTTT
Nextera (V2.B15 only)
Nextera PCR
(p5-V2.A14 & p7-V2.B15)
p7
V2.B15
V2.A14
p5
PCR suppression
Sequenceable fragment
Read 2
Sequence RNA
Read 1
sequence sample BC and UMI

Fig. 2B

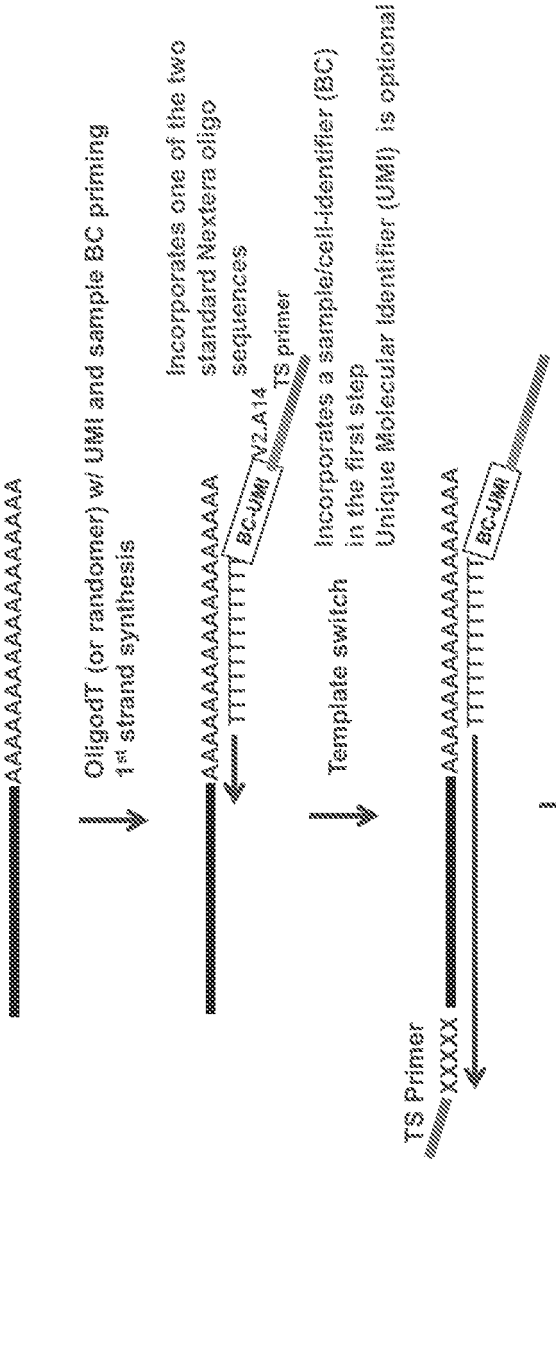

AAAAAAAAAAAAAAAAA
Oligo dT (or randomer) w/ UMI and sample BC priming
1st strand synthesis
Incorporates one of the two
standard Nextera oligo
sequences
AAAAAAAAAAAAAAAAA
TTTTTTTTTTTTTTT
BC-UMI
V2_A14
TS primer
Template switch
Incorporates a sample/cell-identifier (BC)
in the first step
Unique Molecular Identifier (UMI) is optional
TS Primer
XXXXX
AAAAAAAAAAAAAAAAA
TTTTTTTTTTTTTTT
BC-UMI
TTTTTTTTTTTTTTT
BC-UMI
Pool samples
All subsequent steps carried out with multiplexed pool

Fig. 3A

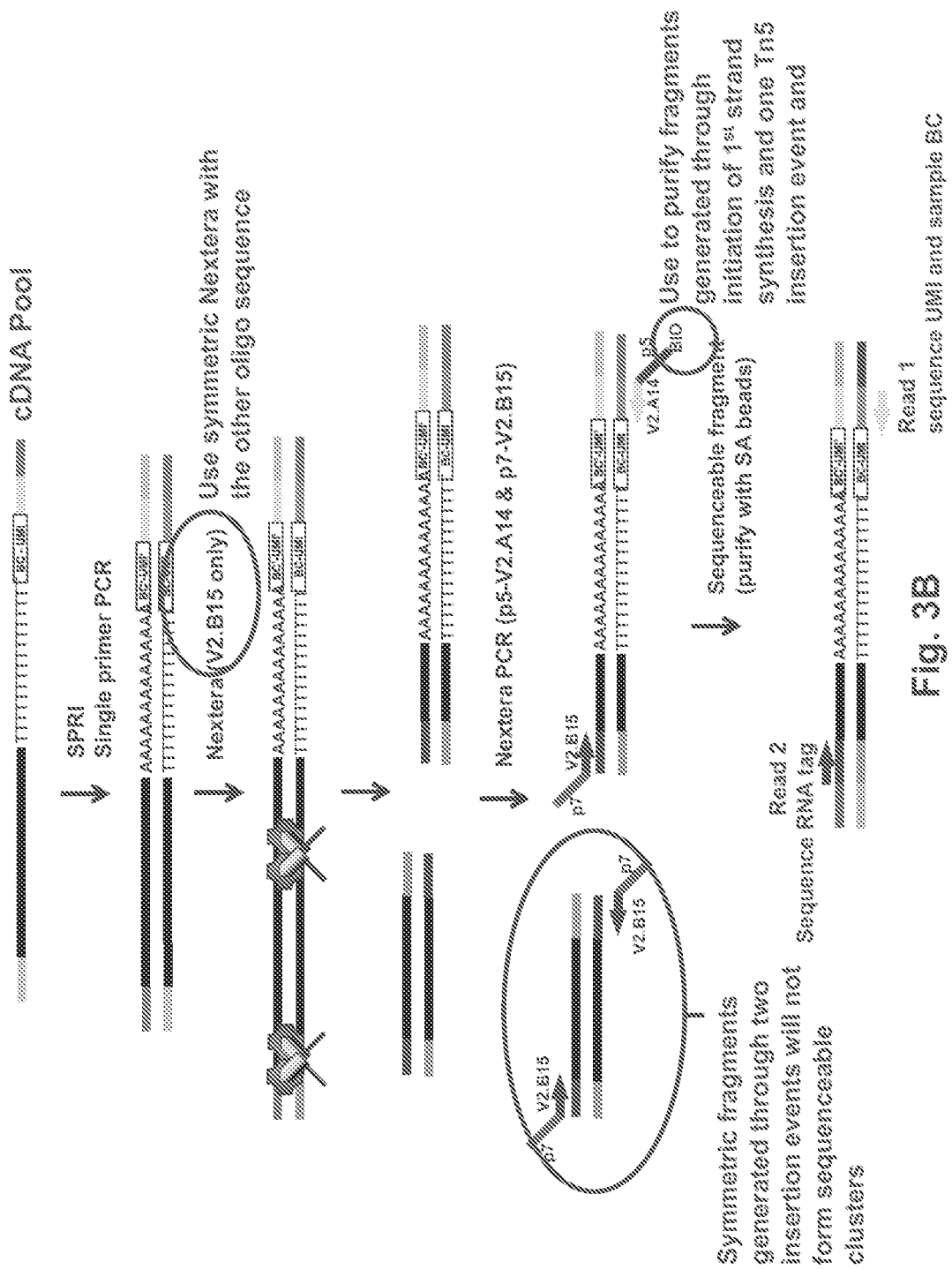

cDNA Pool
SPRI
Single primer PCR
Nextera (V2.B15 only)
Use symmetric Nextera with the other oligo sequence
Nextera PCR (p5-V2.A14 & p7-V2.B15)
Use to purify fragments generated through initiation of 1st strand synthesis and one Tn5 insertion event and
Sequenceable fragments (purify with SA beads)
Read 2
Sequence RNA tag
Read 1
sequence UMI and sample BC
Symmetric fragments generated through two insertion events will not form sequenceable clusters

Fig. 3B

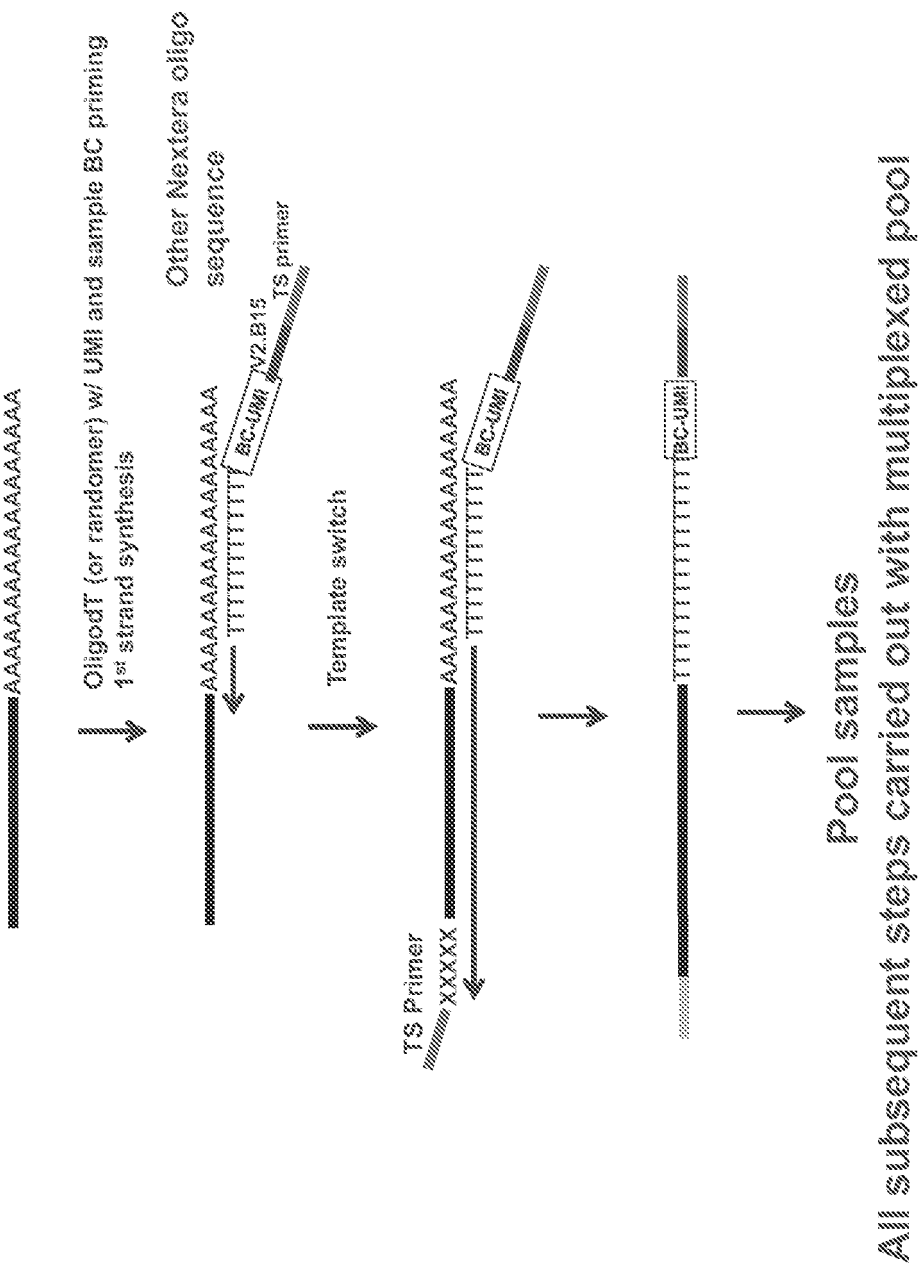

AAAAAAAAAAAAAAAAA
Oligo dT (or randomer) w/ UMI and sample BC priming
1st strand synthesis
Other Nextera oligo sequence
AAAAAAAAAAAAAAAAA
TTTTTTTTTTTTTTTT
BC-UMI
V2.B15
TS primer
Template switch
TS Primer
XXXXX
AAAAAAAAAAAAAAAAA
TTTTTTTTTTTTTTTT
BC-UMI
TTTTTTTTTTTTTTTT
BC-UMI
Pool samples
All subsequent steps carried out with multiplexed pool

Fig. 4A

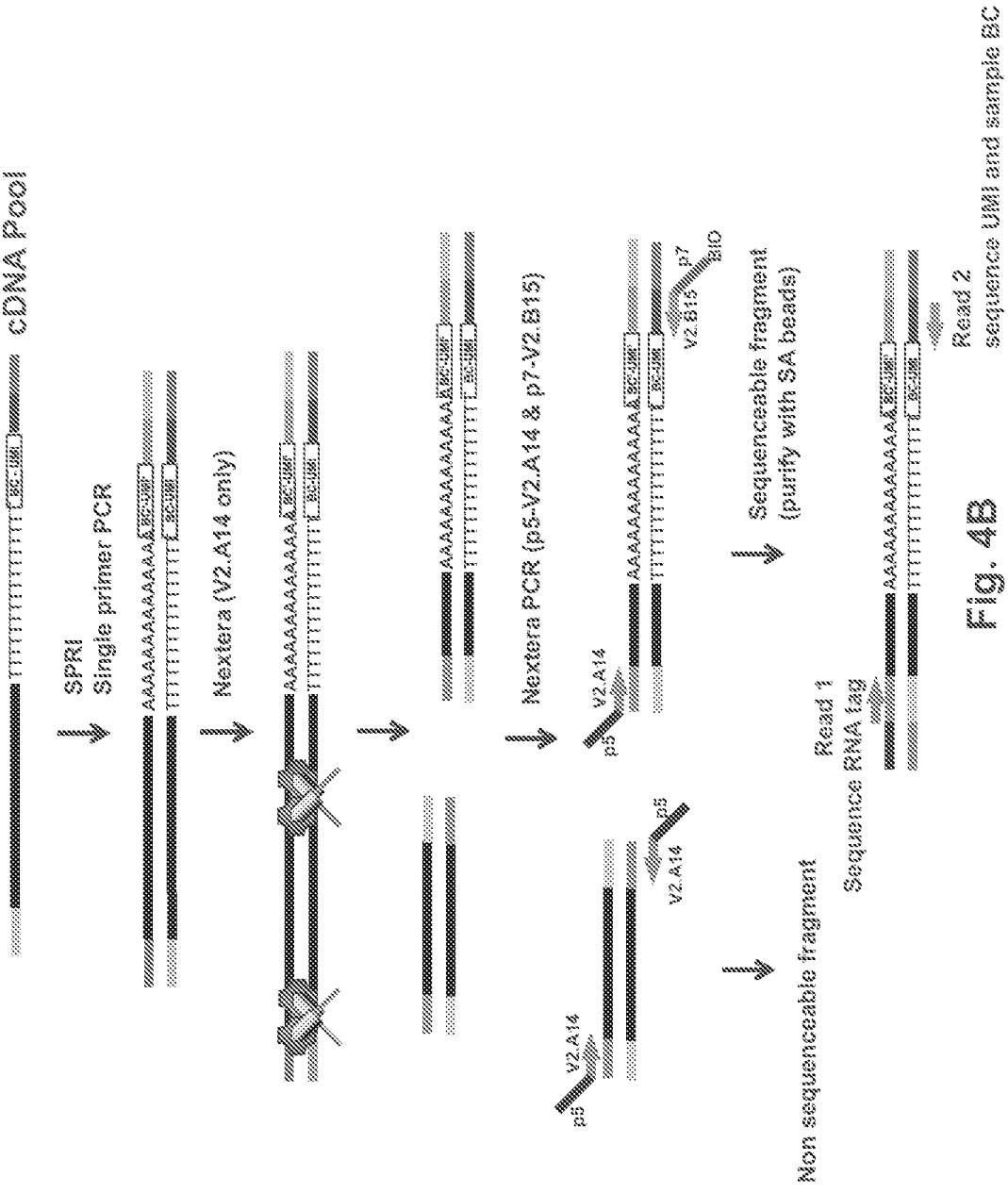

cDNA Pool
SPRI
Single primer PCR
Nextera (V2.A14 only)
Nextera PCR (p5-V2.A14 & p7-V2.B15)
Sequenceable fragment
(purify with SA beads)
Non sequenceable fragment
Read 1
Sequence RNA tag
Read 2
sequence UMI and sample BC
V2.A14
p5
V2.B15
p7
BIO

Fig. 4B

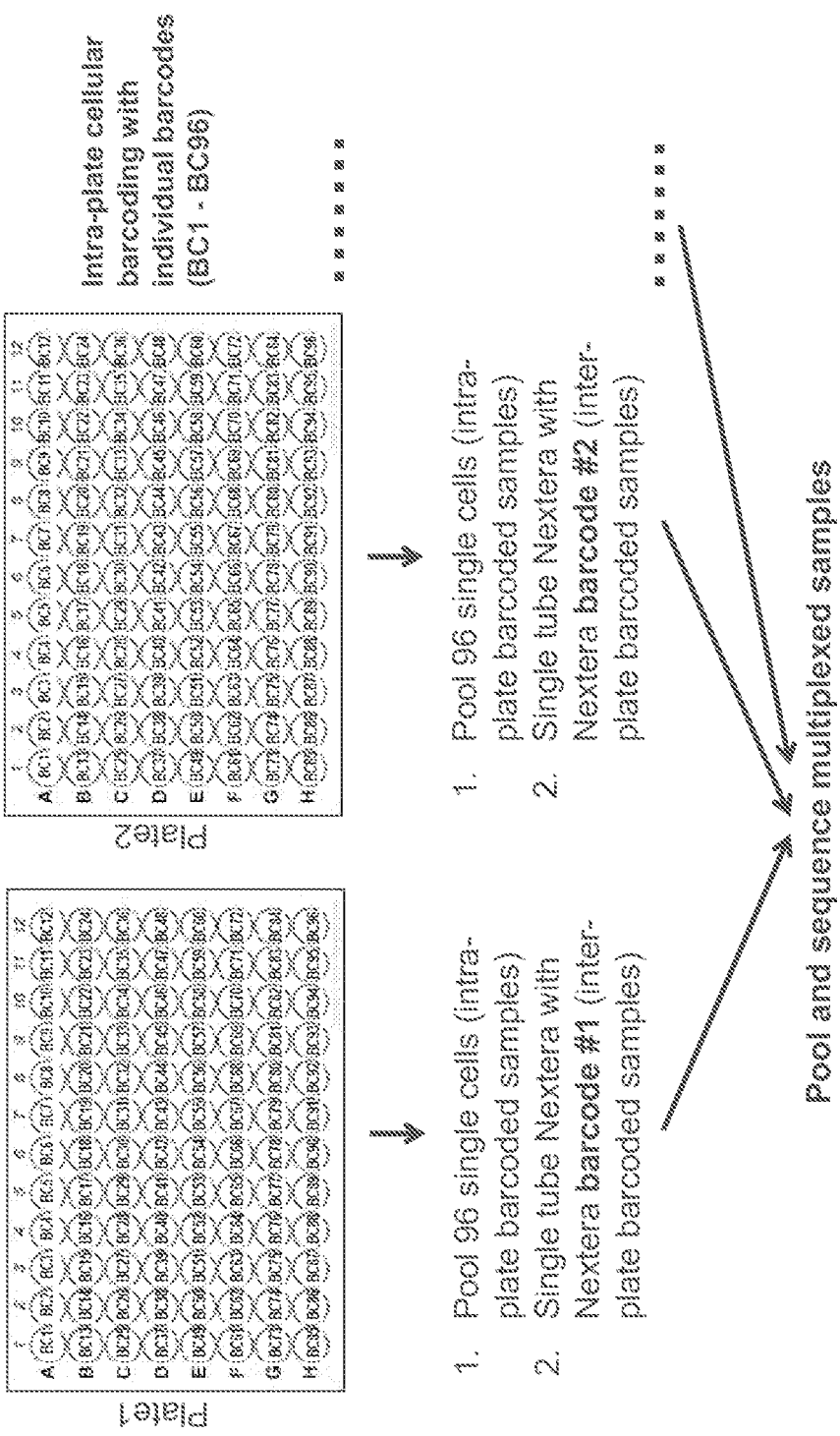

Intra-plate cellular barcoding with individual barcodes (BC1 - BC96)
Plate 1
Plate 2
1. Pool 96 single cells (intra-plate barcoded samples)
2. Single tube Nextera with Nextera barcode #1 (inter-plate barcoded samples)
1. Pool 96 single cells (intra-plate barcoded samples)
2. Single tube Nextera with Nextera barcode #2 (inter-plate barcoded samples)
Pool and sequence multiplexed samples

Fig. 5

Key sequence metrics using 100 pg HBR

Sequence Metric		Statistics			
		High Throughput GEx	STRT		SMART
1st Strand priming method		Oligo-dT	Oligo-dT	Oligo-dT + Randomer	Oligo-dT
Mapping rate	Aligned to Hg19 transcriptome	63%	75%	50%	78%
	Ribosomal	1%	2%	12%	1%
	Mitochondrial	15%	14%	15%	12%
	Other	1%	0%	0%	1%
	Unaligned	20%	9%	23%	8%
Correlation of gene-expression between technical replicates (r)		0.9	0.85	0.72	0.94
No. of genes detected (FPKM>1)		10,021	11,254	11,683	8,492

Fig. 6

Initial data from single cells

Sequence Metric	Statistics			
	High Througput GEx		STRT	
1st Strand priming method	Oligo-dT	Oligo-dT	Oligo-dT + Randomer	
Nextera version	V2.B15	V2.B15/V2.A14	V2.B15/V2.A14	
Mapping rate: Aligned to hg19 / mm10	69%	77%	80%	
Mapping rate: Ribosomal	1%	1%	5%	
Mapping rate: Mitochondria	--	--	--	
Mapping rate: Other	1%	1%	0%	
Mapping rate: Unaligned	29%	21%	14%	
Correlation of gene-expression between technical replicates (r)	0.95	0.94	0.60*	
No. of genes detected (FPKM>1)	6,904	7,789	8,727	

* Unusually low correlation due to one outlier.

Fig. 7

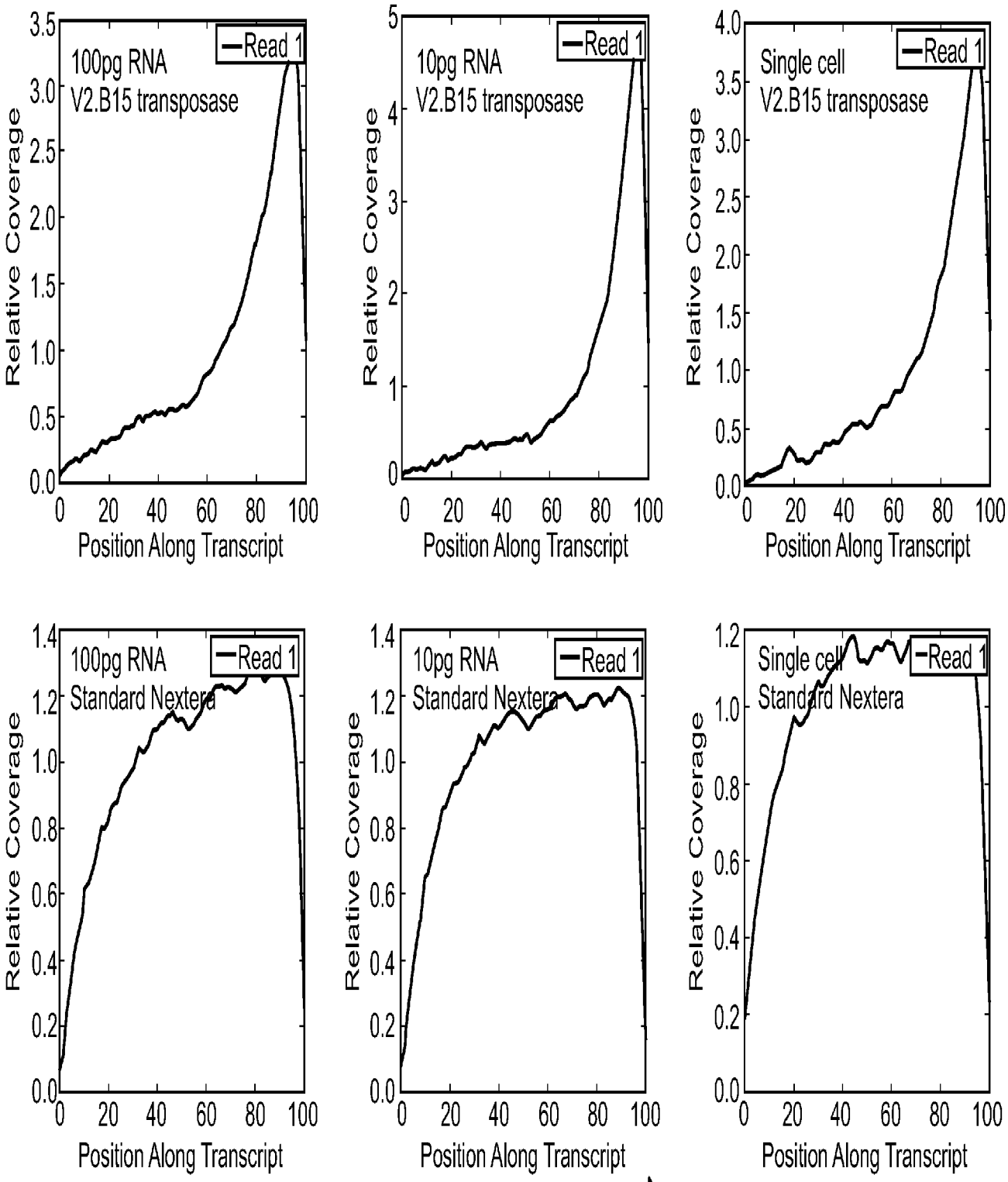

100pg RNA
V2.B15 transposase
Read 1
10pg RNA
V2.B15 transposase
Read 1
Single cell
V2.B15 transposase
Read 1
100pg RNA
Standard Nextera
Read 1
10pg RNA
Standard Nextera
Read 1
Single cell
Standard Nextera
Read 1
Relative Coverage
Position Along Transcript
5'-3' coverage

Fig. 8

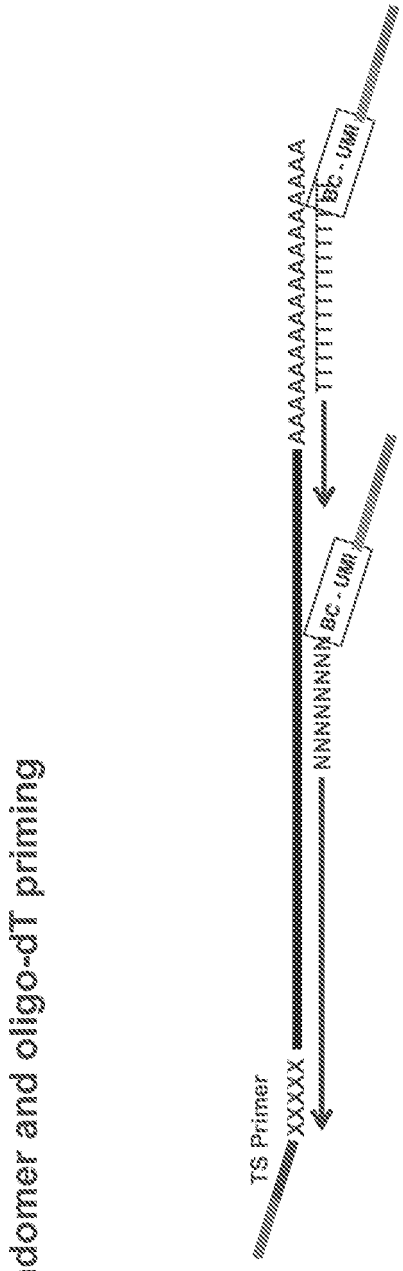

Randomer and oligo-dT priming
TS Primer
XXXXX
NNNNNNNN
BC + UMI
AAAAAAAAAAAAAAAAAA
TTTTTTTTTTTTTTTTTT
BC + UMI

Fig. 9A

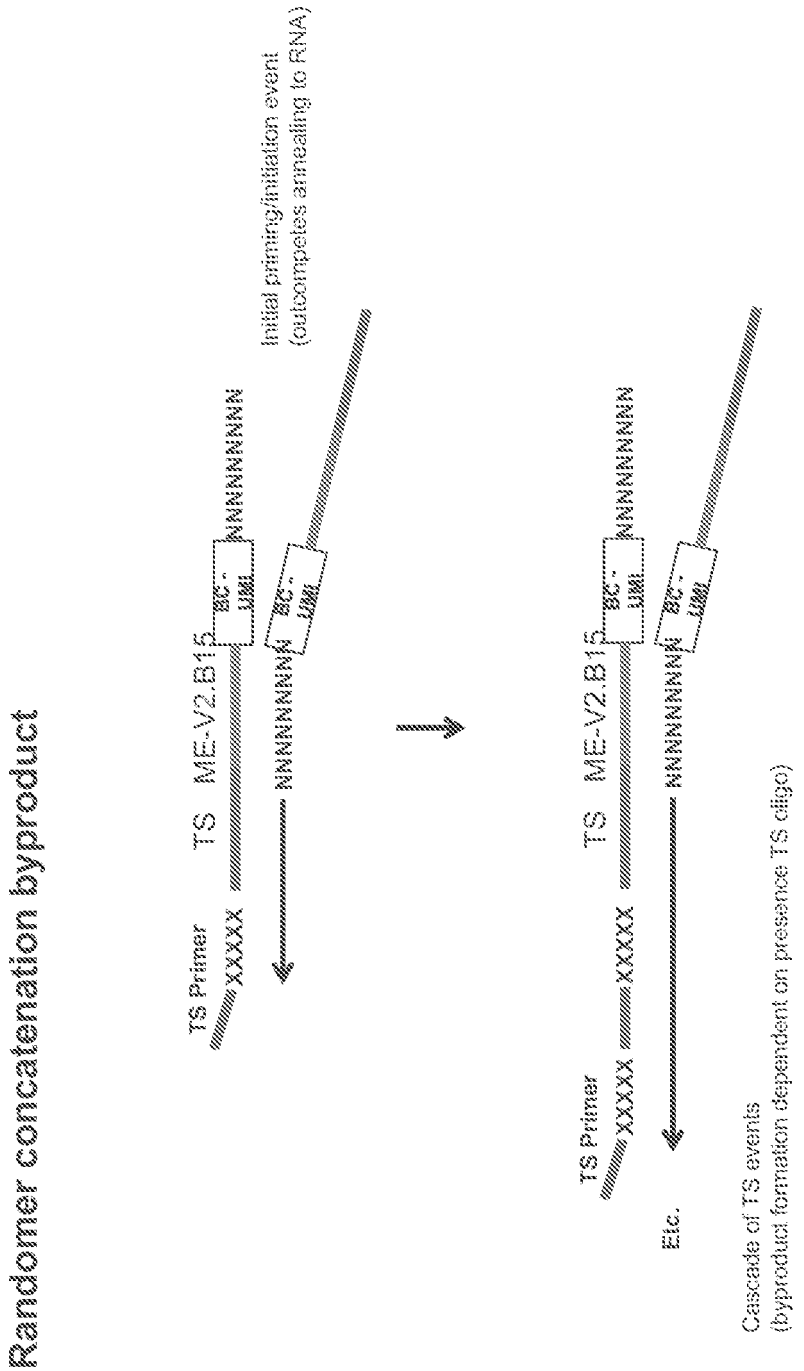

Randomer concatenation byproduct
TS Primer
XXXXX
TS
ME-V2.B15
BC: UMI
NNNNNNNNN
NNNNNNNNN
BC: UMI
Initial priming/initiation event
(outcompetes annealing to RNA)
TS Primer
XXXXX
TS
ME-V2.B15
BC: UMI
NNNNNNNNN
NNNNNNNNN
BC: UMI
Etc.
Cascade of TS events
(byproduct formation dependent on presence TS oligo)

Fig. 9B

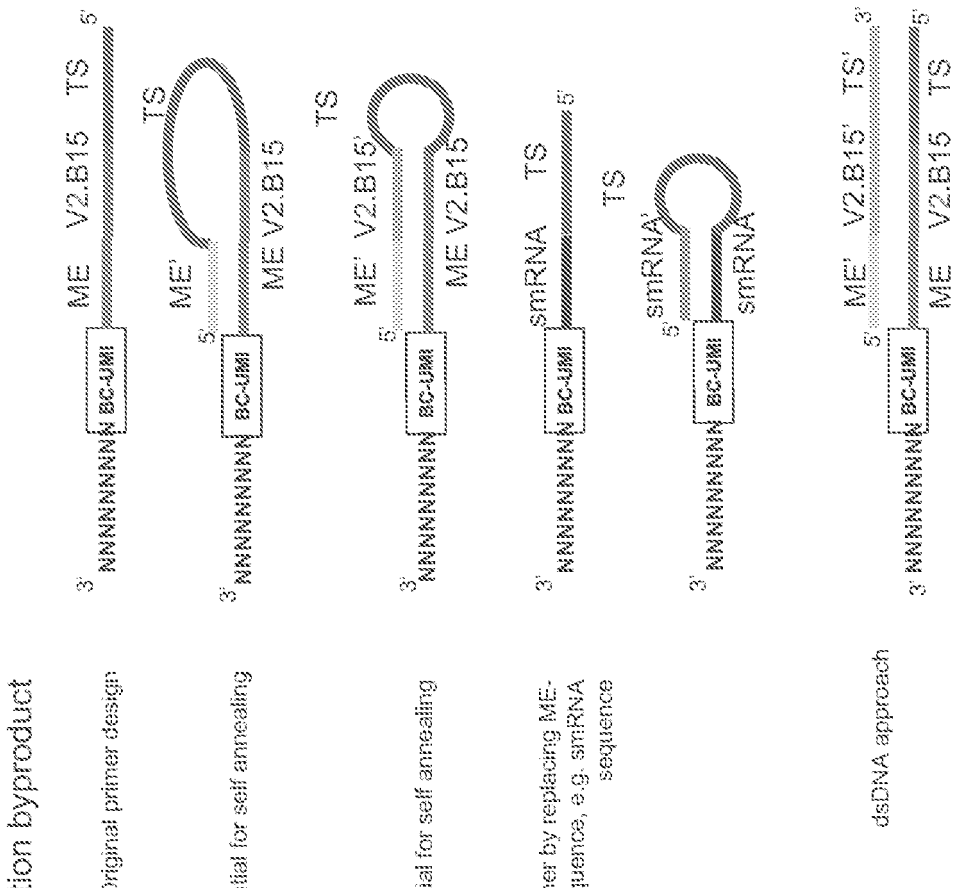

Possible strategies to reduce concatenation byproduct
N9 primers or N6 primers
Original primer design
Hairpin 1 to reduce potential for self annealing
Hairpin 2 to reduce potential for self annealing
Reduce length of primer by replacing ME-V2.A14 with shorter sequence, e.g. smRNA sequence
dsDNA approach
ME V2.B15 TS
ME' V2.B15' TS'
smRNA TS
smRNA'
NNNNNNNNN
BC-UMI

Fig. 10

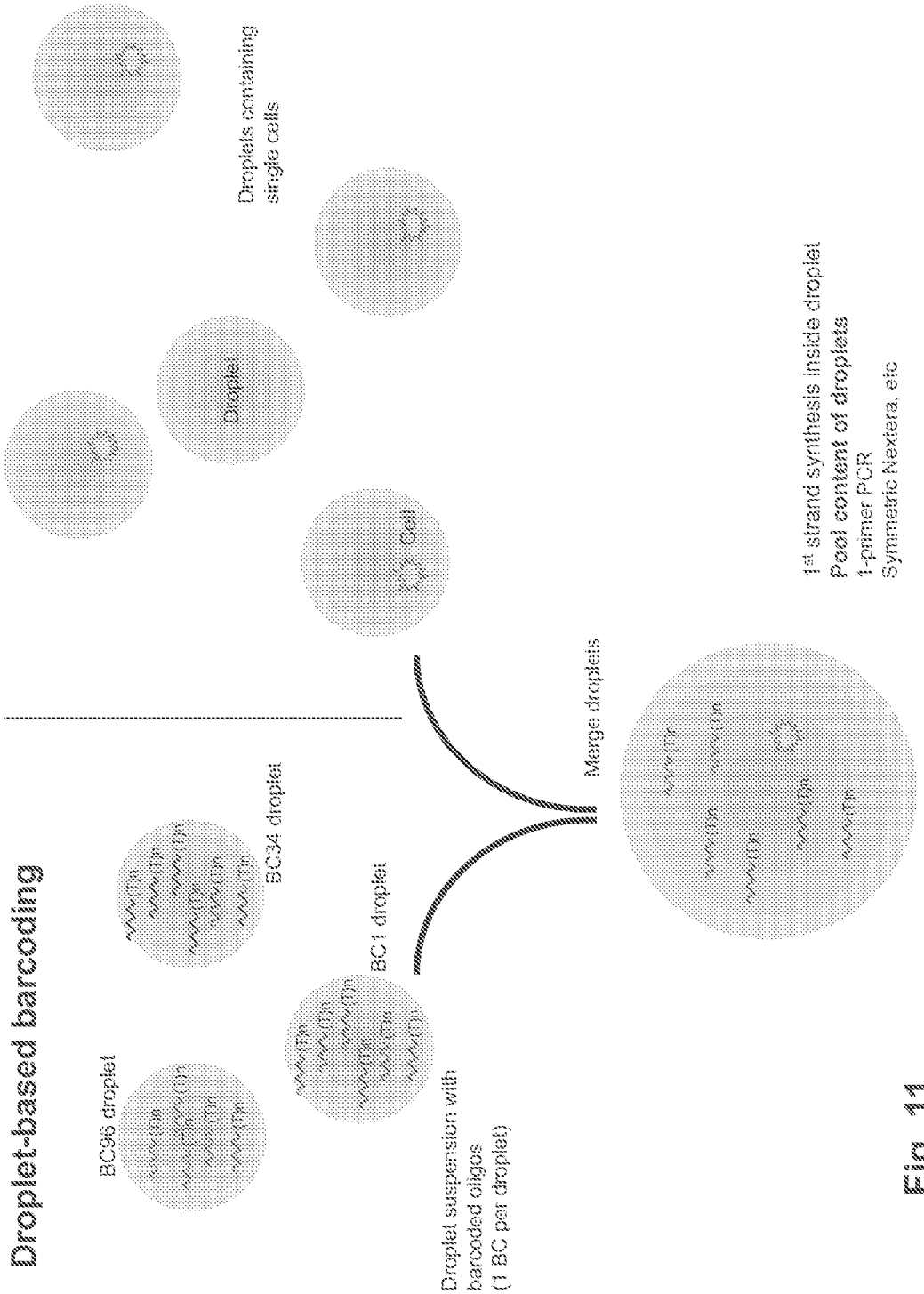

Droplet-based barcoding
BC96 droplet
BC34 droplet
BC1 droplet
Droplet suspension with barcoded oligos (1 BC per droplet)
Merge droplets
Cell
Droplet
Droplets containing single cells
1st strand synthesis inside droplet
Pool content of droplets
1-primer PCR
Symmetric Nextera, etc

Fig. 11

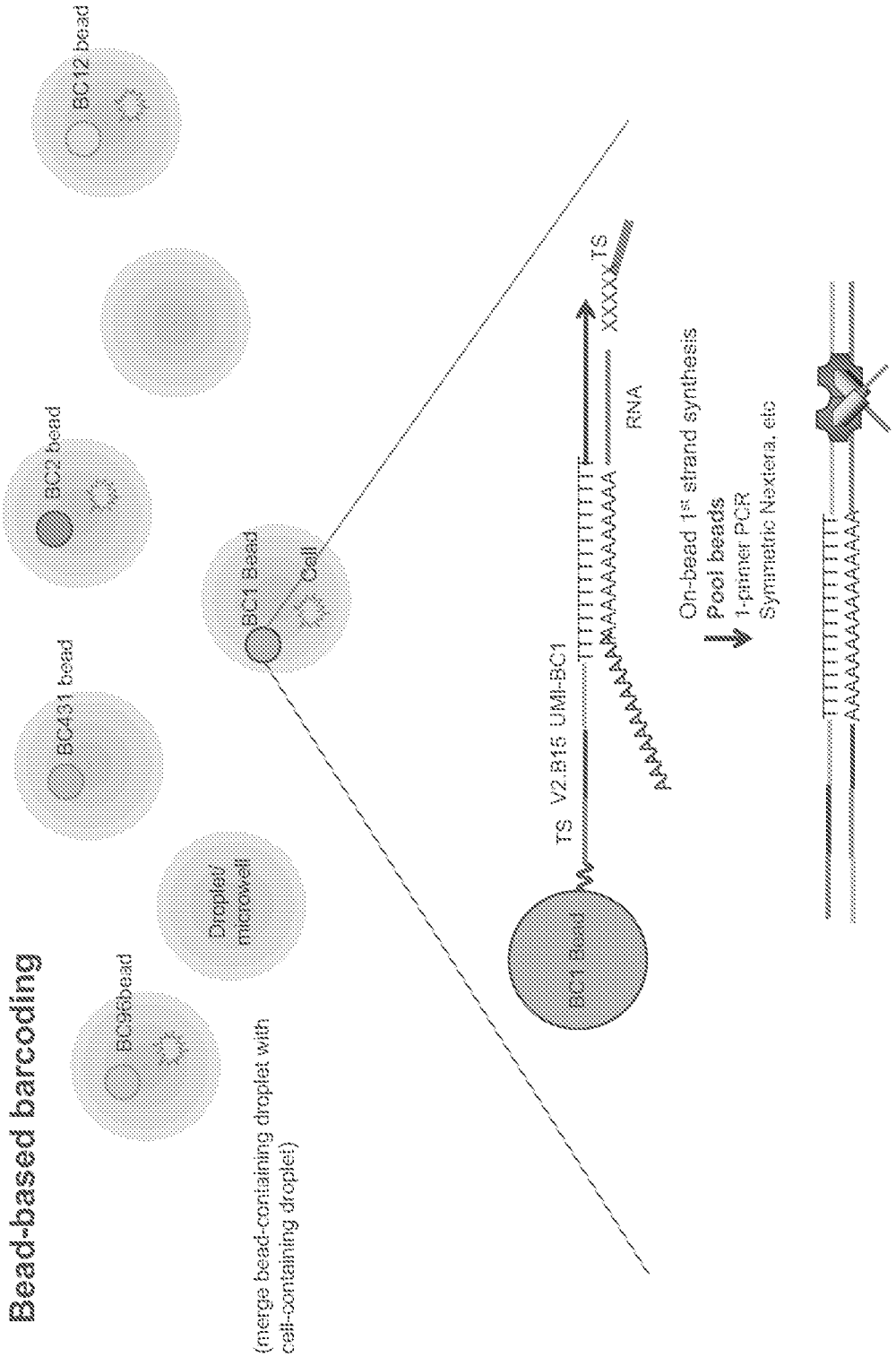

Bead-based barcoding
BC96bead
BC431 bead
BC2 bead
BC12 bead
Droplet/ microwell
(merge bead-containing droplet with cell-containing droplet)
BC1 Bead
Cell
TS V2.B16 UMI-BC1
RNA
XXXX TS
On-bead 1st strand synthesis
Pool beads
1-primer PCR
Symmetric Nextera, etc

Fig. 12