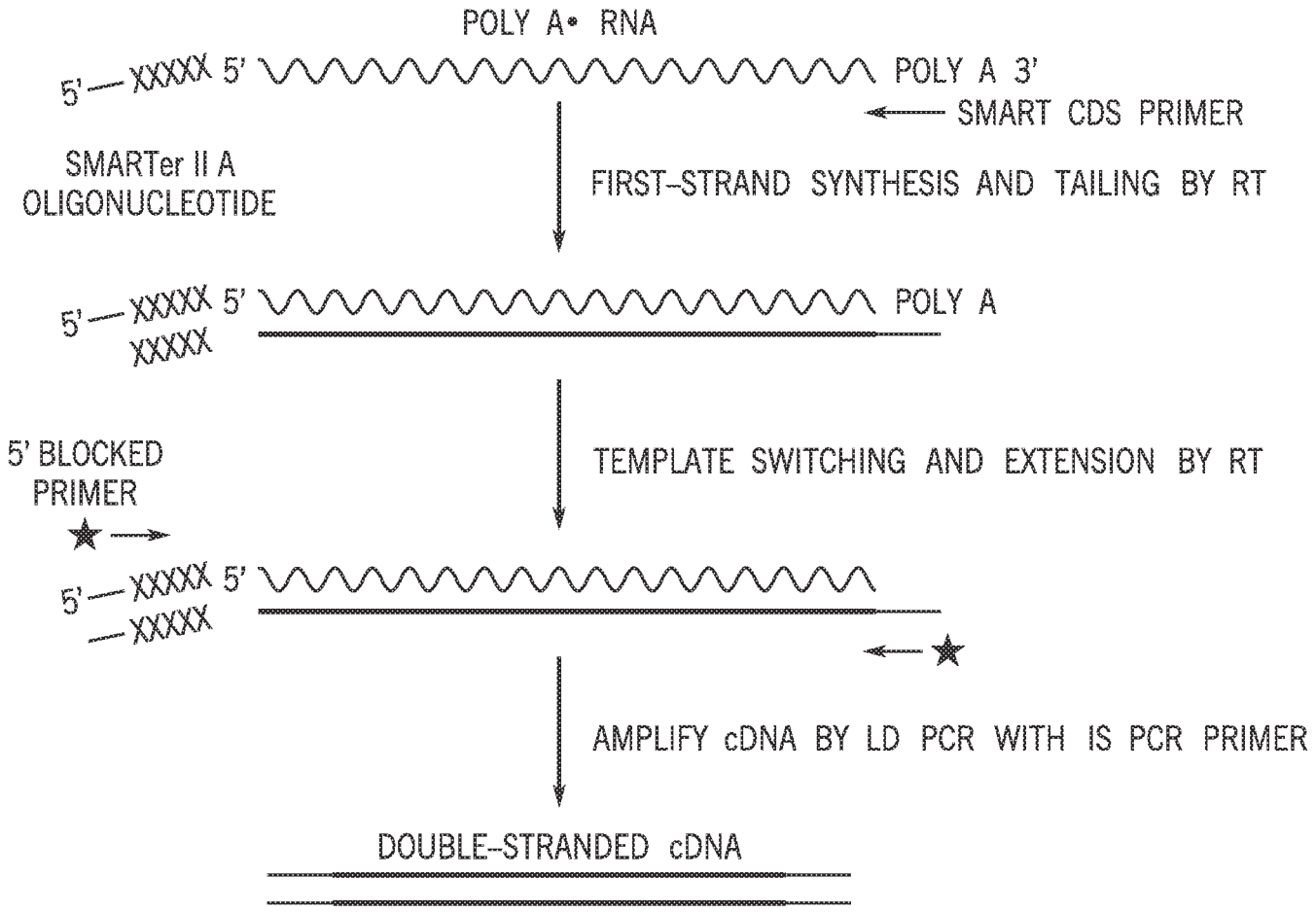

POLY A+ RNA
5'— XXXXX 5'
POLY A 3'
SMART CDS PRIMER
SMARTer II A
OLIGONUCLEOTIDE
FIRST-STRAND SYNTHESIS AND TAILING BY RT
5'— XXXXX 5'
XXXXX
POLY A
5' BLOCKED
PRIMER
TEMPLATE SWITCHING AND EXTENSION BY RT
5'— XXXXX 5'
— XXXXX
AMPLIFY cDNA BY LD PCR WITH IS PCR PRIMER
DOUBLE-STRANDED cDNA

FIG. 1

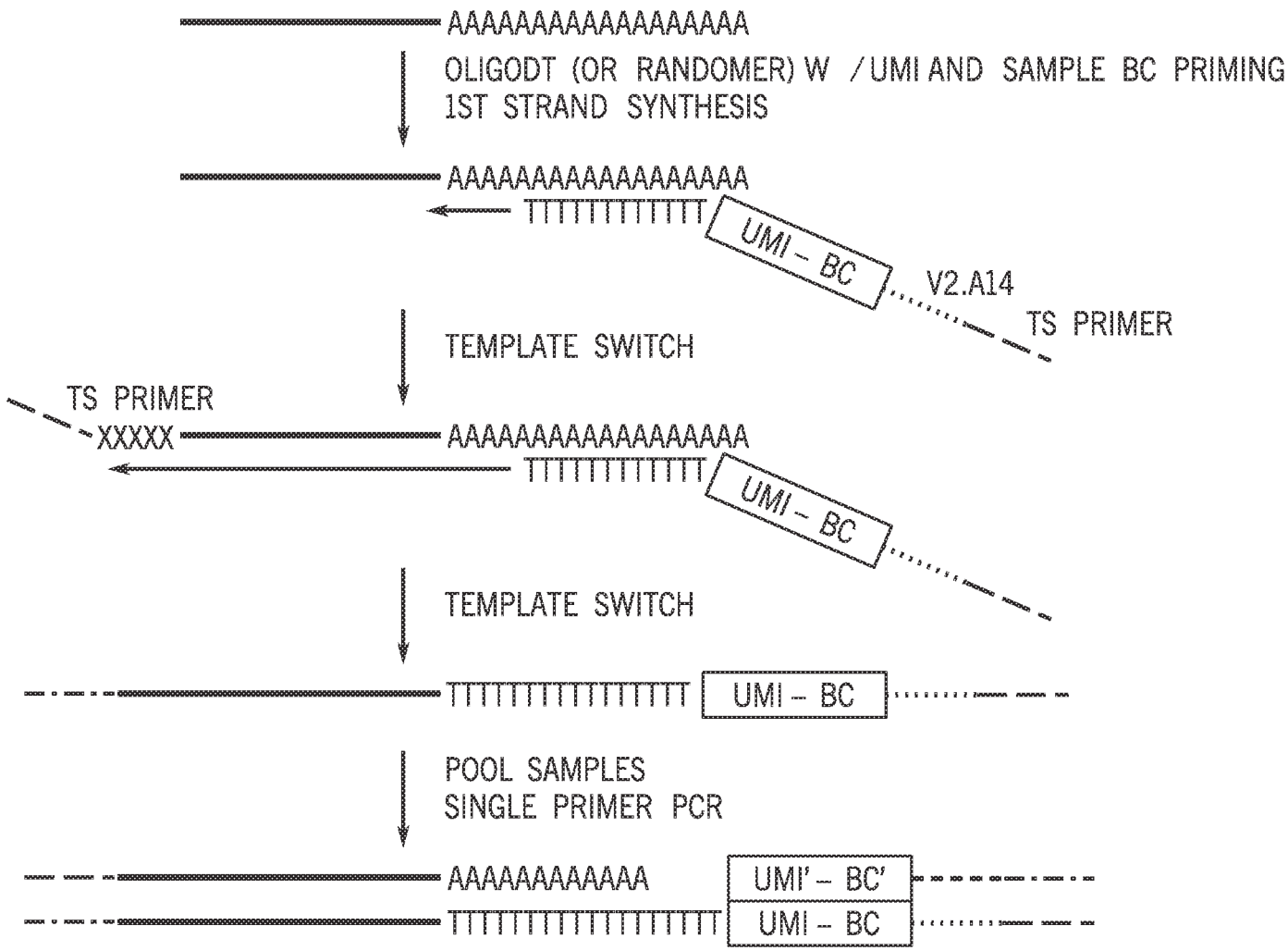

AAAAAAAAAAAAAAAAAA
OLIGODT (OR RANDOMER) W /UMI AND SAMPLE BC PRIMING
1ST STRAND SYNTHESIS
AAAAAAAAAAAAAAAAAA
TTTTTTTTTTTT
UMI – BC
V2.A14
TS PRIMER
TEMPLATE SWITCH
TS PRIMER
XXXXX
AAAAAAAAAAAAAAAAAA
TTTTTTTTTTTT
UMI – BC
TEMPLATE SWITCH
TTTTTTTTTTTTTTTT
UMI – BC
POOL SAMPLES
SINGLE PRIMER PCR
AAAAAAAAAAA
UMI' – BC'
TTTTTTTTTTTTTTTTT
UMI – BC

FIG. 2A

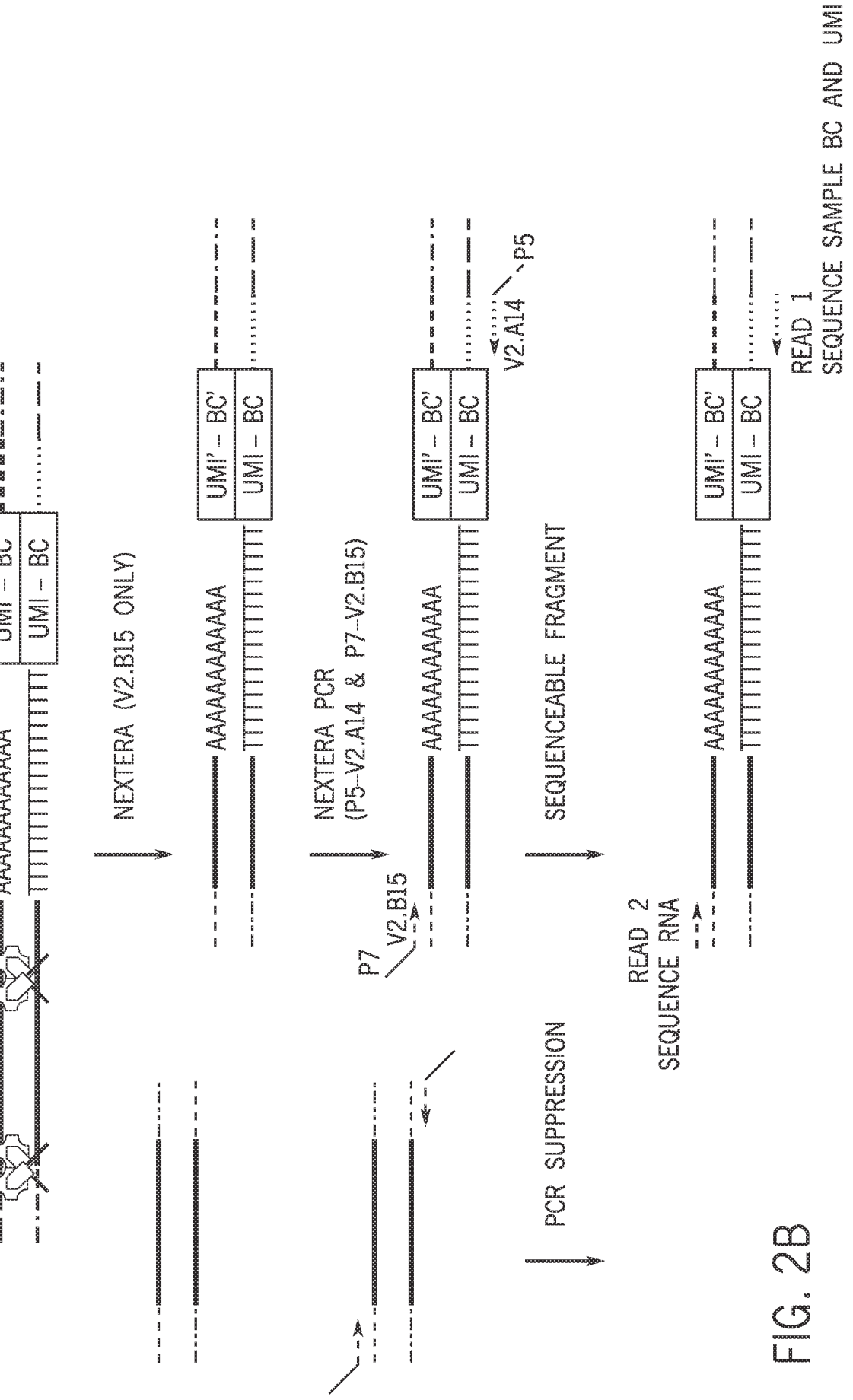

AAAAAAAAAA
TTTTTTTTTTTTTTTT
UMI' – BC'
UMI – BC
NEXTERA (V2.B15 ONLY)
AAAAAAAAAA
TTTTTTTTTTTTTTTT
UMI' – BC'
UMI – BC
NEXTERA PCR
(P5–V2.A14 & P7–V2.B15)
P7
V2.B15
AAAAAAAAAA
TTTTTTTTTTTTTTTT
UMI' – BC'
UMI – BC
V2.A14
P5
SEQUENCEABLE FRAGMENT
PCR SUPPRESSION
READ 2
SEQUENCE RNA
AAAAAAAAAA
TTTTTTTTTTTTTTTT
UMI' – BC'
UMI – BC
READ 1
SEQUENCE SAMPLE BC AND UMI

FIG. 2B

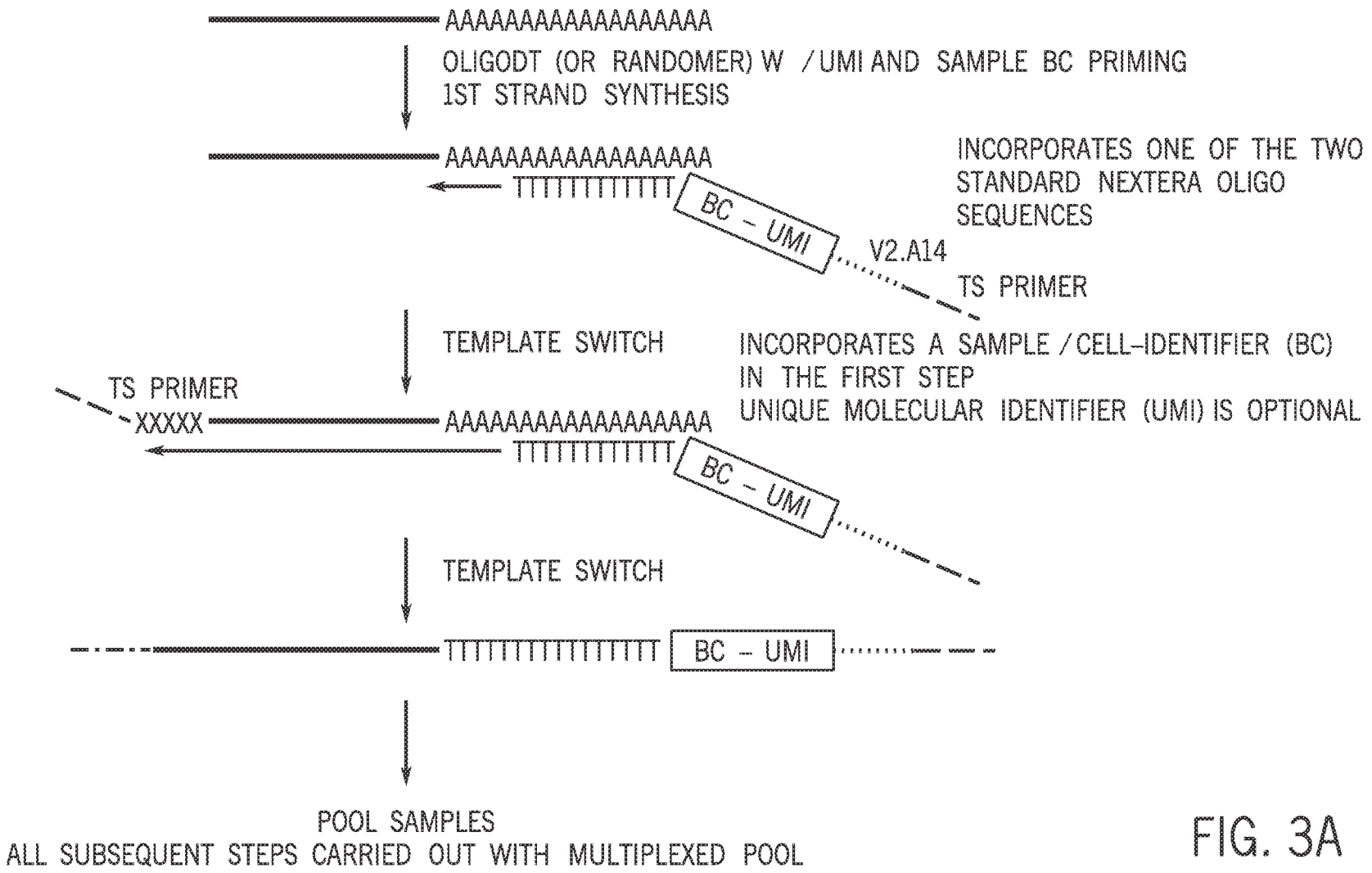

AAAAAAAAAAAAAAAAAA
OLIGODT (OR RANDOMER) W /UMI AND SAMPLE BC PRIMING
1ST STRAND SYNTHESIS
AAAAAAAAAAAAAAAAAA
TTTTTTTTTTTT
BC – UMI
V2.A14
INCORPORATES ONE OF THE TWO STANDARD NEXTERA OLIGO SEQUENCES
TS PRIMER
TEMPLATE SWITCH
INCORPORATES A SAMPLE /CELL–IDENTIFIER (BC) IN THE FIRST STEP
UNIQUE MOLECULAR IDENTIFIER (UMI) IS OPTIONAL
TS PRIMER
XXXXX
AAAAAAAAAAAAAAAAAA
TTTTTTTTTTTT
BC – UMI
TEMPLATE SWITCH
TTTTTTTTTTTTTTTT
BC – UMI
POOL SAMPLES
ALL SUBSEQUENT STEPS CARRIED OUT WITH MULTIPLEXED POOL

FIG. 3A

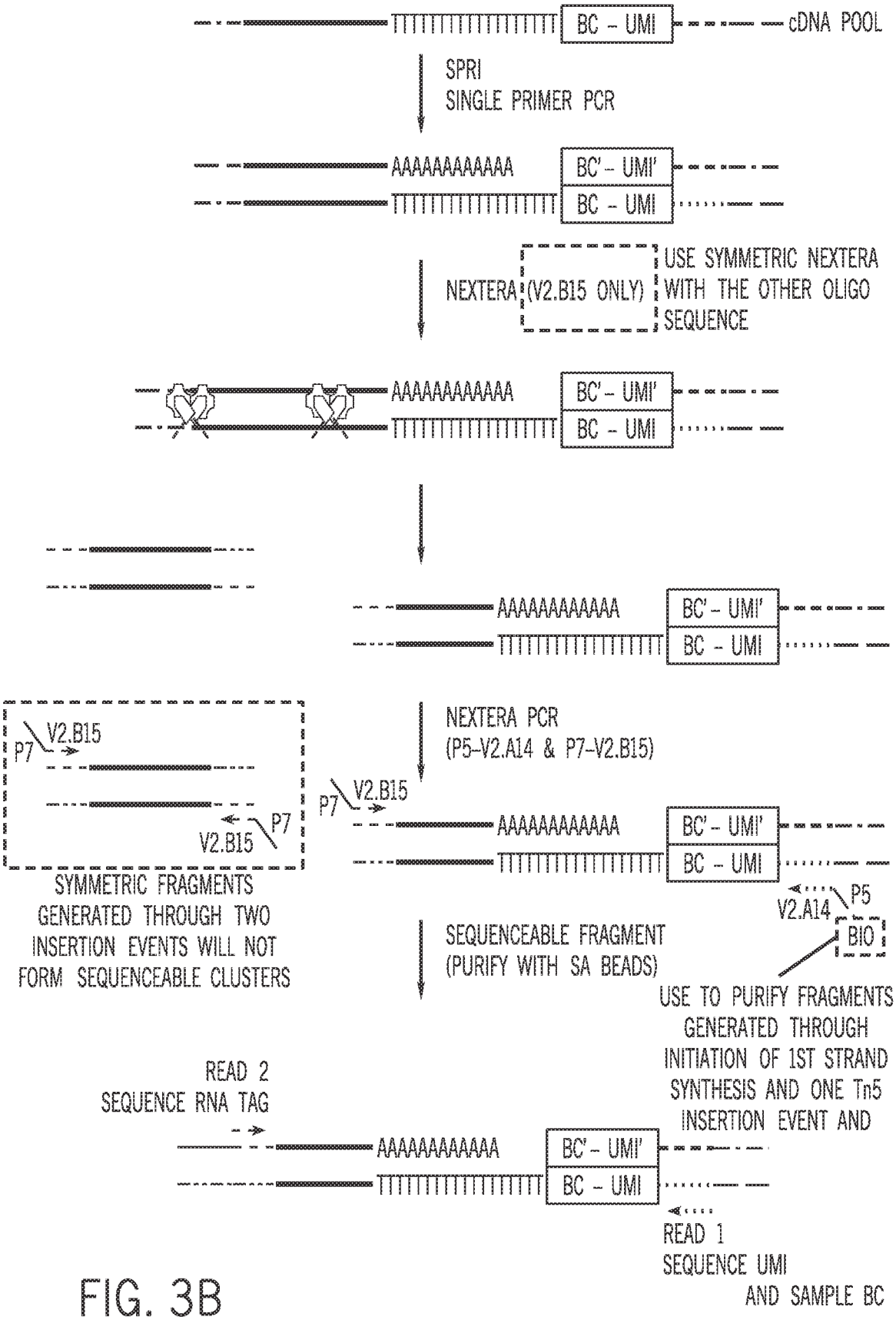

TTTTTTTTTTTTTTTTT
BC – UMI
cDNA POOL
SPRI
SINGLE PRIMER PCR
AAAAAAAAAAAA
BC' – UMI'
NEXTERA
(V2.B15 ONLY)
USE SYMMETRIC NEXTERA WITH THE OTHER OLIGO SEQUENCE
P7
V2.B15
NEXTERA PCR
(P5–V2.A14 & P7–V2.B15)
SYMMETRIC FRAGMENTS GENERATED THROUGH TWO INSERTION EVENTS WILL NOT FORM SEQUENCEABLE CLUSTERS
SEQUENCEABLE FRAGMENT
(PURIFY WITH SA BEADS)
V2.A14
P5
BIO
USE TO PURIFY FRAGMENTS GENERATED THROUGH INITIATION OF 1ST STRAND SYNTHESIS AND ONE Tn5 INSERTION EVENT AND
READ 2
SEQUENCE RNA TAG
READ 1
SEQUENCE UMI
AND SAMPLE BC

FIG. 3B

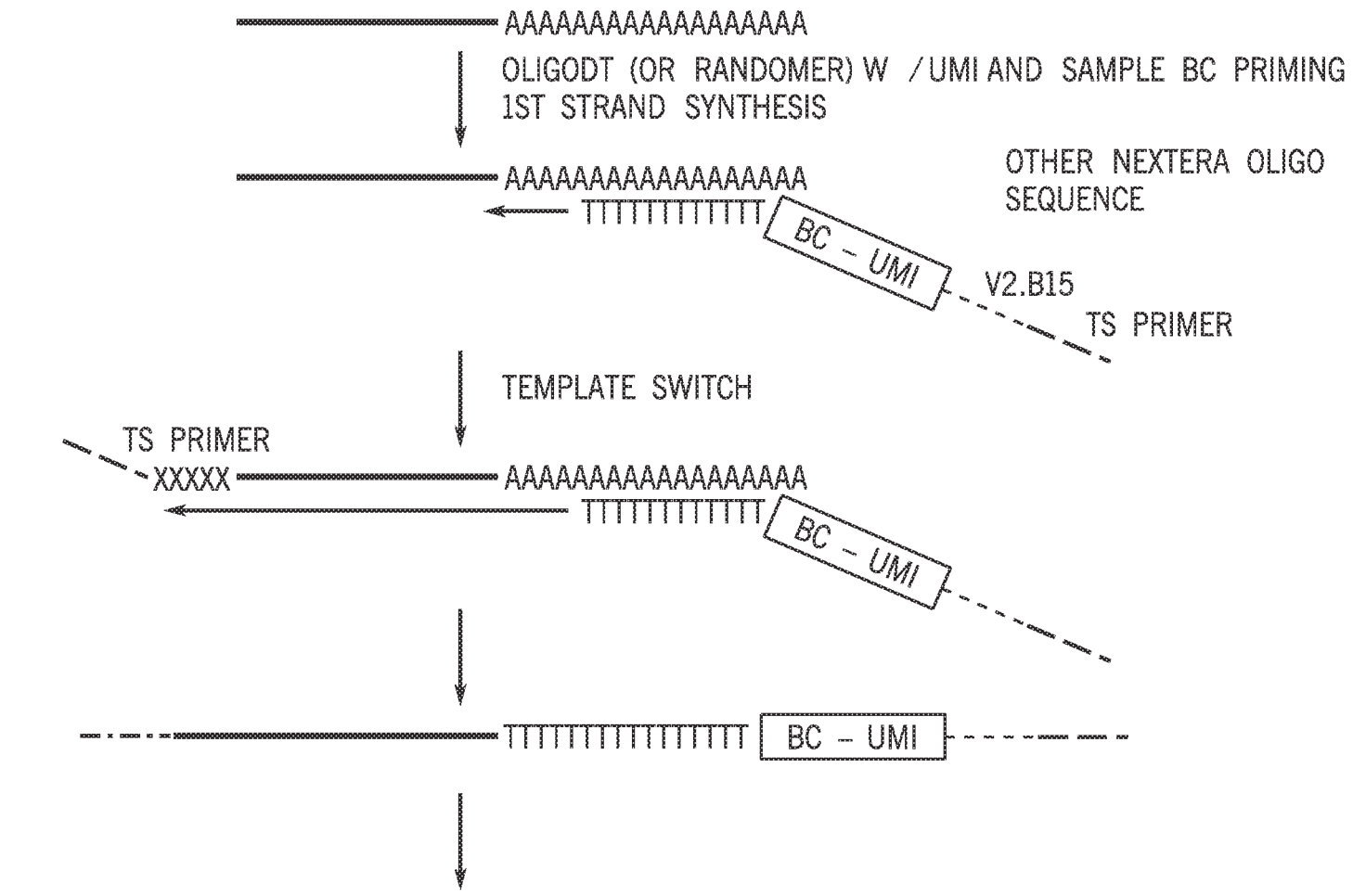

AAAAAAAAAAAAAAAAAA
OLIGODT (OR RANDOMER) W /UMI AND SAMPLE BC PRIMING
1ST STRAND SYNTHESIS
AAAAAAAAAAAAAAAAAA
TTTTTTTTTTTT
BC – UMI
OTHER NEXTERA OLIGO SEQUENCE
V2.B15
TS PRIMER
TEMPLATE SWITCH
TS PRIMER
XXXXX
AAAAAAAAAAAAAAAAAA
TTTTTTTTTTTT
BC – UMI
TTTTTTTTTTTTTTTT
BC – UMI
POOL SAMPLES
ALL SUBSEQUENT STEPS CARRIED OUT WITH MULTIPLEXED POOL

FIG. 4A

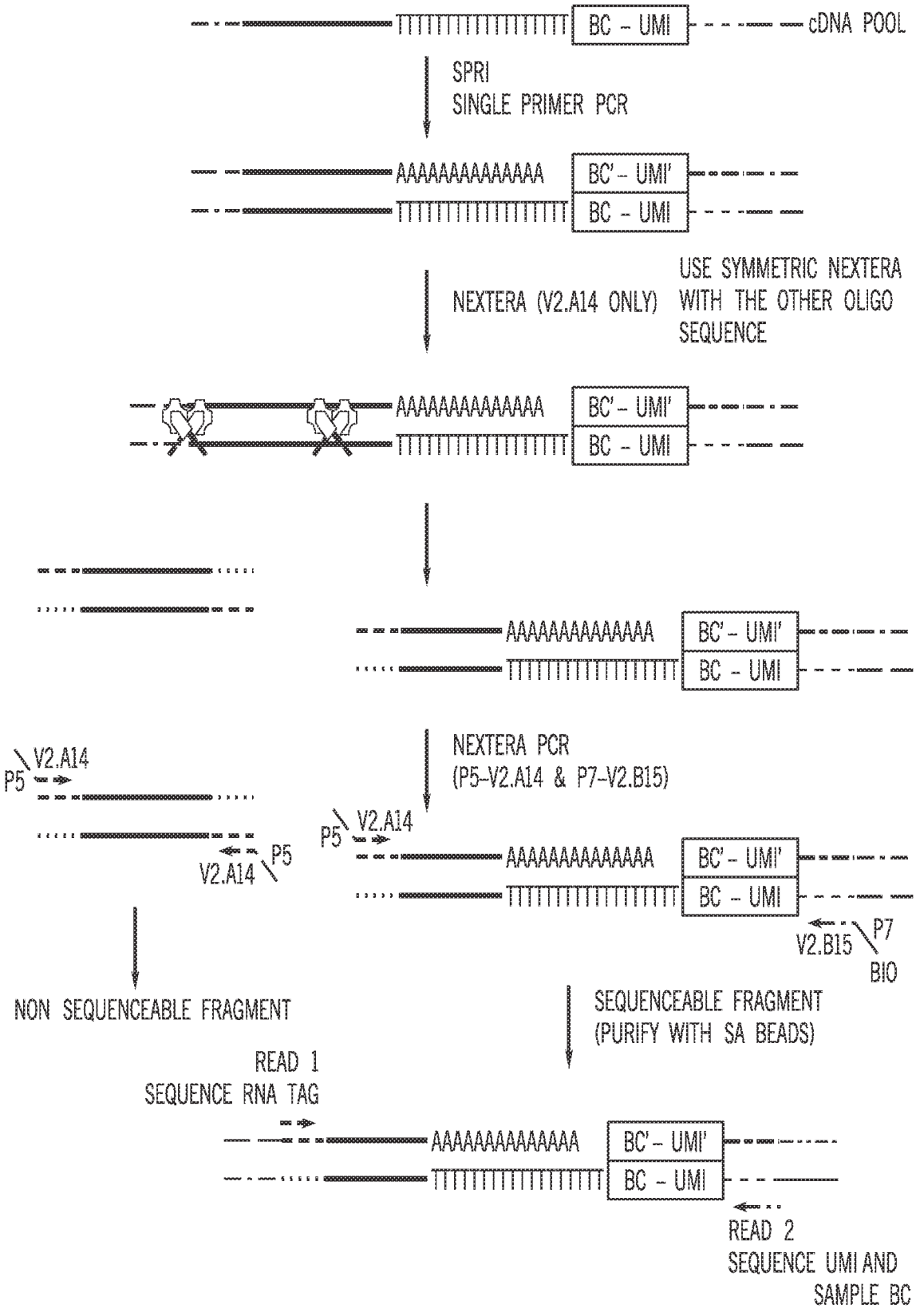

TTTTTTTTTTTTTTTTT
BC – UMI
cDNA POOL
SPRI
SINGLE PRIMER PCR
AAAAAAAAAAAAAA
BC' – UMI'
TTTTTTTTTTTTTTTTT
BC – UMI
NEXTERA (V2.A14 ONLY)
USE SYMMETRIC NEXTERA WITH THE OTHER OLIGO SEQUENCE
P5
V2.A14
NEXTERA PCR
(P5–V2.A14 & P7–V2.B15)
V2.B15
P7
BIO
NON SEQUENCEABLE FRAGMENT
SEQUENCEABLE FRAGMENT
(PURIFY WITH SA BEADS)
READ 1
SEQUENCE RNA TAG
READ 2
SEQUENCE UMI AND
SAMPLE BC

FIG. 4B

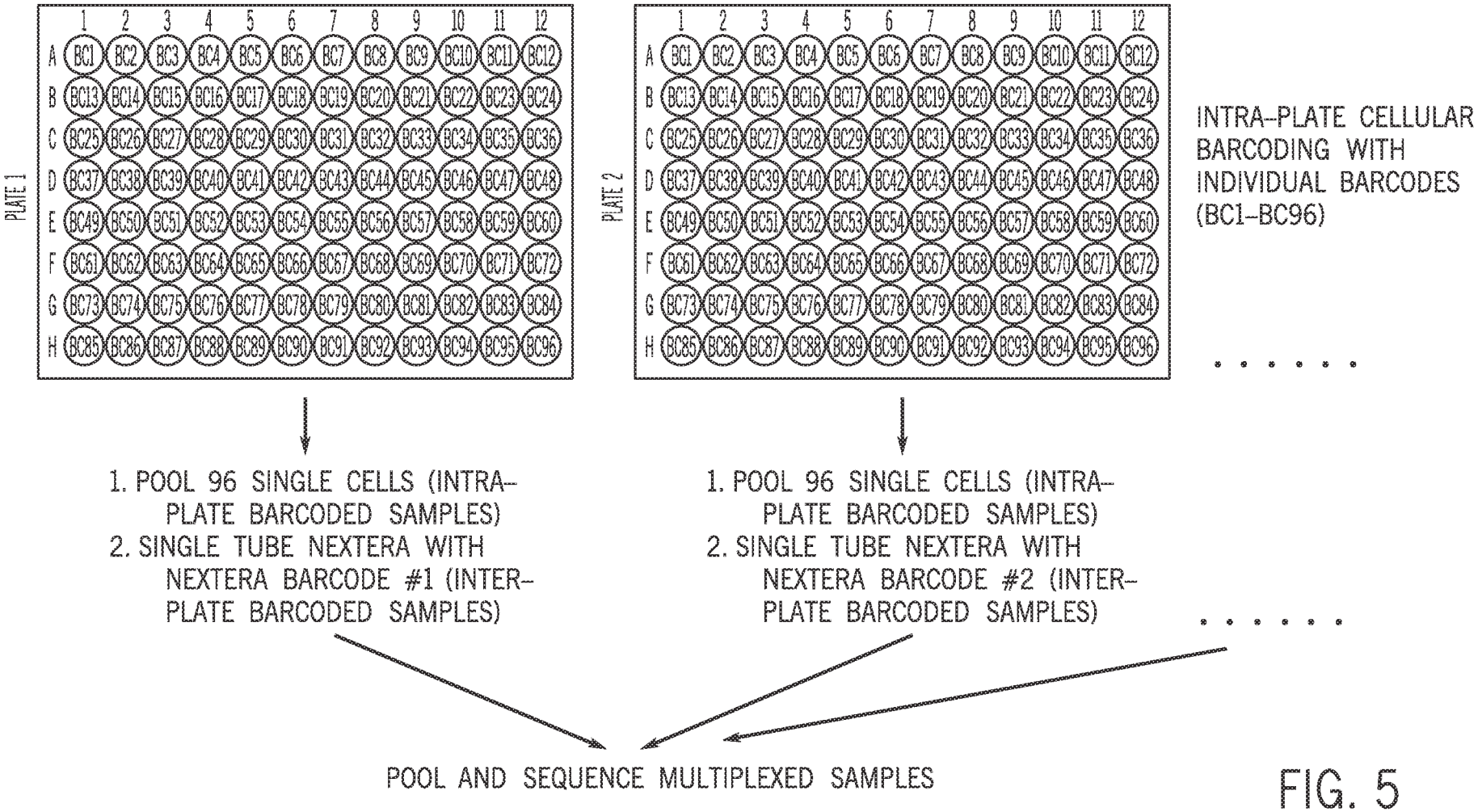

PLATE 1
PLATE 2
1 2 3 4 5 6 7 8 9 10 11 12
A BC1 BC2 BC3 BC4 BC5 BC6 BC7 BC8 BC9 BC10 BC11 BC12
B BC13 BC14 BC15 BC16 BC17 BC18 BC19 BC20 BC21 BC22 BC23 BC24
C BC25 BC26 BC27 BC28 BC29 BC30 BC31 BC32 BC33 BC34 BC35 BC36
D BC37 BC38 BC39 BC40 BC41 BC42 BC43 BC44 BC45 BC46 BC47 BC48
E BC49 BC50 BC51 BC52 BC53 BC54 BC55 BC56 BC57 BC58 BC59 BC60
F BC61 BC62 BC63 BC64 BC65 BC66 BC67 BC68 BC69 BC70 BC71 BC72
G BC73 BC74 BC75 BC76 BC77 BC78 BC79 BC80 BC81 BC82 BC83 BC84
H BC85 BC86 BC87 BC88 BC89 BC90 BC91 BC92 BC93 BC94 BC95 BC96
INTRA-PLATE CELLULAR BARCODING WITH INDIVIDUAL BARCODES (BC1-BC96)
. . . . . .
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 THROUGPUT 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%
	RIBOSOMAL	1%	1%	5%
	MITOCHONDRIA	–	–	–
	OTHER	1%	1%	0%
	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

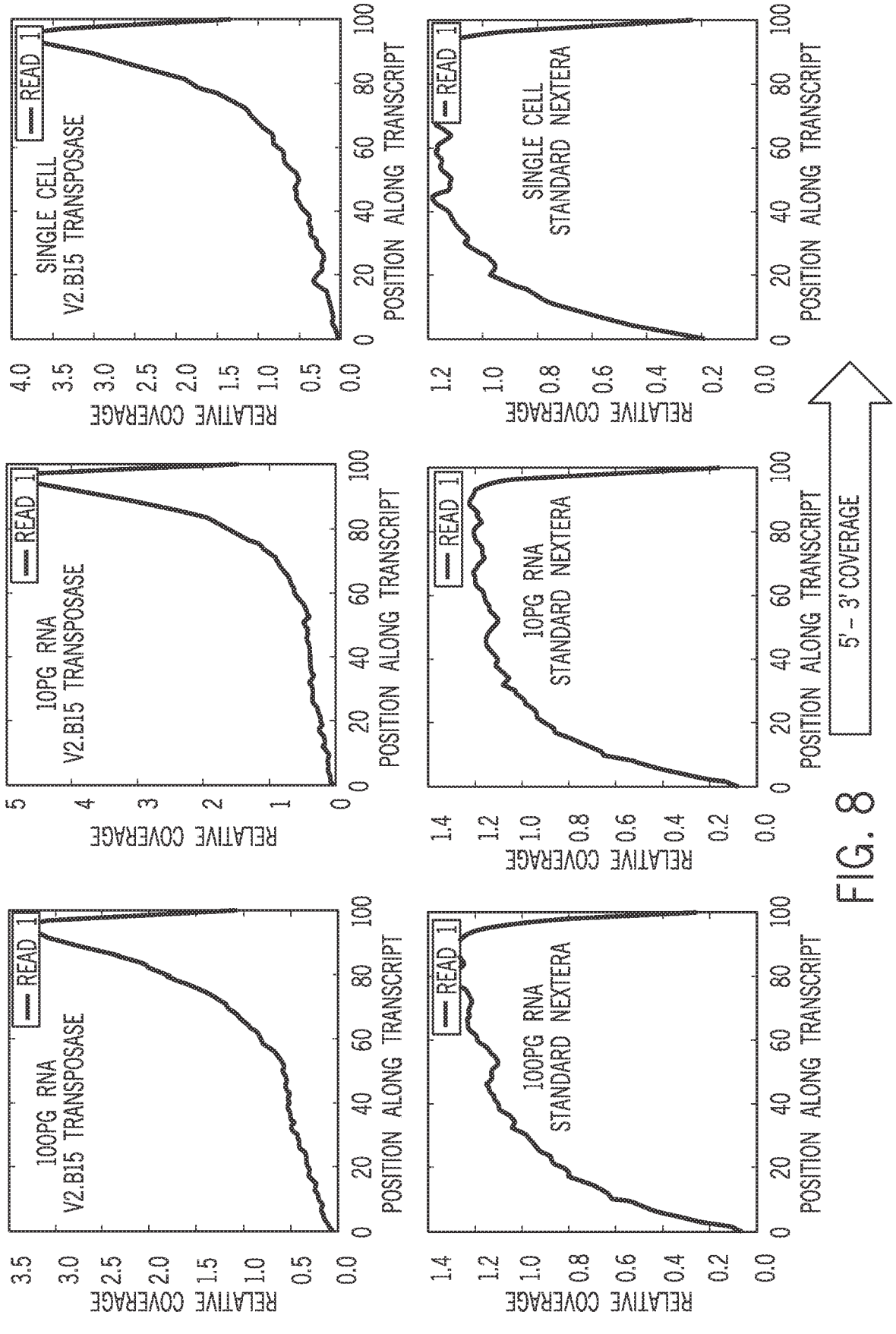

100PG RNA
V2.B15 TRANSPOSASE
READ 1
RELATIVE COVERAGE
POSITION ALONG TRANSCRIPT
10PG RNA
V2.B15 TRANSPOSASE
READ 1
RELATIVE COVERAGE
POSITION ALONG TRANSCRIPT
SINGLE CELL
V2.B15 TRANSPOSASE
READ 1
RELATIVE COVERAGE
POSITION ALONG TRANSCRIPT
100PG RNA
STANDARD NEXTERA
READ 1
RELATIVE COVERAGE
POSITION ALONG TRANSCRIPT
10PG RNA
STANDARD NEXTERA
READ 1
RELATIVE COVERAGE
POSITION ALONG TRANSCRIPT
SINGLE CELL
STANDARD NEXTERA
READ 1
RELATIVE COVERAGE
POSITION ALONG TRANSCRIPT
5' – 3' COVERAGE

FIG. 8

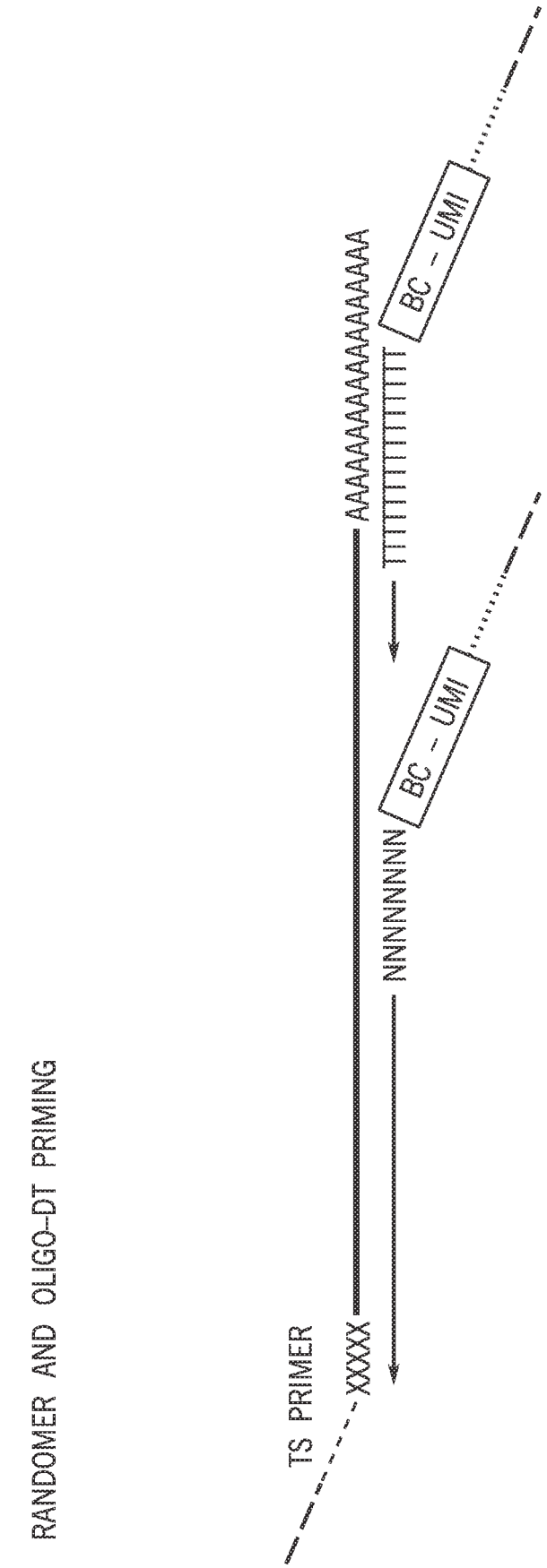

RANDOMER AND OLIGO-DT PRIMING
TS PRIMER
XXXX
AAAAAAAAAAAAAAA
TTTTTTTTTTTTTTT
NNNNNNNN
BC – UMI
BC – UMI

FIG. 9A

RANDOMER CONCATENATION BYPRODUCT

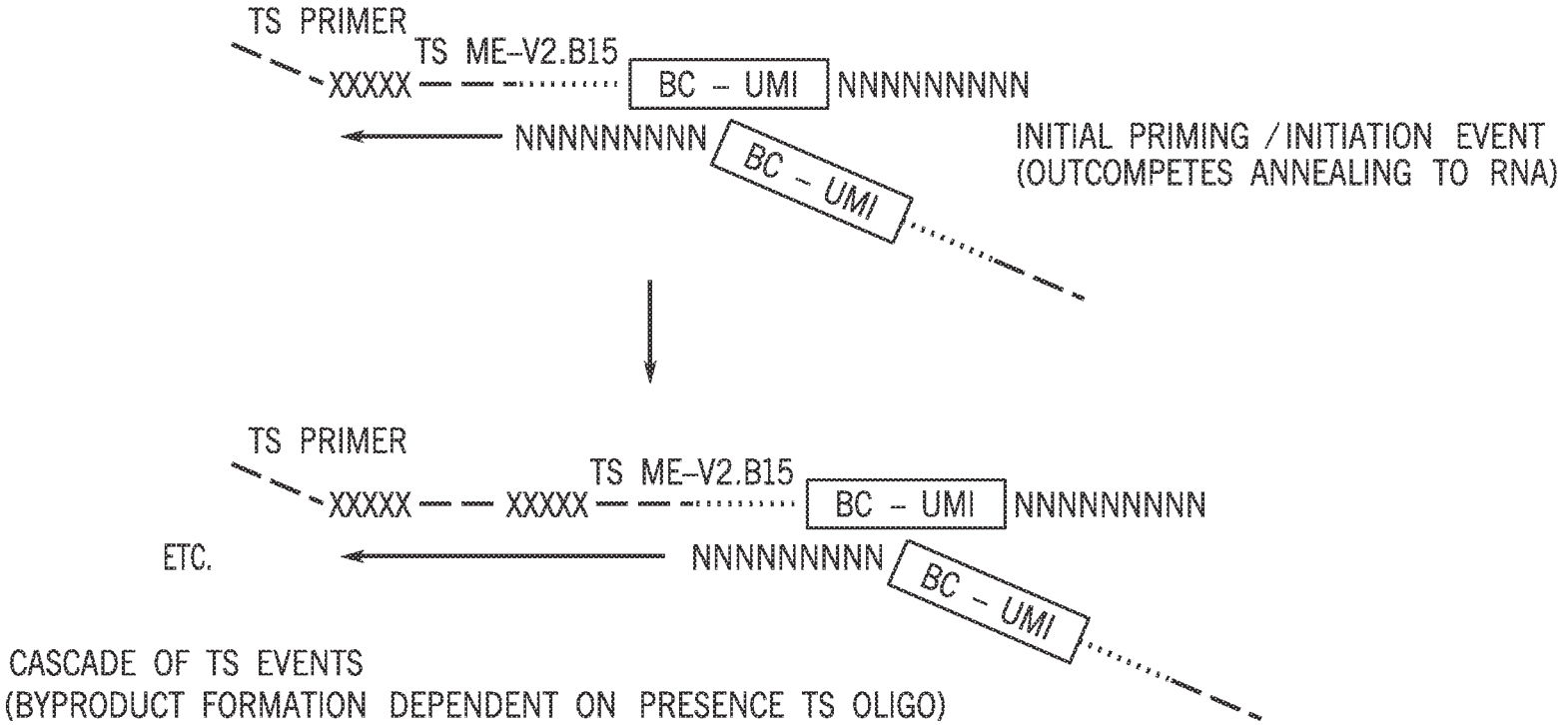


FIG. 9B

POSSIBLE STRATEGIES TO REDUCE CONCATENATION BYPRODUCT

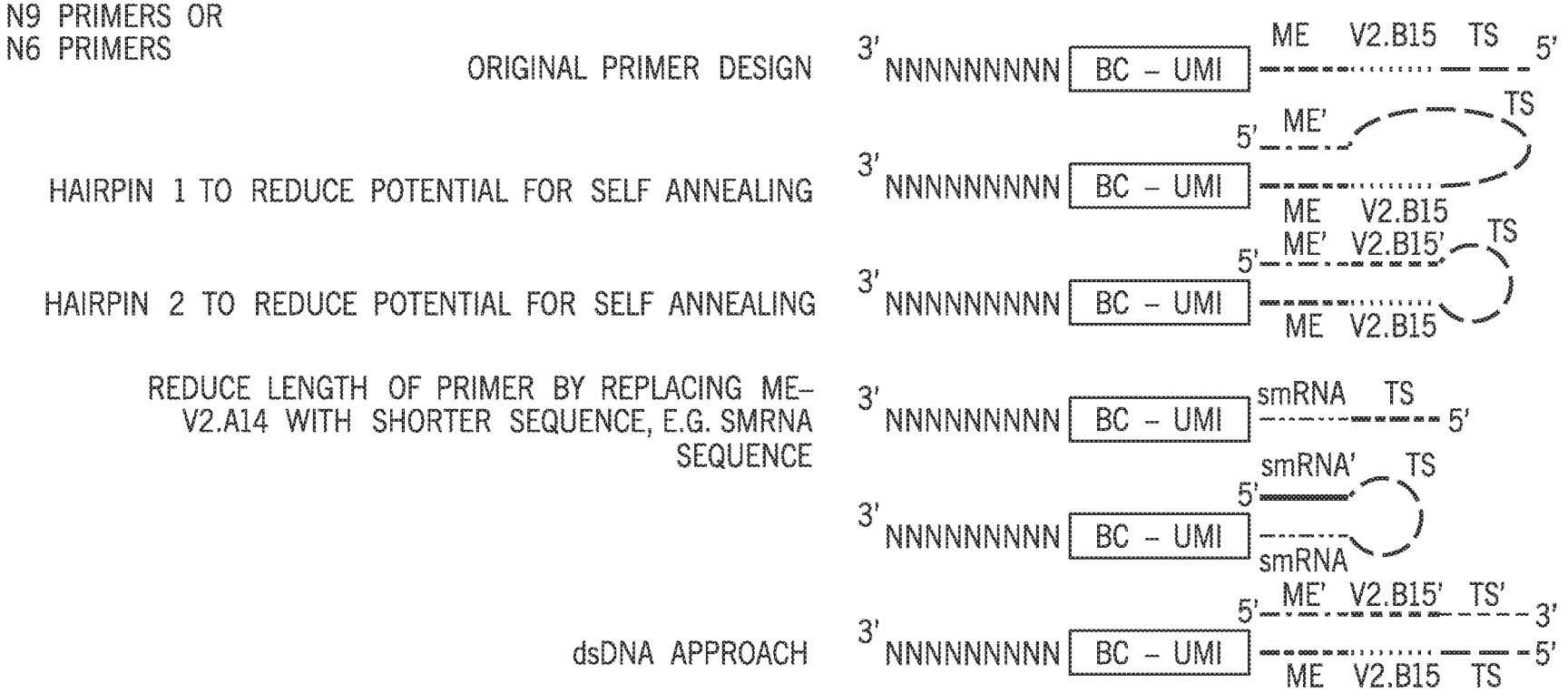


FIG. 10

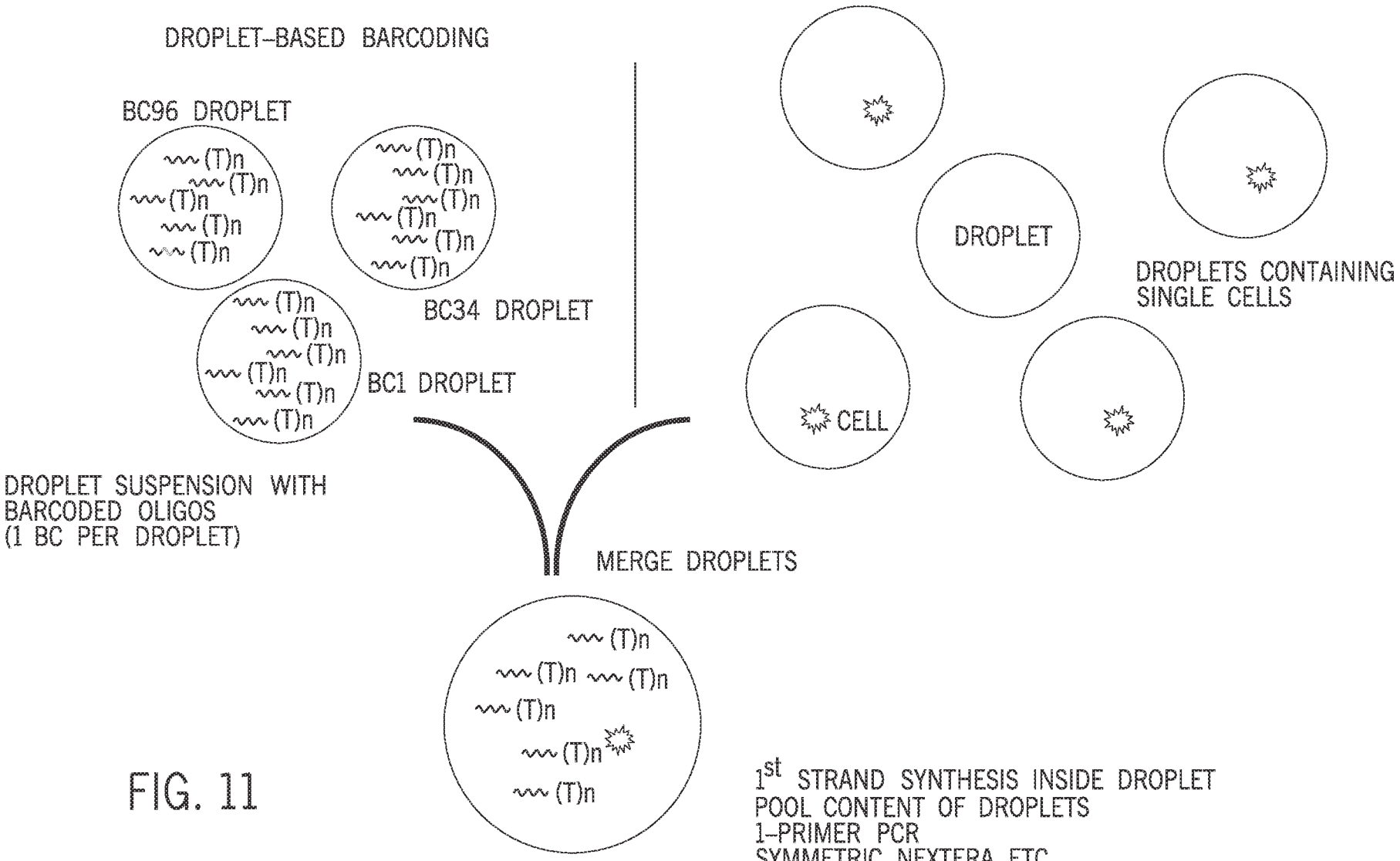

DROPLET–BASED BARCODING
BC96 DROPLET
(T)n
BC34 DROPLET
BC1 DROPLET
DROPLET SUSPENSION WITH
BARCODED OLIGOS
(1 BC PER DROPLET)
MERGE DROPLETS
1st STRAND SYNTHESIS INSIDE DROPLET
POOL CONTENT OF DROPLETS
1–PRIMER PCR
SYMMETRIC NEXTERA, ETC
DROPLET
CELL
DROPLETS CONTAINING
SINGLE CELLS

FIG. 11

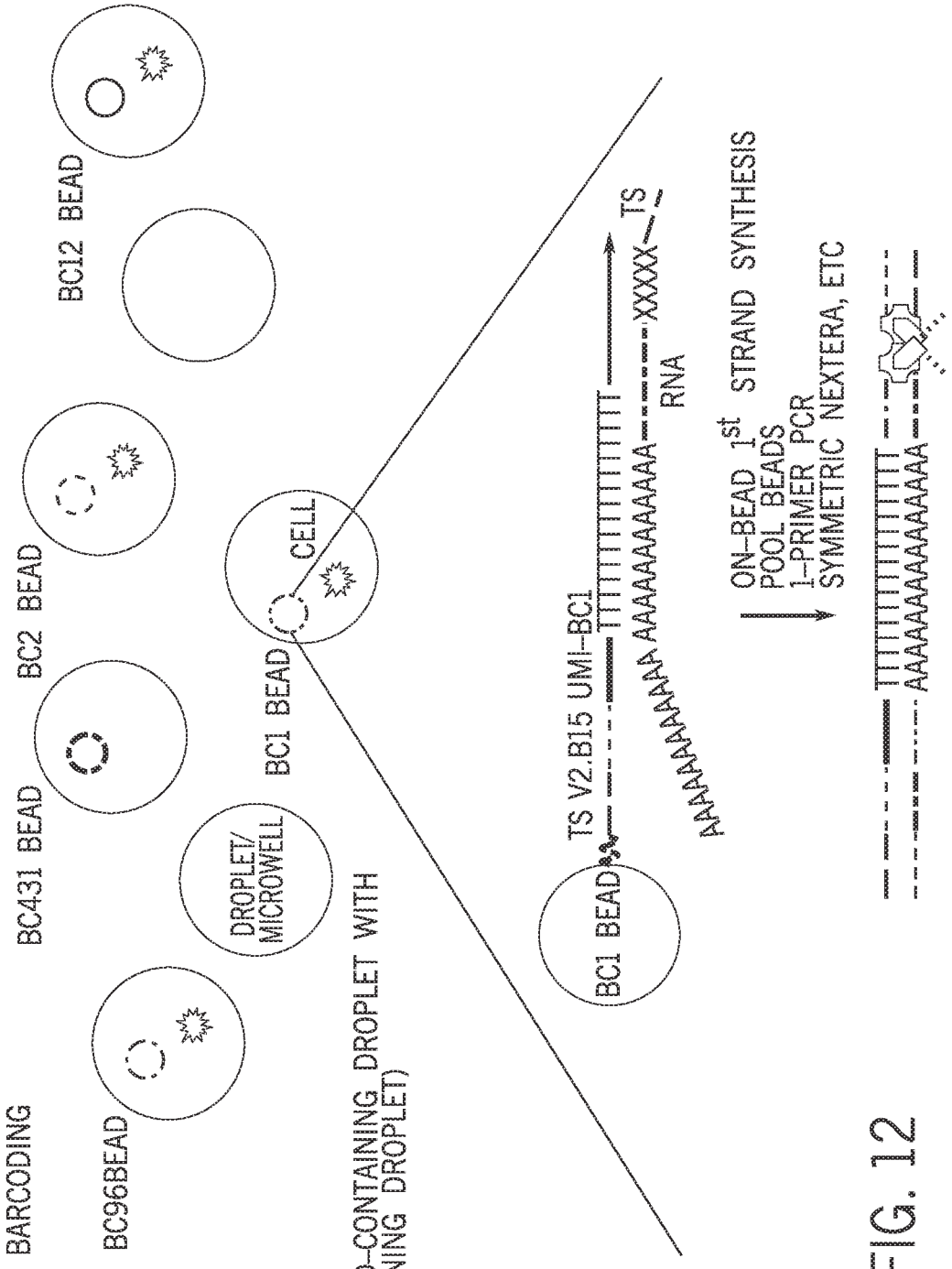

BEAD-BASED BARCODING
BC96BEAD
BC431 BEAD
BC2 BEAD
BC12 BEAD
DROPLET/ MICROWELL
BC1 BEAD
CELL
(MERGE BEAD-CONTAINING DROPLET WITH CELL-CONTAINING DROPLET)
TS V2.B15 UMI-BC1
BC1 BEAD
AAAAAAAAAAAAAAA
XXXX
TS
RNA
ON-BEAD 1st STRAND SYNTHESIS
POOL BEADS
1-PRIMER PCR
SYMMETRIC NEXTERA, ETC

FIG. 12

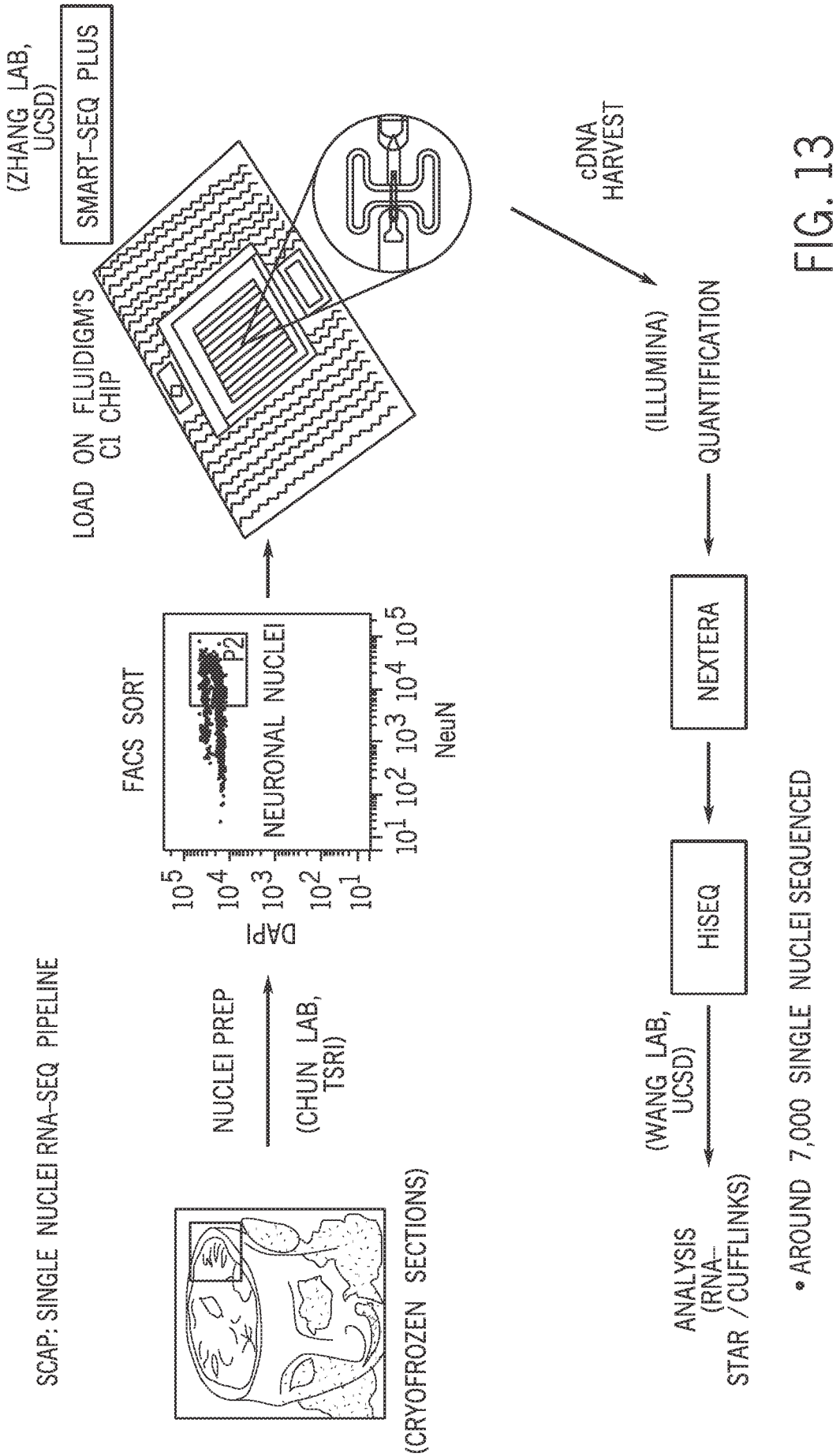

SCAP: SINGLE NUCLEI RNA-SEQ PIPELINE
(CRYOFROZEN SECTIONS)
NUCLEI PREP
(CHUN LAB, TSRI)
FACS SORT
DAPI
NeuN
P2
NEURONAL NUCLEI
LOAD ON FLUIDIGM'S C1 CHIP
(ZHANG LAB, UCSD)
SMART-SEQ PLUS
cDNA HARVEST
(ILLUMINA)
QUANTIFICATION
NEXTERA
HISEQ
(WANG LAB, UCSD)
ANALYSIS (RNA-STAR /CUFFLINKS)
• AROUND 7,000 SINGLE NUCLEI SEQUENCED

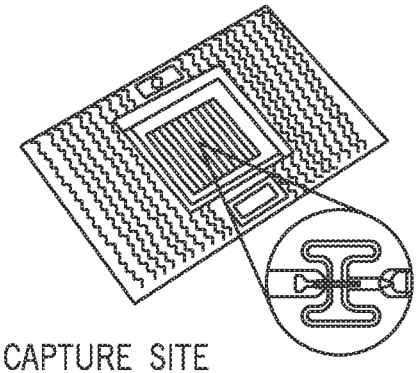
FIG. 13

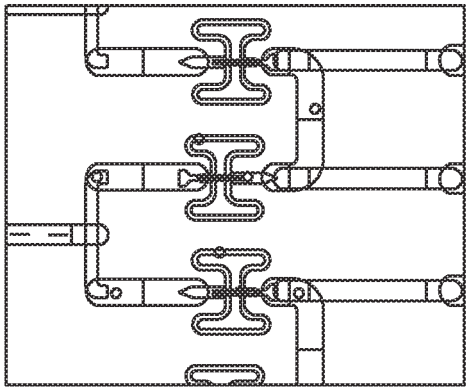
cDNA FROM SINGLE NUCLEI

OVERVIEW OF FLUIDIGM'S C1 SYSTEM FOR RNA-SEQ

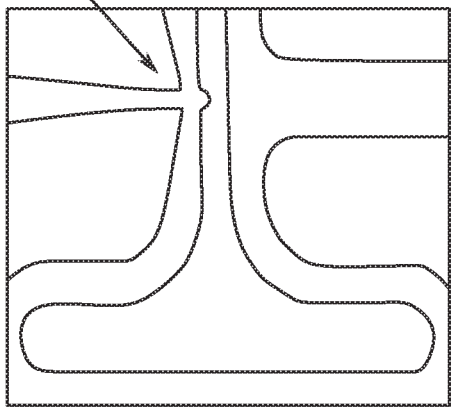
-TRANSCRIPTOME PROFILING FROM NUCLEI RATHER THAN WHOLE NEURONS
  - -TECHNICALLY DIFFICULT TO ISOLATE NEURONS IN HIGH THROUGHPUT
  - -RNA CONTAMINATION FROM NEIGHBORING NEURONAL SYNAPTIC RNA

-C1 SYSTEM DESIGNED FOR 96 NANO-LITER REACTION VOLUMES
  - -NUCLEI LOADING AND CAPTURE
  - -WASH, LYSIS
  - -cDNA SYNTHESIS
  - -PCR AMPLIFICATION
  - -cDNA HARVEST





NUCLEI IN CAPTURE SITE



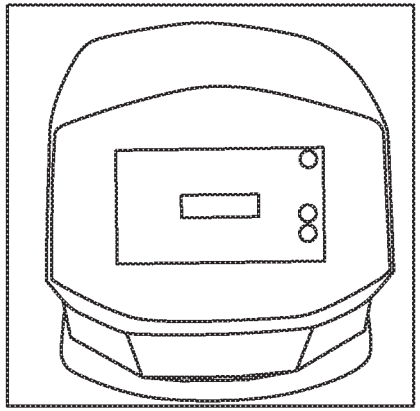


FIG. 14

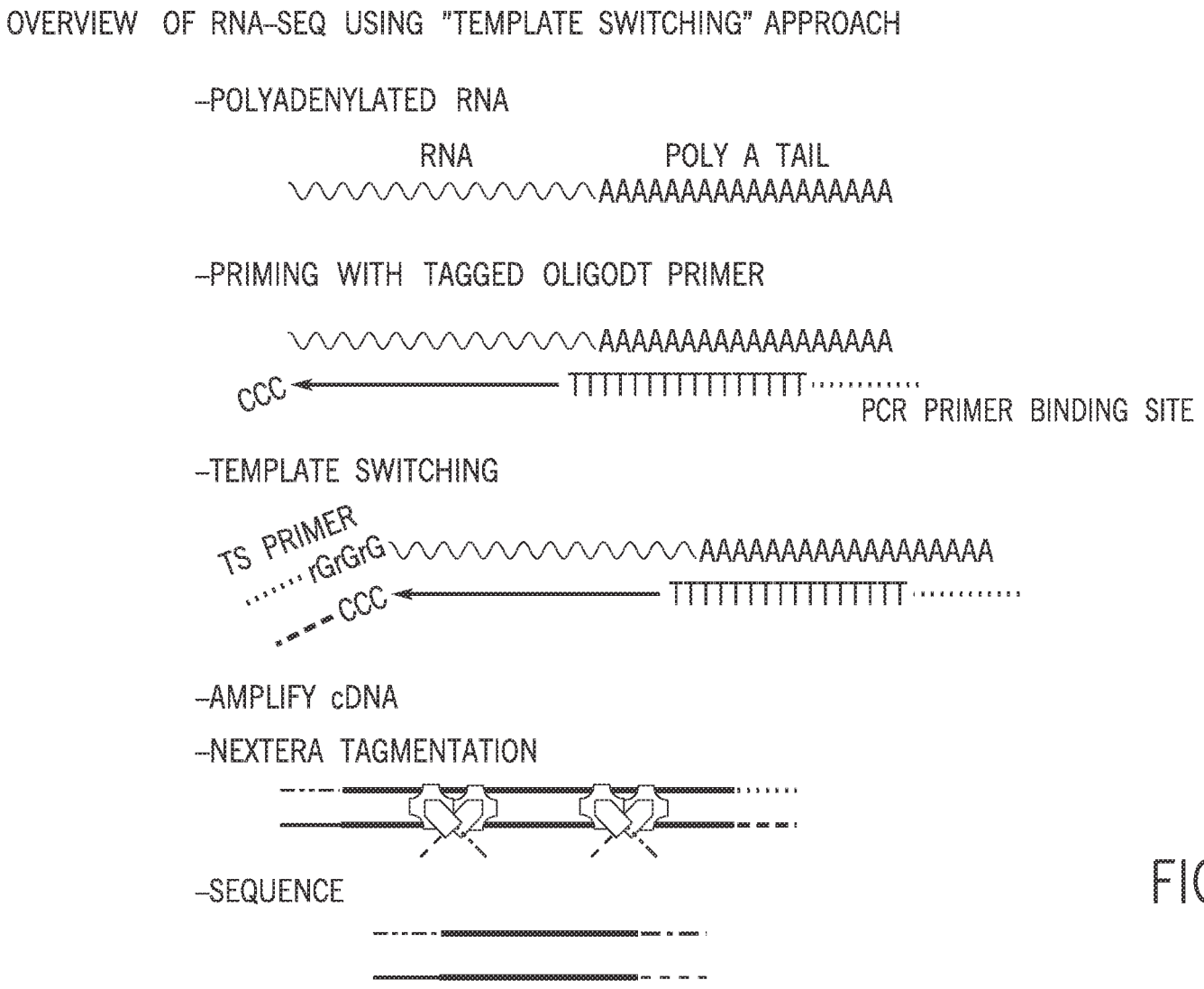

OVERVIEW OF RNA-SEQ USING "TEMPLATE SWITCHING" APPROACH
–POLYADENYLATED RNA
RNA
POLY A TAIL
AAAAAAAAAAAAAAAAA
–PRIMING WITH TAGGED OLIGODT PRIMER
AAAAAAAAAAAAAAAAA
CCC
TTTTTTTTTTTTTTT
PCR PRIMER BINDING SITE
–TEMPLATE SWITCHING
TS PRIMER
rGrGrG
AAAAAAAAAAAAAAAAA
CCC
TTTTTTTTTTTTTTT
–AMPLIFY cDNA
–NEXTERA TAGMENTATION
–SEQUENCE

FIG. 15

SMART–PLUS: IMPROVING ASSAY SENSITIVITY AND GENE–BODY COVERAGE

–SMART–PLUS: ADDING OF A RANDOM PRIMER FOR cDNA SYNTHESIS WITH IDENTICAL PCR PRIMING SITES AS SMART OLIGODT cDNA SYNTHESIS PRIMER

–ADVANTAGES:
- –ENHANCED LIBRARY PREP FROM DEGRADED RNA
- –SUPERIOR UNIFORMITY OF TRANSCRIPT COVERAGE
- –LOW RIBOSOMAL AMPLIFICATION

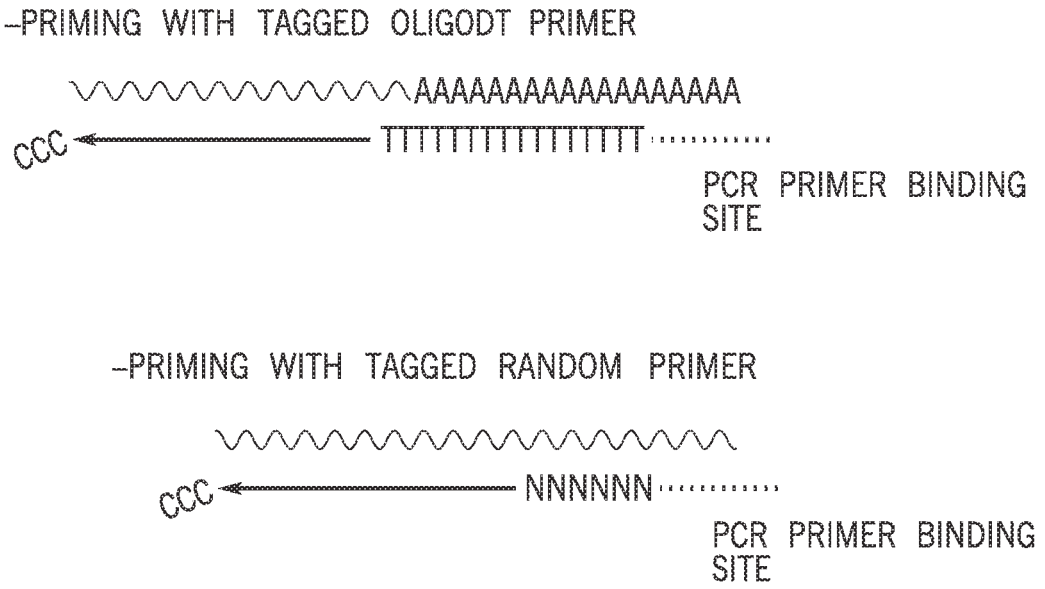


FIG. 16

ADVANTAGES OF SMART–PLUS

- ASSAY PERFORMANCE HAS BEEN VALIDATED ON A DATASET COMPILED FROM >1,000 HIGH QUALITY SINGLE NUCLEI (A)
- ENHANCED REPRESENTATION OF 5′ REGIONS OF GENE–BODIES (B)

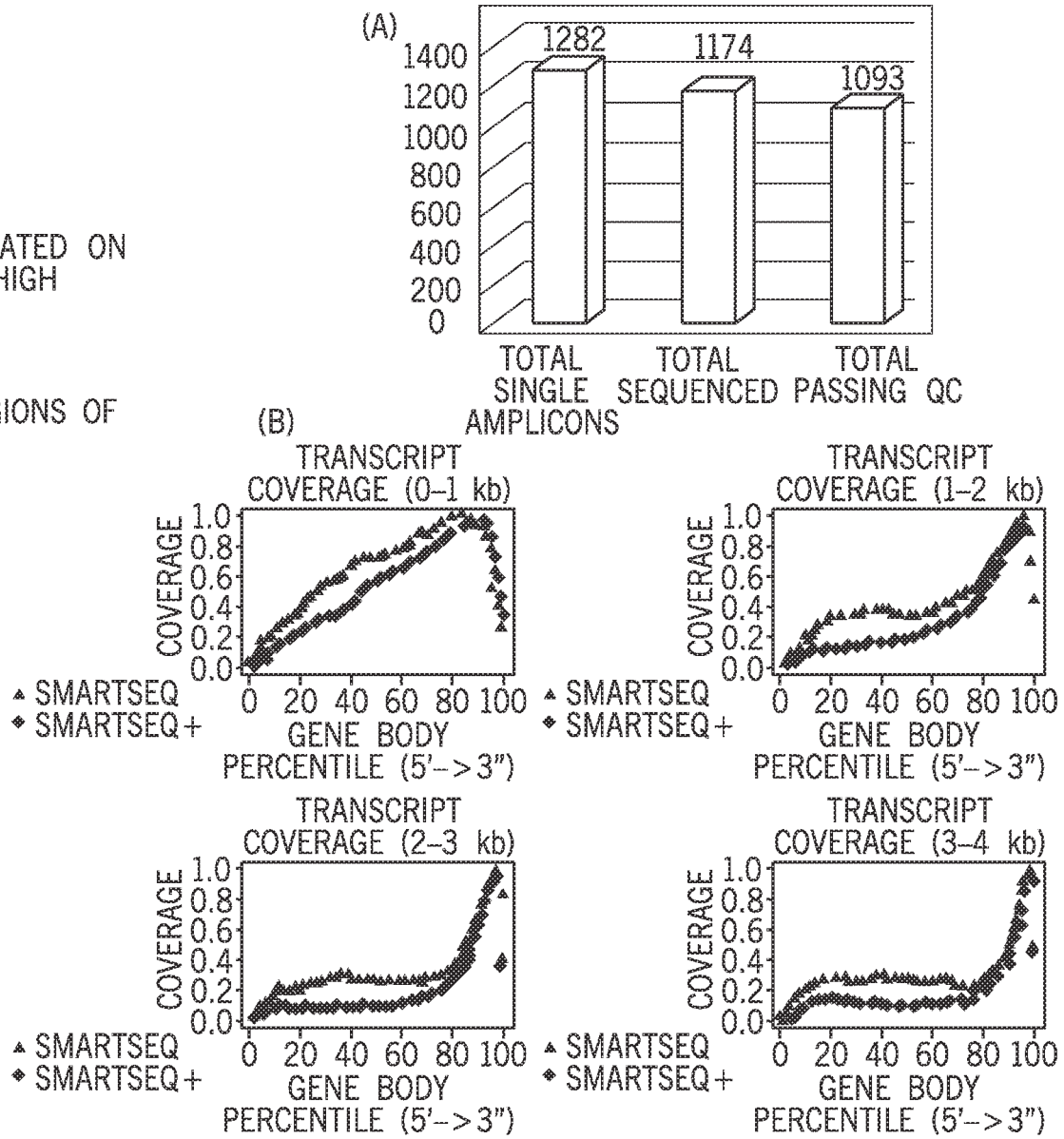


FIG. 17

SMART–SEQ PLUS: INCREASING ASSAY SENSITIVITY

- APPROXIMATELY 1.5x INCREASE IN SENSITIVITY (NUMBER OF GENES IDENTIFIED BY RNA–SEQ) USING SMART–SEQ PLUS (FIG A)

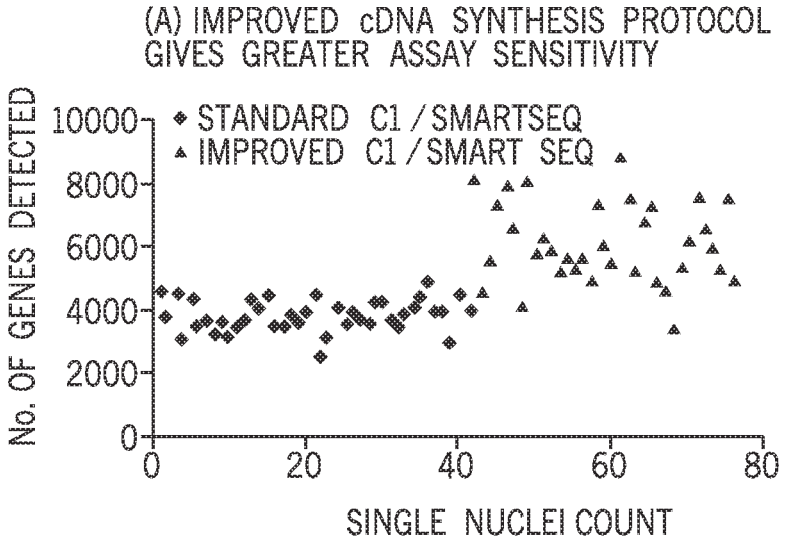


FIG. 18