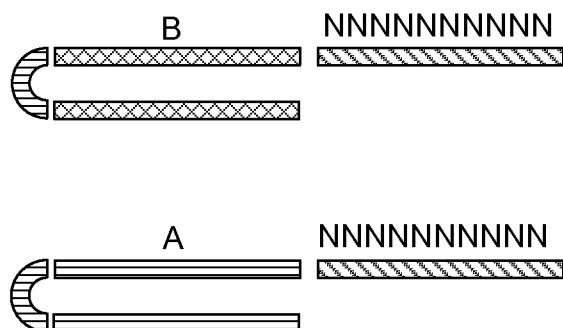


1 / 43

Degenerate-tailed hairpin sequencing linkers



A- Sequencer specific tag1

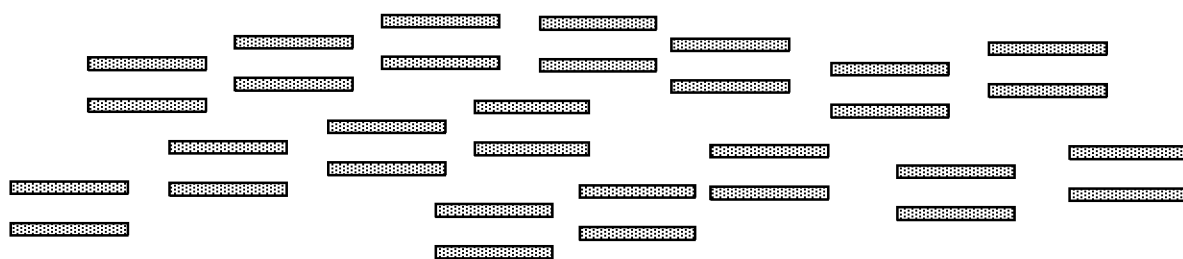
B- Sequencer specific tag2

Rest. Site: Optional restriction site/specific enzyme cutting site
(dUTP, phosphothiorate possible)

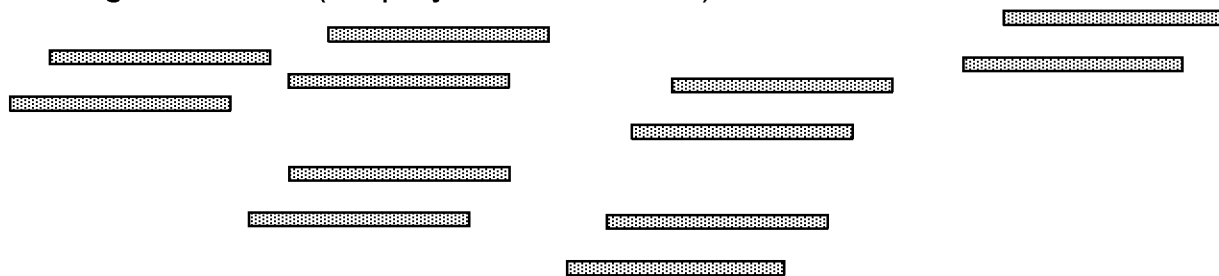
NNNNNNNNNN- Degenerate primer region-10mer, 20mer, 3' end protected

FIG. 1A

Fragment DNA (general nuclease)

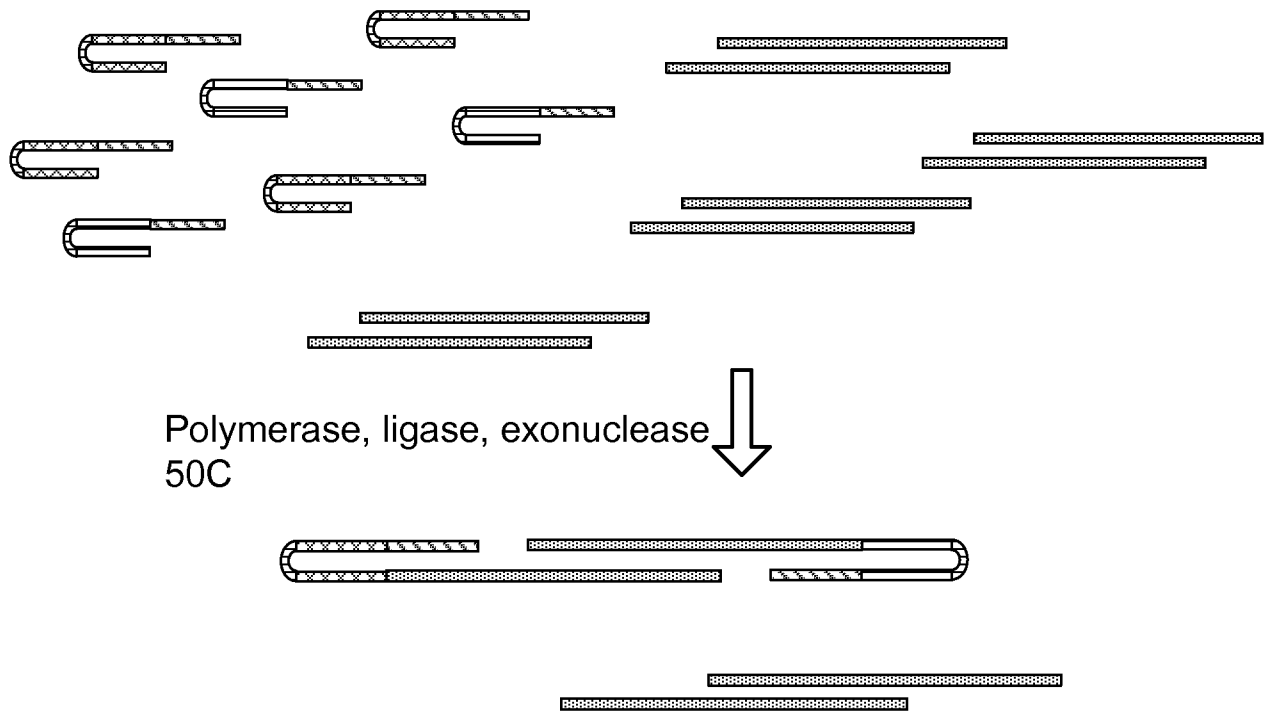


Digest 5' ends (T4 polymerase, Exo III)

**FIG. 1B**

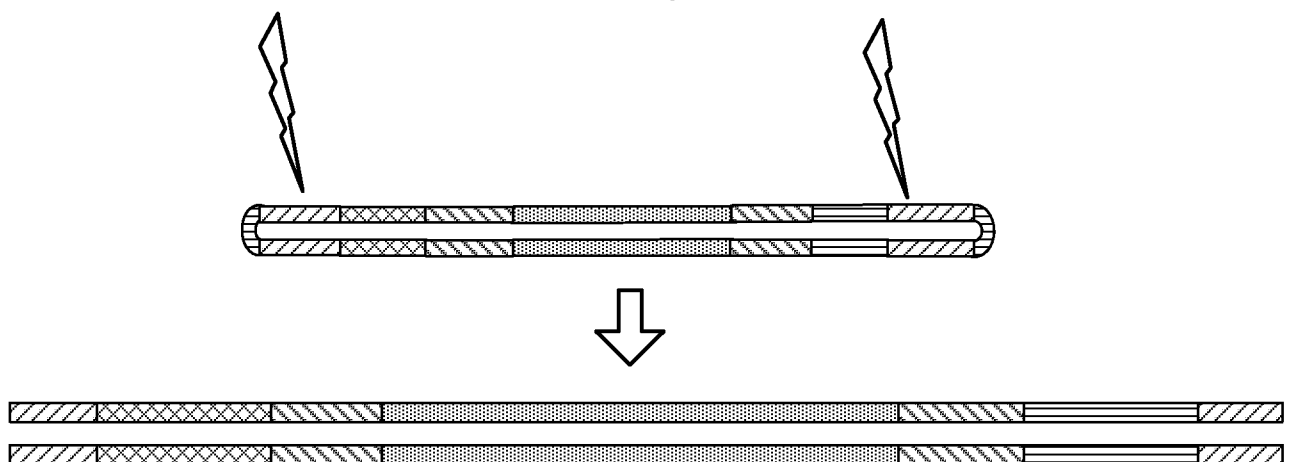
2 / 43

Add degenerate-hairpin sequencing linker

**FIG. 1C**

Linearize circular DNA with restriction enzyme

Restriction Enzyme

**FIG. 1D**

3 / 43

Degenerate-tailed sequencing linker



A- Sequencer specific tag1

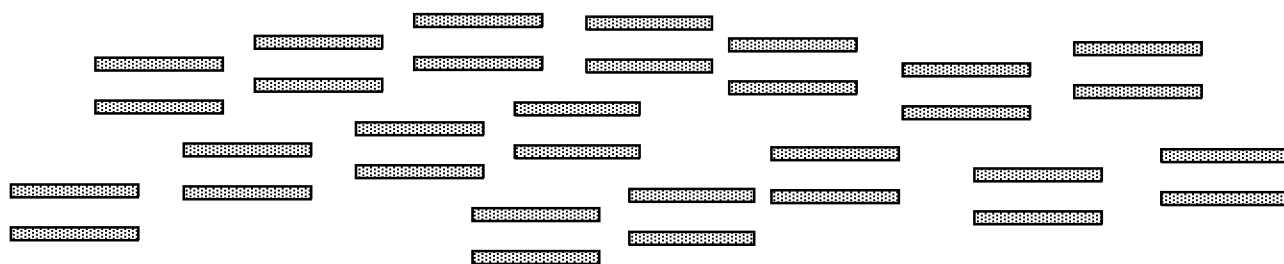
B- Sequencer specific tag2

Rest. Site: restriction site/specific enzyme cutting site (dUTP possible)

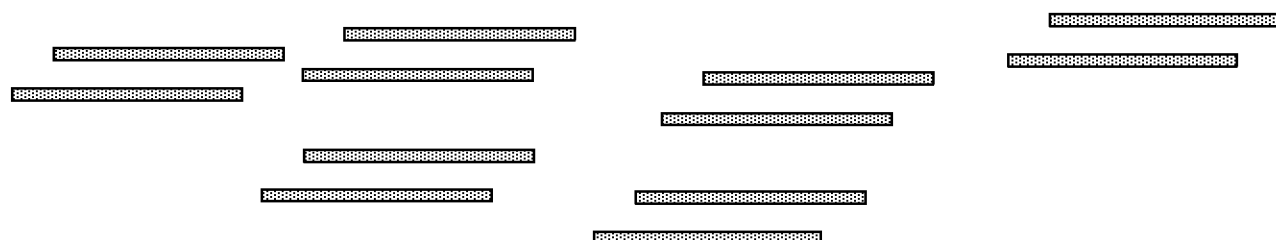
NNNNNNNNNN- Degenerate primer region-10mer, 20mer

FIG. 2A

Fragment DNA (general nuclease)



Digest 5' ends (T4 polymerase, Exo III)

**FIG. 2B**

4 / 43

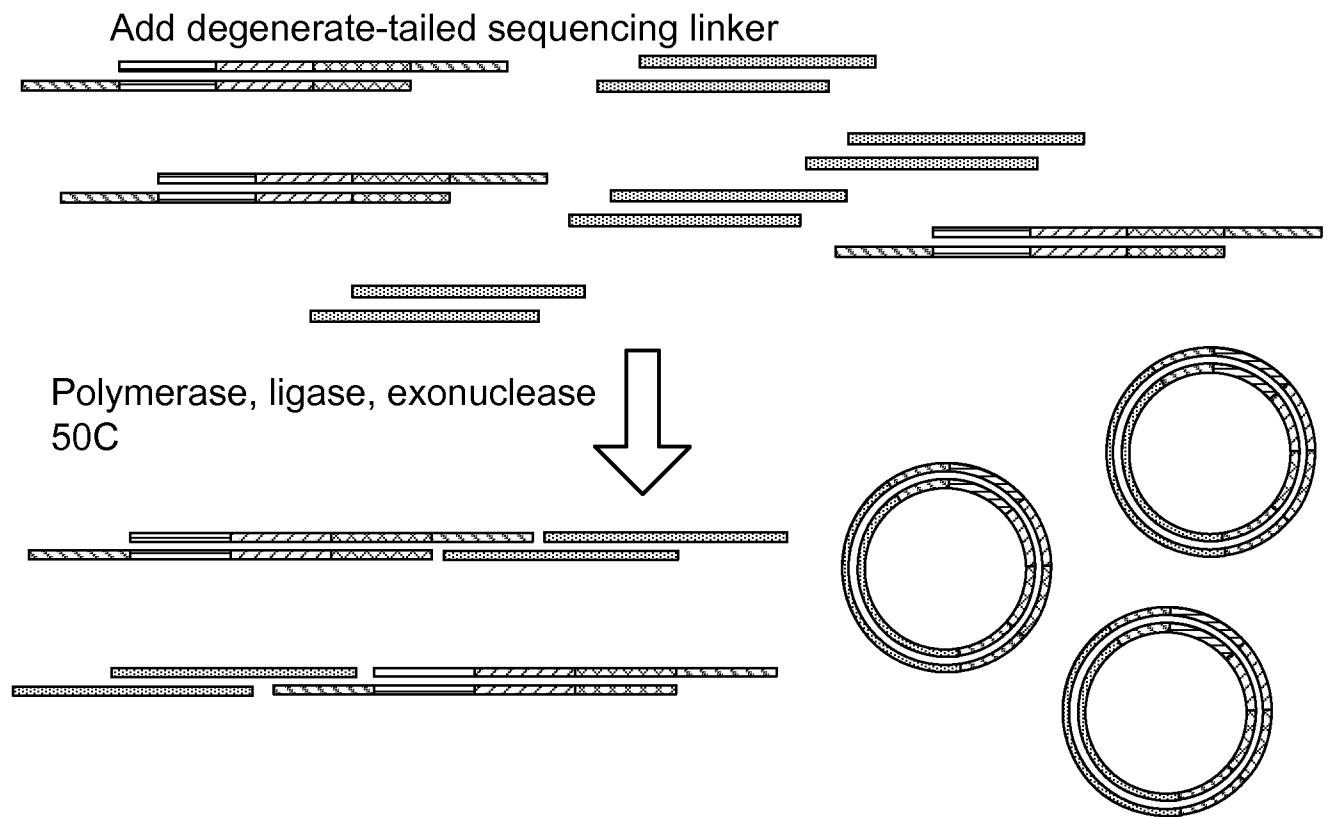


FIG. 2C

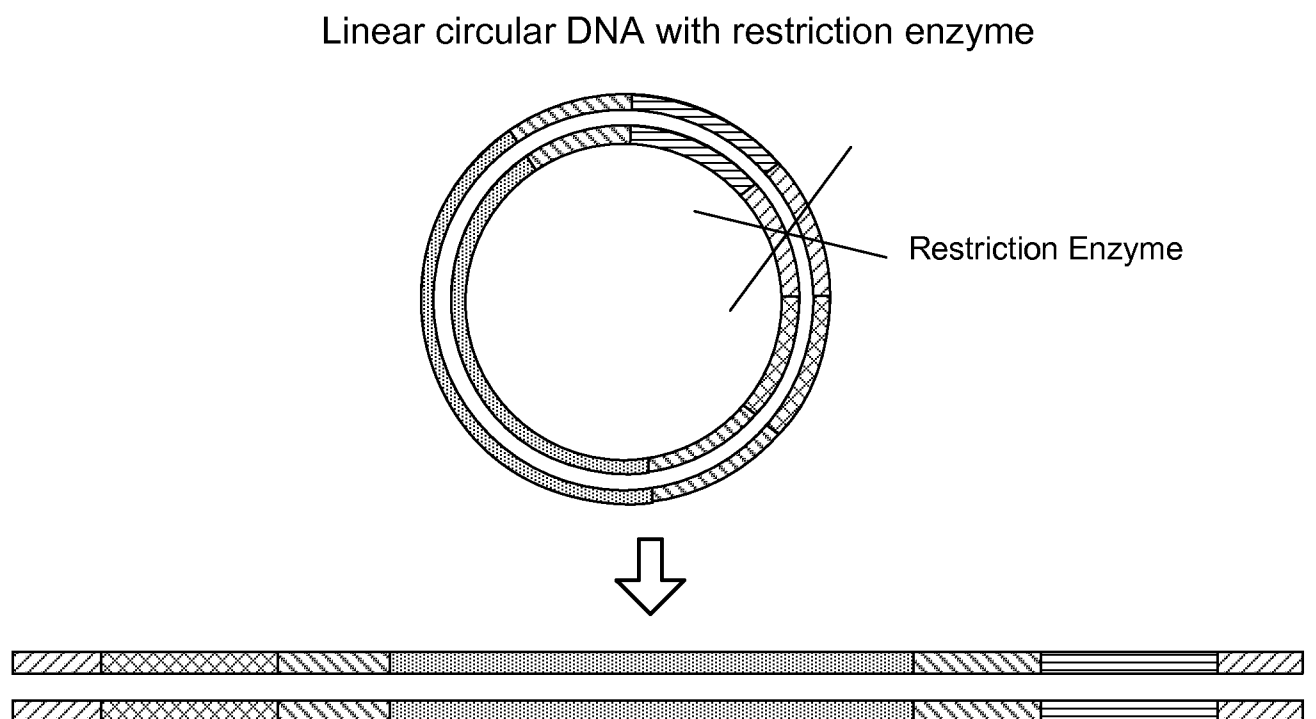


FIG. 2D

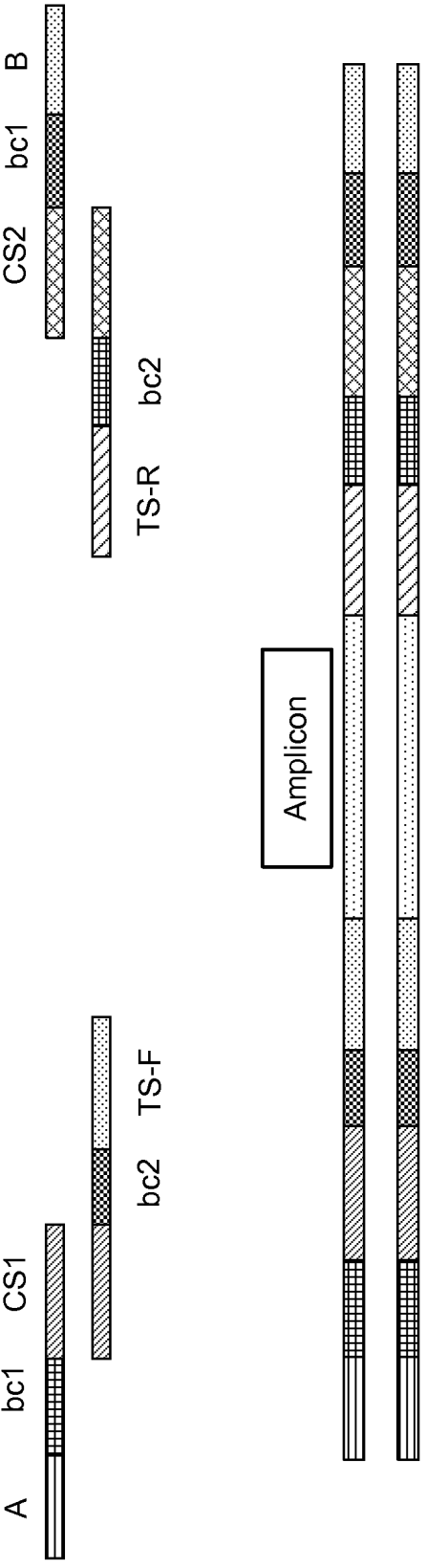


FIG. 3

5 / 43

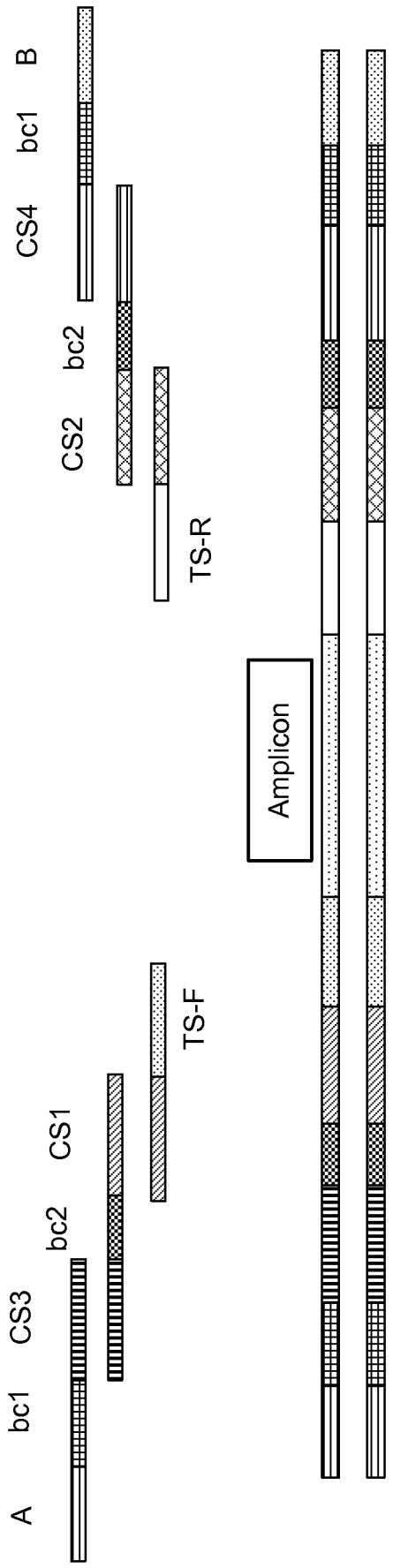


FIG. 4

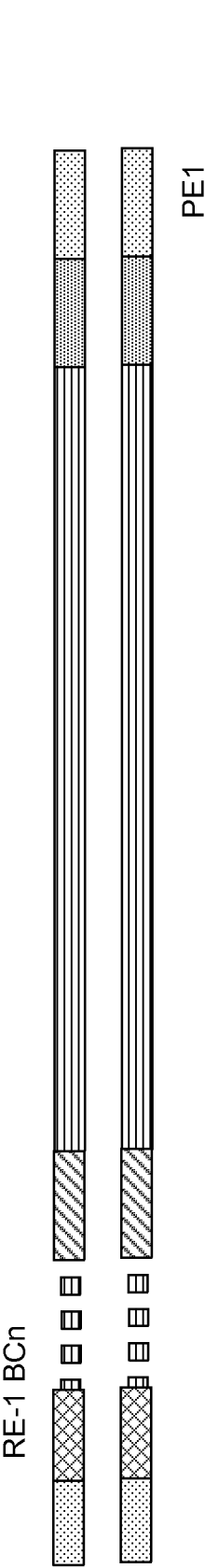


FIG. 5A

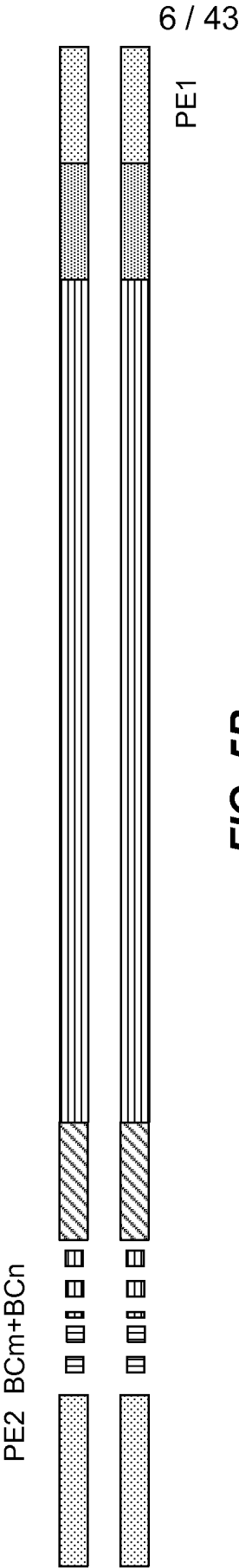


FIG. 5B

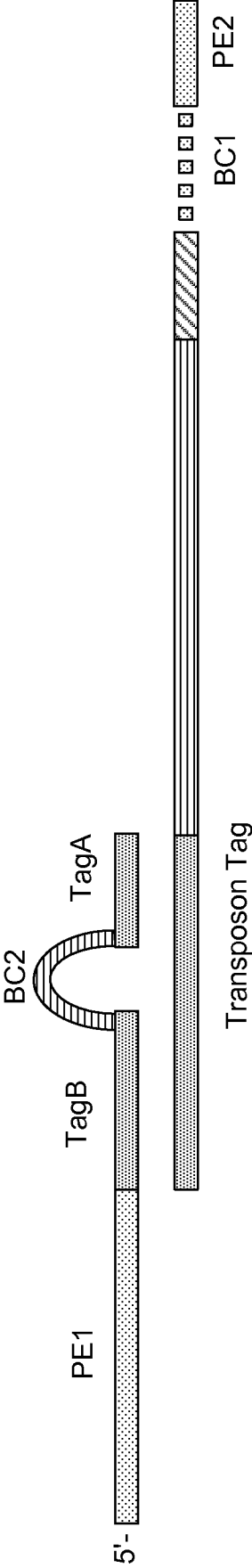


FIG. 6

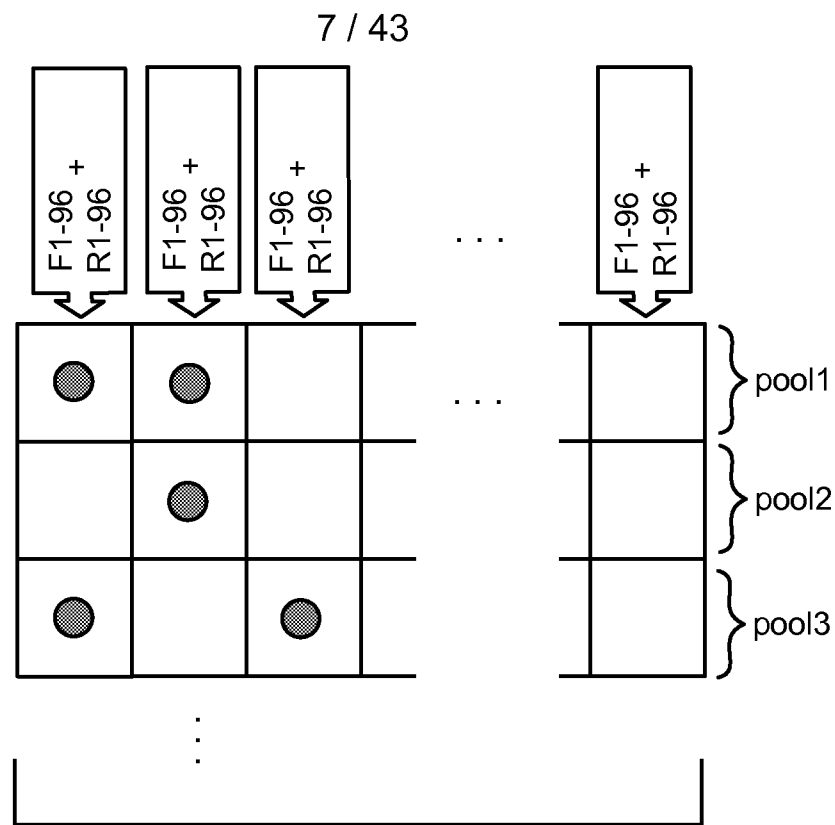


FIG. 7A

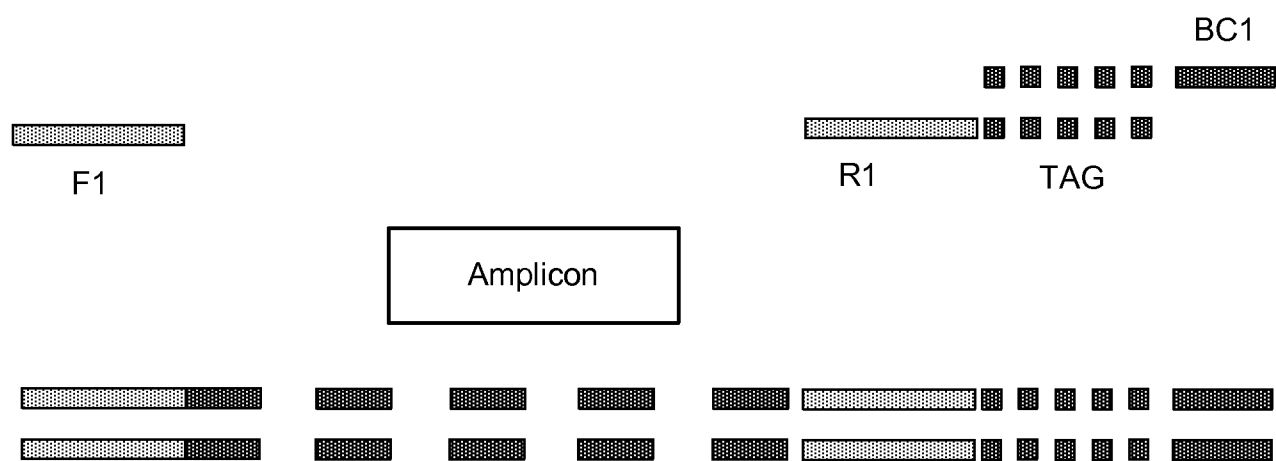


FIG. 7B

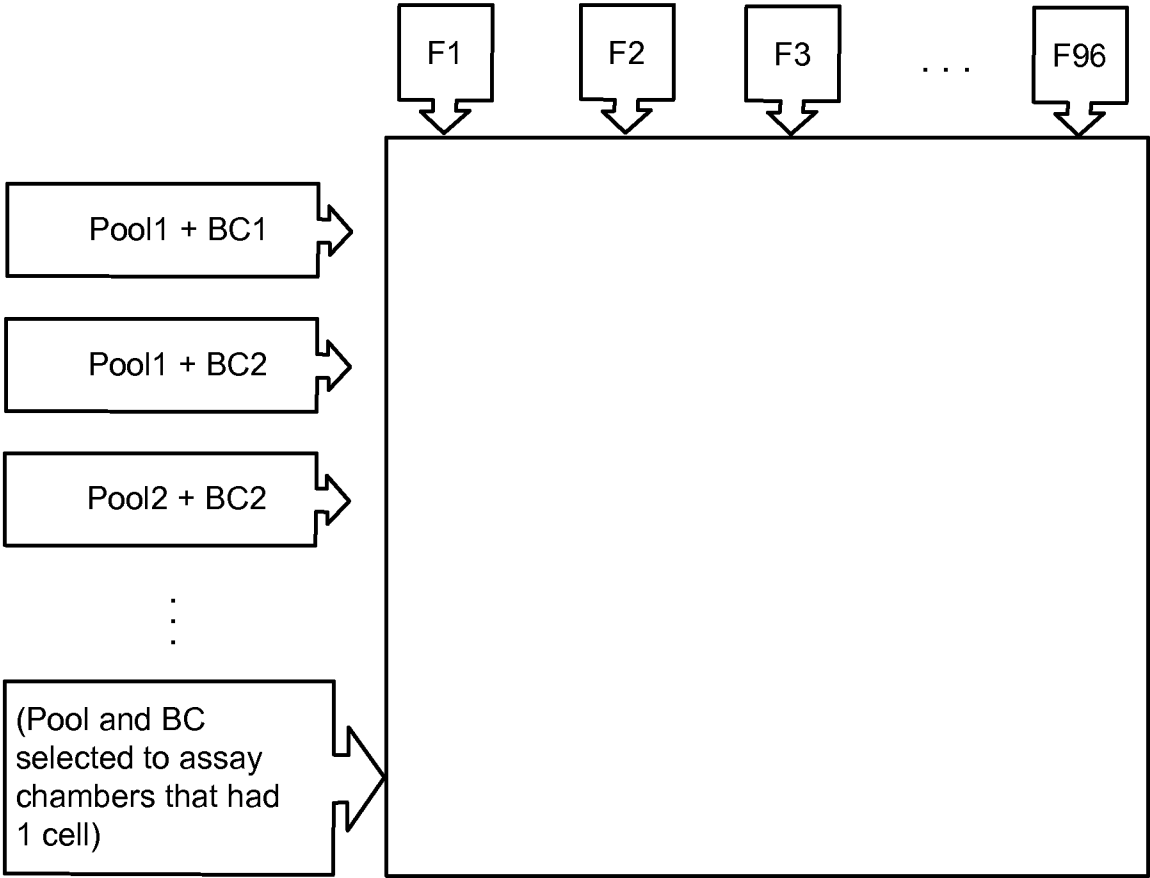


FIG. 7C

9 / 43

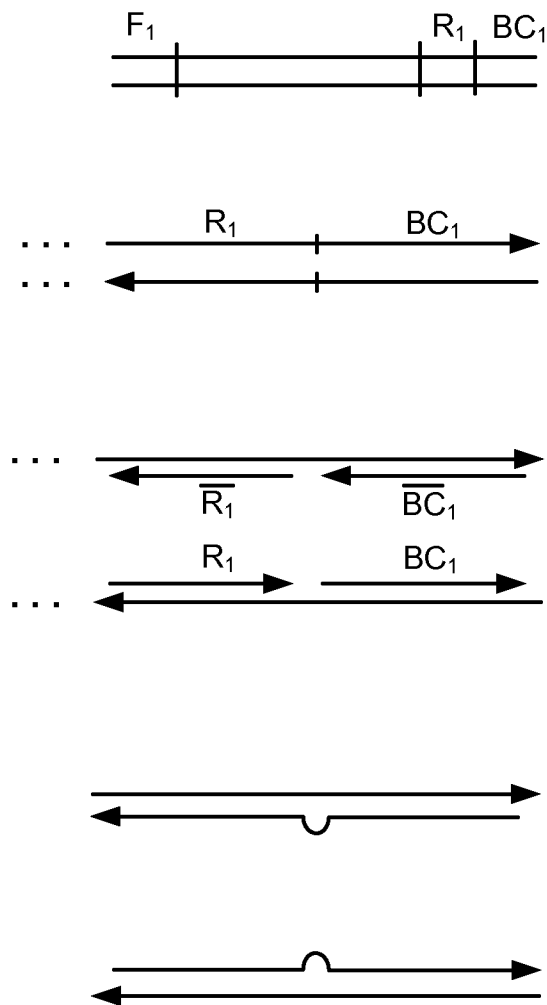


FIG. 8A

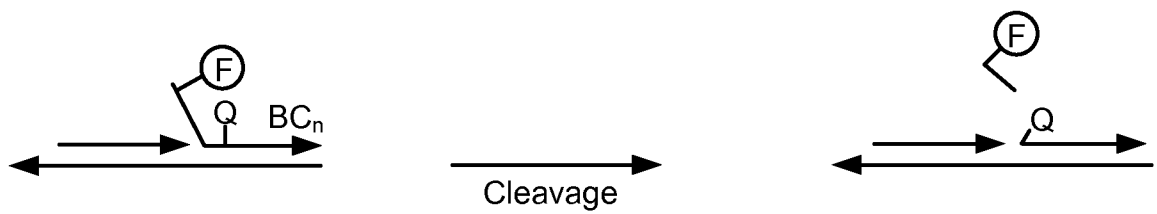


FIG. 8B

10 / 43

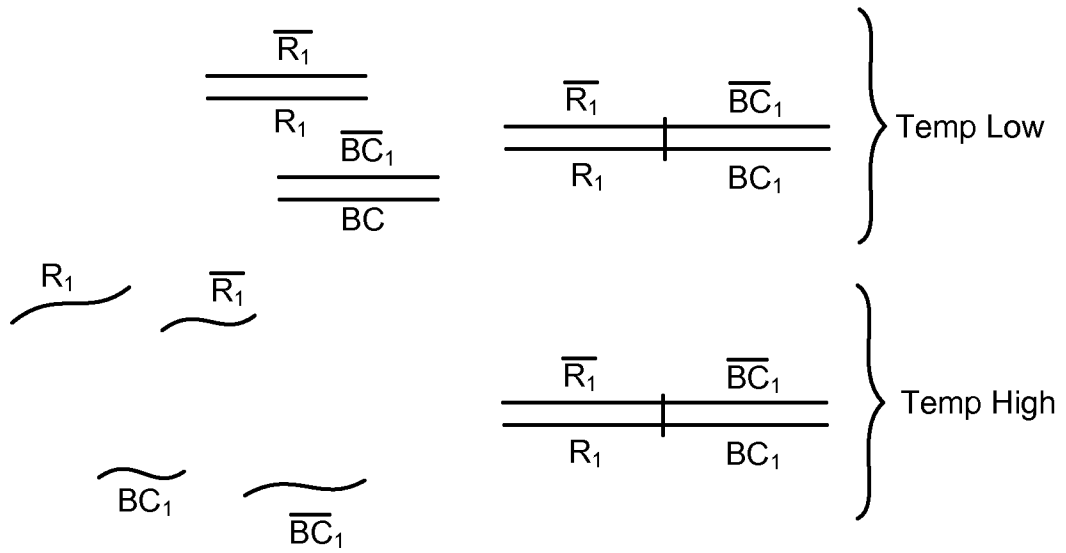


FIG. 8C

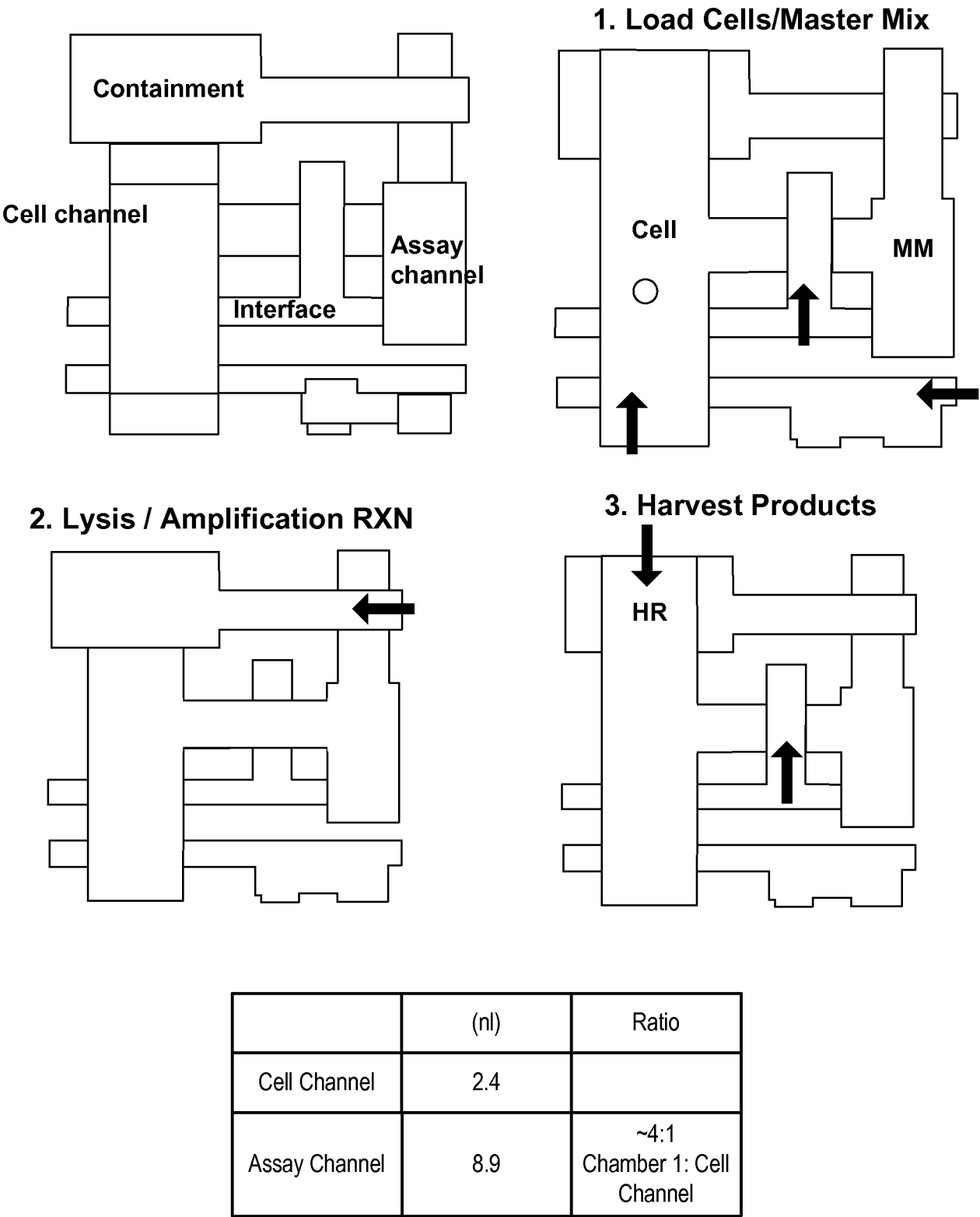
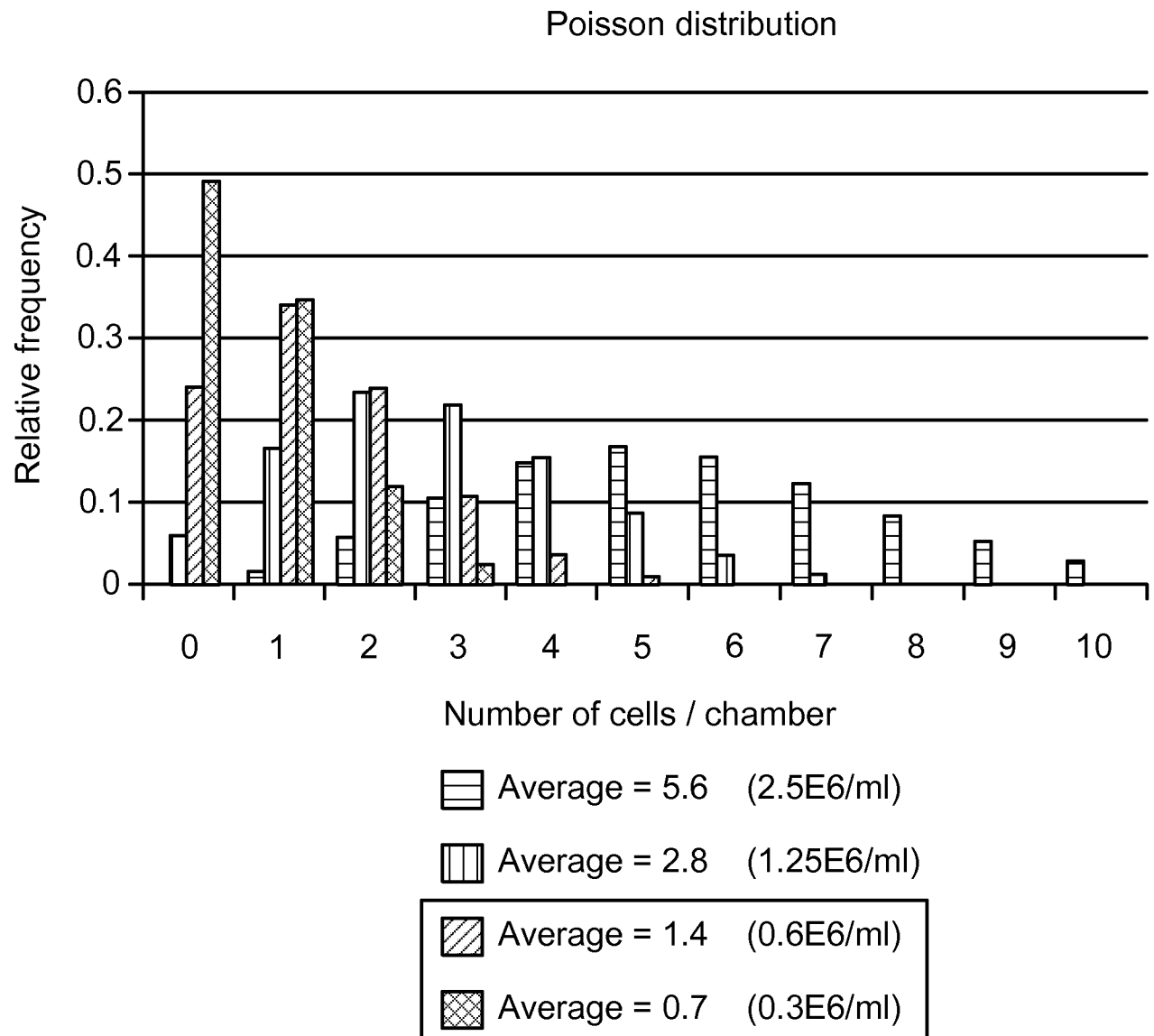


FIG. 9

12 / 43

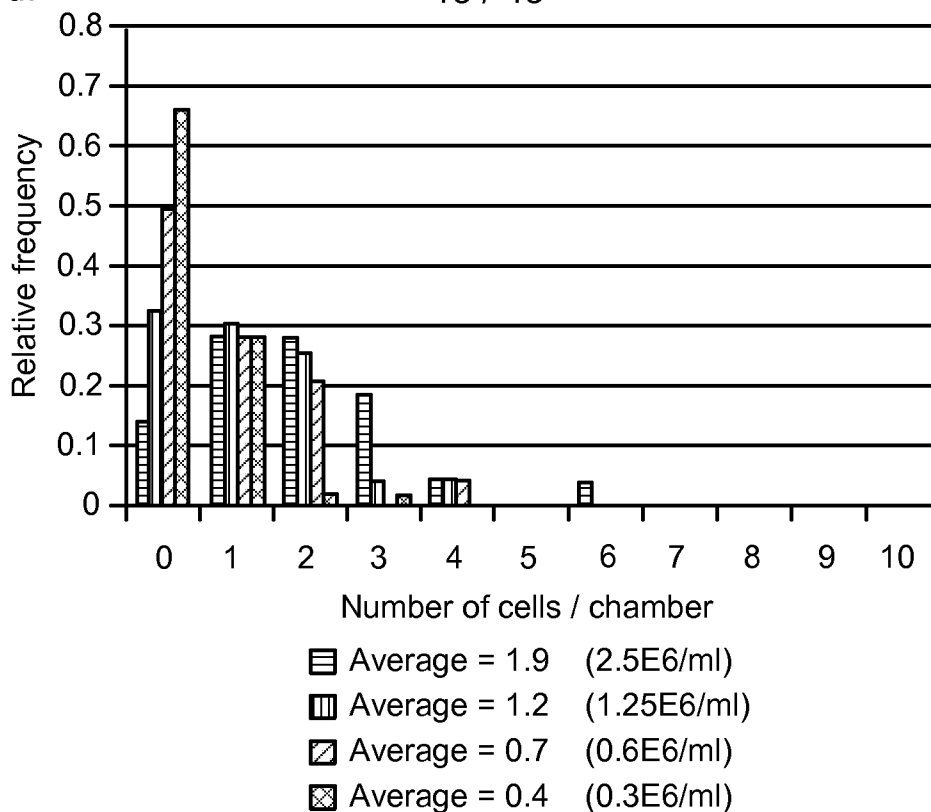


The “best” theoretical cell density for single cells:
 ~ 1 per chamber on average = $1 / 2.4 \text{ nl} = \sim 0.5\text{E}6/\text{ml}$

FIG. 10

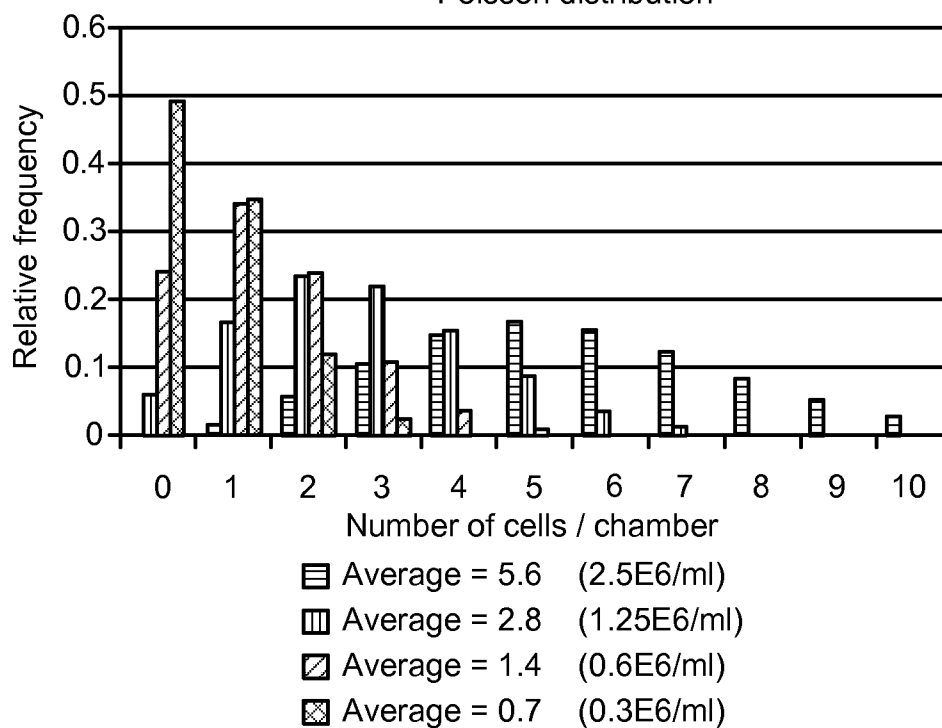
Measured:

13 / 43

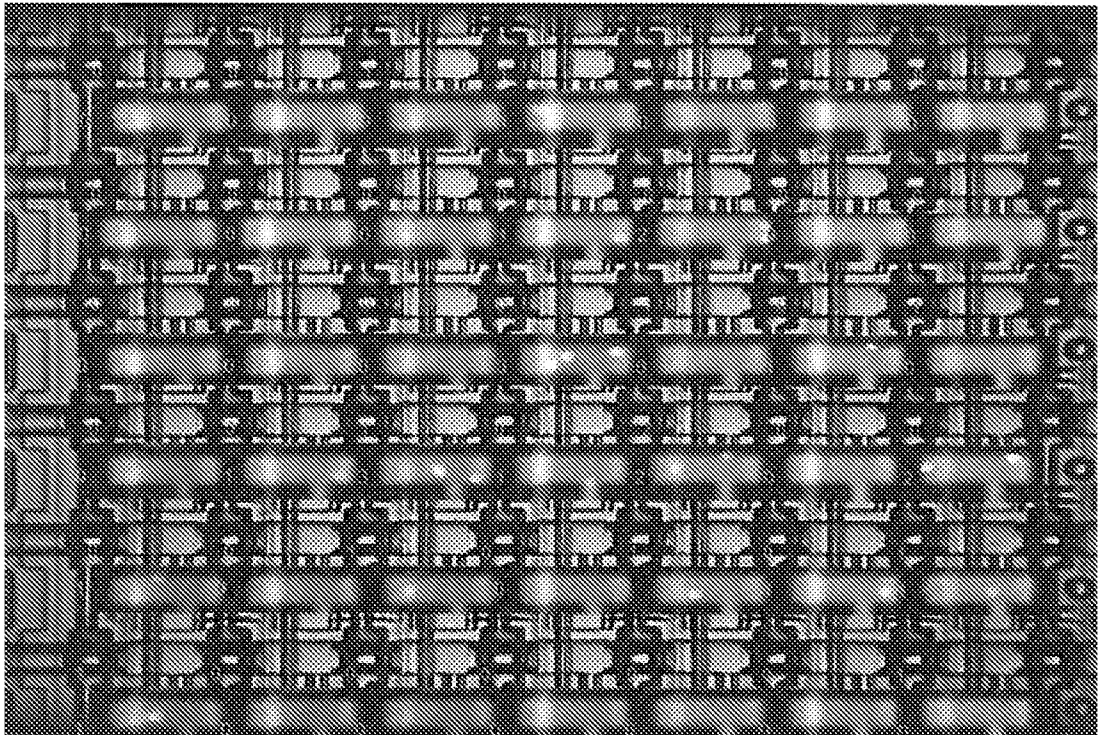
**FIG. 11A**

Theoretical:

Poisson distribution

**FIG. 11B**

0.15E6 /ml
Expect
0-1
chamber

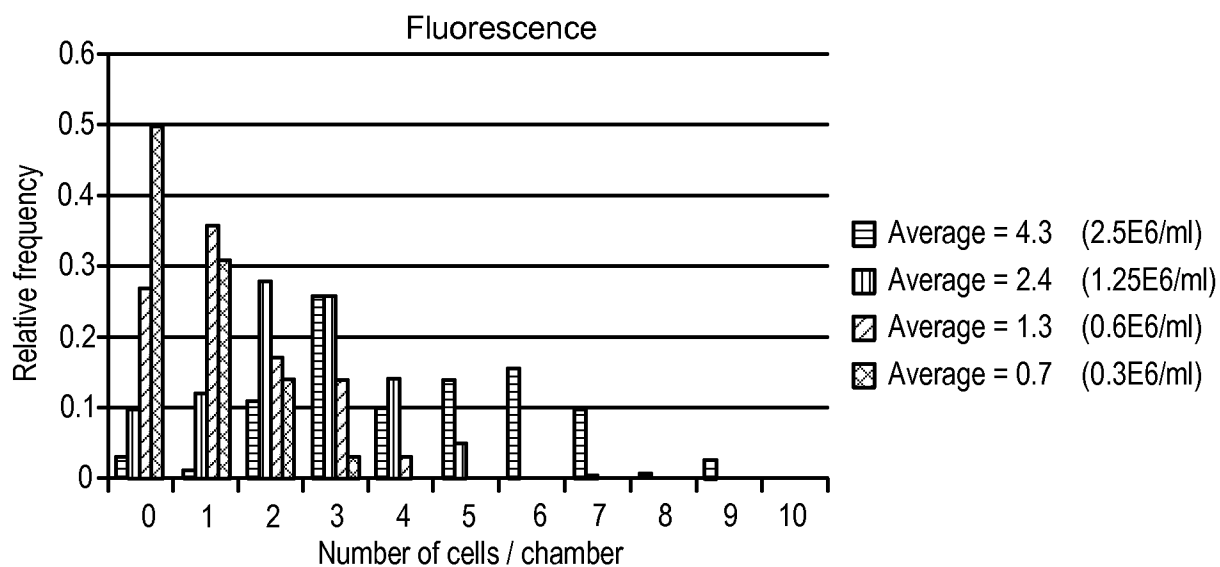
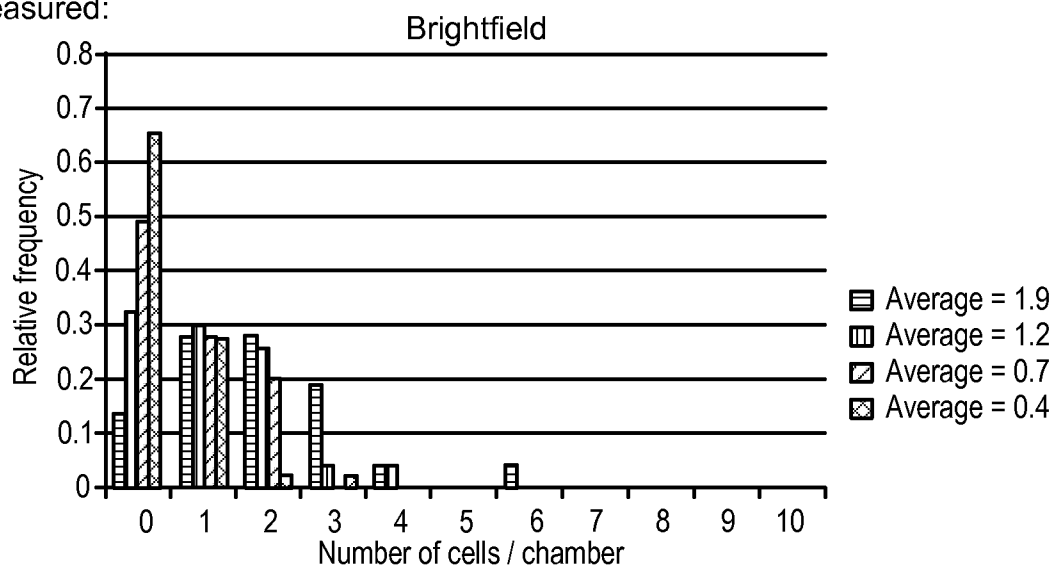


	Brightfield	Fluorescence
Average cells/ chamber	0.29	0.52

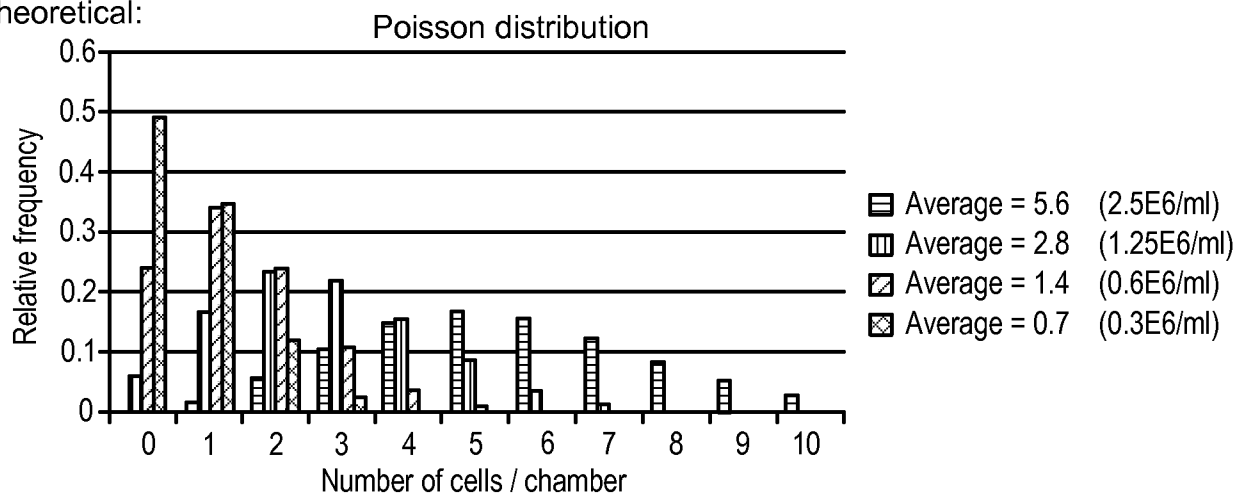
FIG. 12A

15 / 43

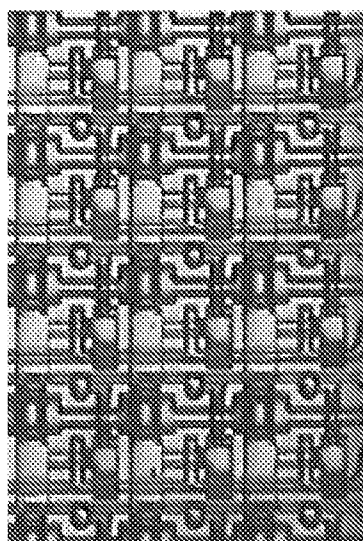
Measured:



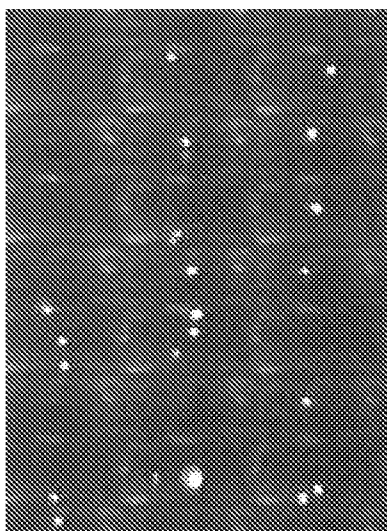
Theoretical:

**FIG. 12B**

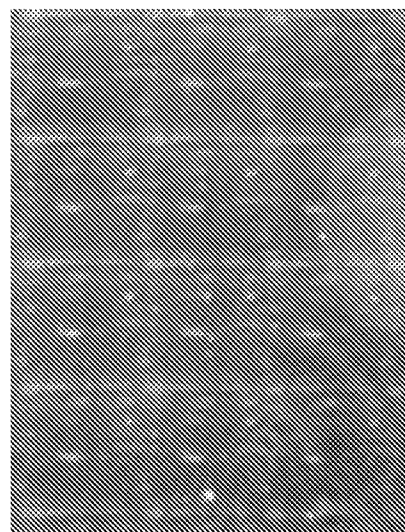
16 / 43



Brightfield



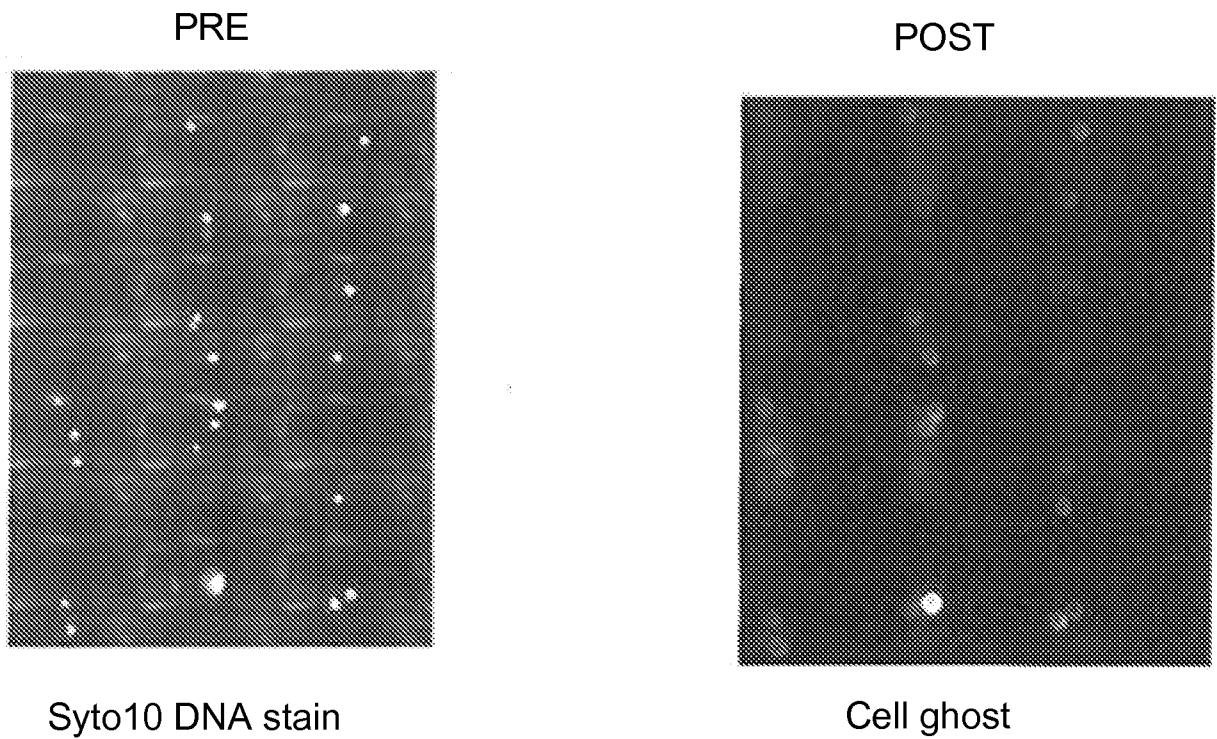
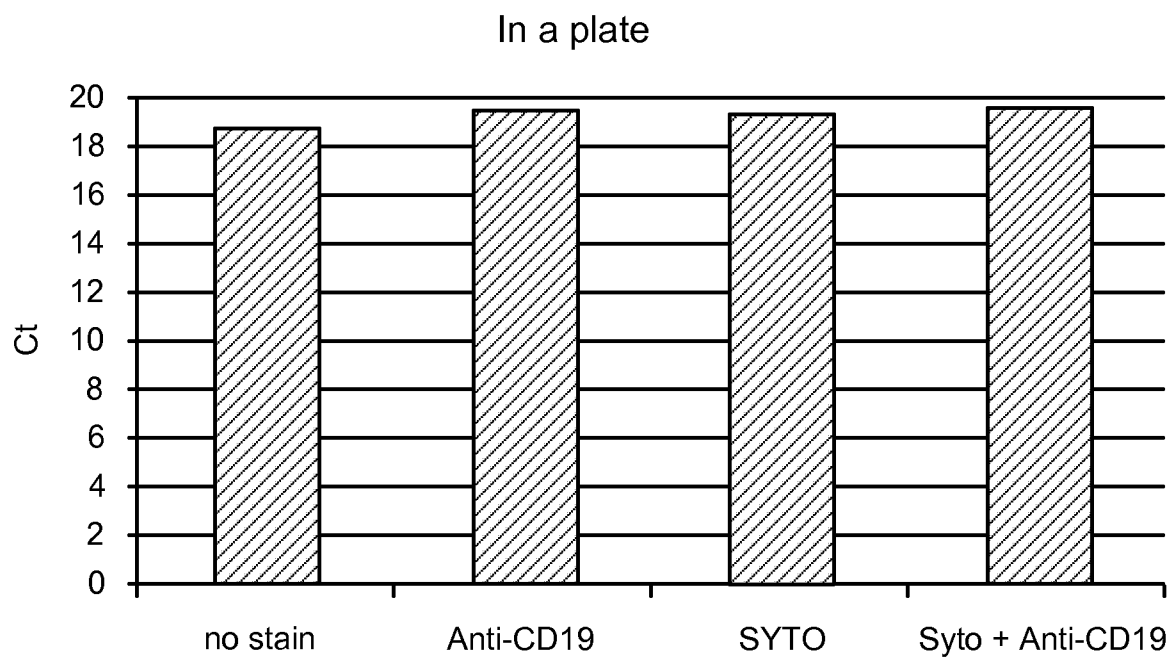
Syto10 DNA stain



Cy5 antibody

FIG. 13

17 / 43

**FIG. 14A****FIG. 14B**

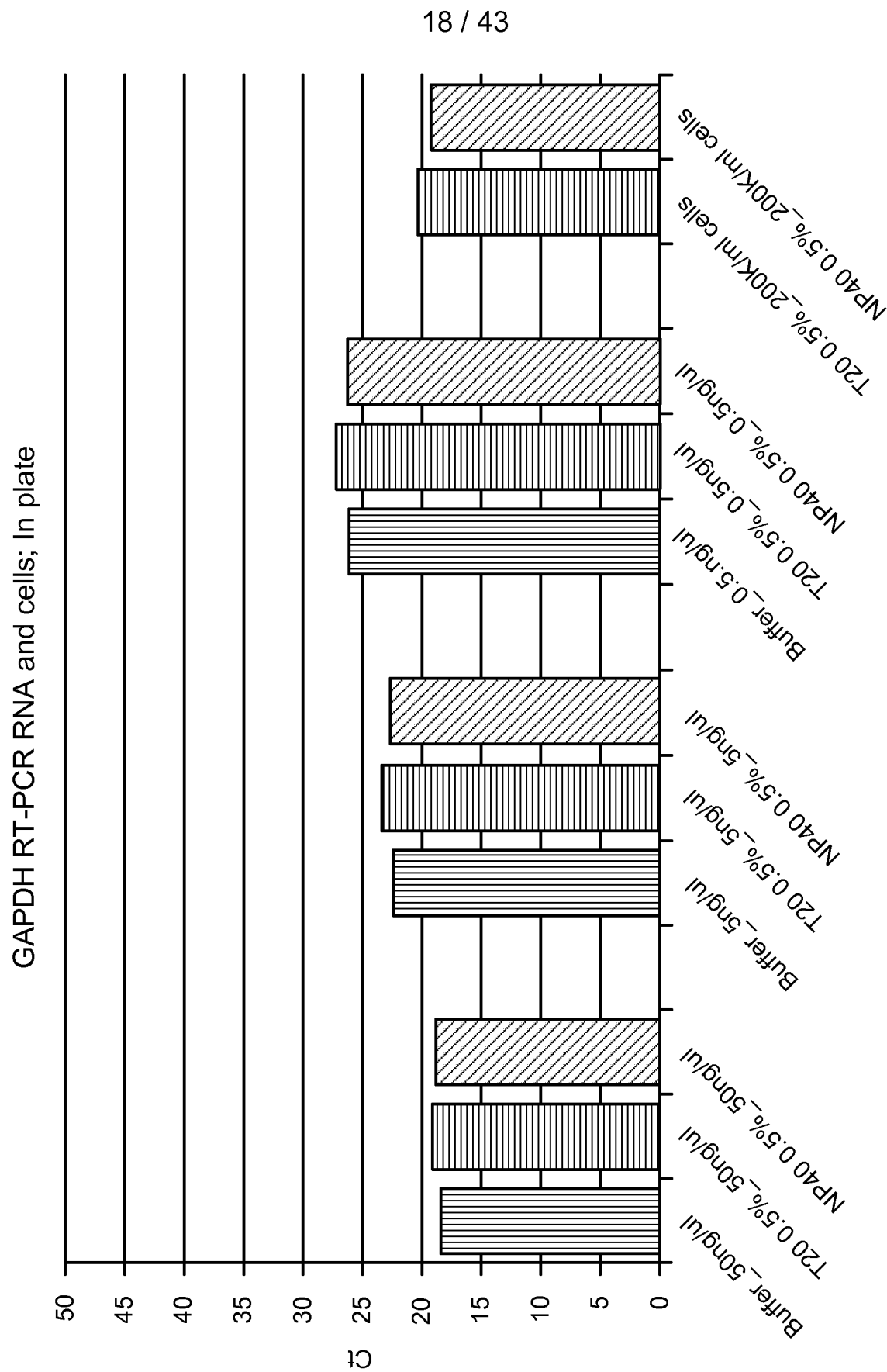
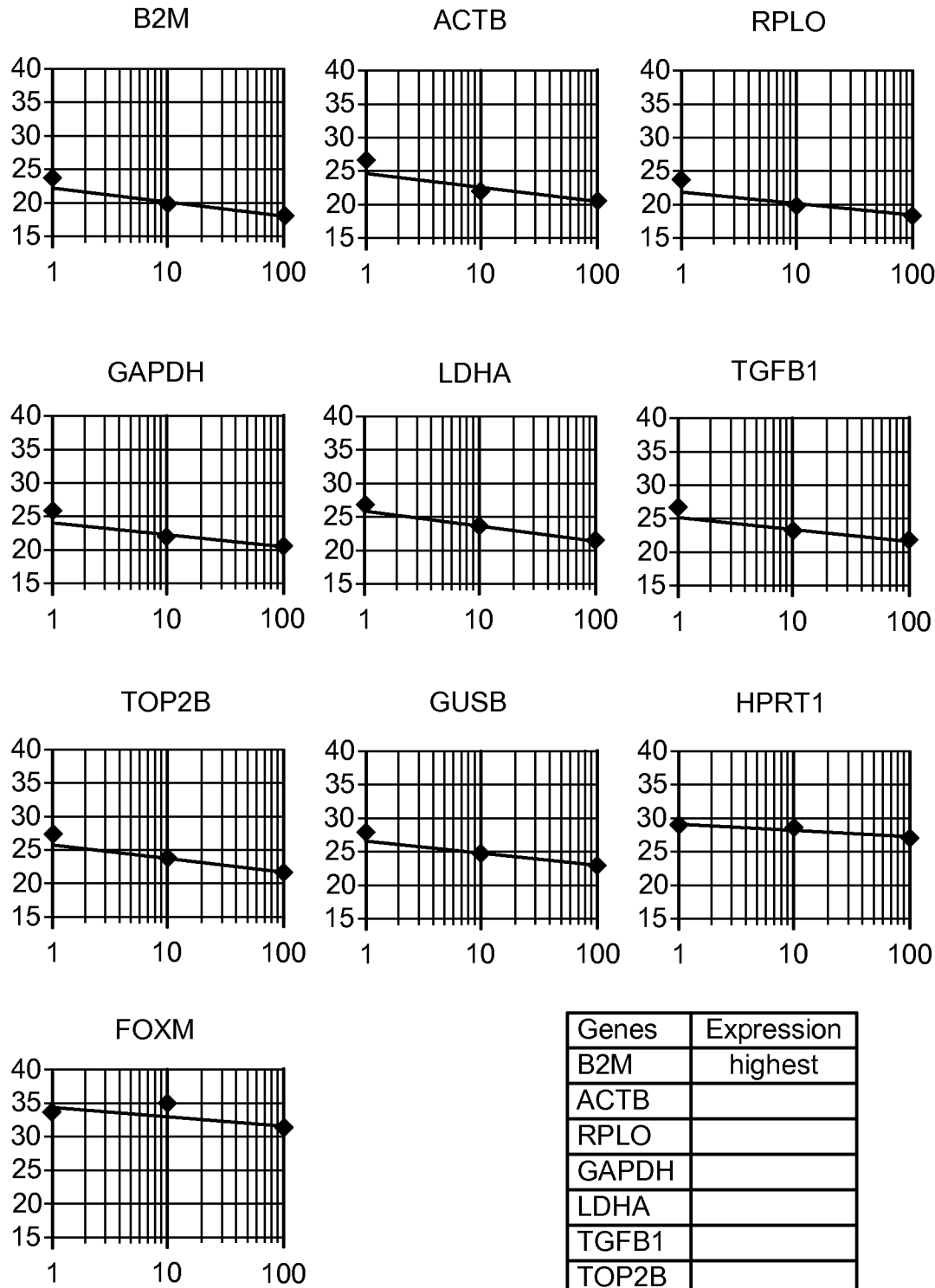


FIG. 15

19 / 43
ACTB



Genes	Expression
B2M	highest
ACTB	
RPLO	
GAPDH	
LDHA	
TGFB1	
TOP2B	
GUSB	
HPRT1	
FOXM	
TFRC	lowest

FIG. 16

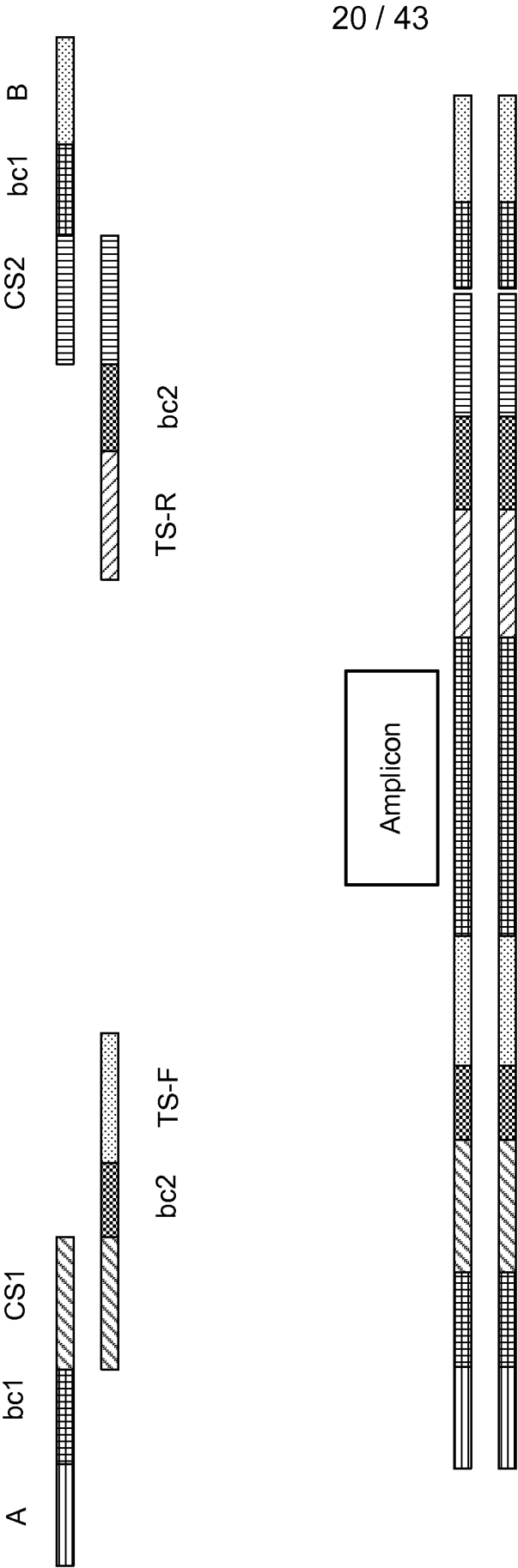
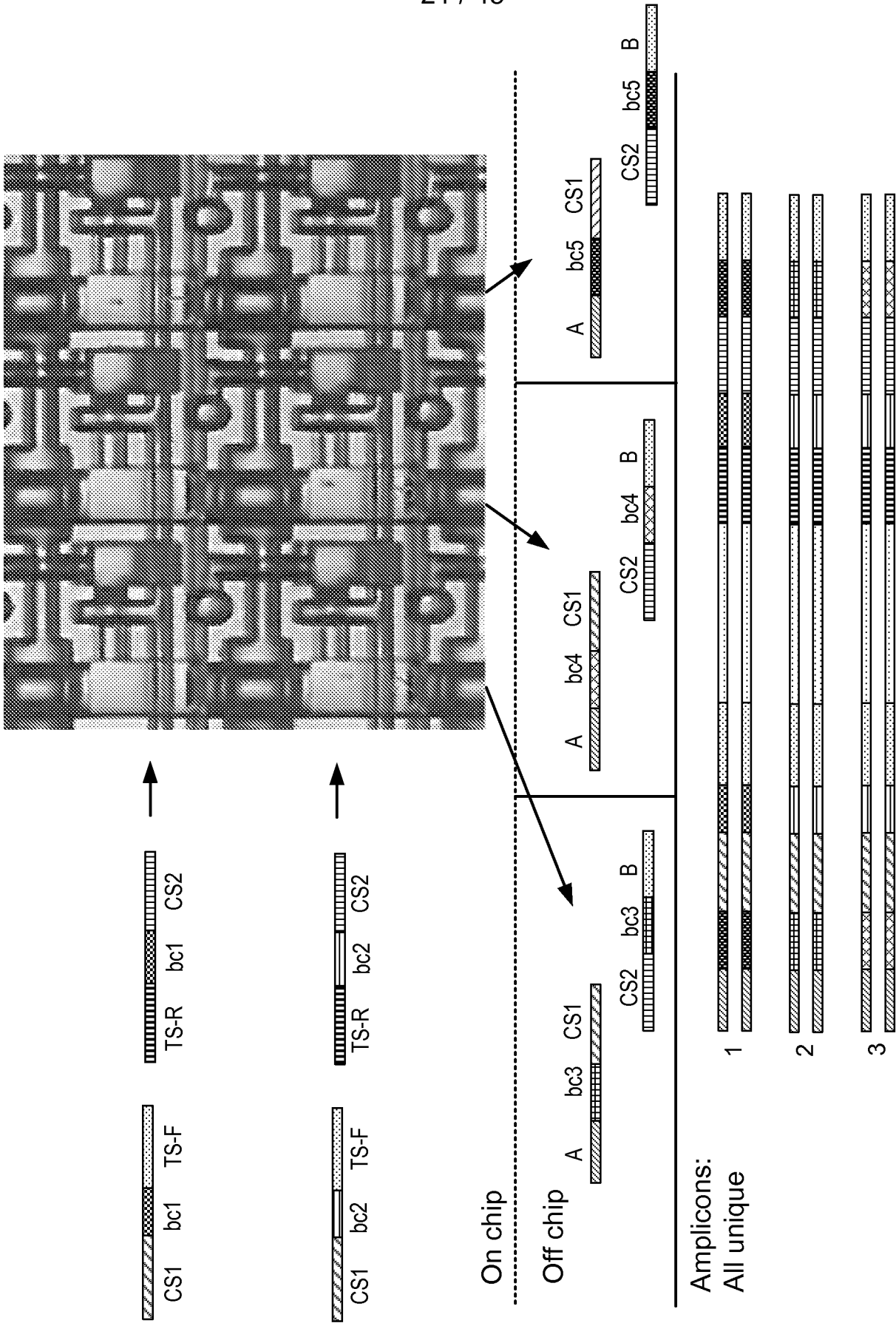
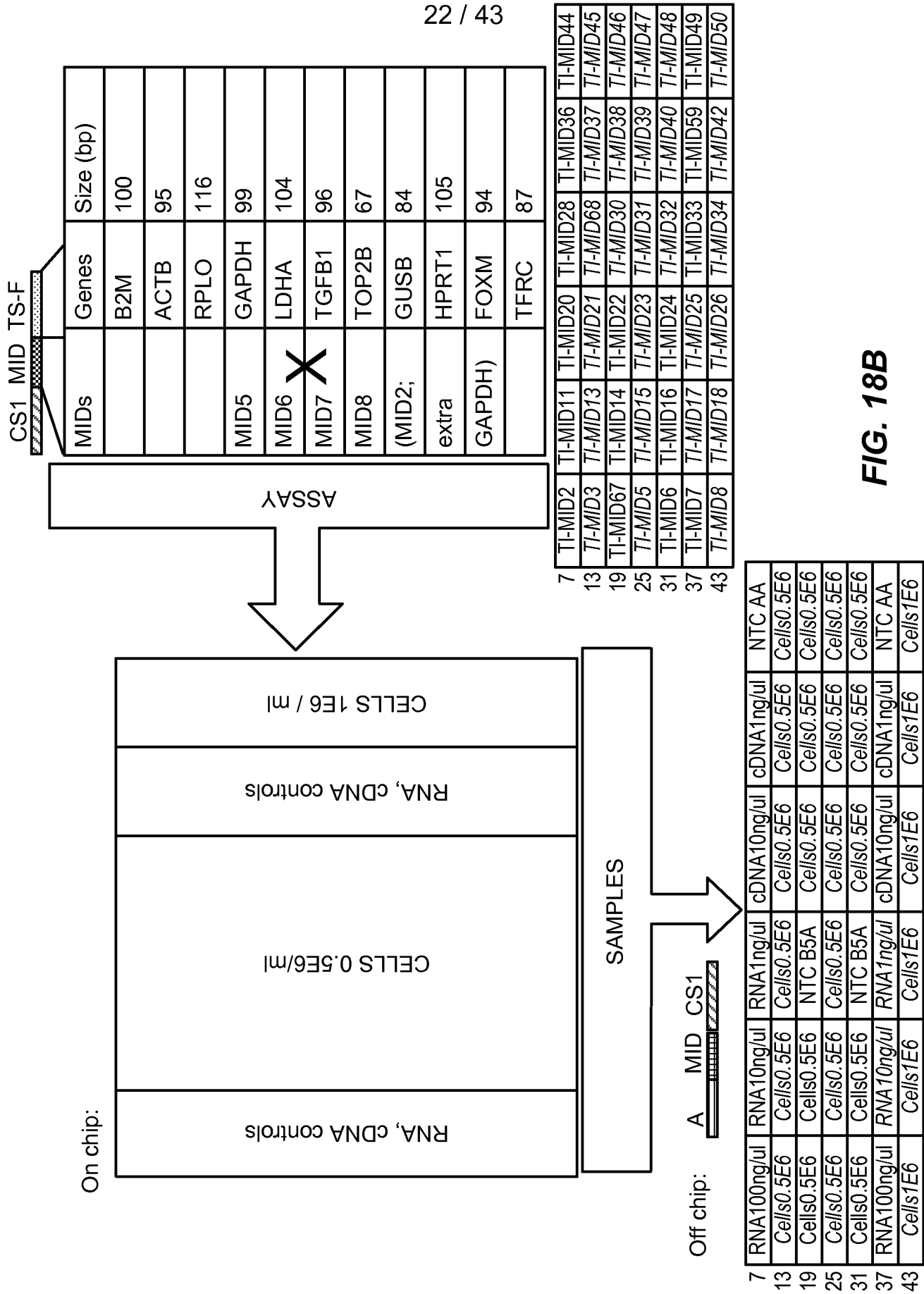


FIG. 17





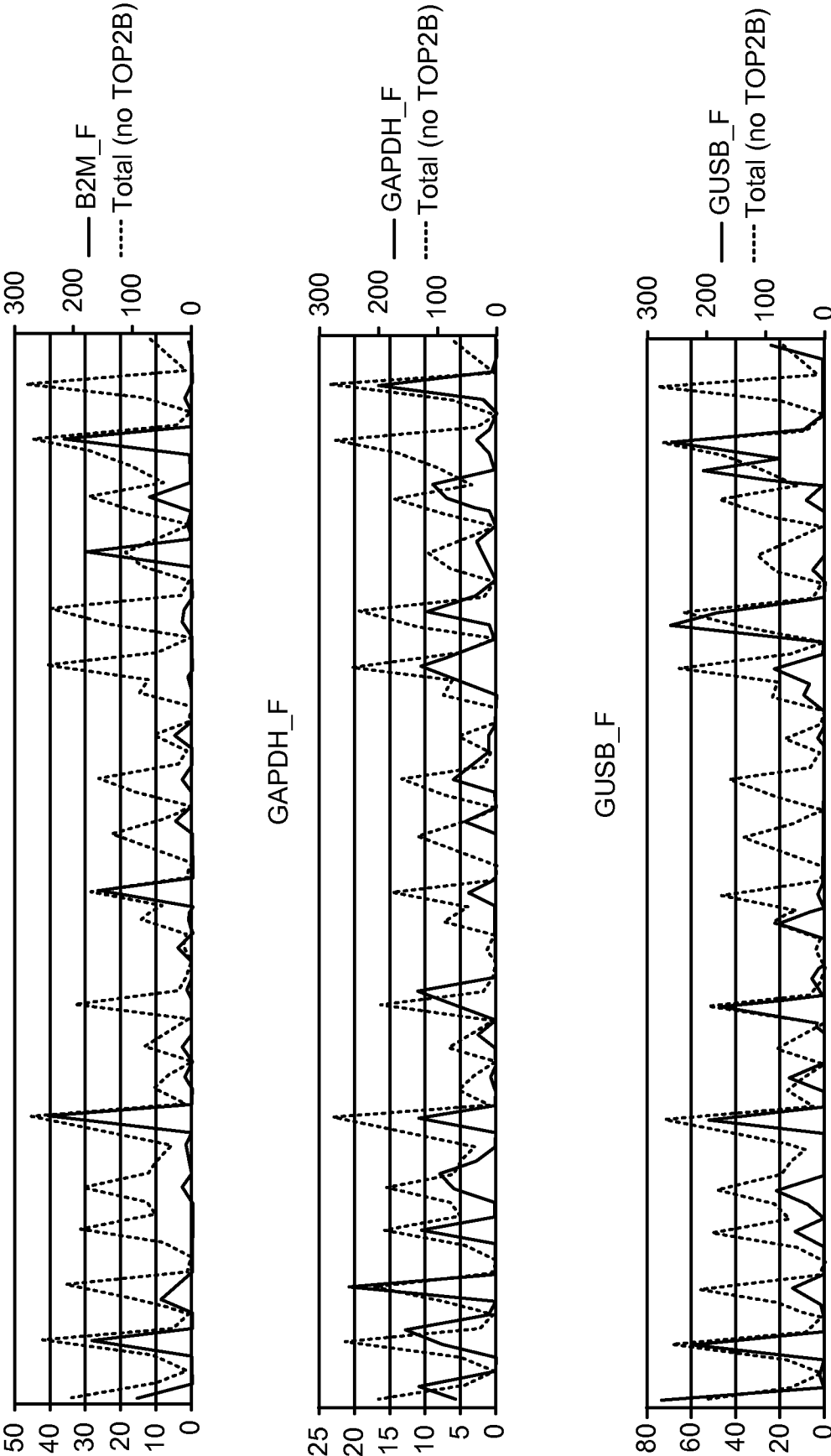


FIG. 19

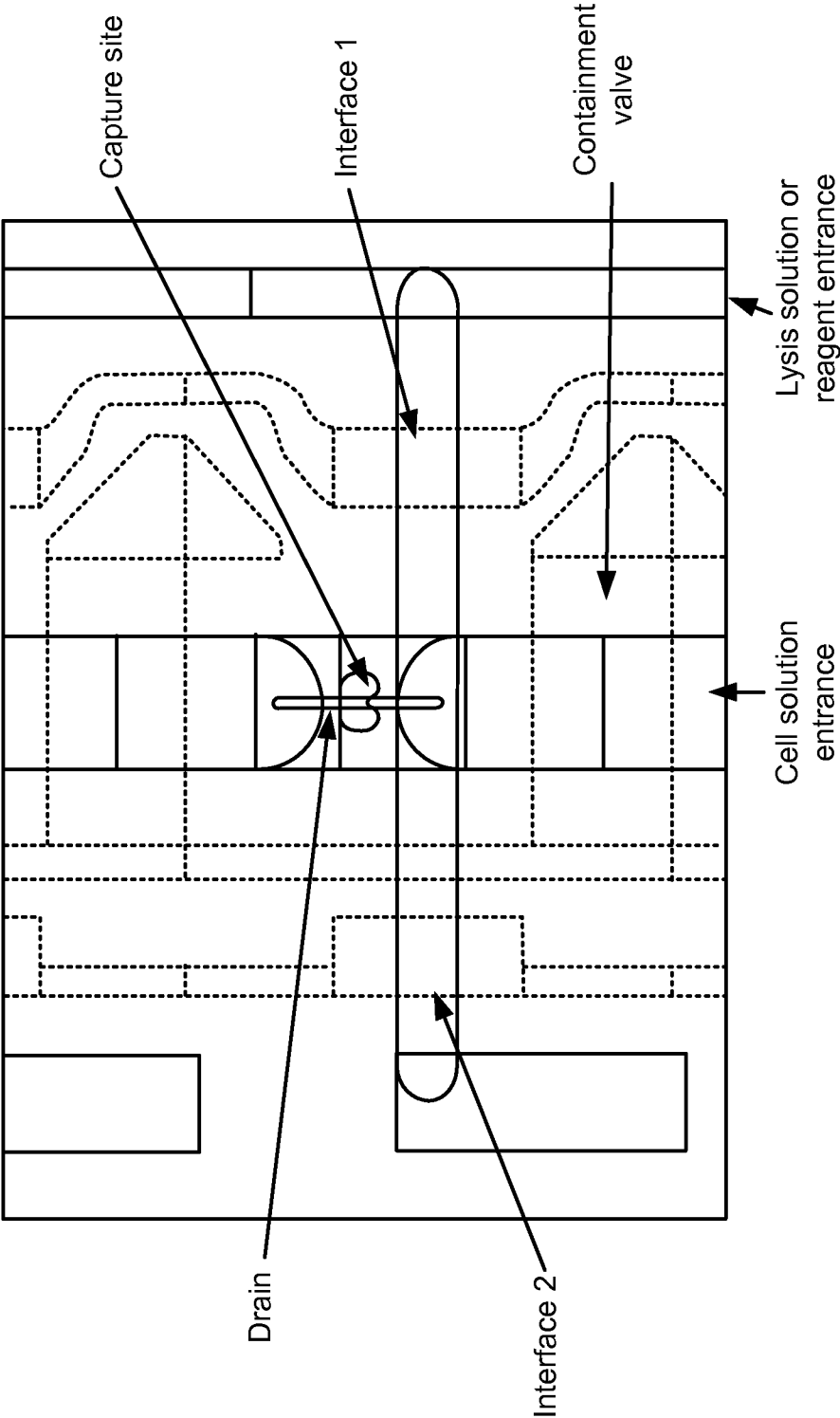


FIG. 20A

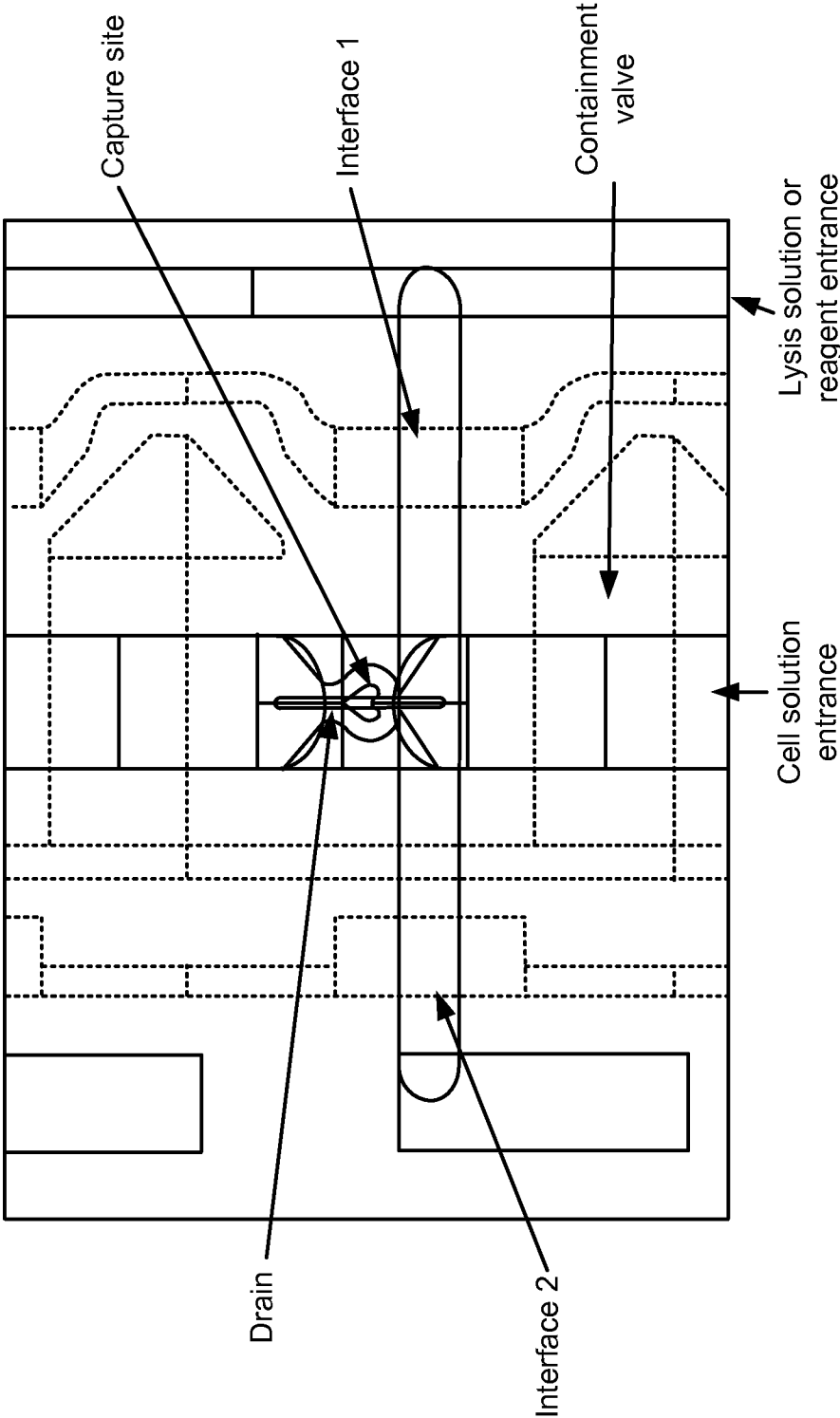


FIG. 20B

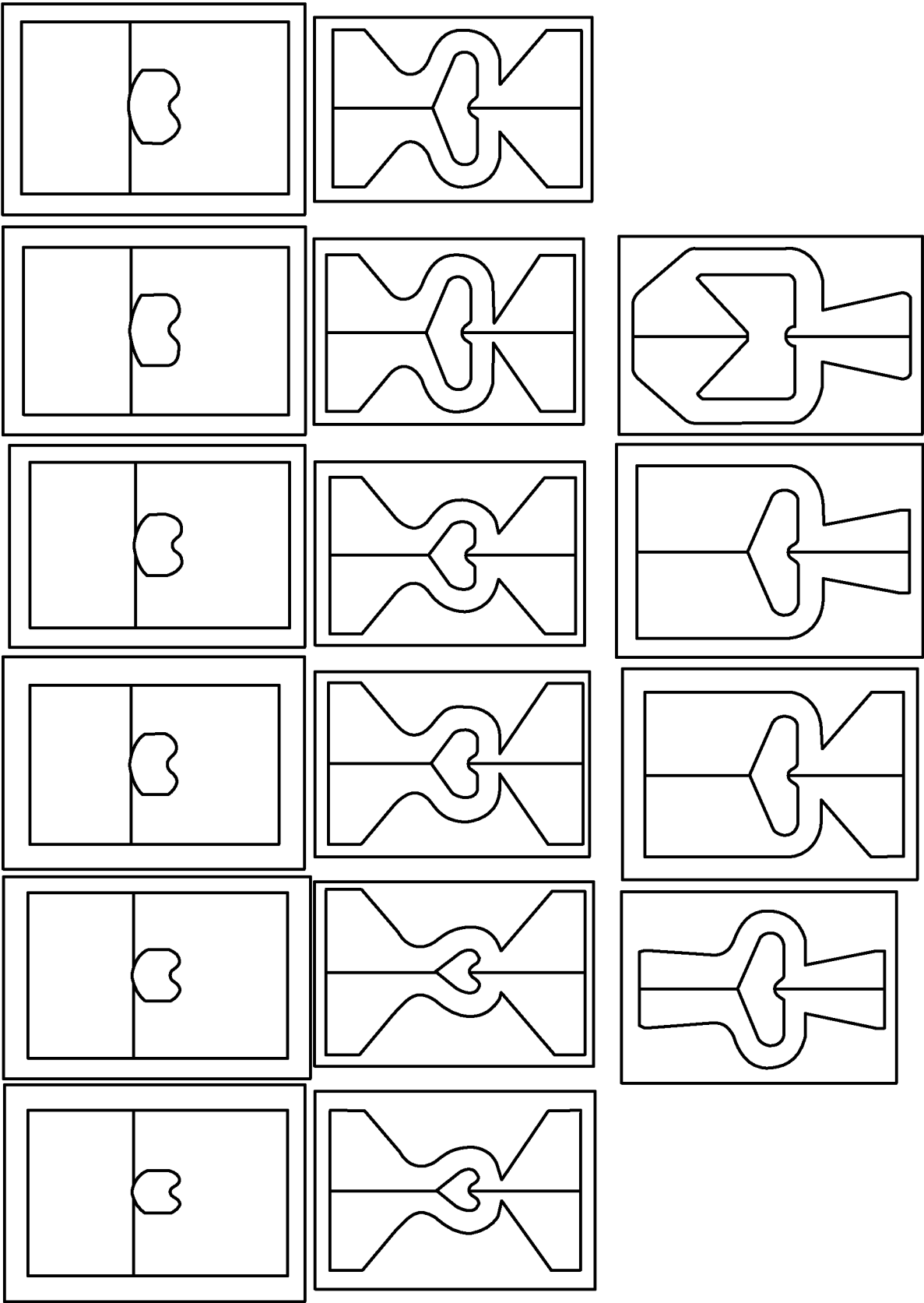


FIG. 21

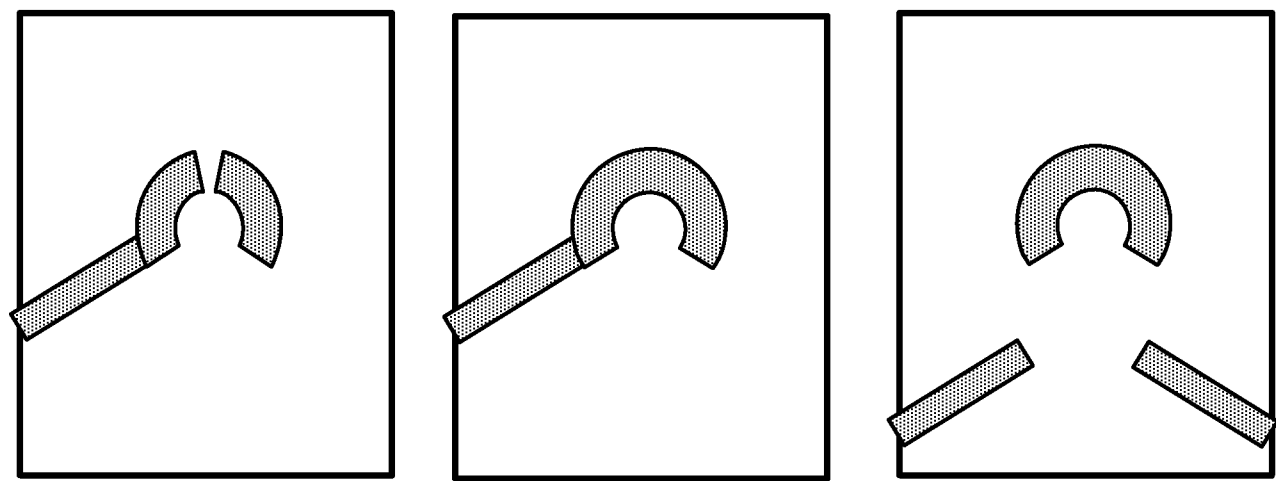


FIG. 22A

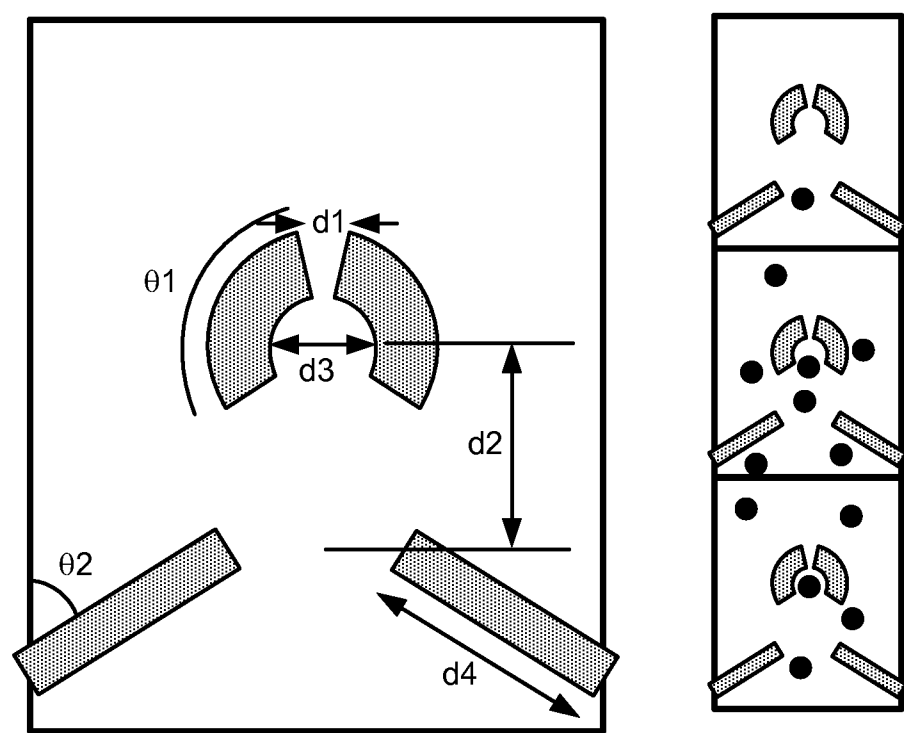


FIG. 22B

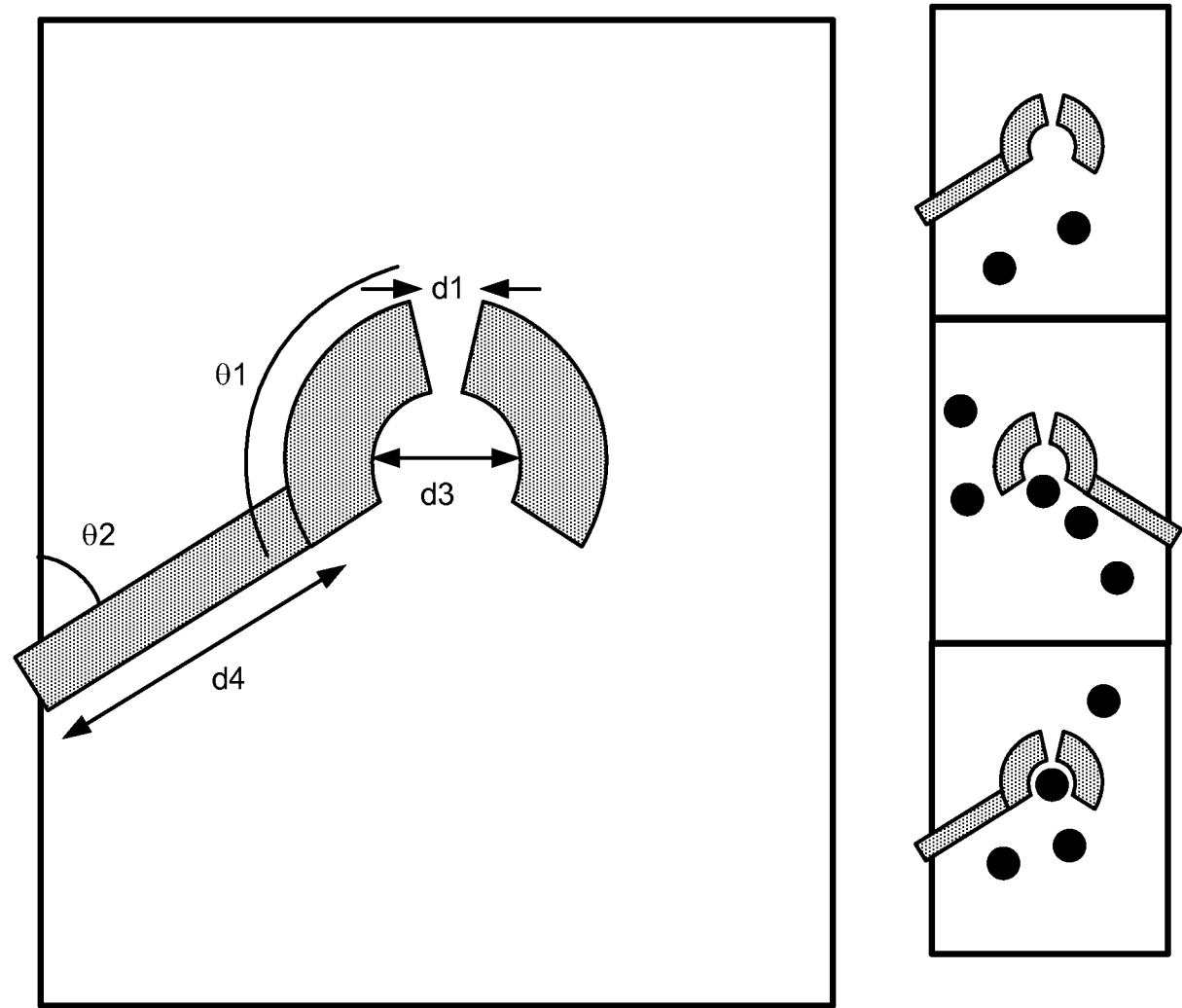


FIG. 22C

29 / 43

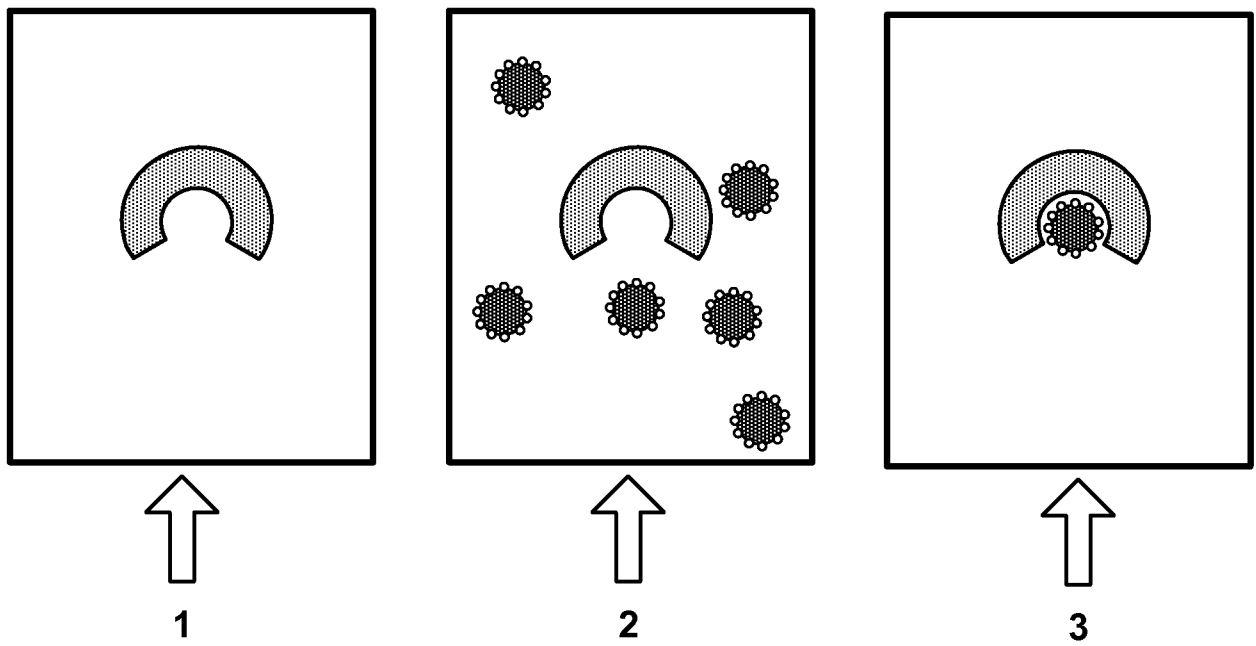


FIG. 23A

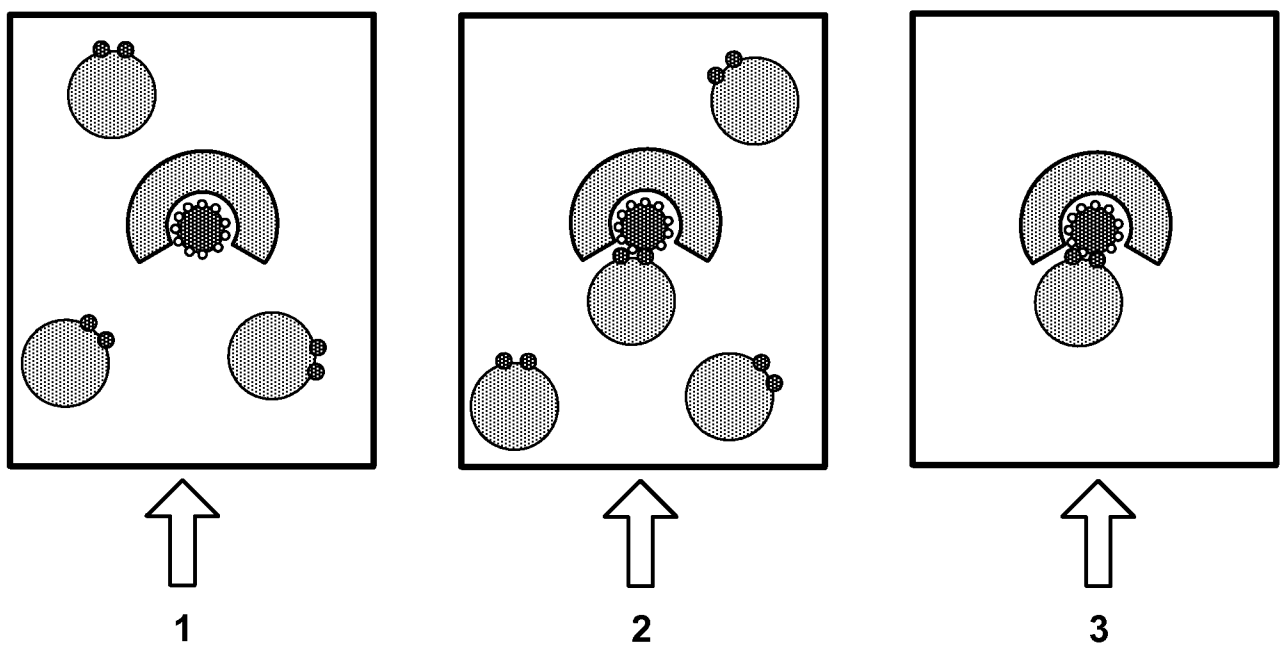


FIG. 23B

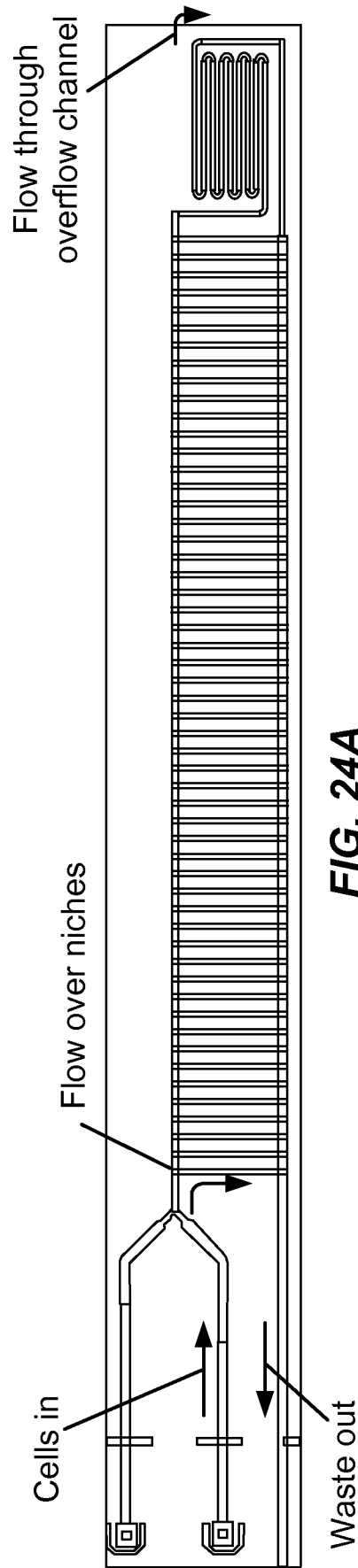


FIG. 24A

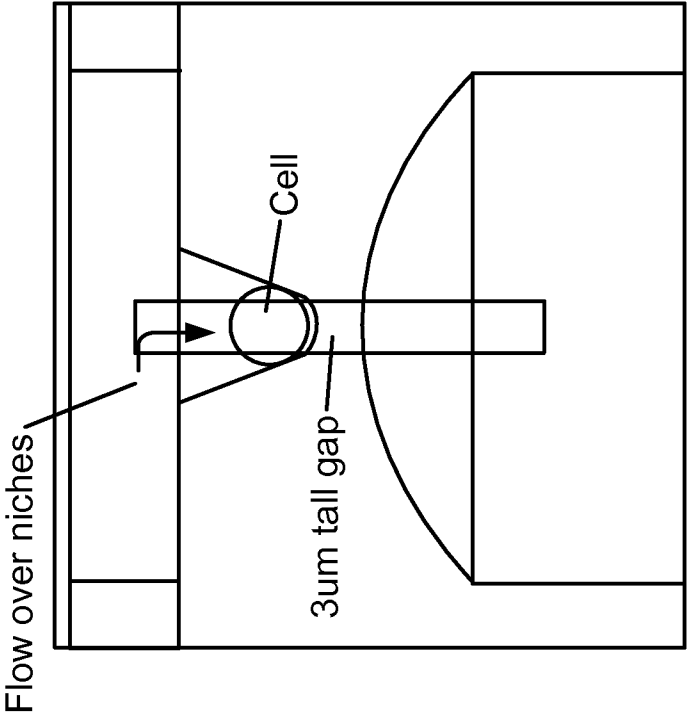


FIG. 24B

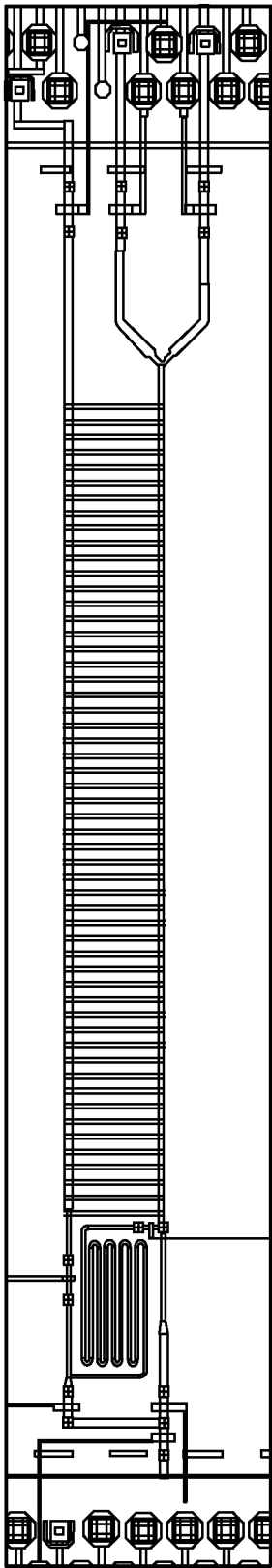


FIG. 24C

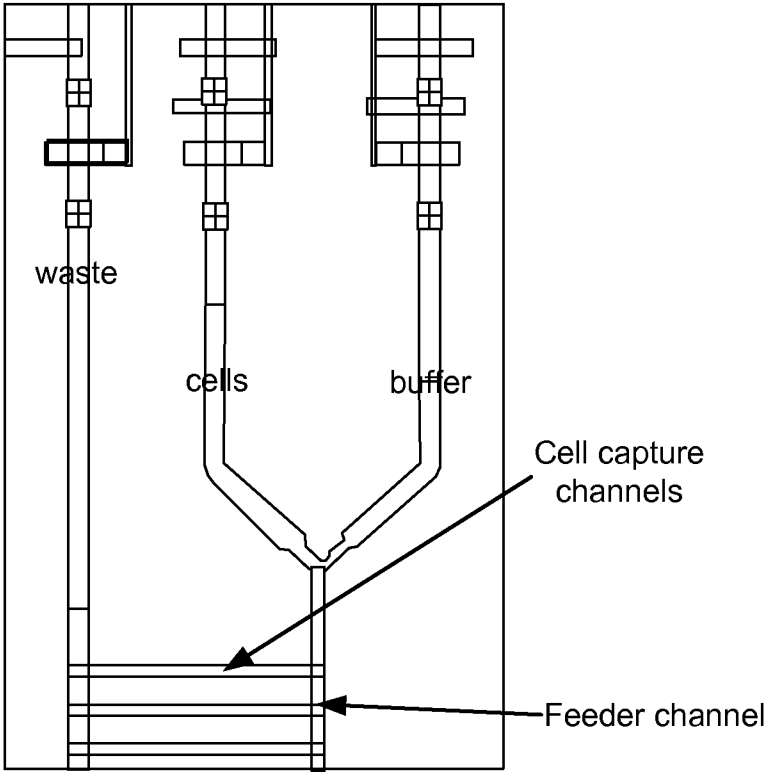


FIG. 24D

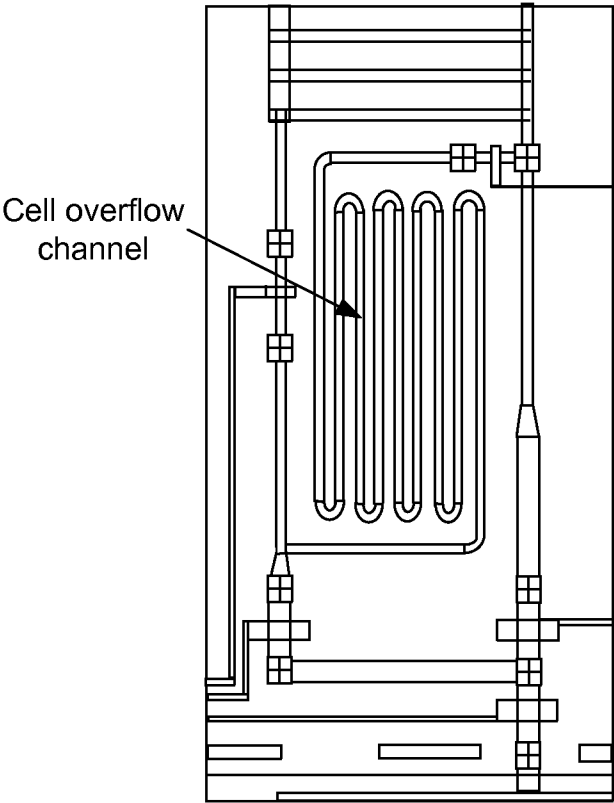


FIG. 24E

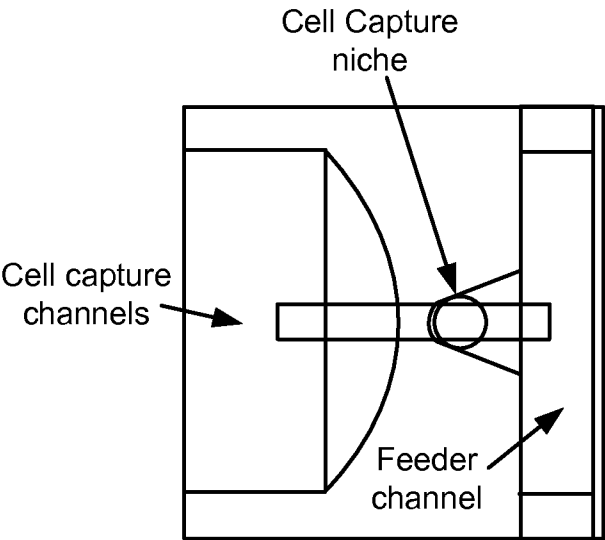


FIG. 24F

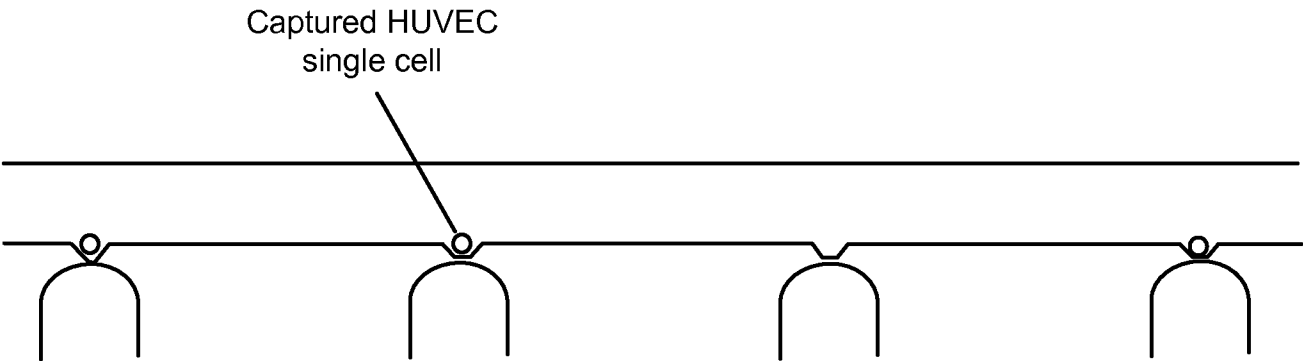
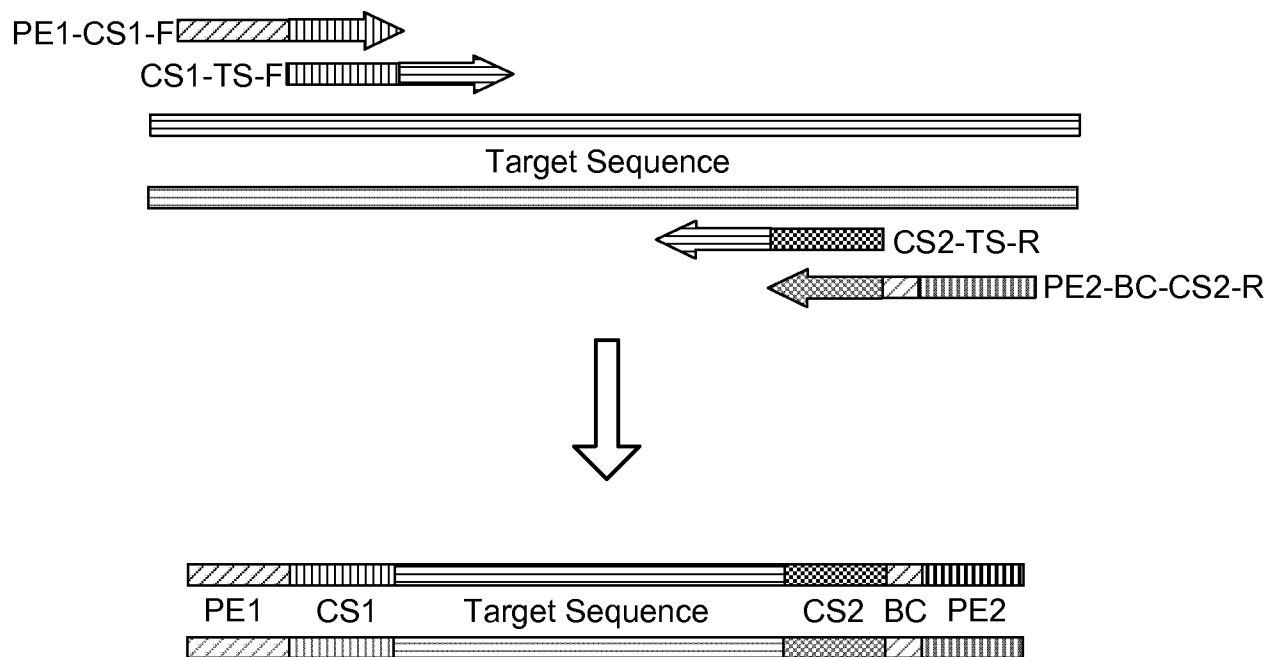
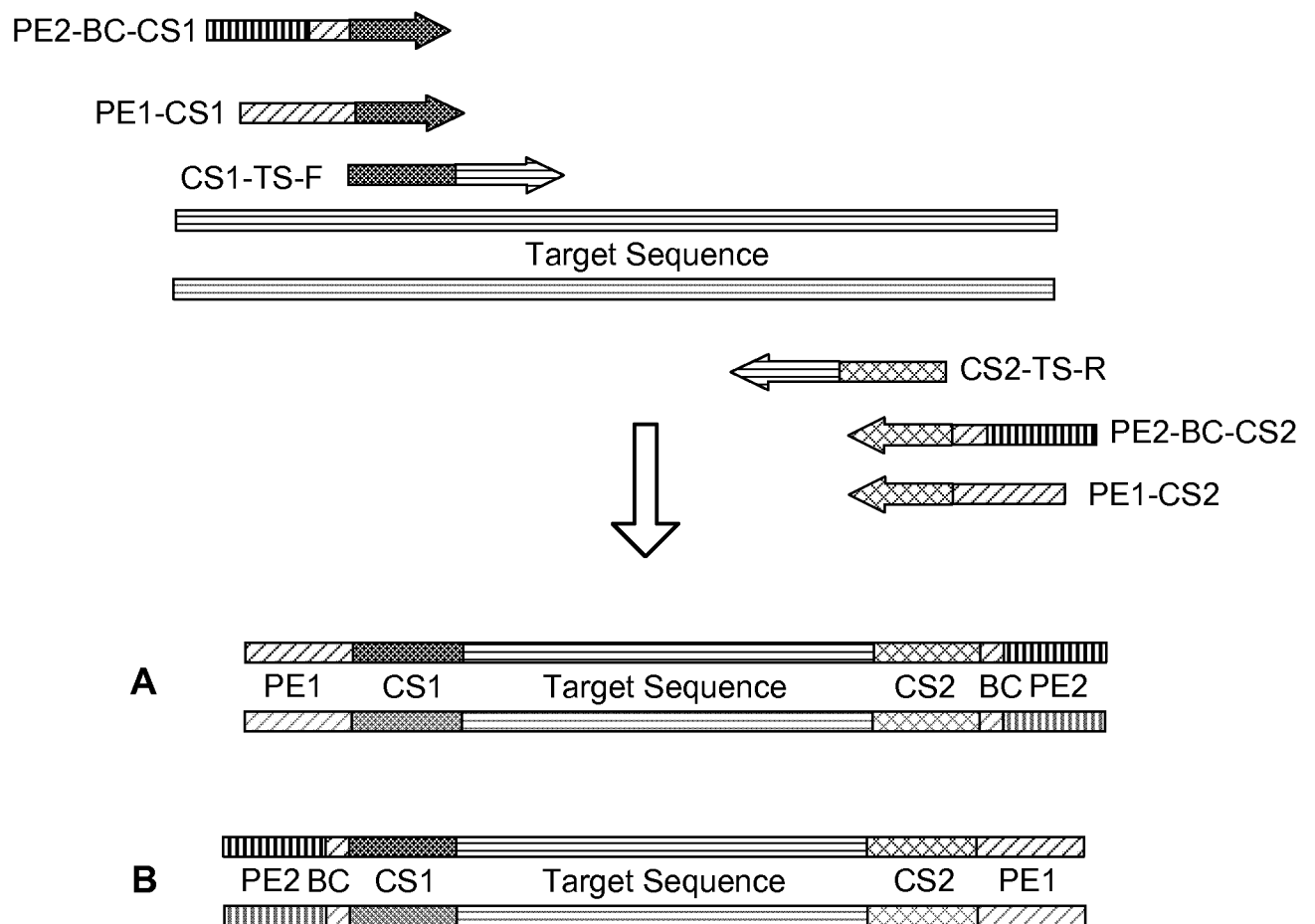
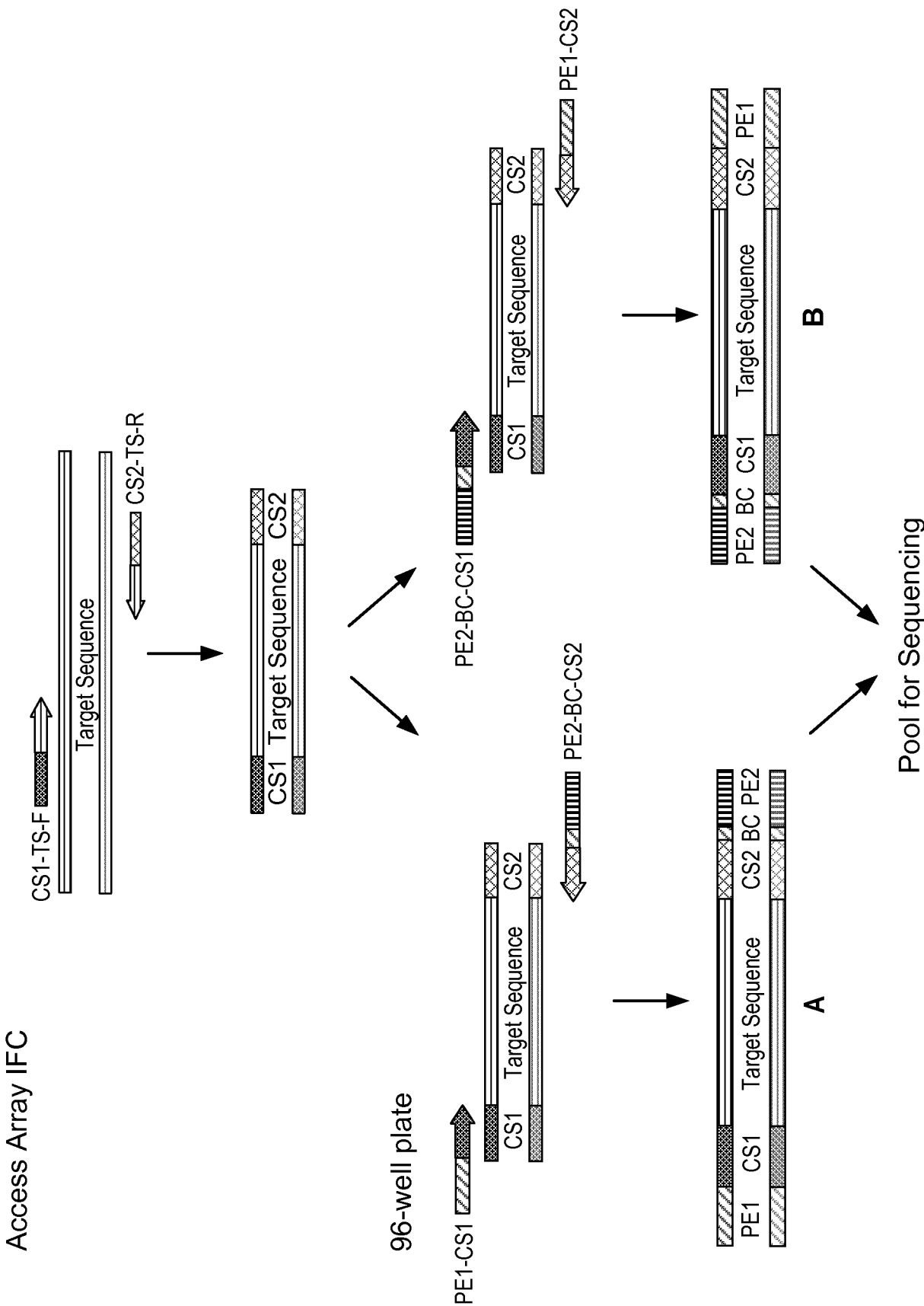


FIG. 24G

33 / 43

A**B****FIG. 25**



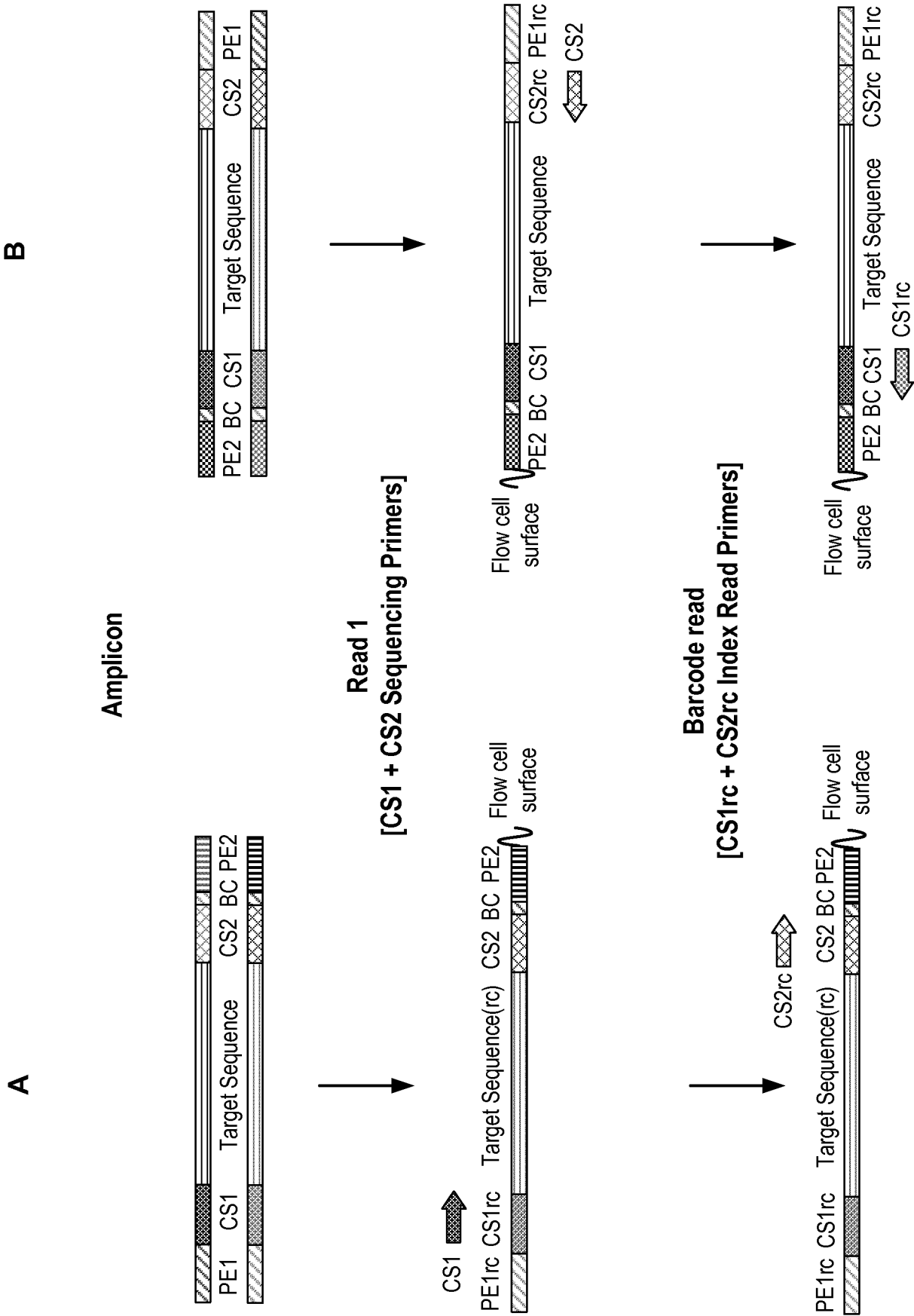


FIG. 27

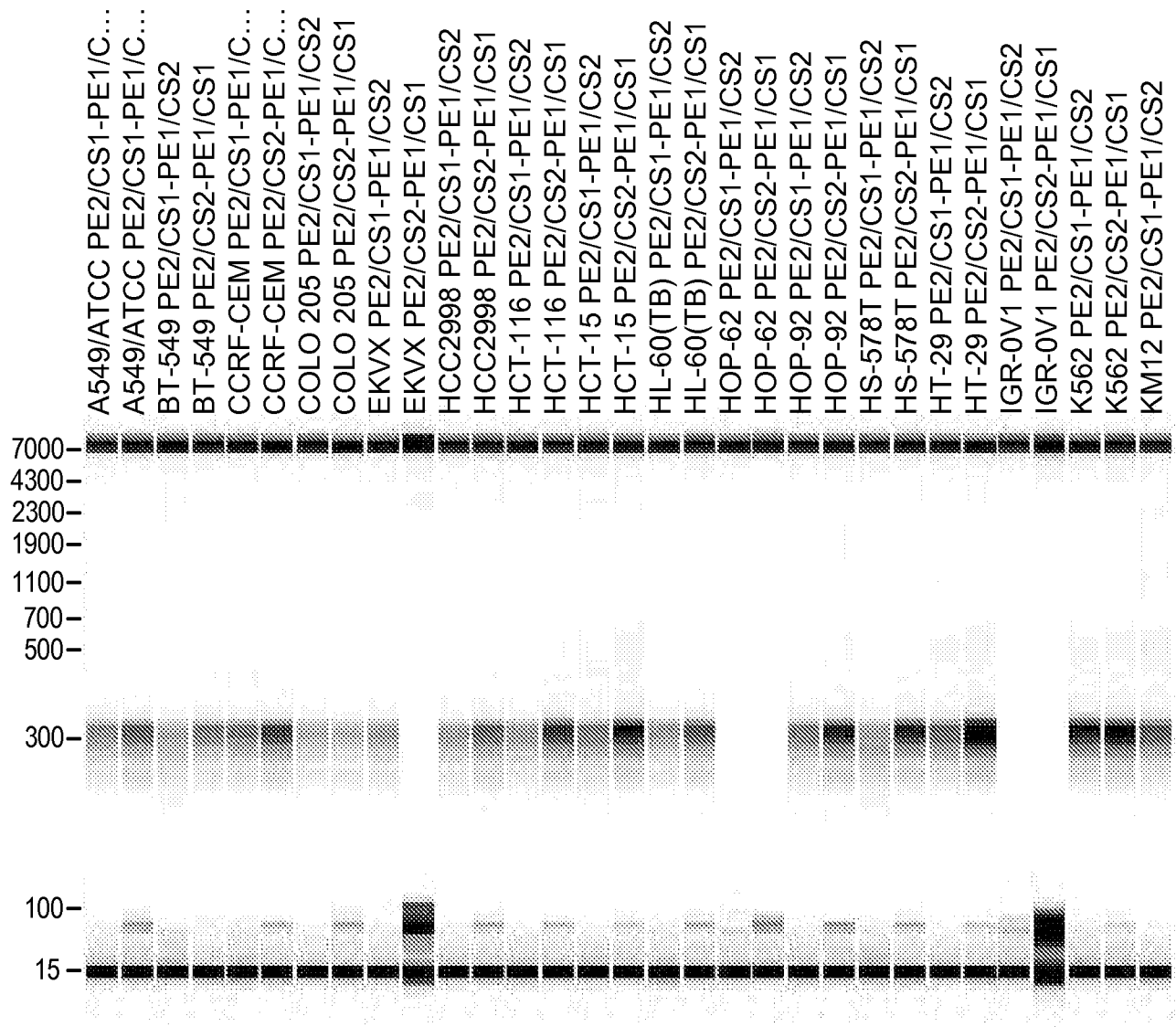


FIG. 28

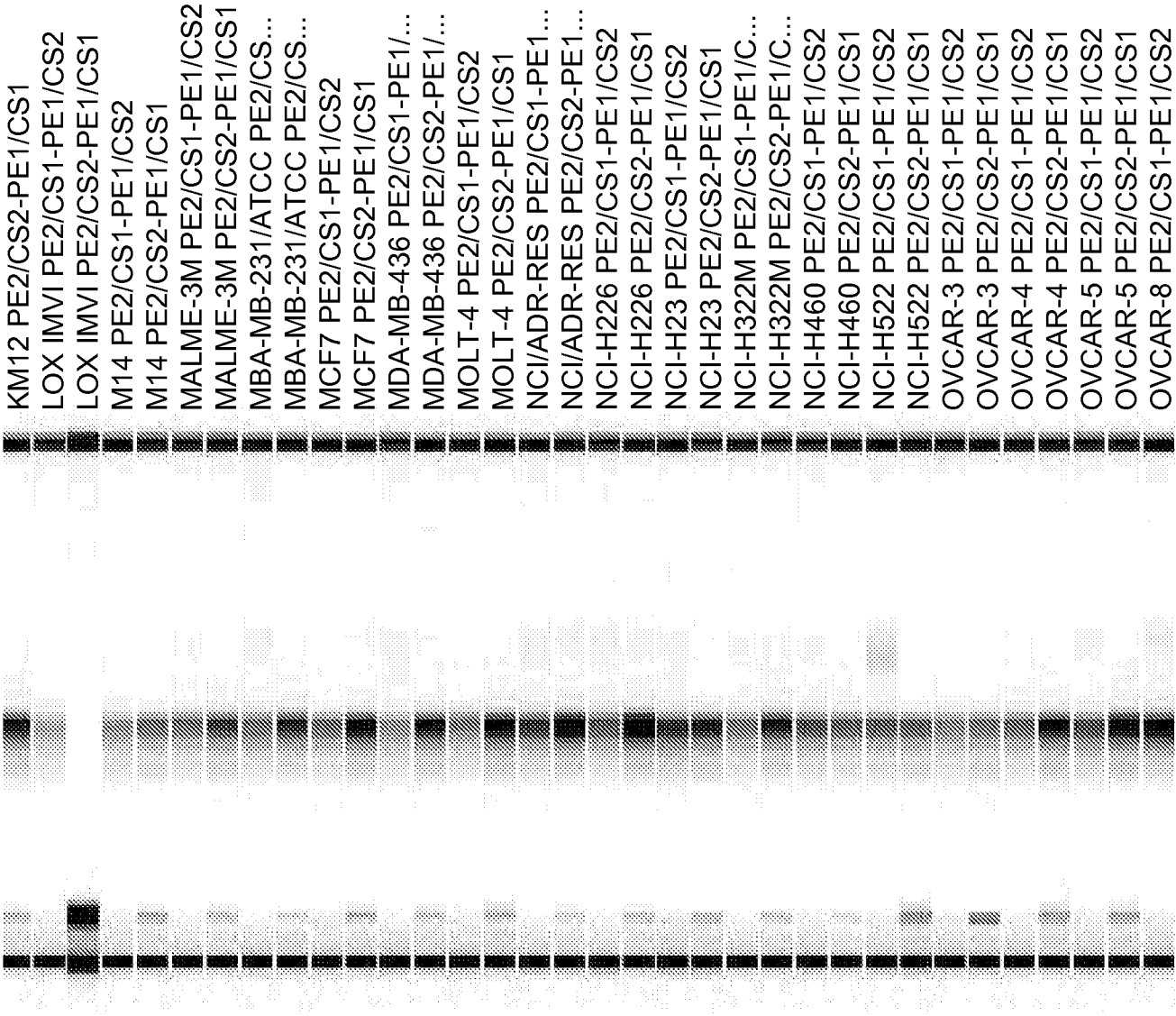


FIG. 28
(Continued)

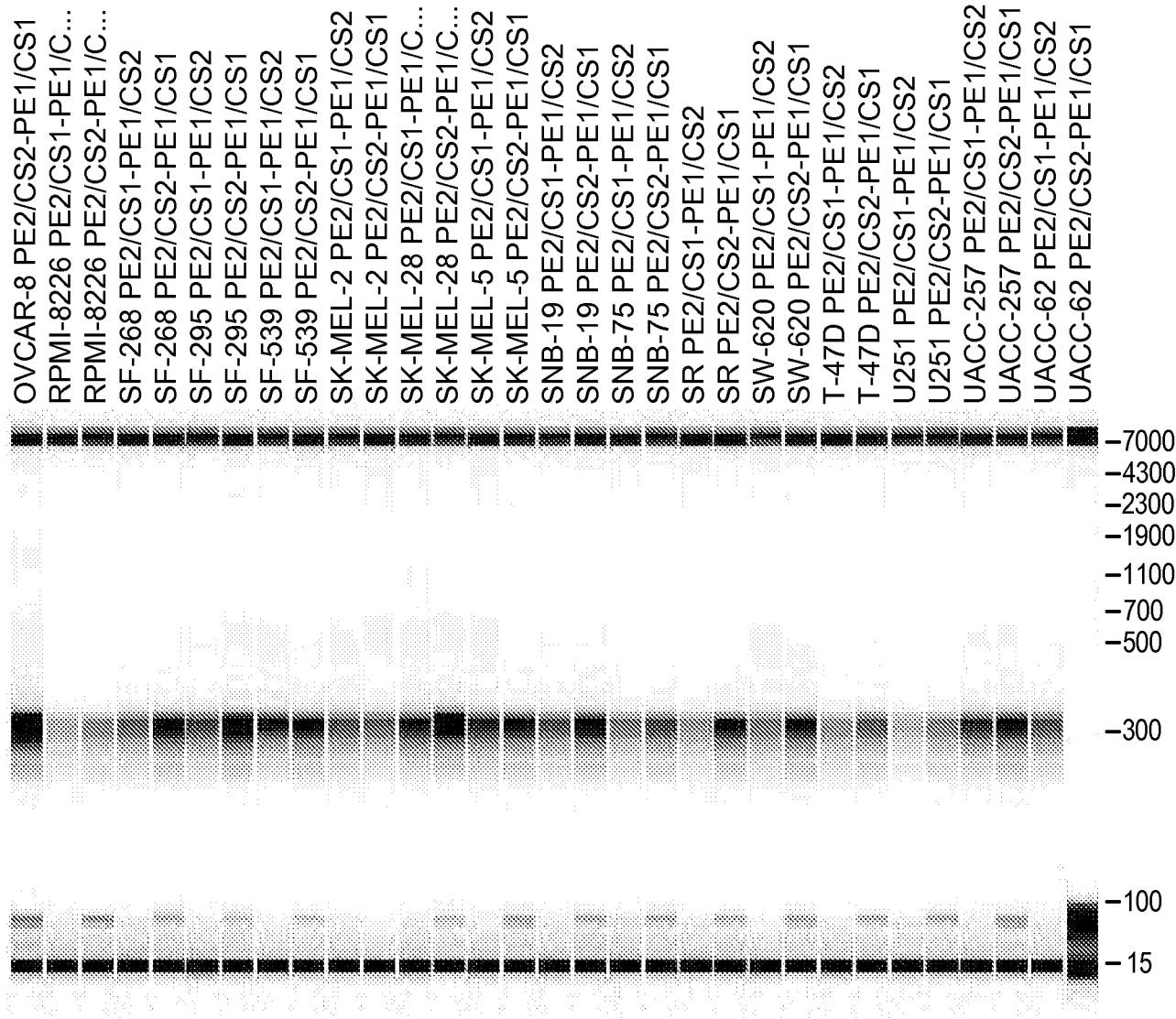


FIG. 28
(Continued)

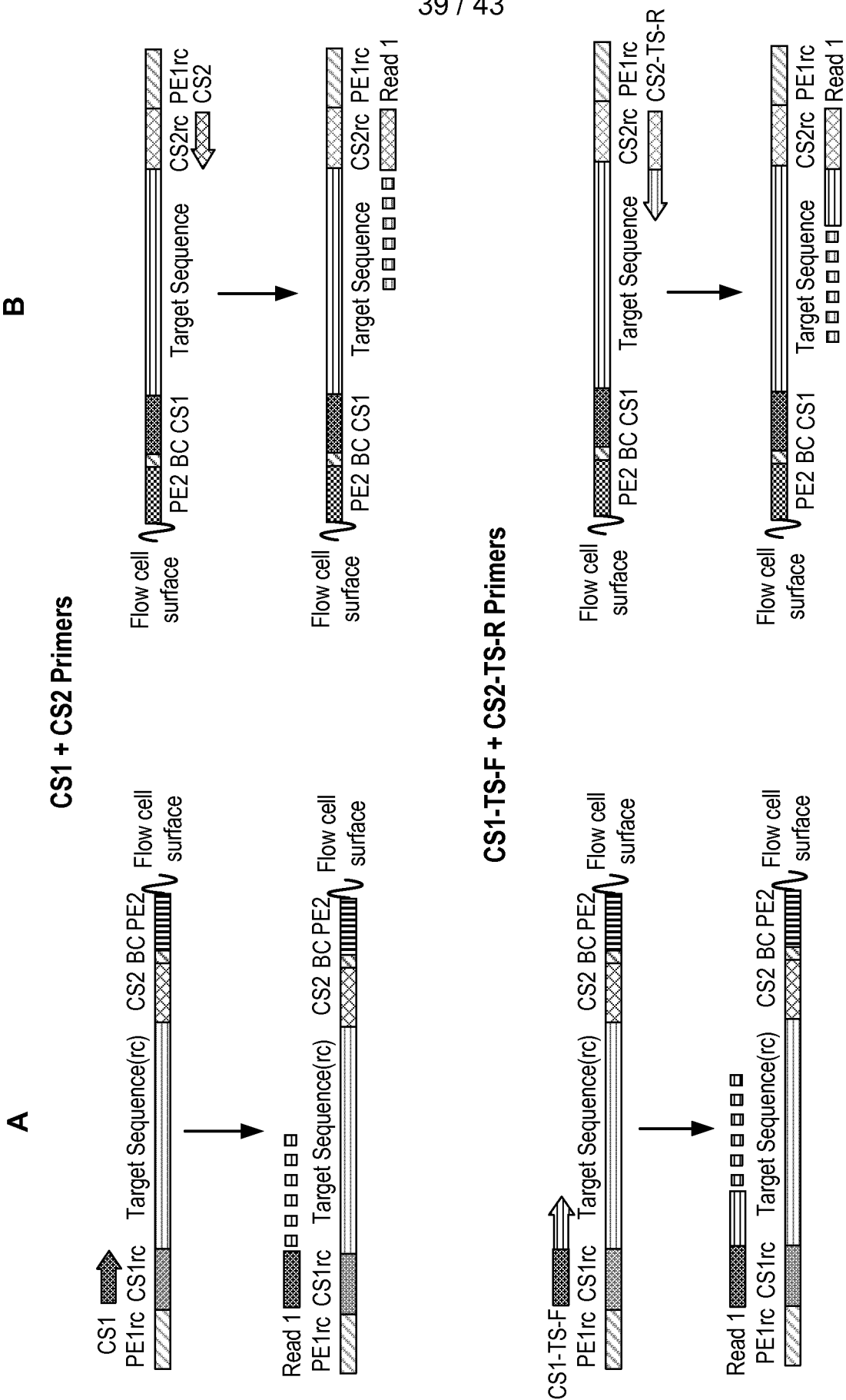


FIG. 29

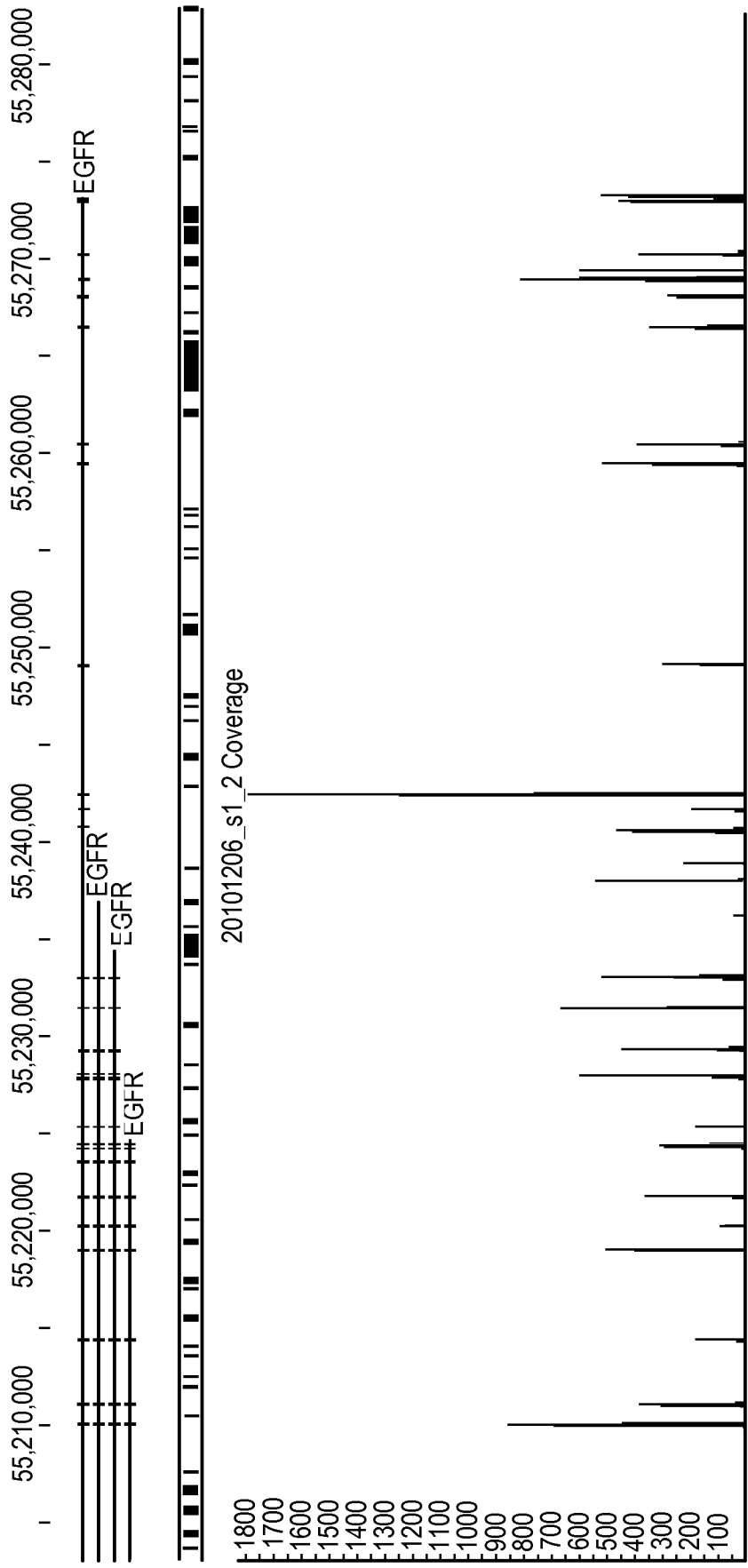
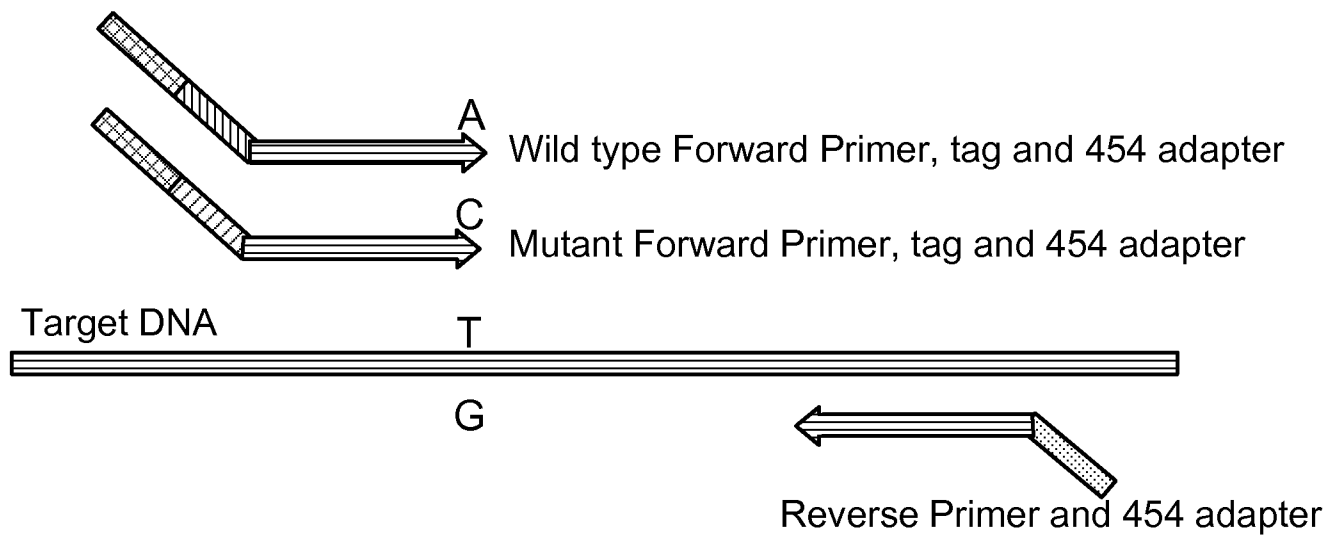


FIG. 30

41 / 43

**FIG. 31A**

Cycle 1 Wild type detection by priming on WT tag and extending all bases



Cycle 2 Mutant detection by priming on Mutant tag and extending all bases



Mutant – Few of these

FIG. 31B

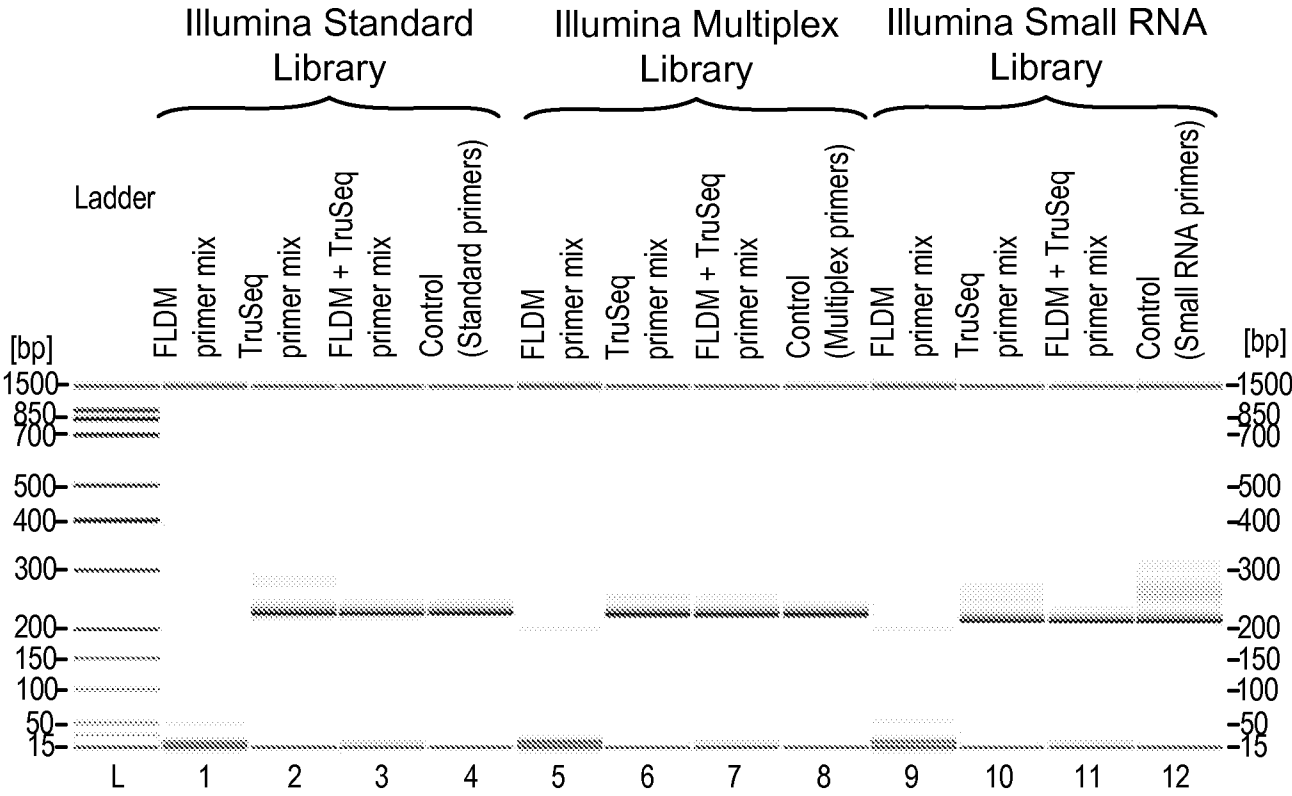


FIG. 32

Access Array Library

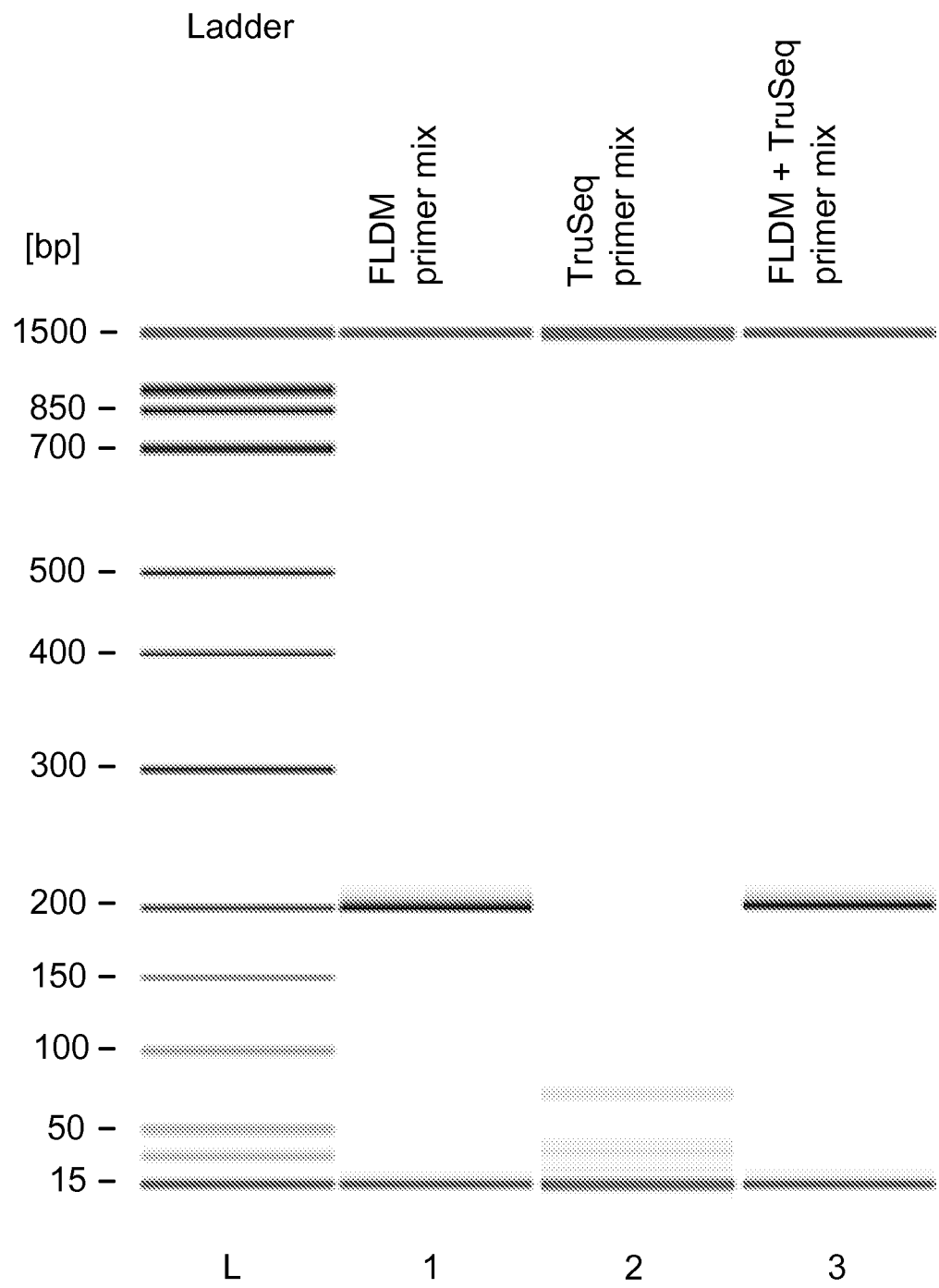


FIG. 33