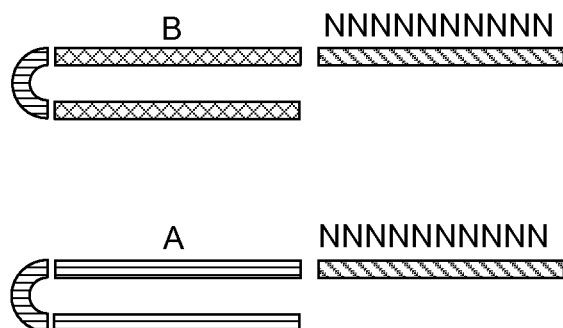


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Degenerate-tailed hairpin sequencing linkers



A- Sequencer specific tag1

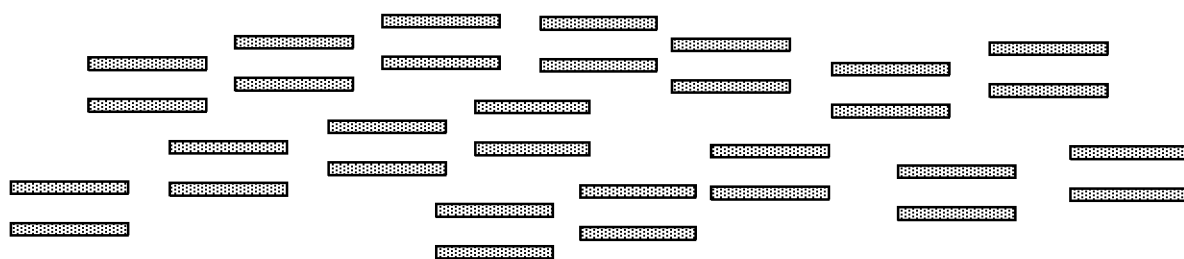
B- Sequencer specific tag2

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

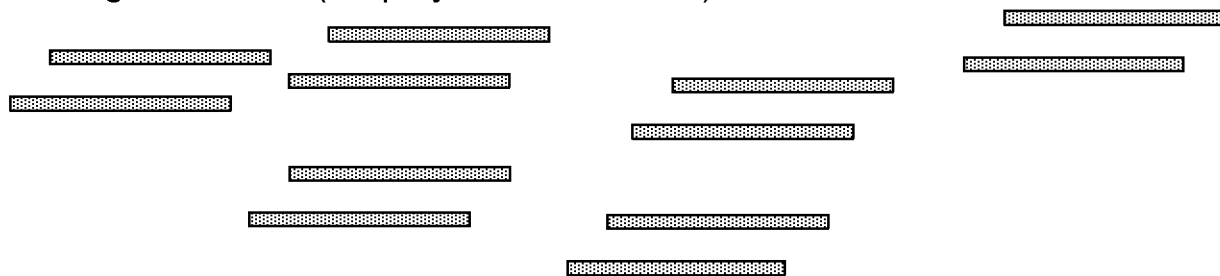
NNNNNNNNNN- Degenerate primer region-10mer, 20mer, 3' end protected

FIG. 1A

Fragment DNA (general nuclease)

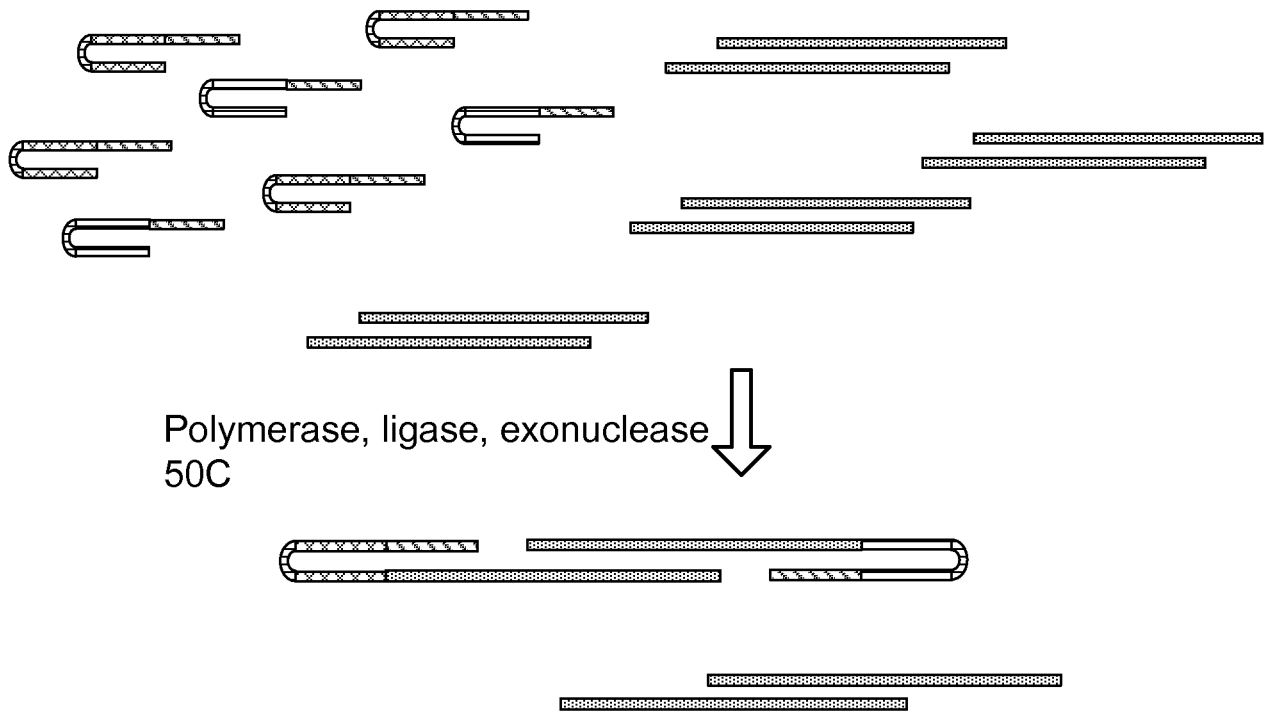


Digest 5' ends (T4 polymerase, Exo III)

**FIG. 1B**

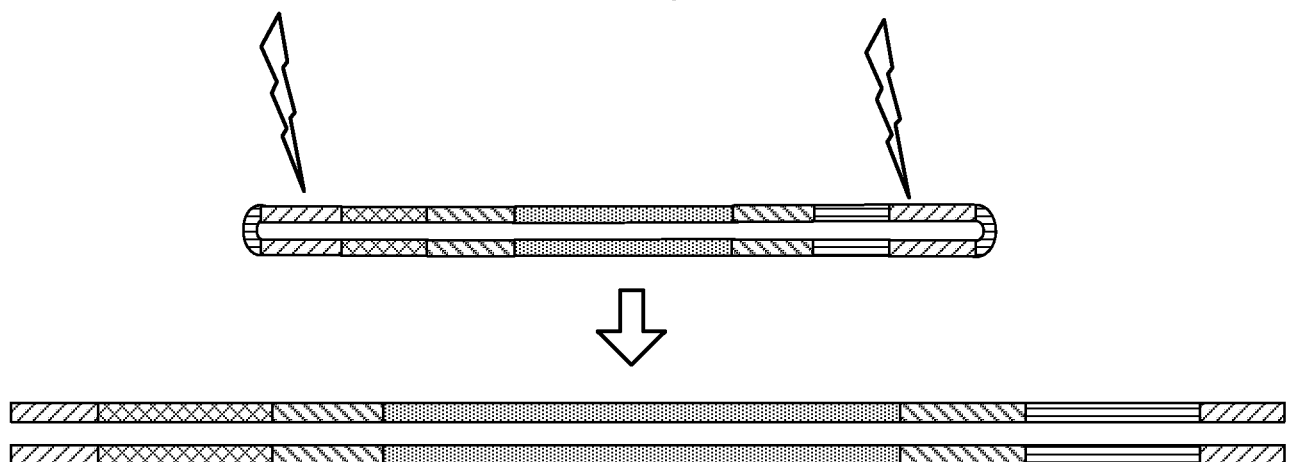
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Add degenerate-hairpin sequencing linker

**FIG. 1C**

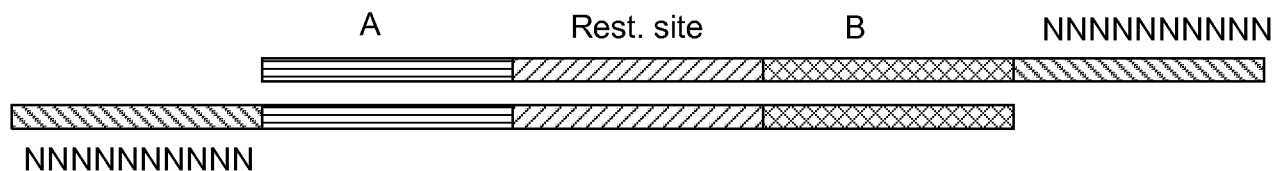
Linearize circular DNA with restriction enzyme

Restriction Enzyme

**FIG. 1D**

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Degenerate-tailed sequencing linker



A- Sequencer specific tag1

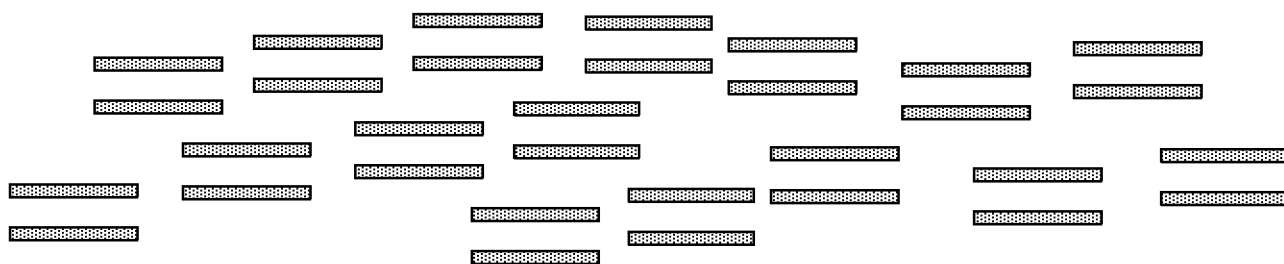
B- Sequencer specific tag2

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

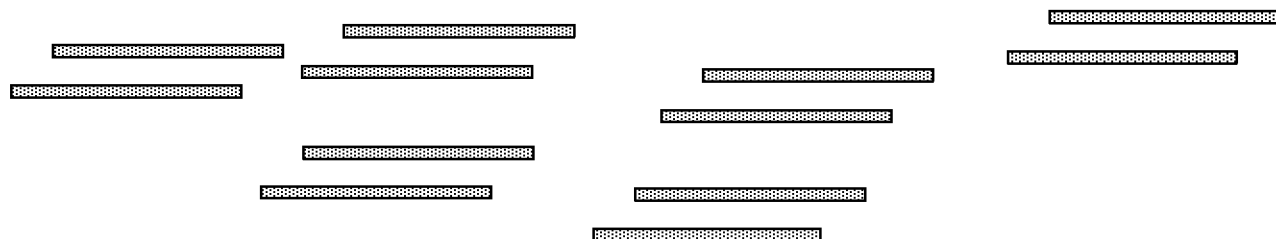
NNNNNNNNNN- Degenerate primer region-10mer, 20mer

FIG. 2A

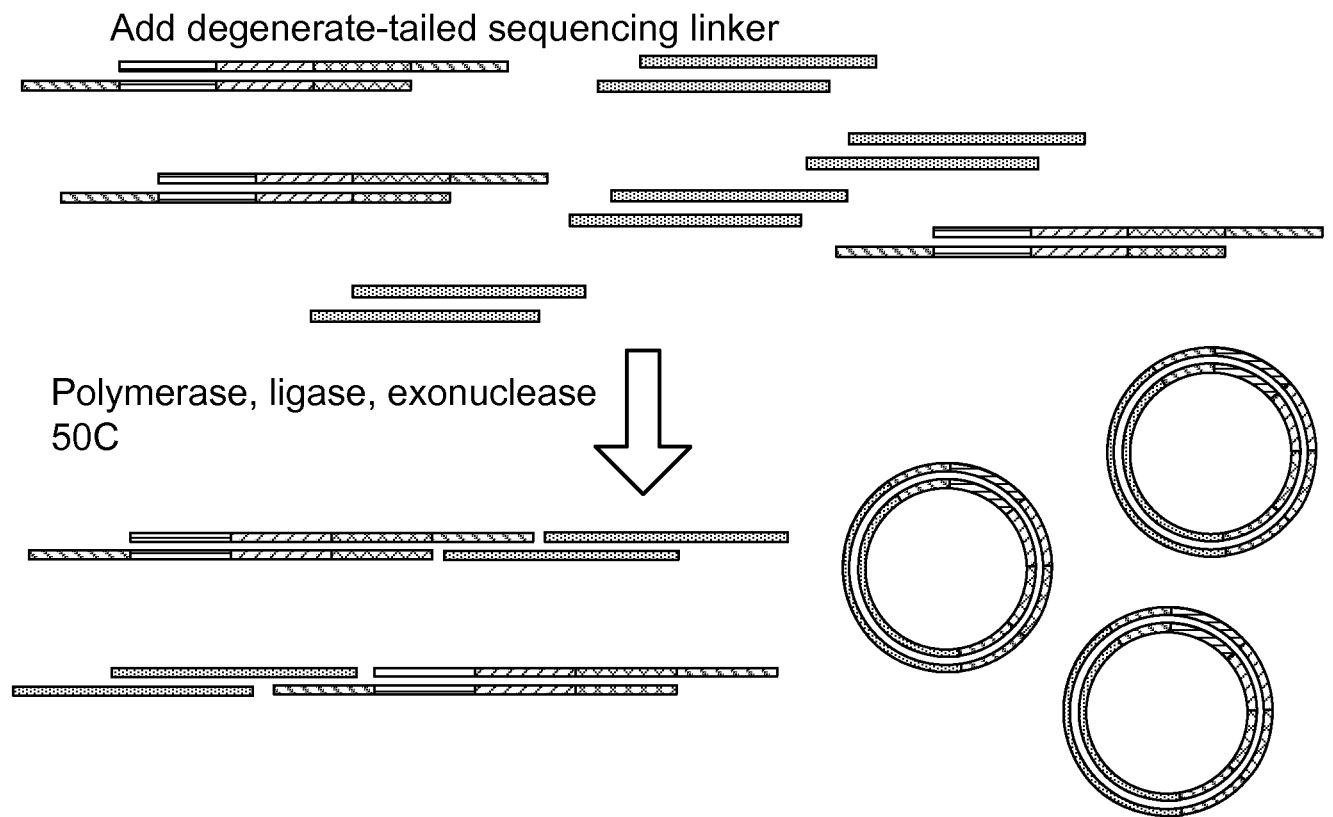
Fragment DNA (general nuclease)



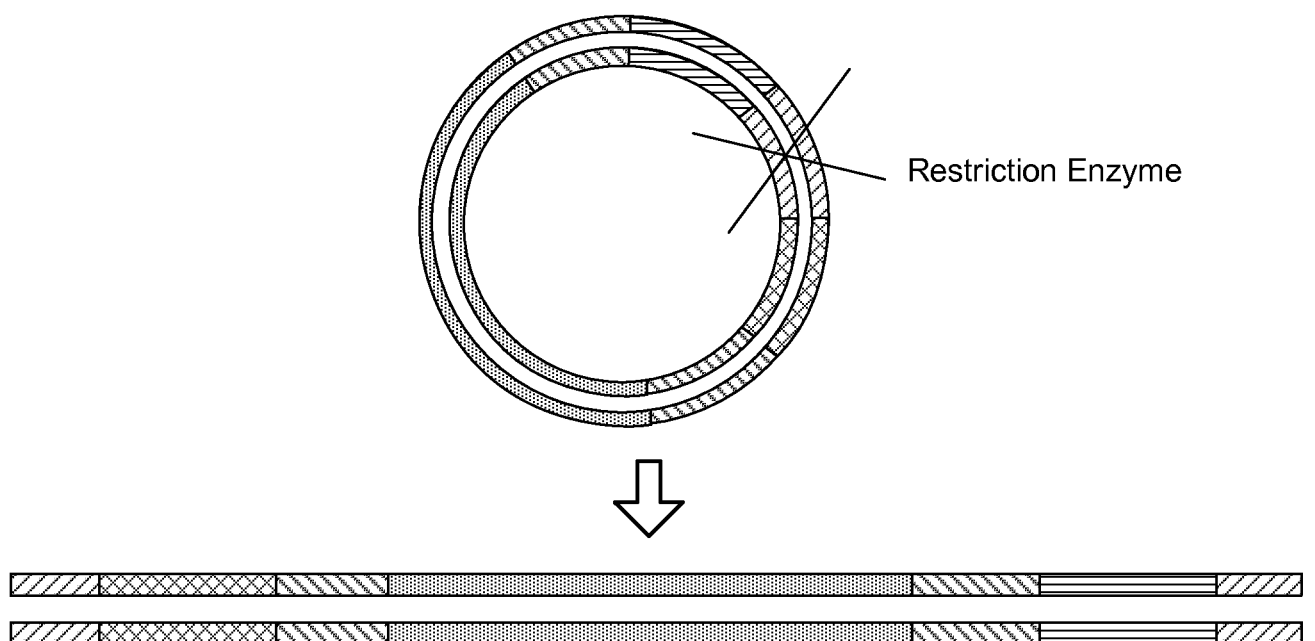
Digest 5' ends (T4 polymerase, Exo III)

**FIG. 2B**

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**FIG. 2C**

Linear circular DNA with restriction enzyme

**FIG. 2D**

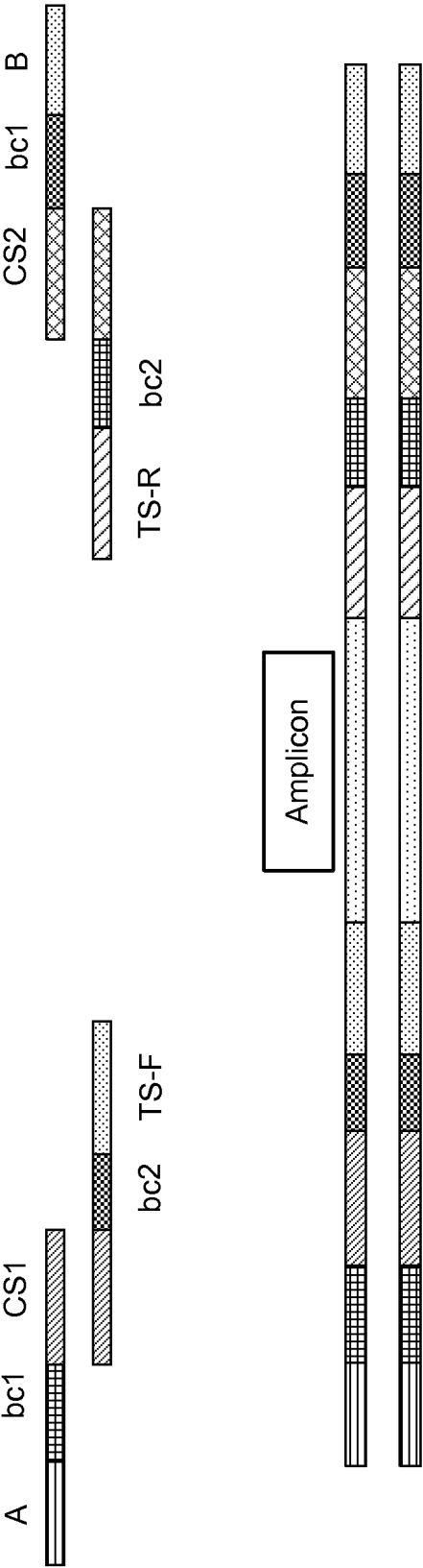


FIG. 3

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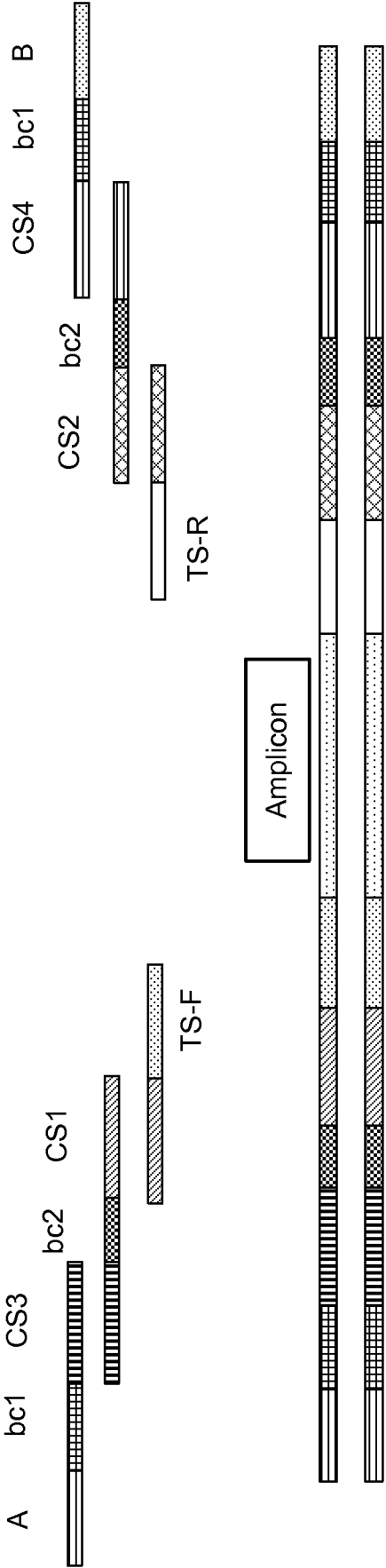


FIG. 4

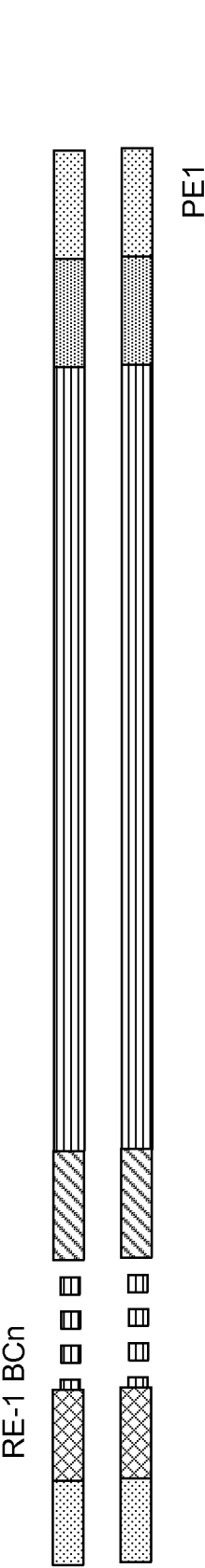


FIG. 5A

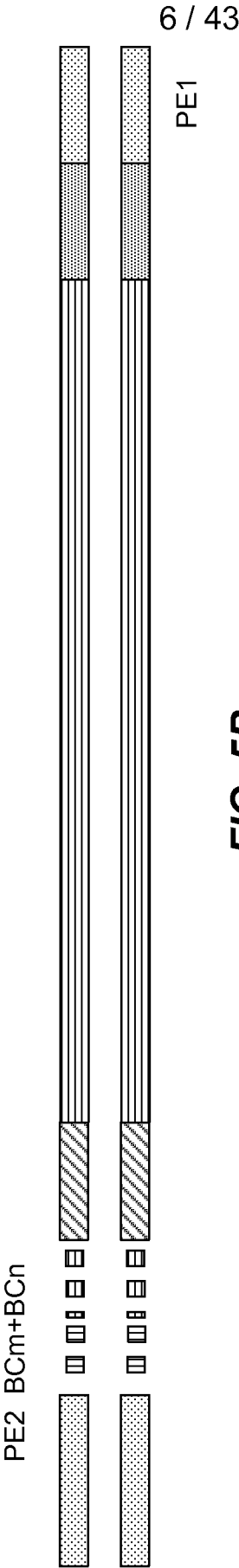


FIG. 5B

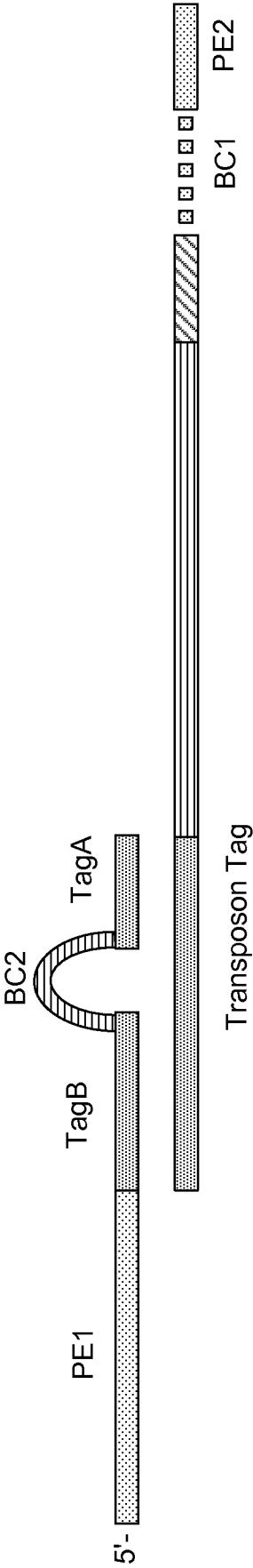


FIG. 6

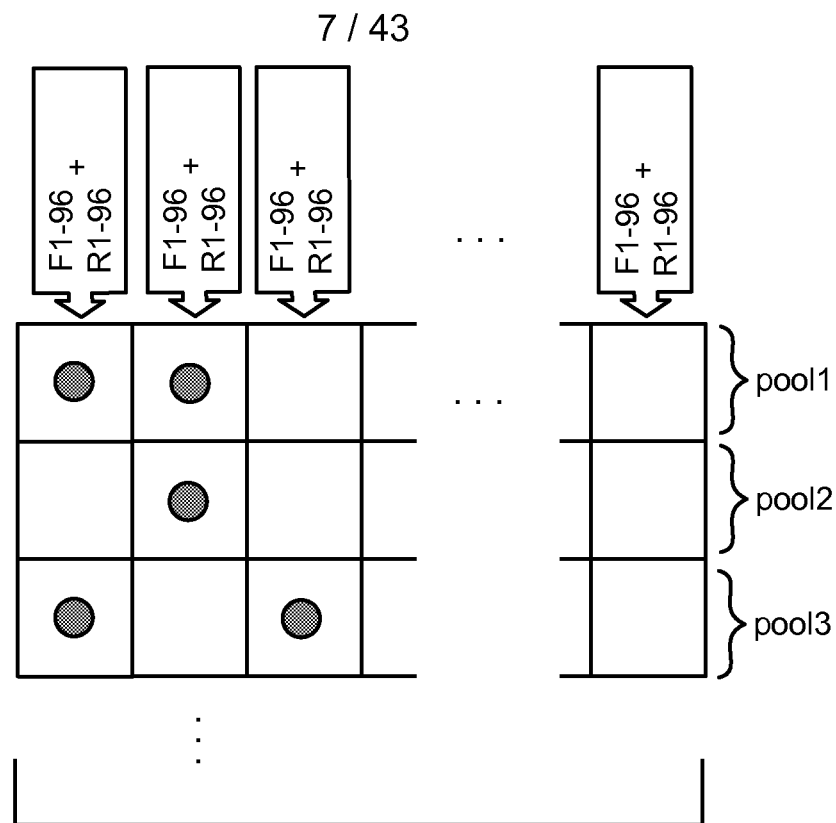


FIG. 7A

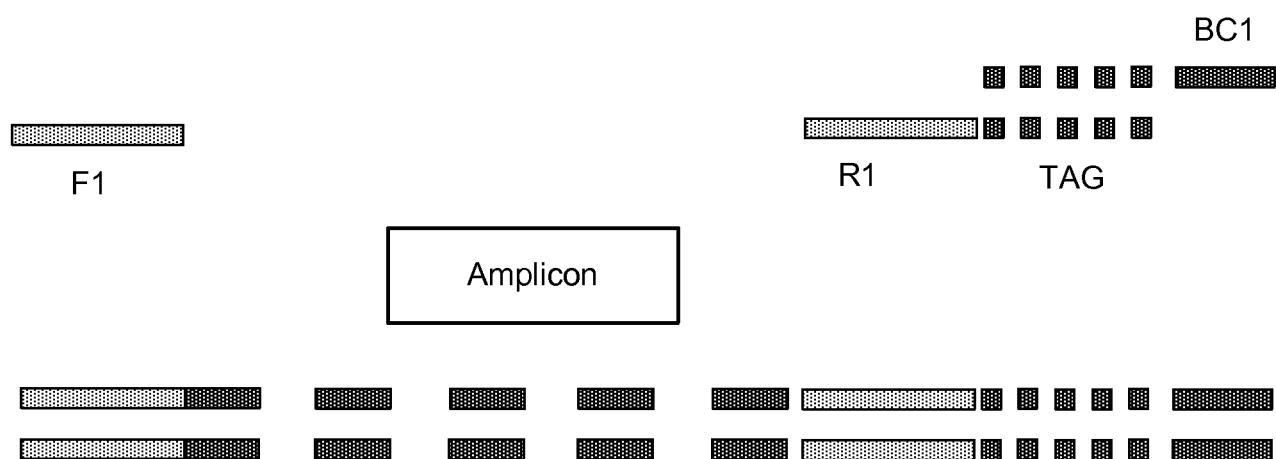


FIG. 7B

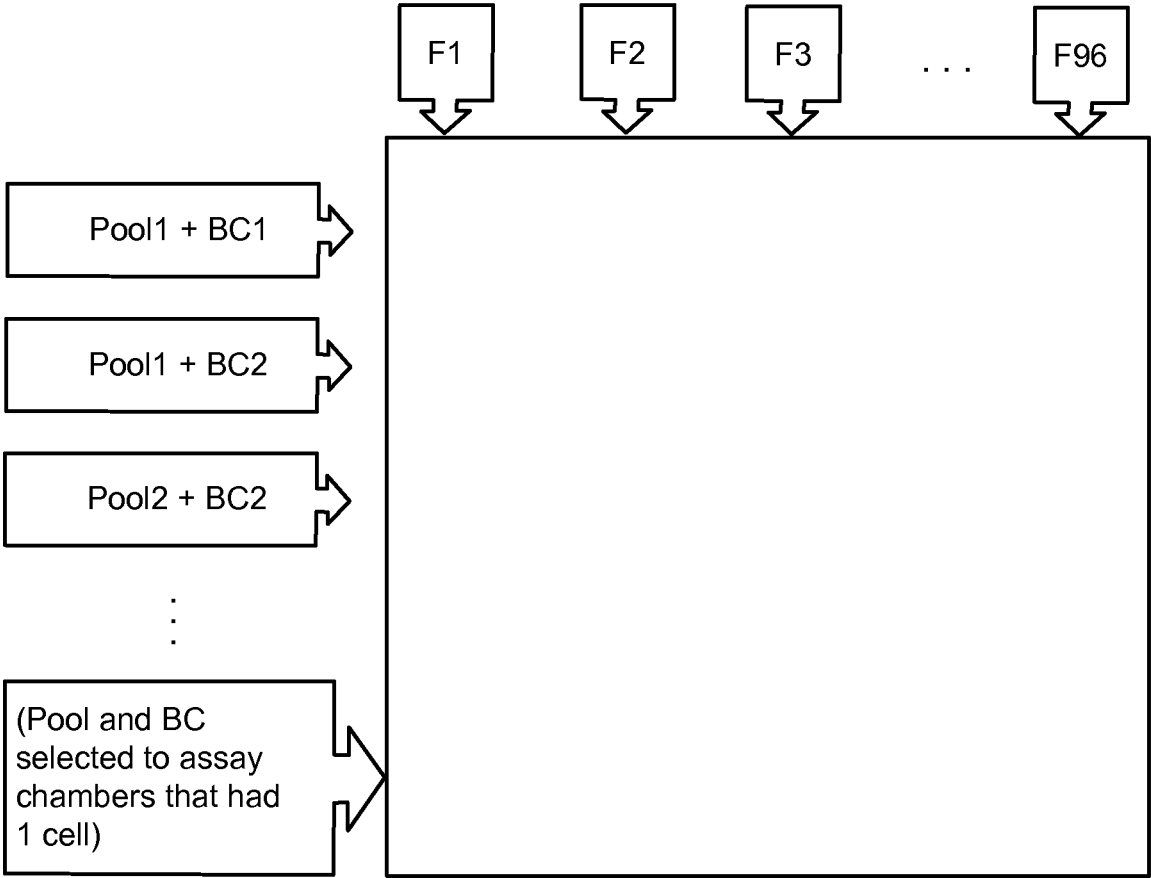


FIG. 7C

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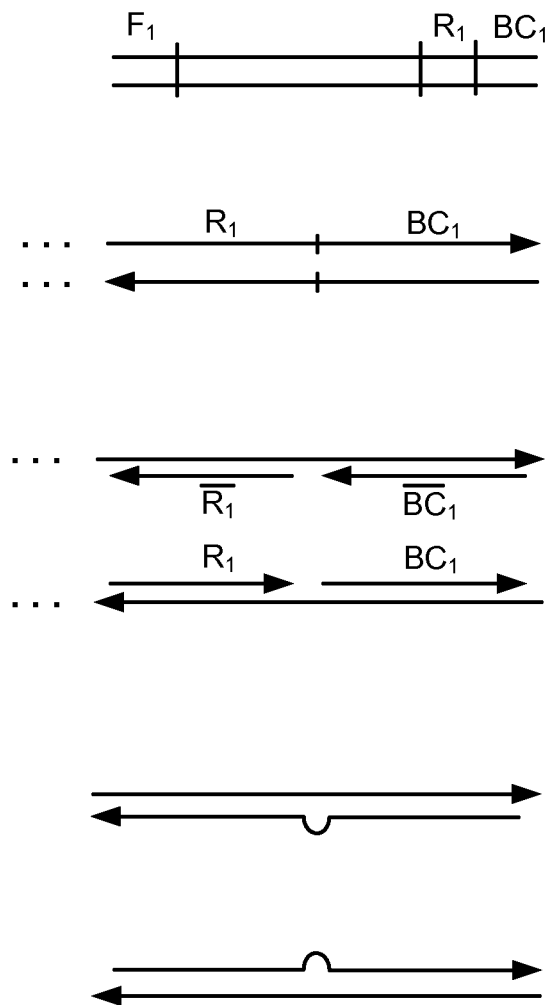


FIG. 8A

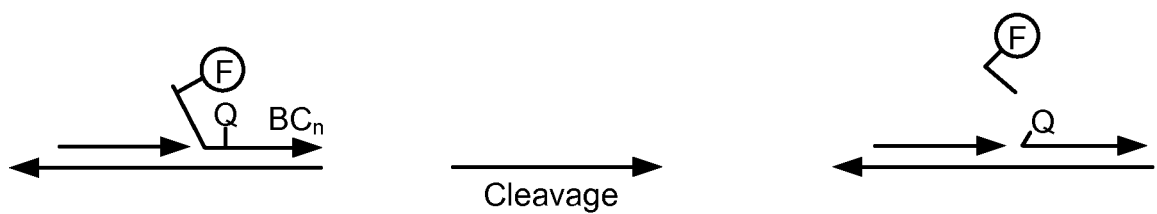


FIG. 8B

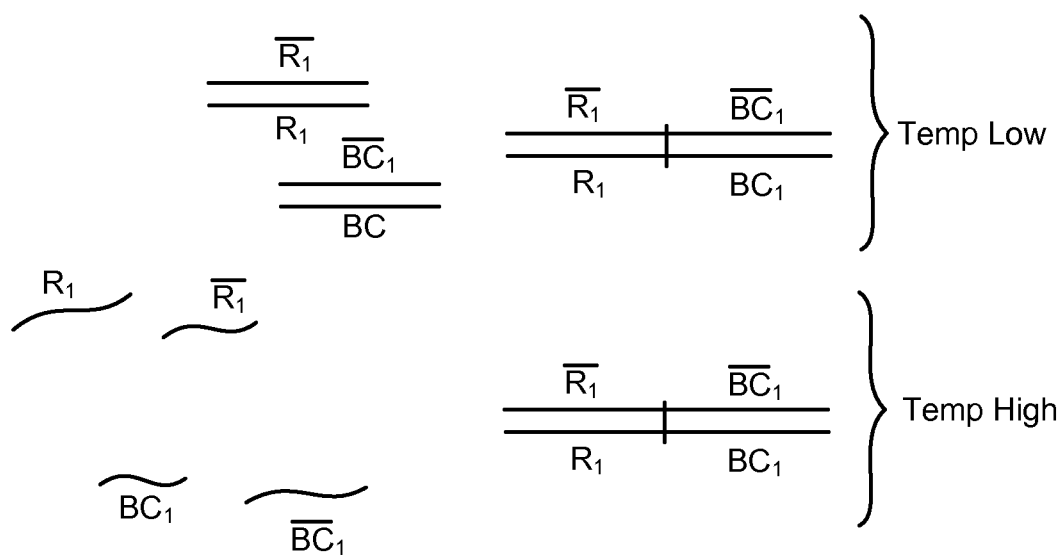
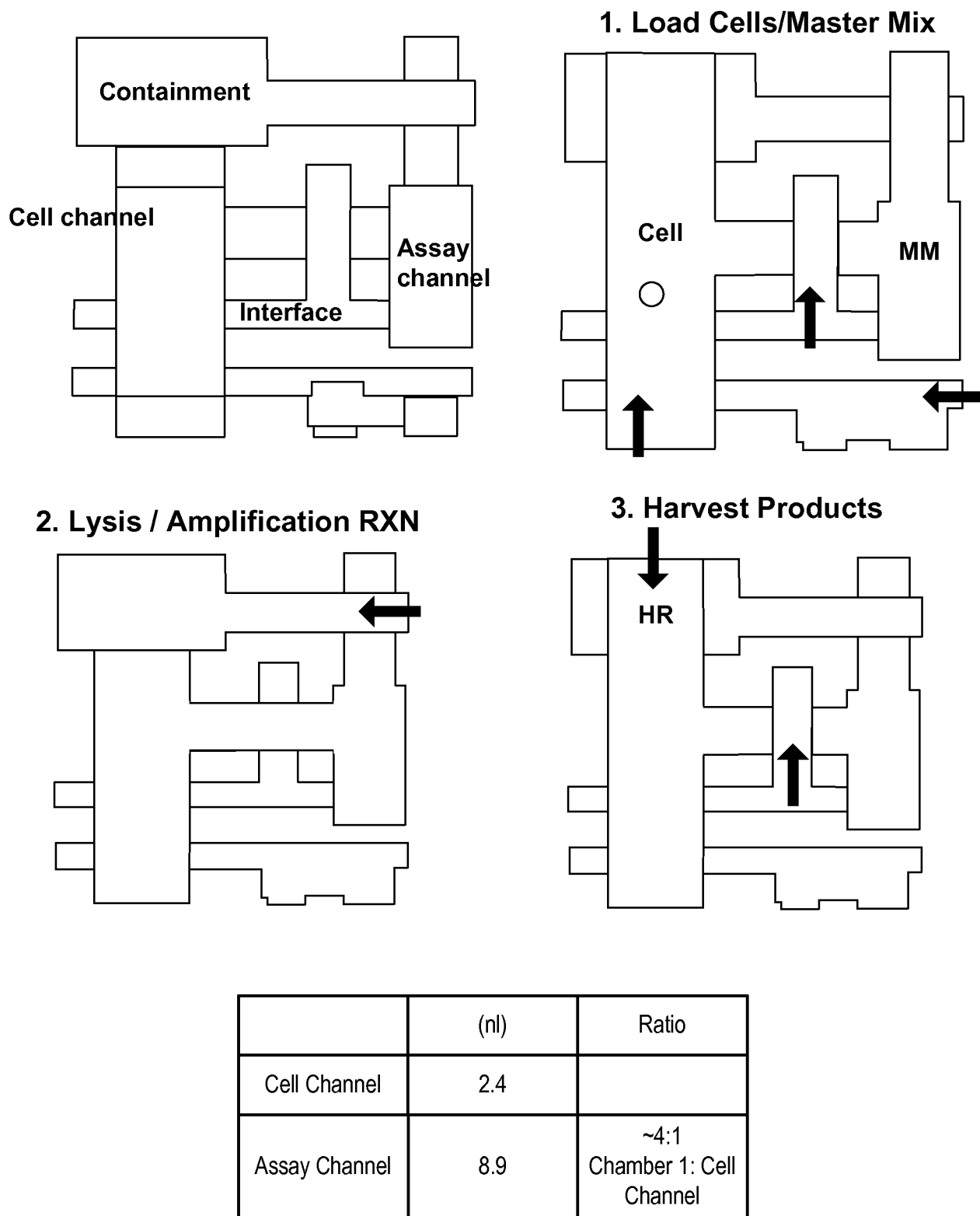
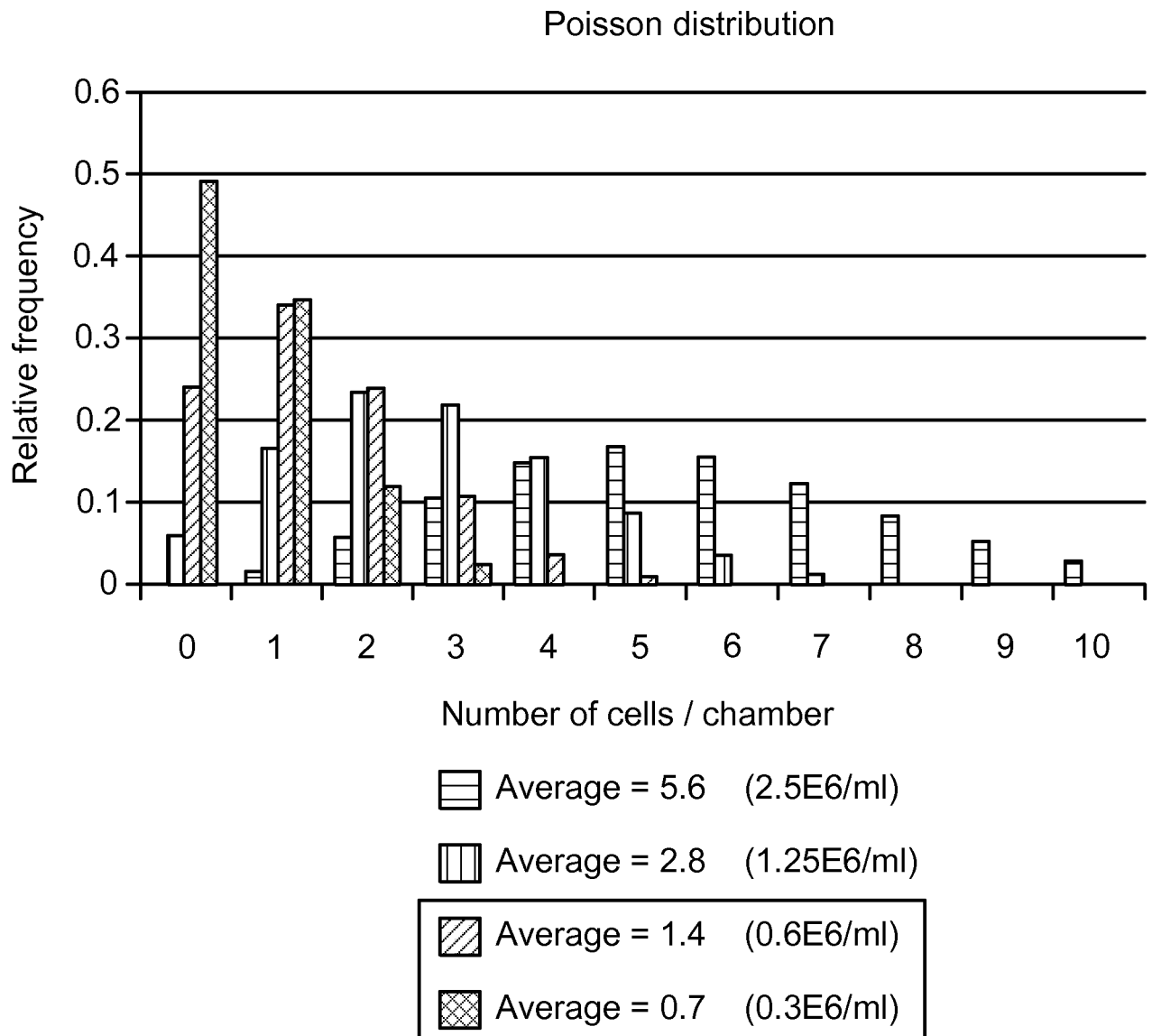


FIG. 8C

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**FIG. 9**

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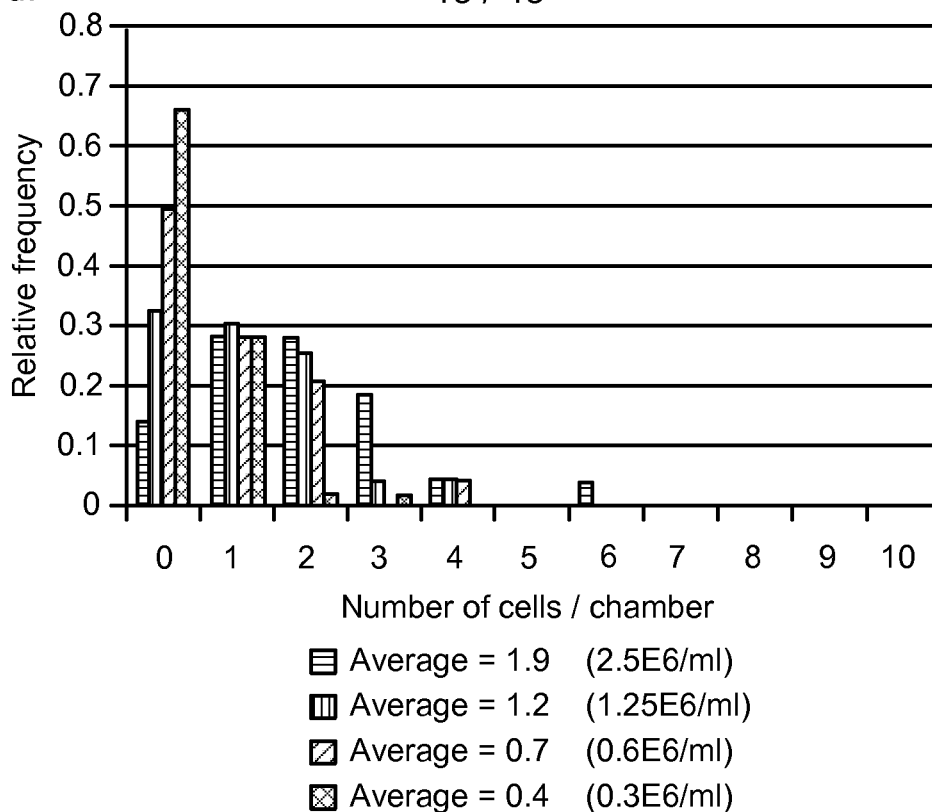


The “best” theoretical cell density for single cells:
~1 per chamber on average = $1 / 2.4 \text{ nl} = \sim 0.5\text{E}6/\text{ml}$

FIG. 10

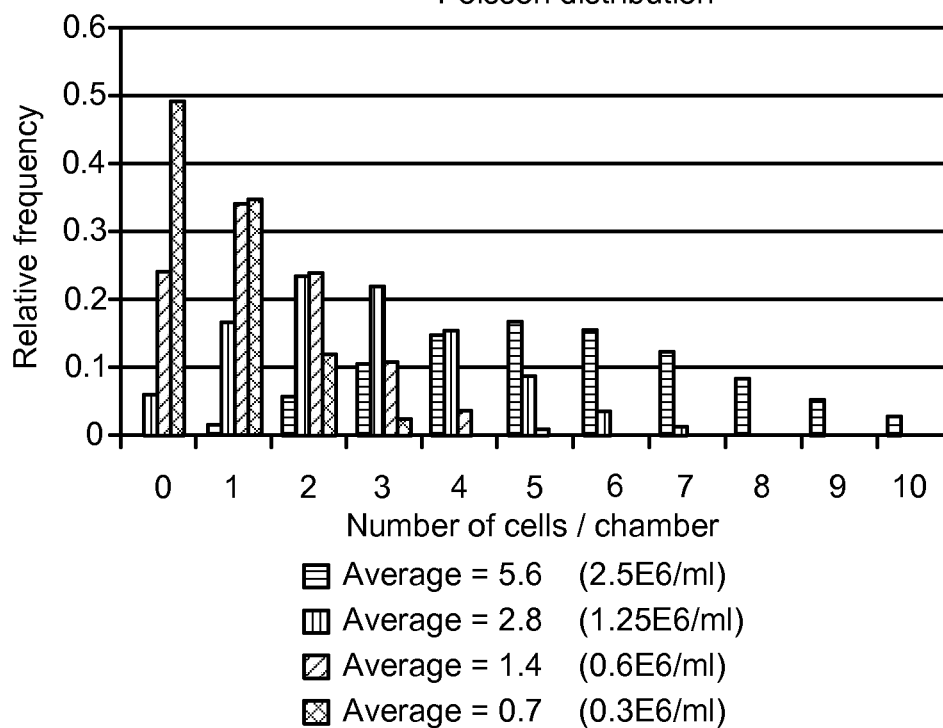
Measured:

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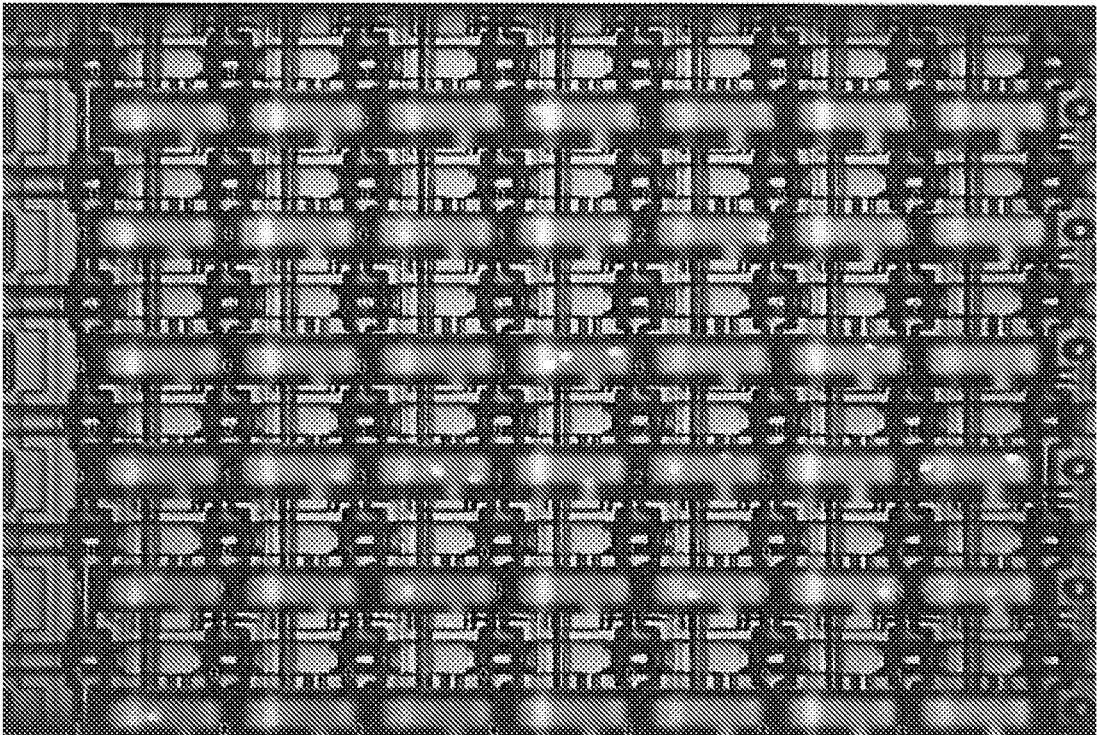
**FIG. 11A**

Theoretical:

Poisson distribution

**FIG. 11B**

0.15E6 /ml
Expect
0-1
chamber

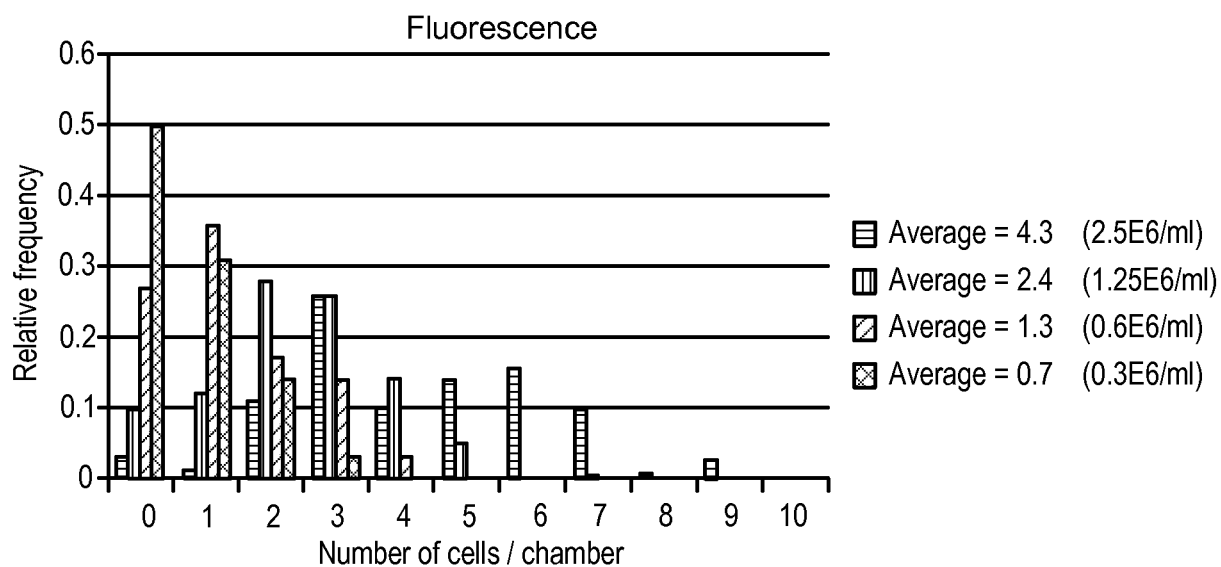
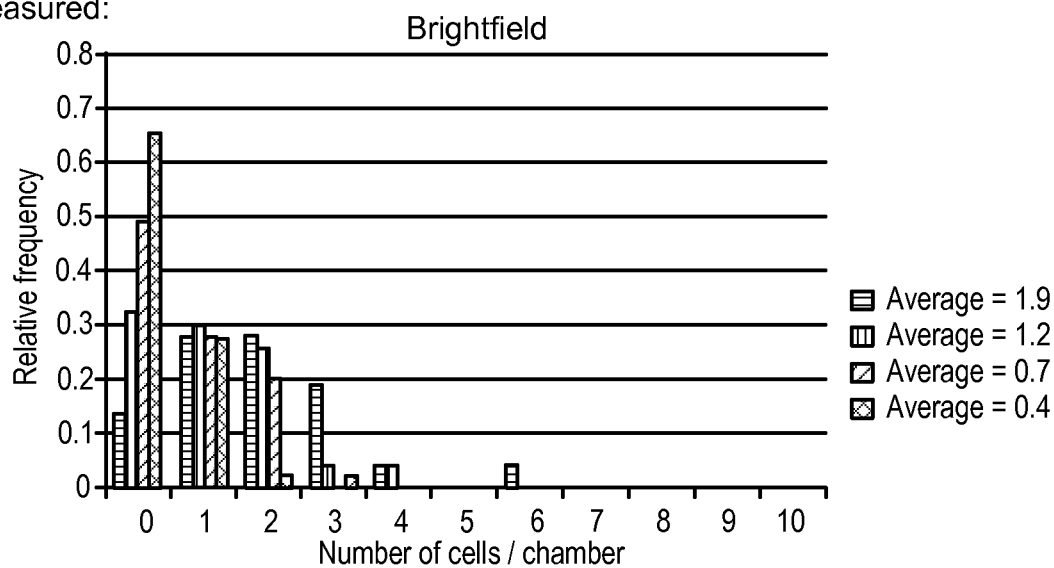


	Brightfield	Fluorescence
Average cells/ chamber	0.29	0.52

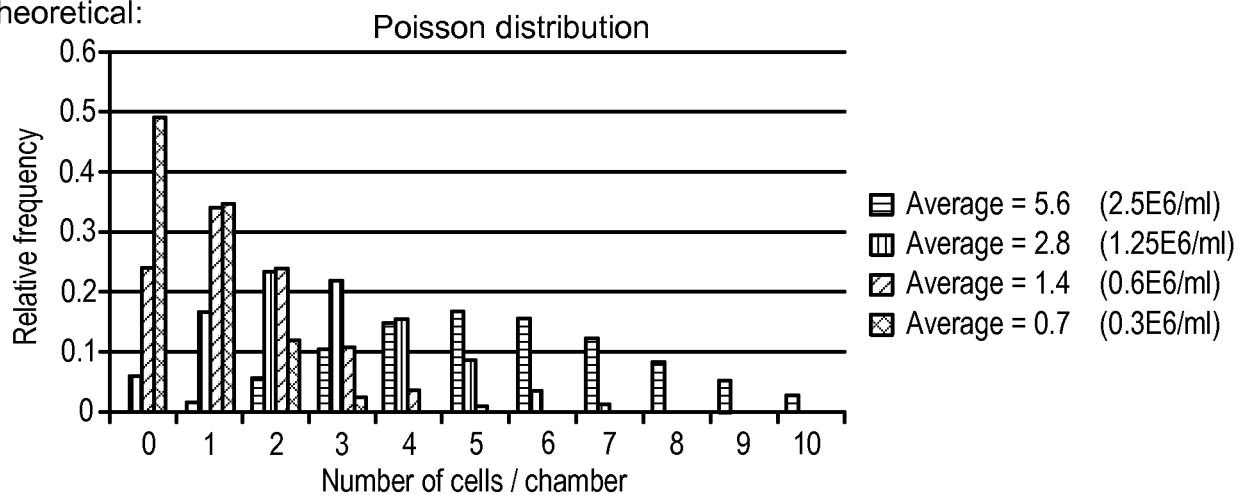
FIG. 12A

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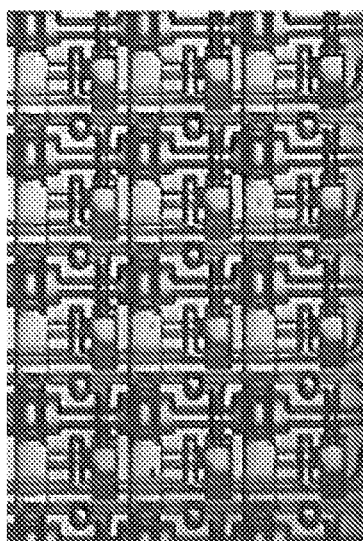
Measured:



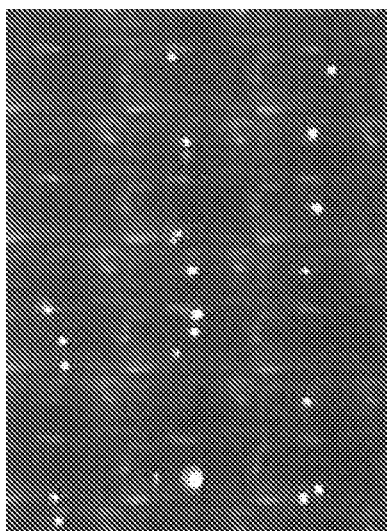
Theoretical:

**FIG. 12B**

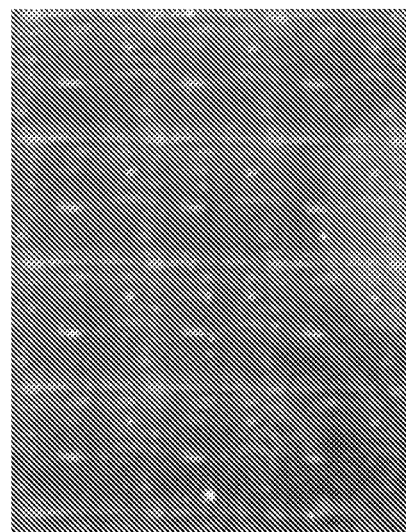
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Brightfield



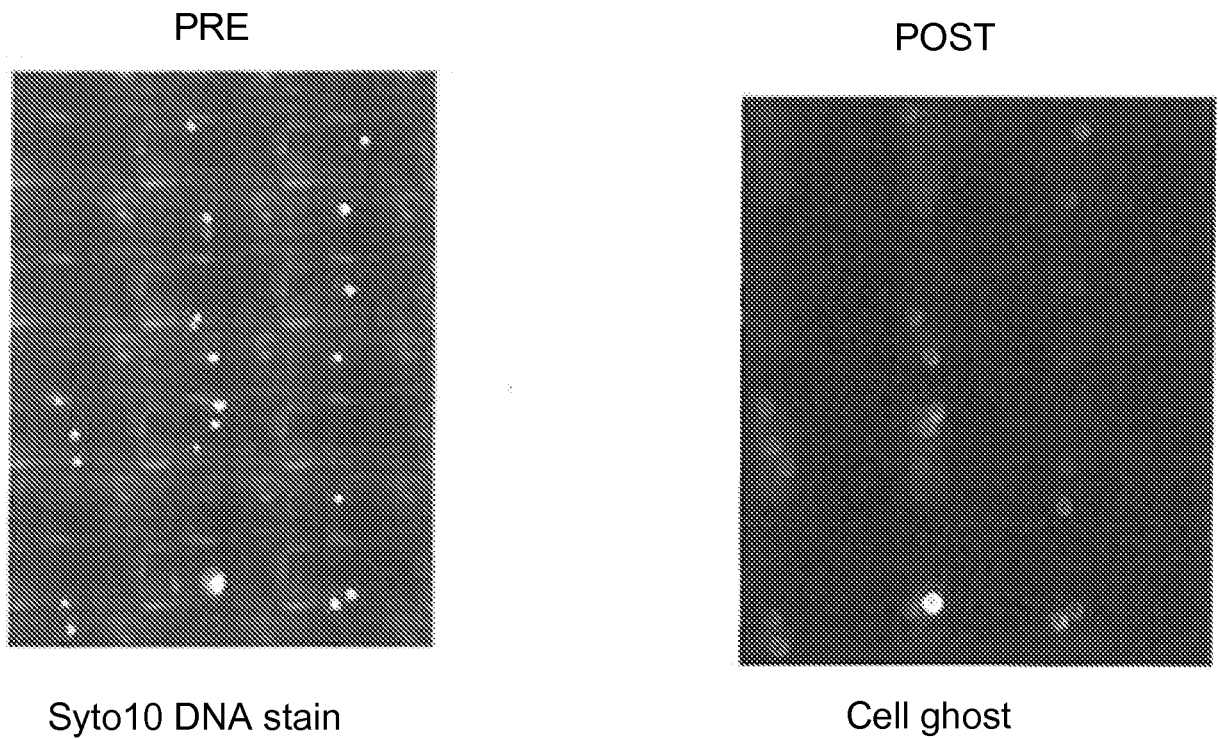
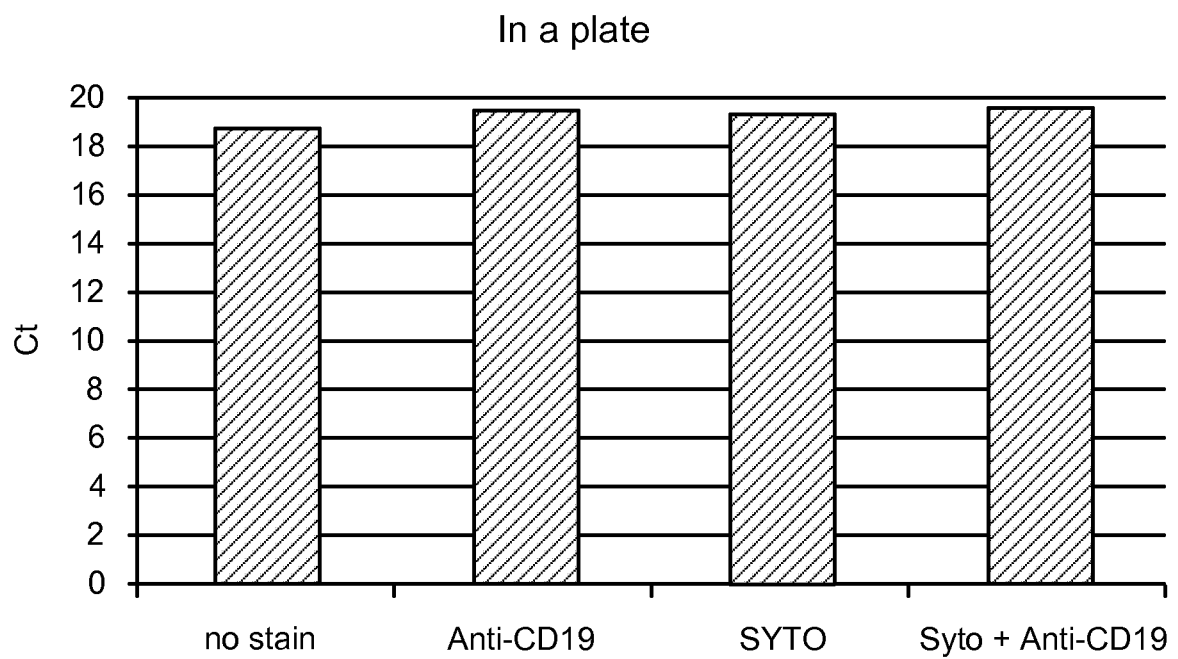
Syto10 DNA stain



Cy5 antibody

FIG. 13

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

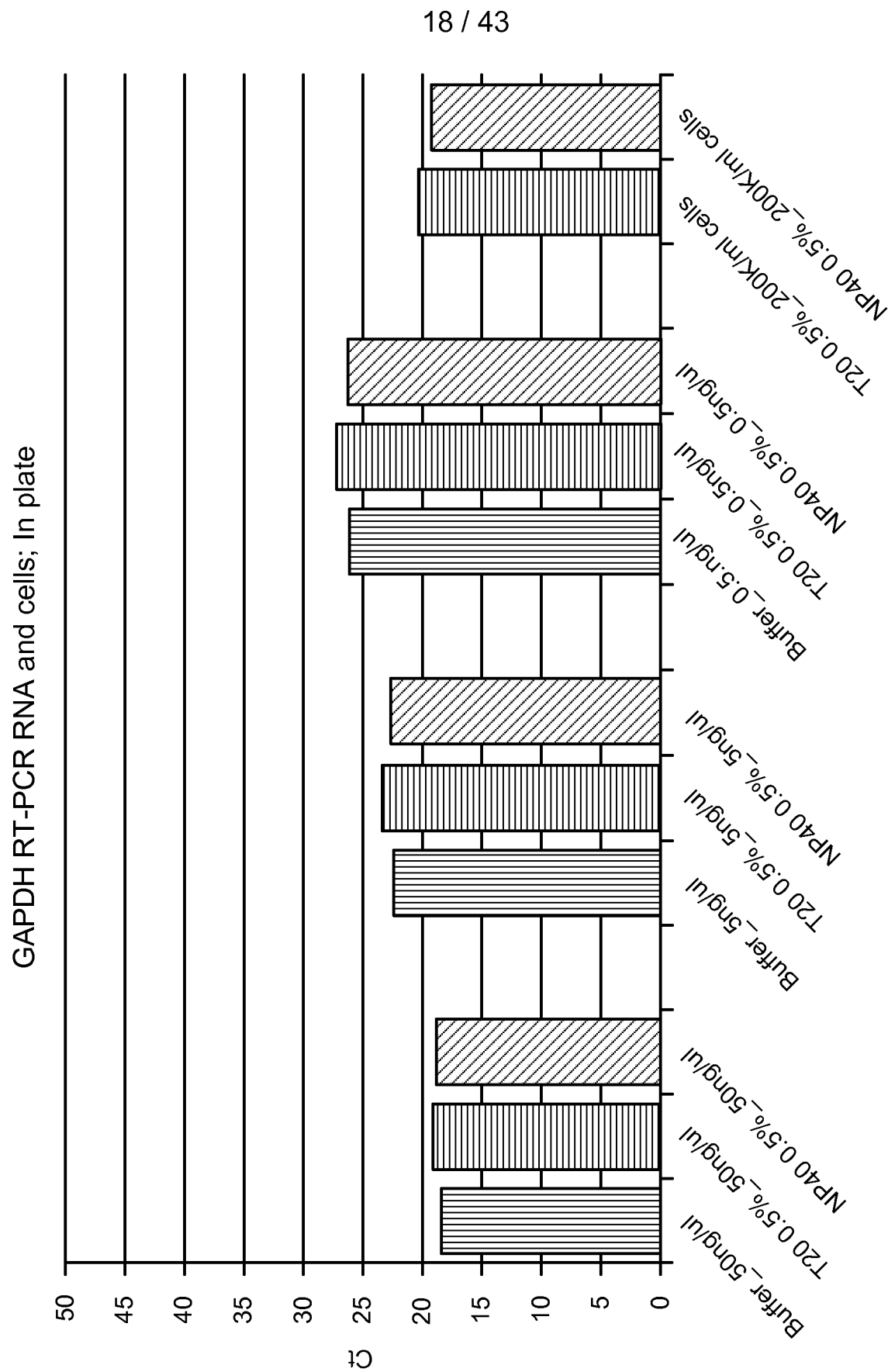
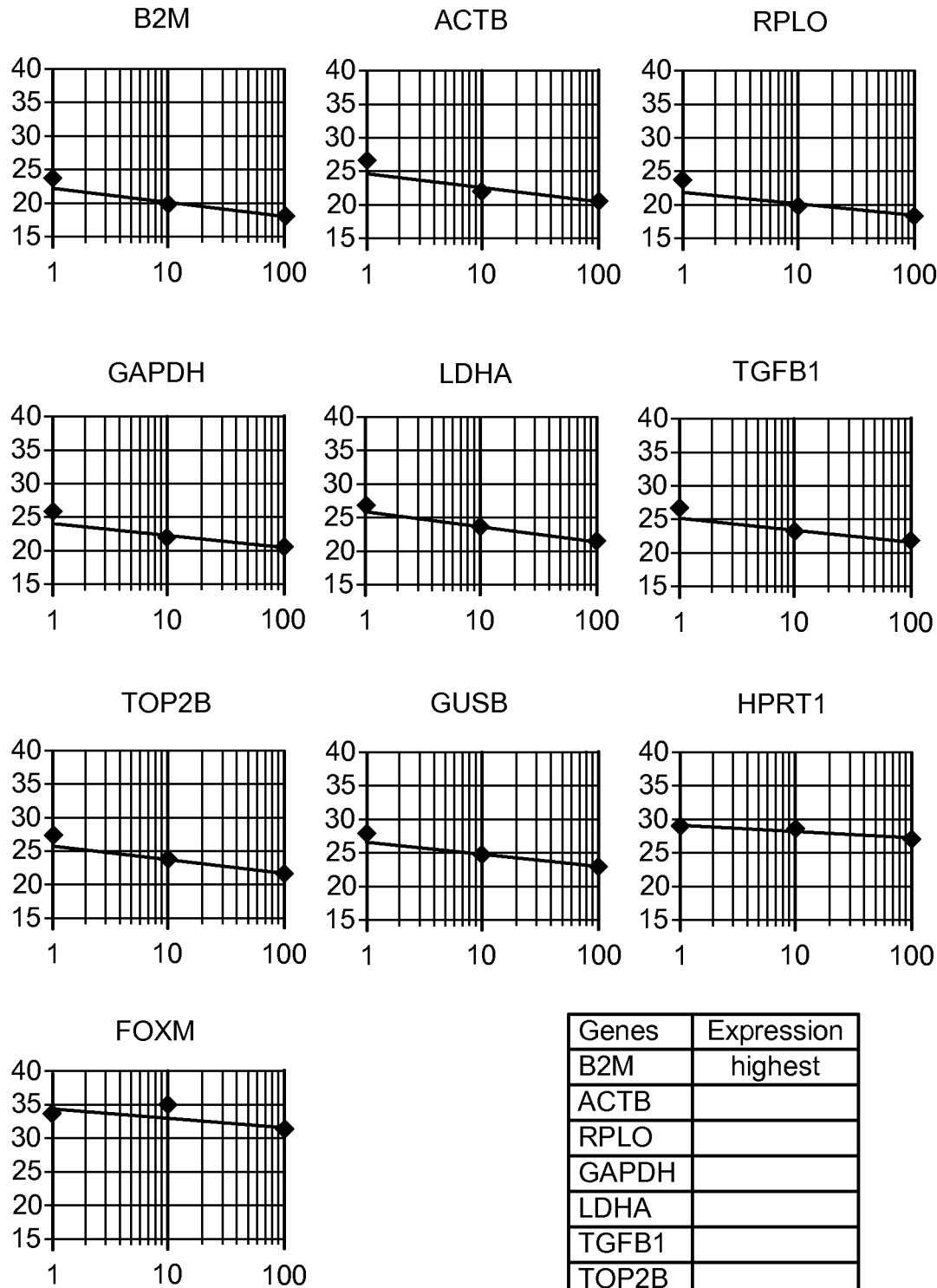


FIG. 15

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ACTB



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

FIG. 16

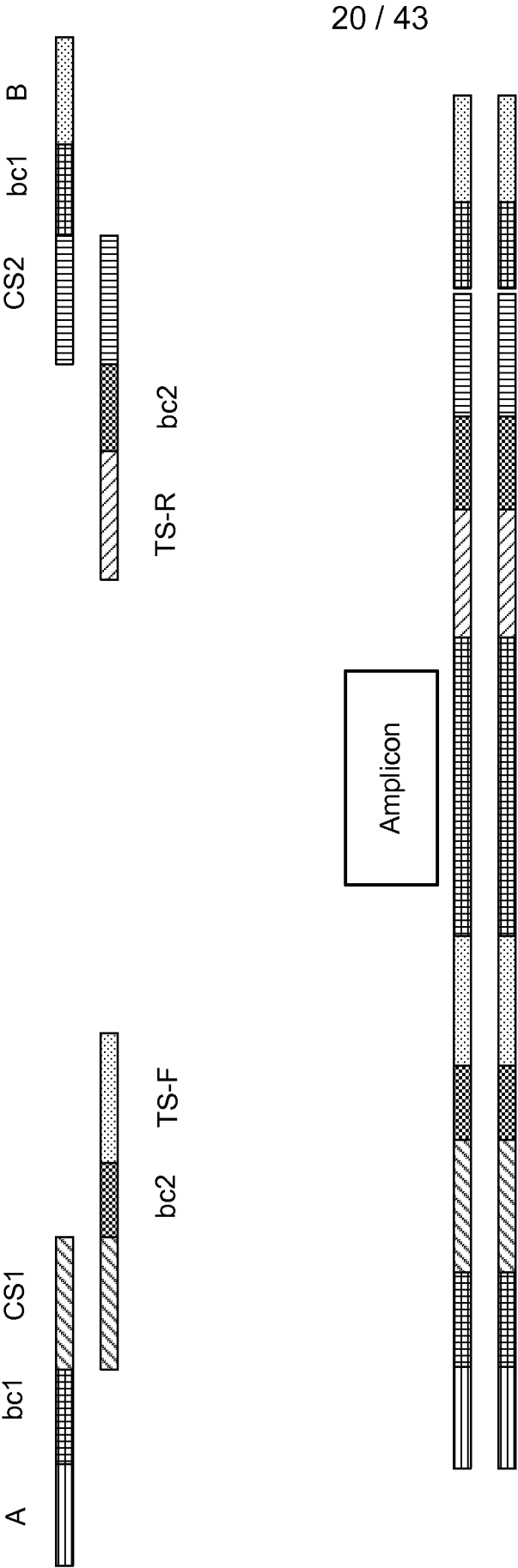
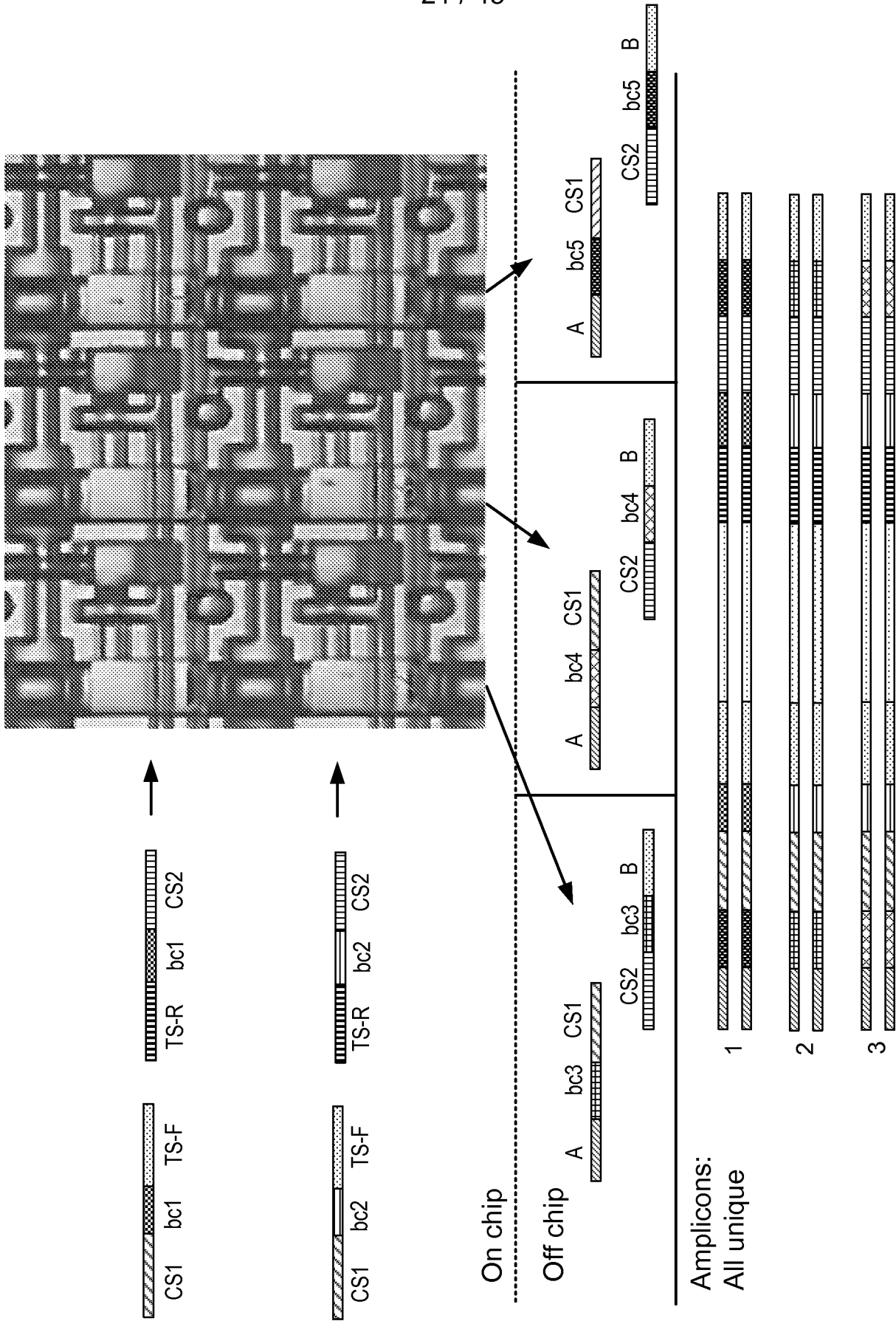


FIG. 17



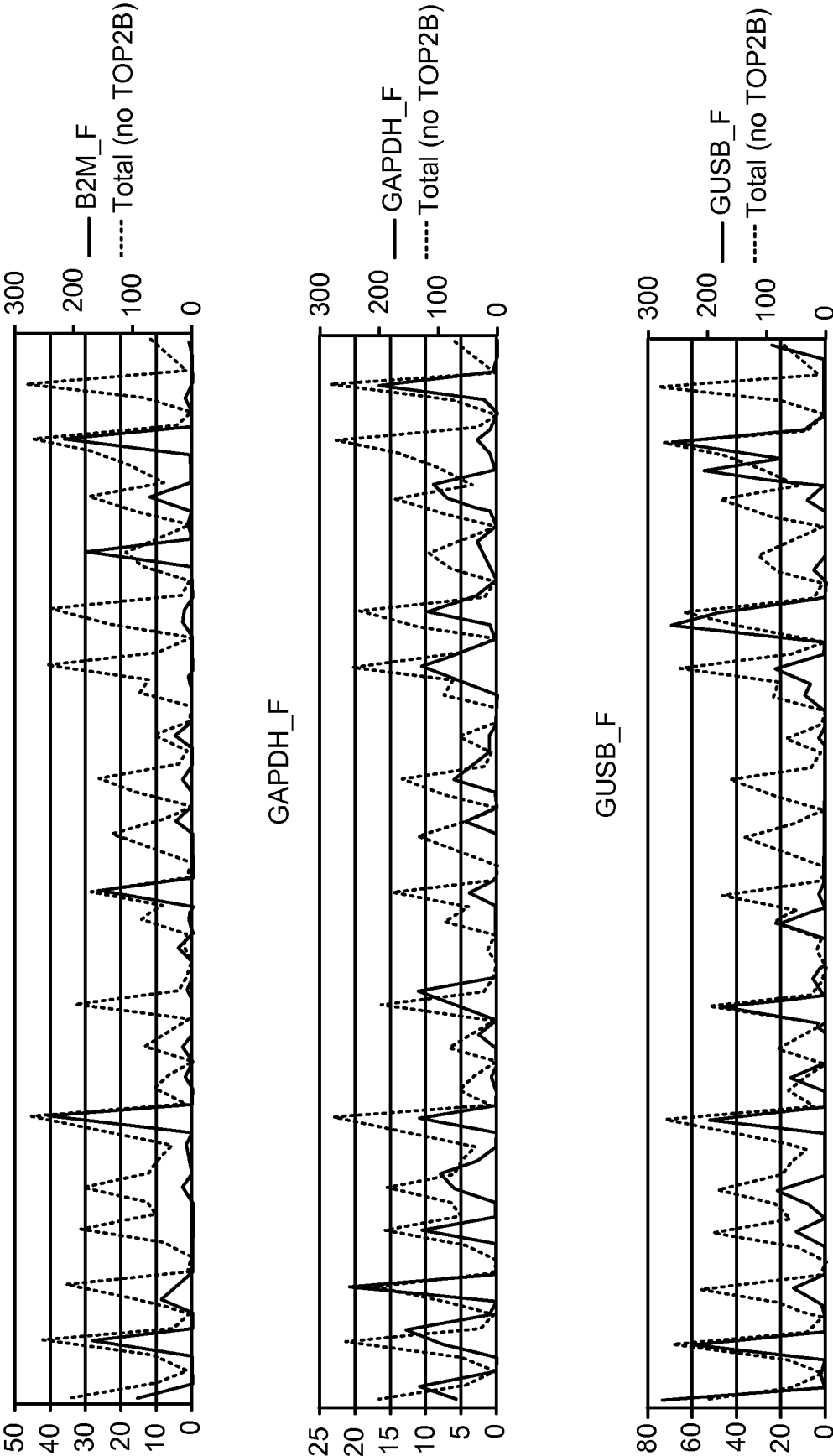


FIG. 19

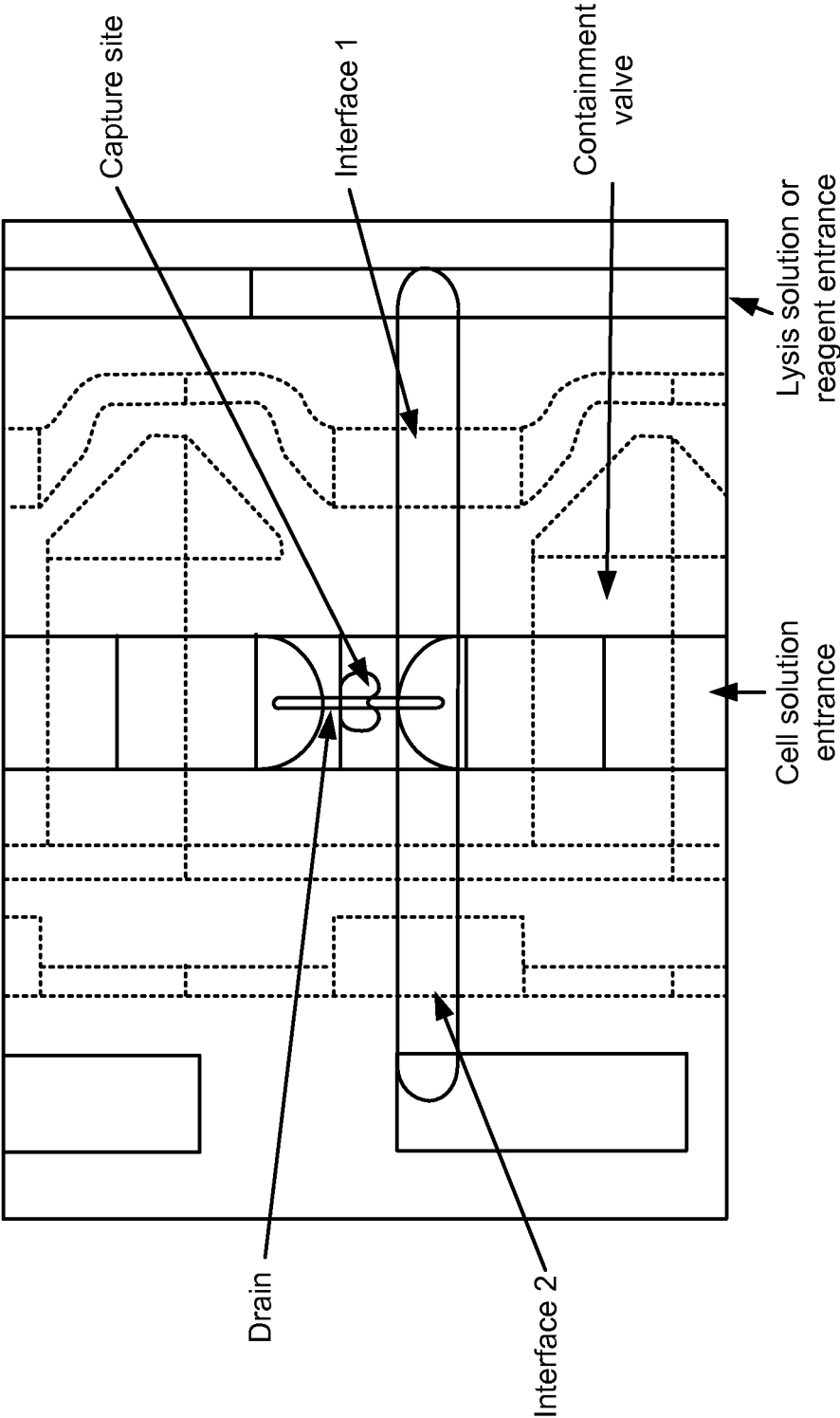


FIG. 20A

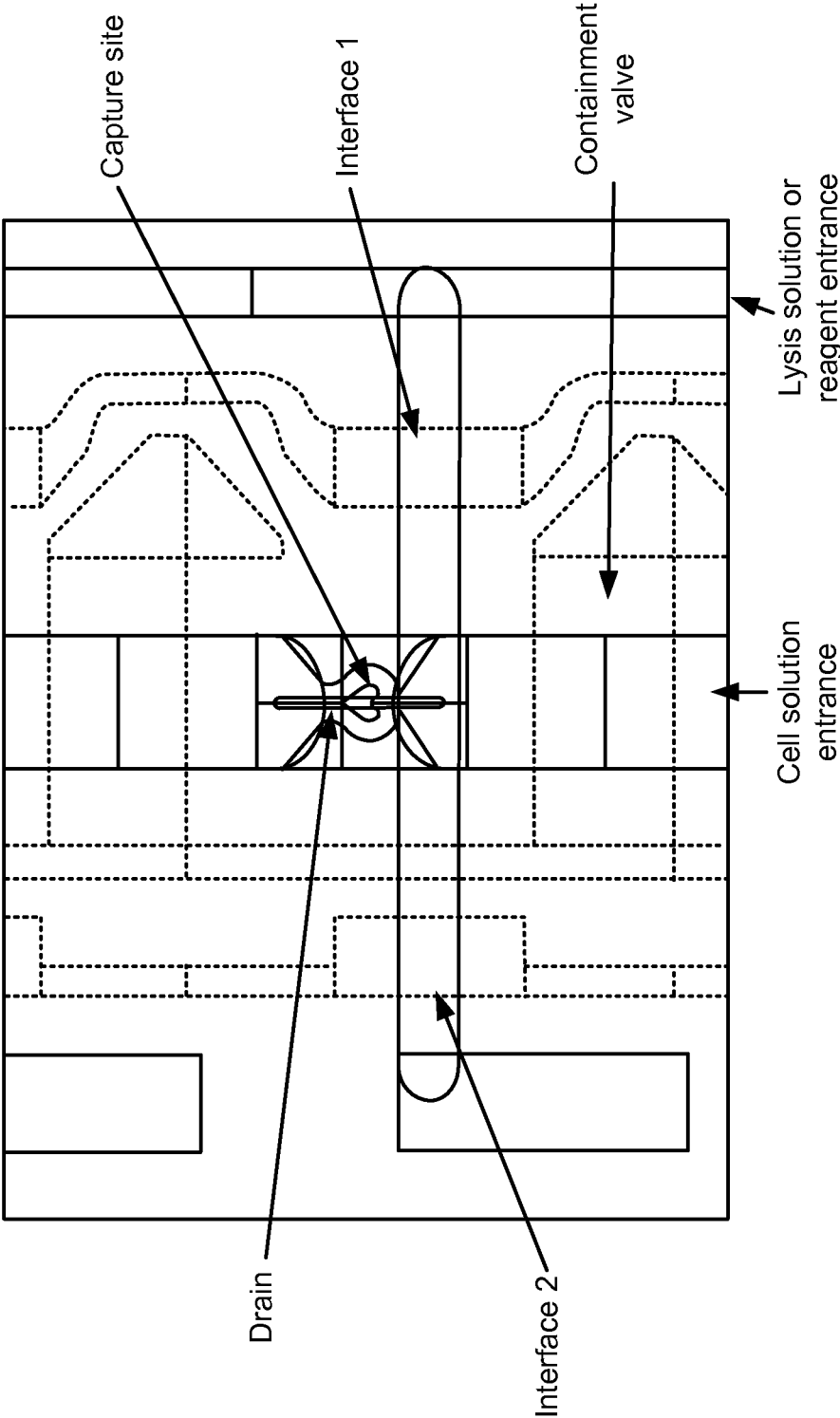


FIG. 20B

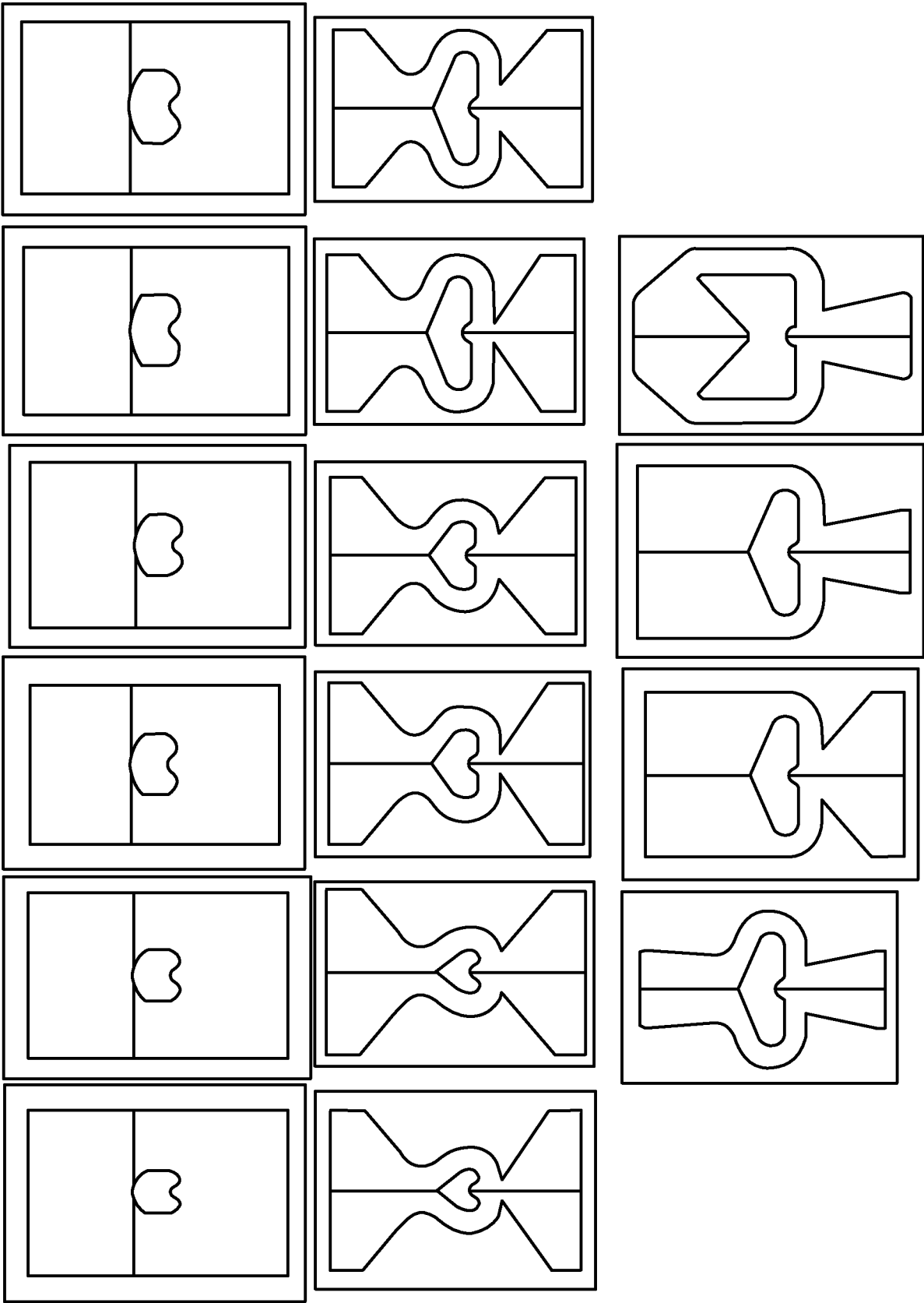


FIG. 21

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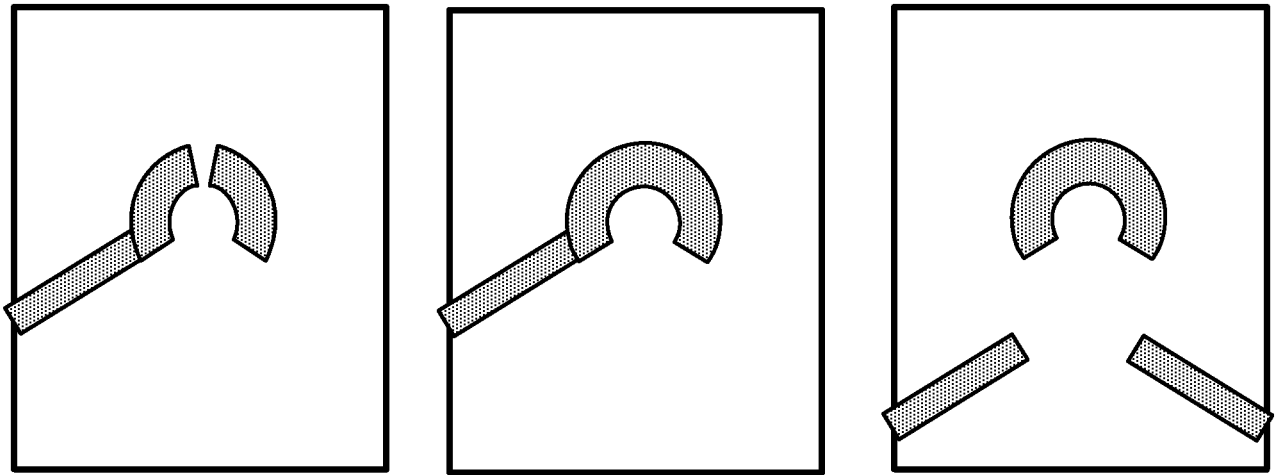


FIG. 22A

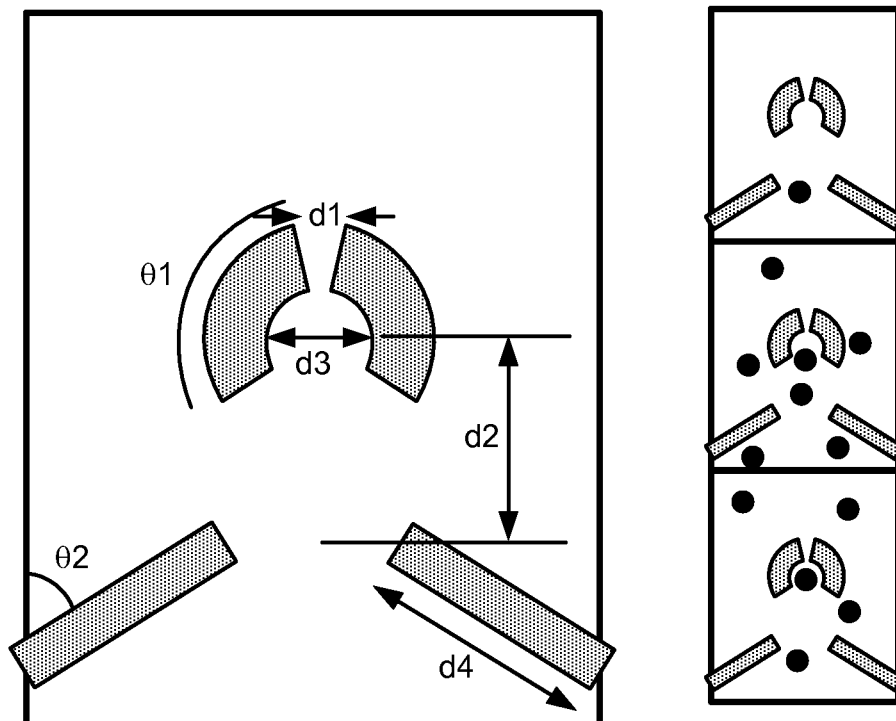


FIG. 22B

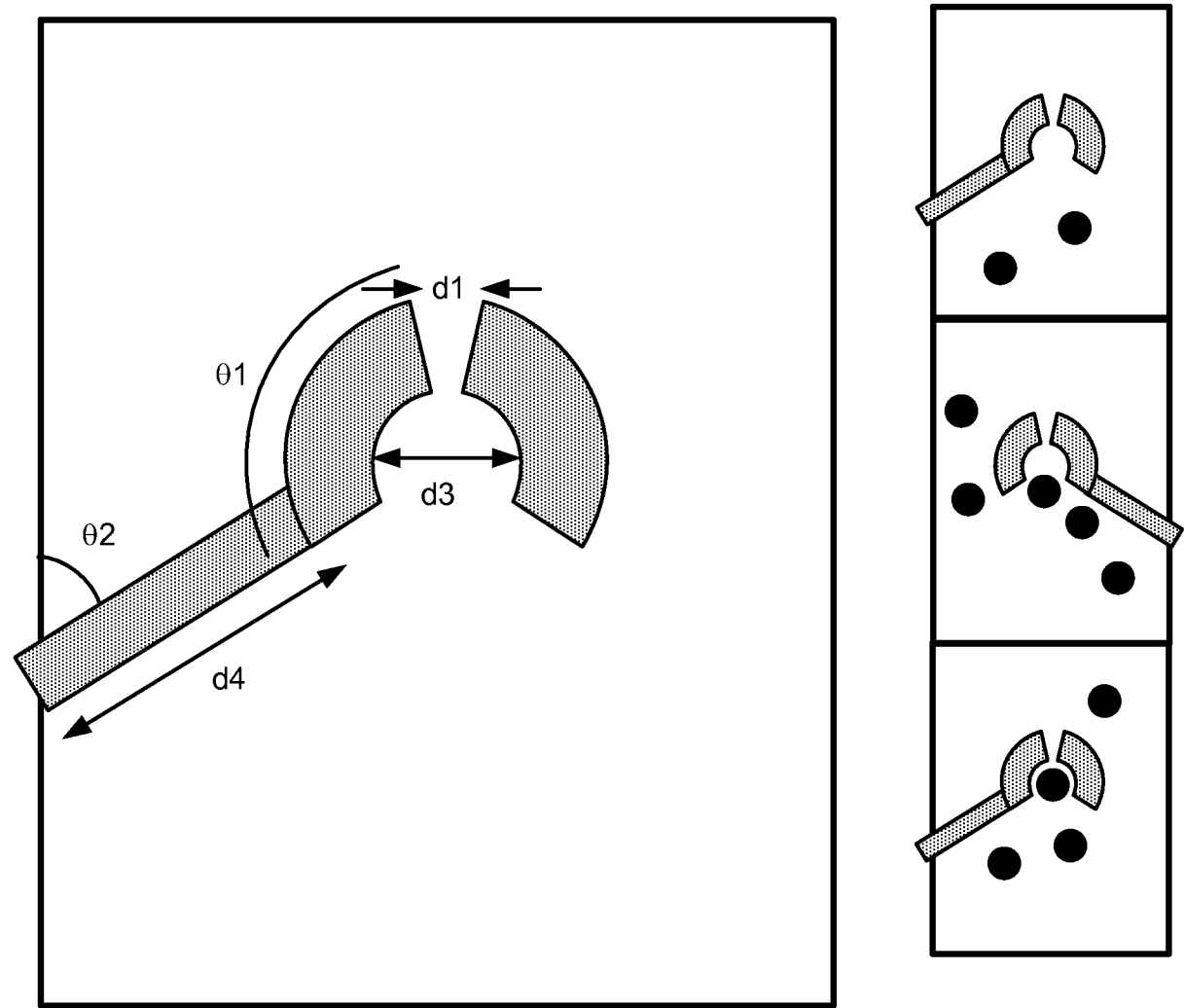


FIG. 22C

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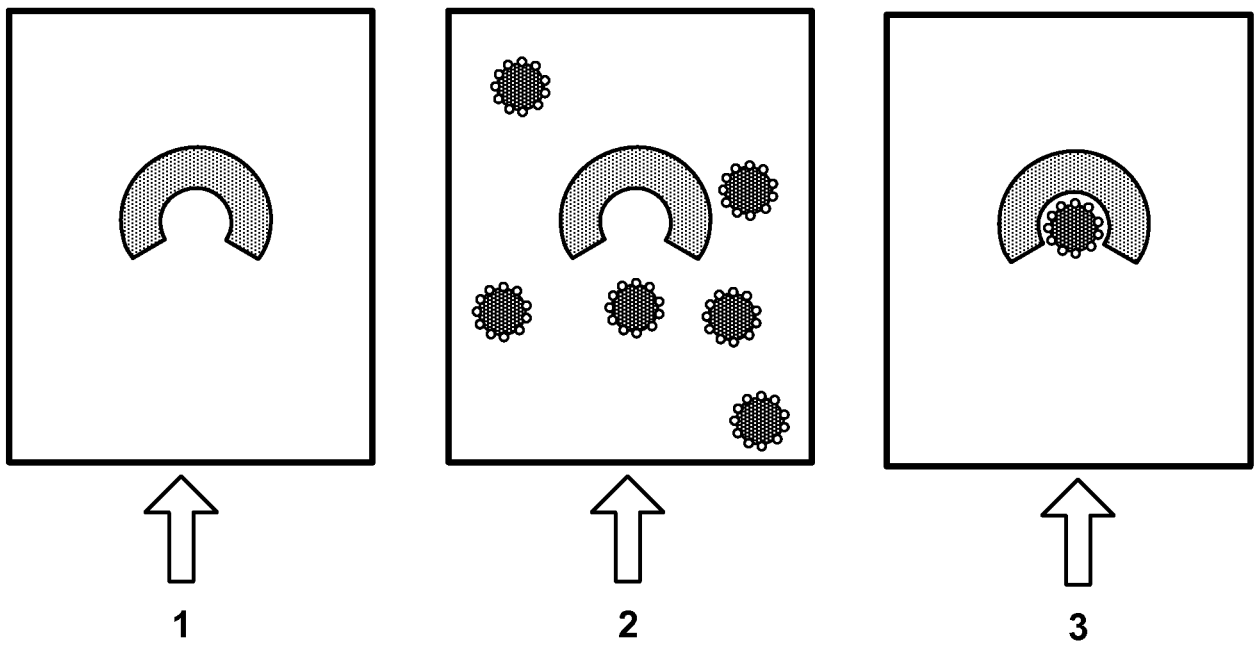


FIG. 23A

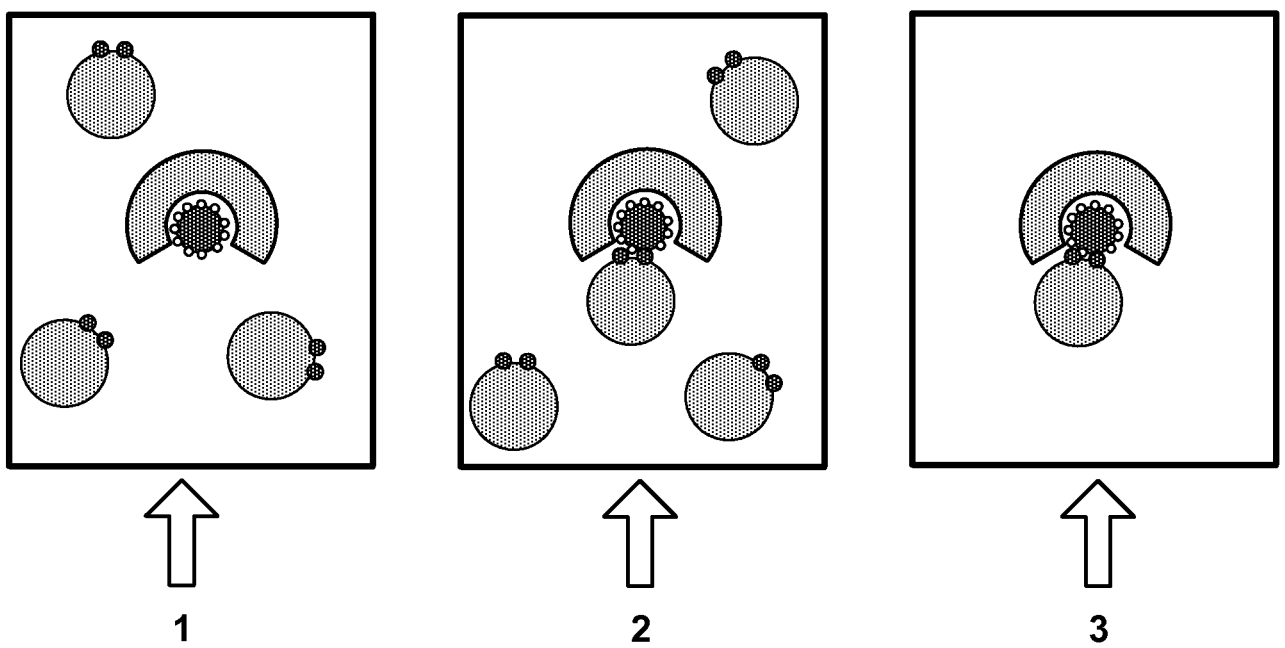


FIG. 23B

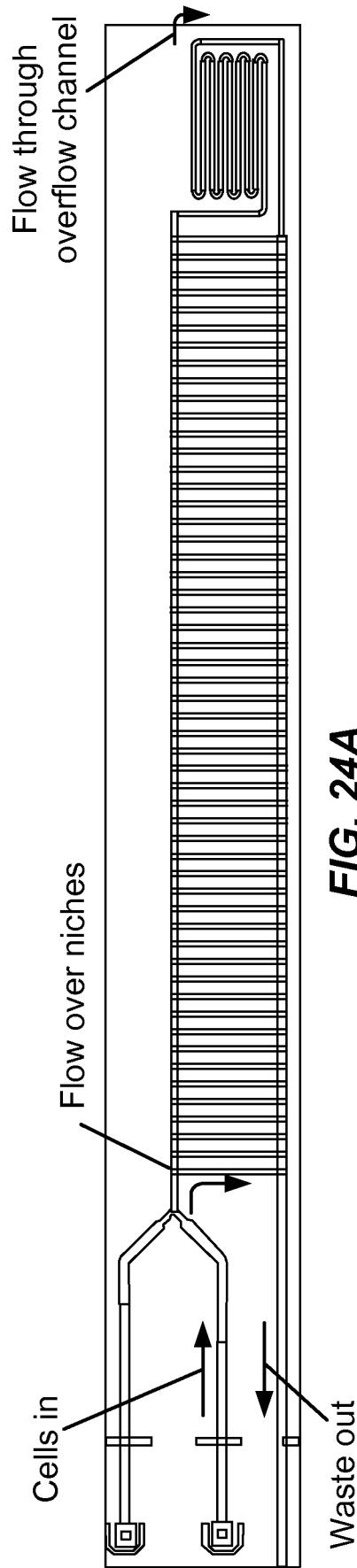


FIG. 24A

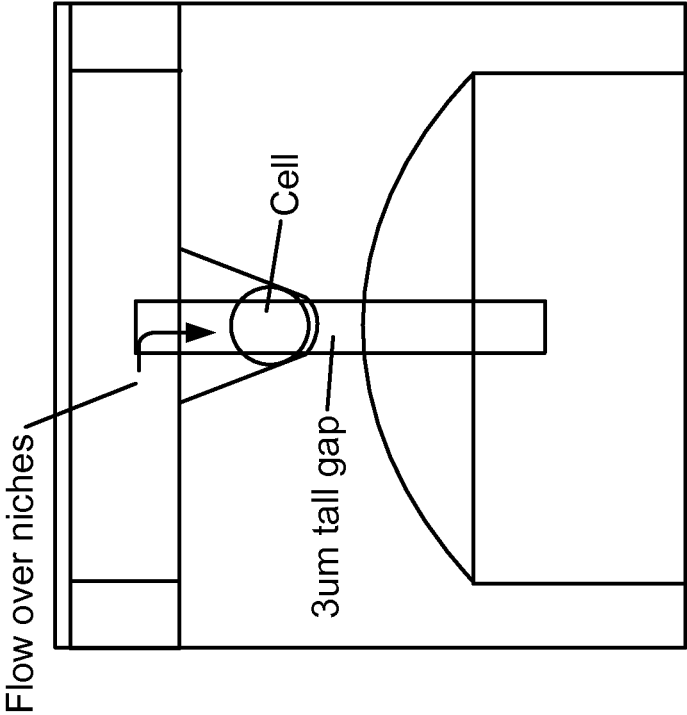


FIG. 24B

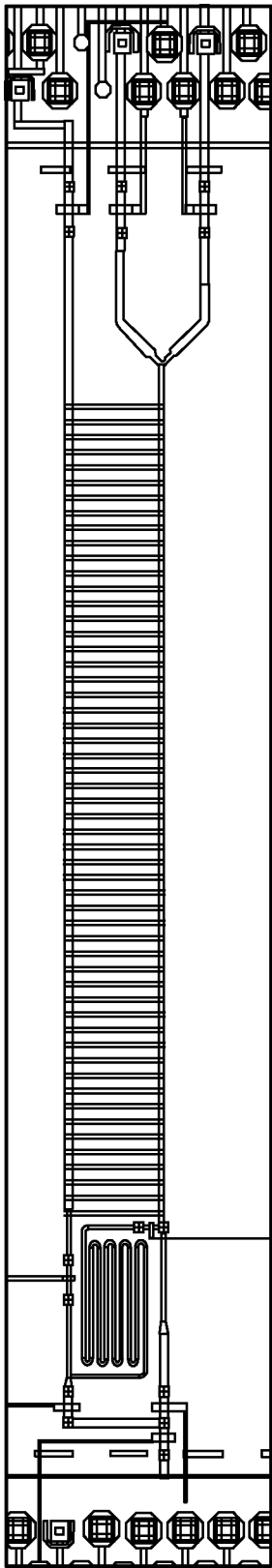


FIG. 24C

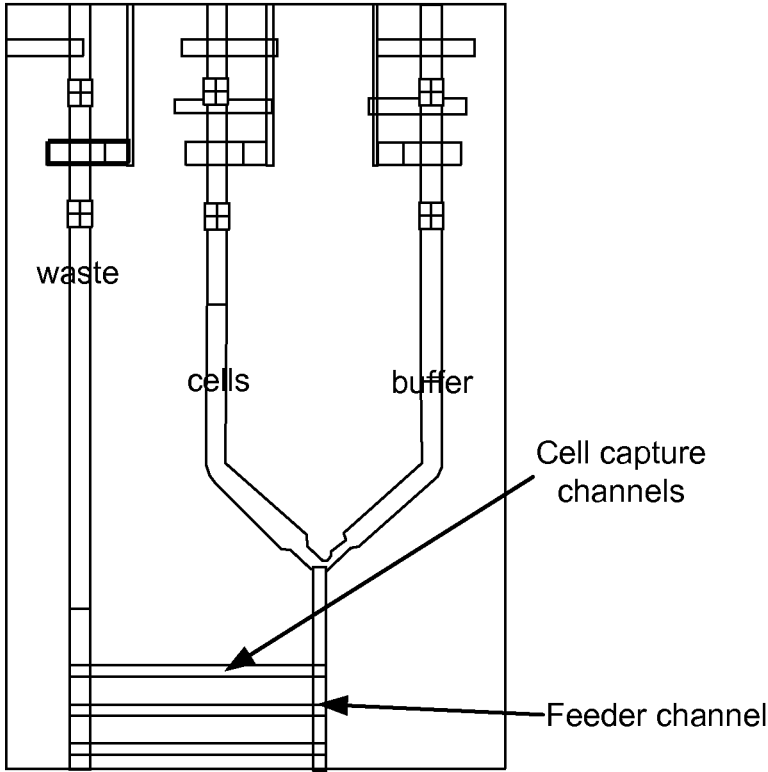


FIG. 24D

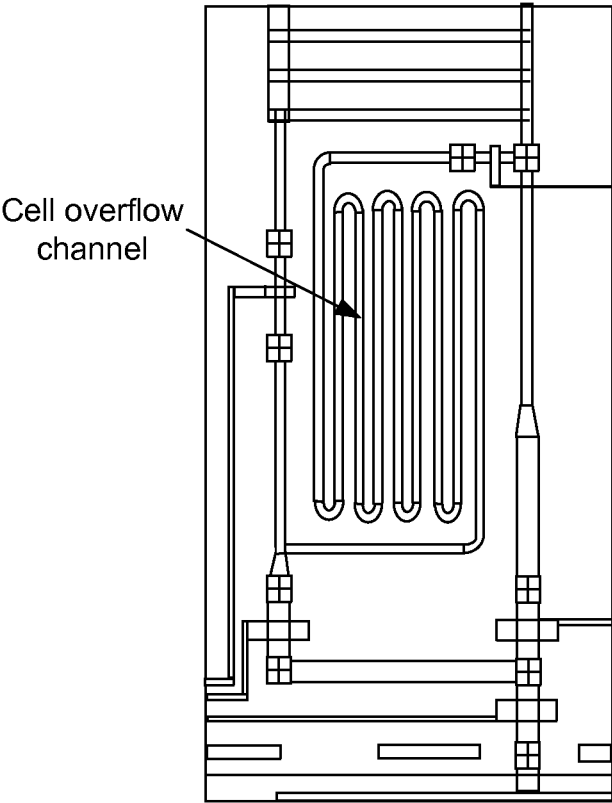


FIG. 24E

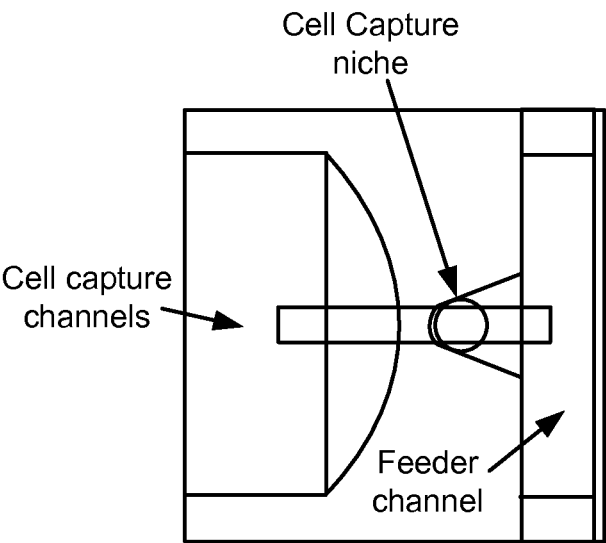


FIG. 24F

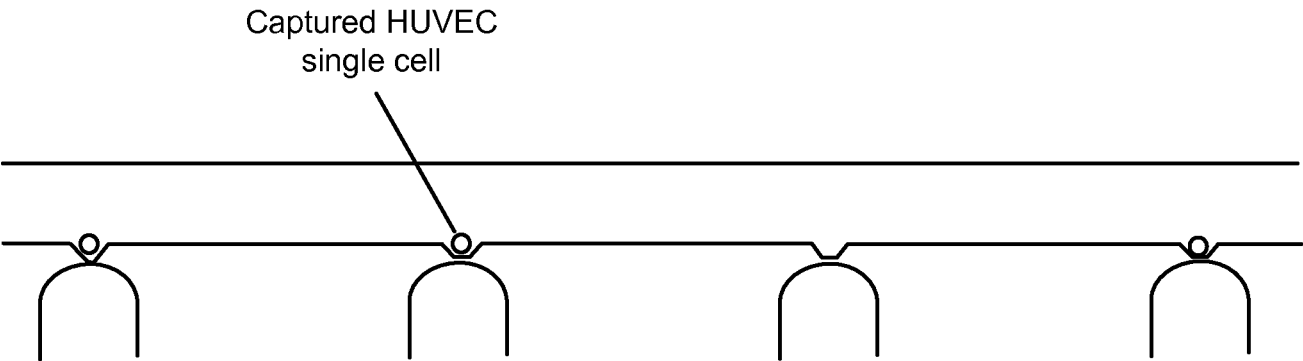
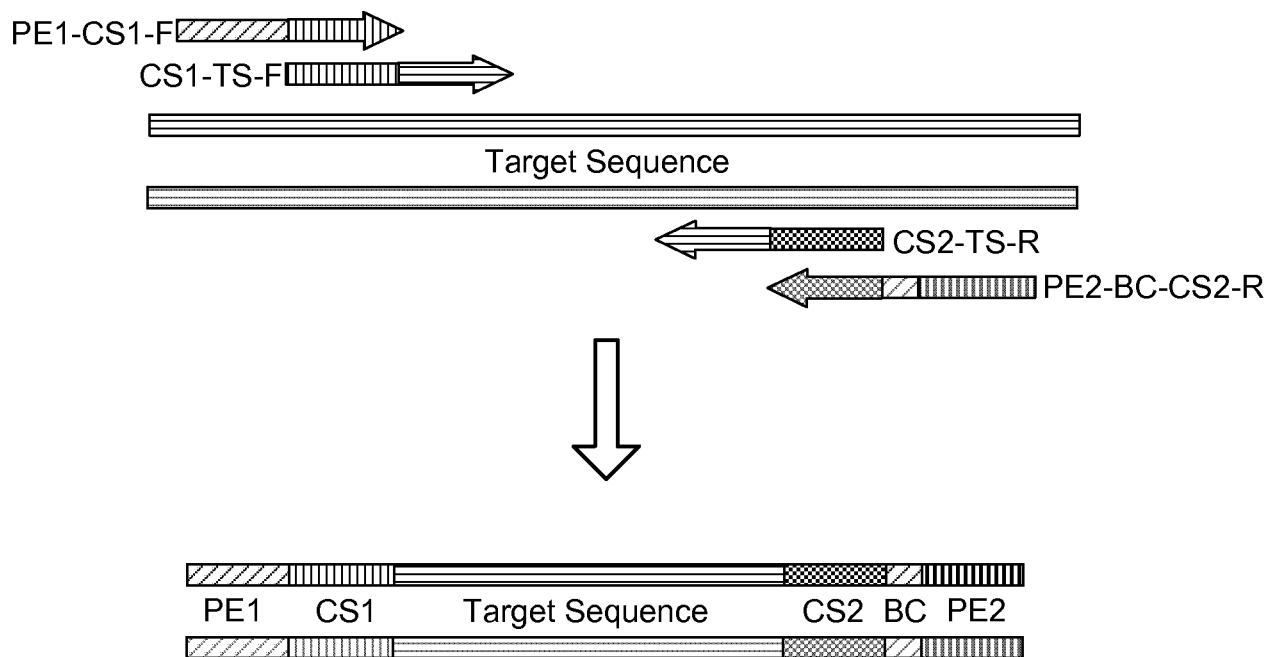
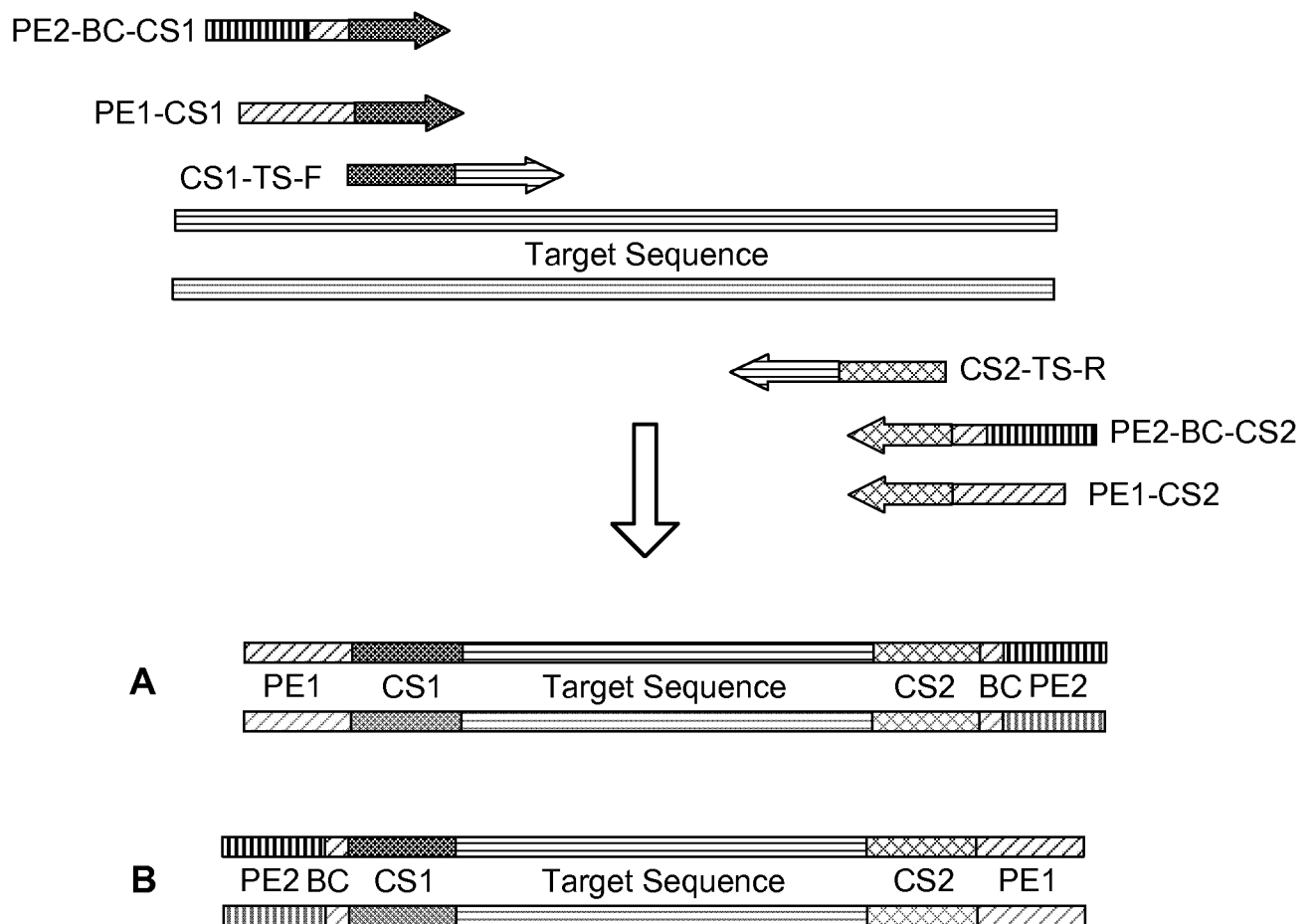


FIG. 24G

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

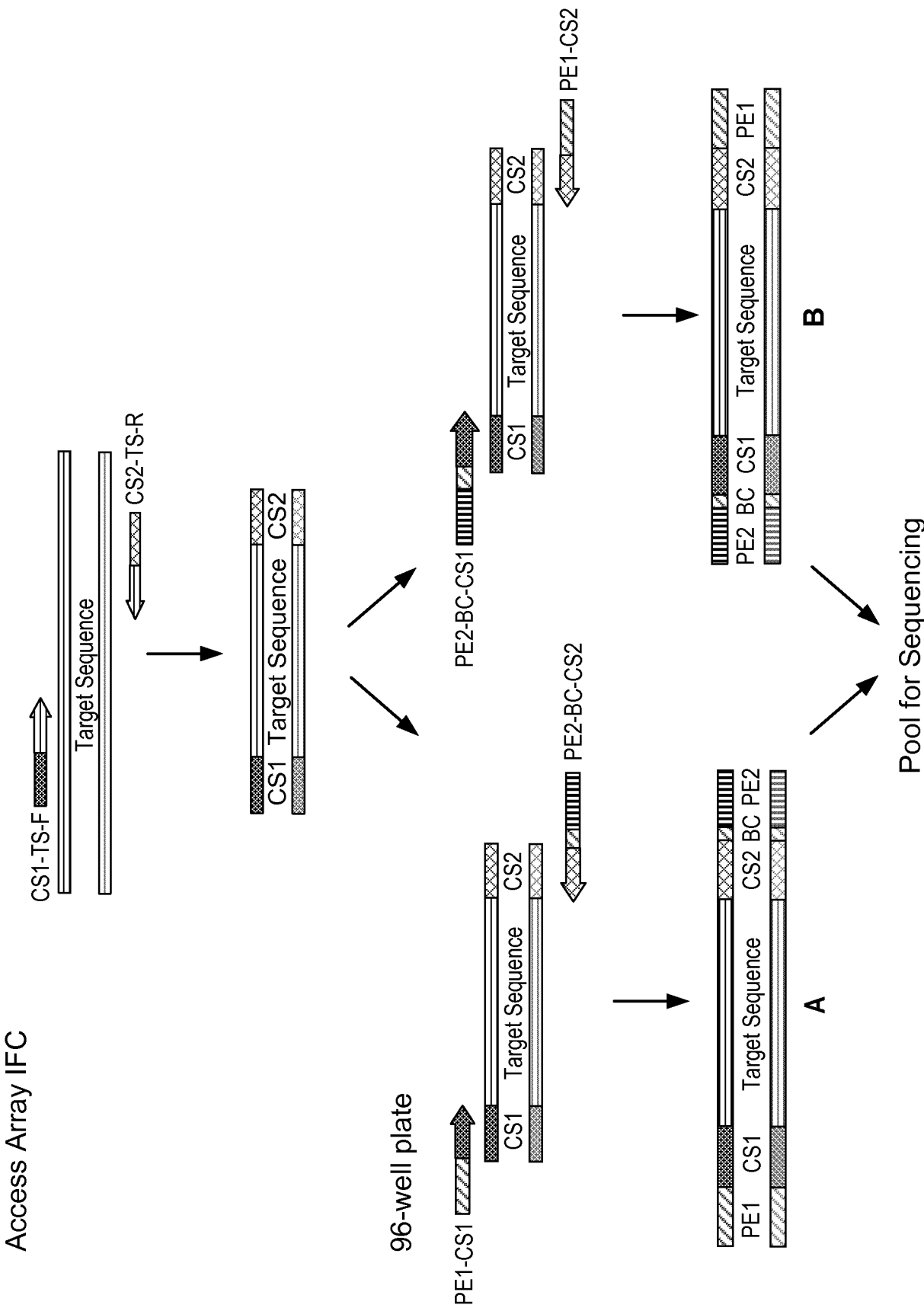


FIG. 26

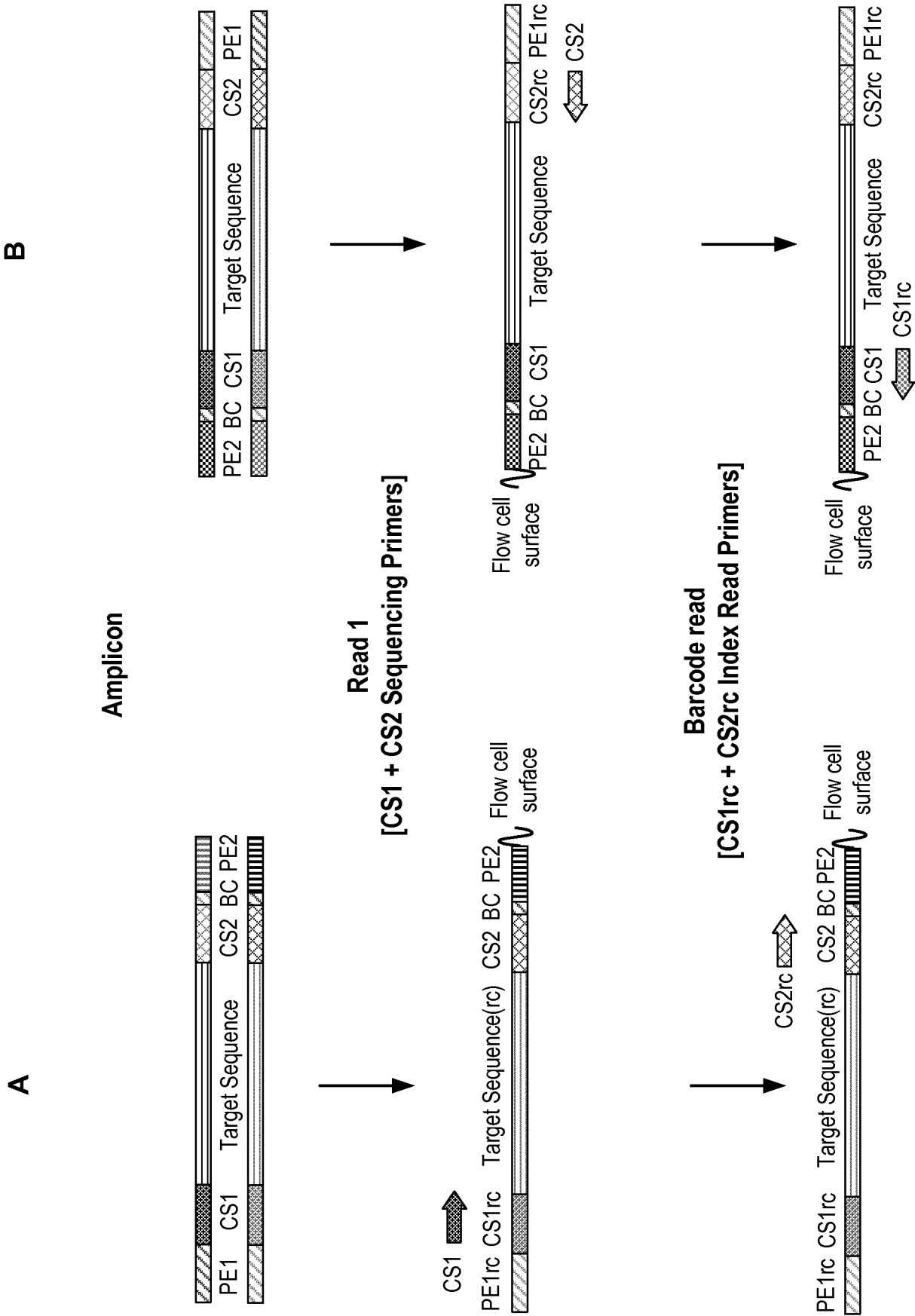


FIG. 27

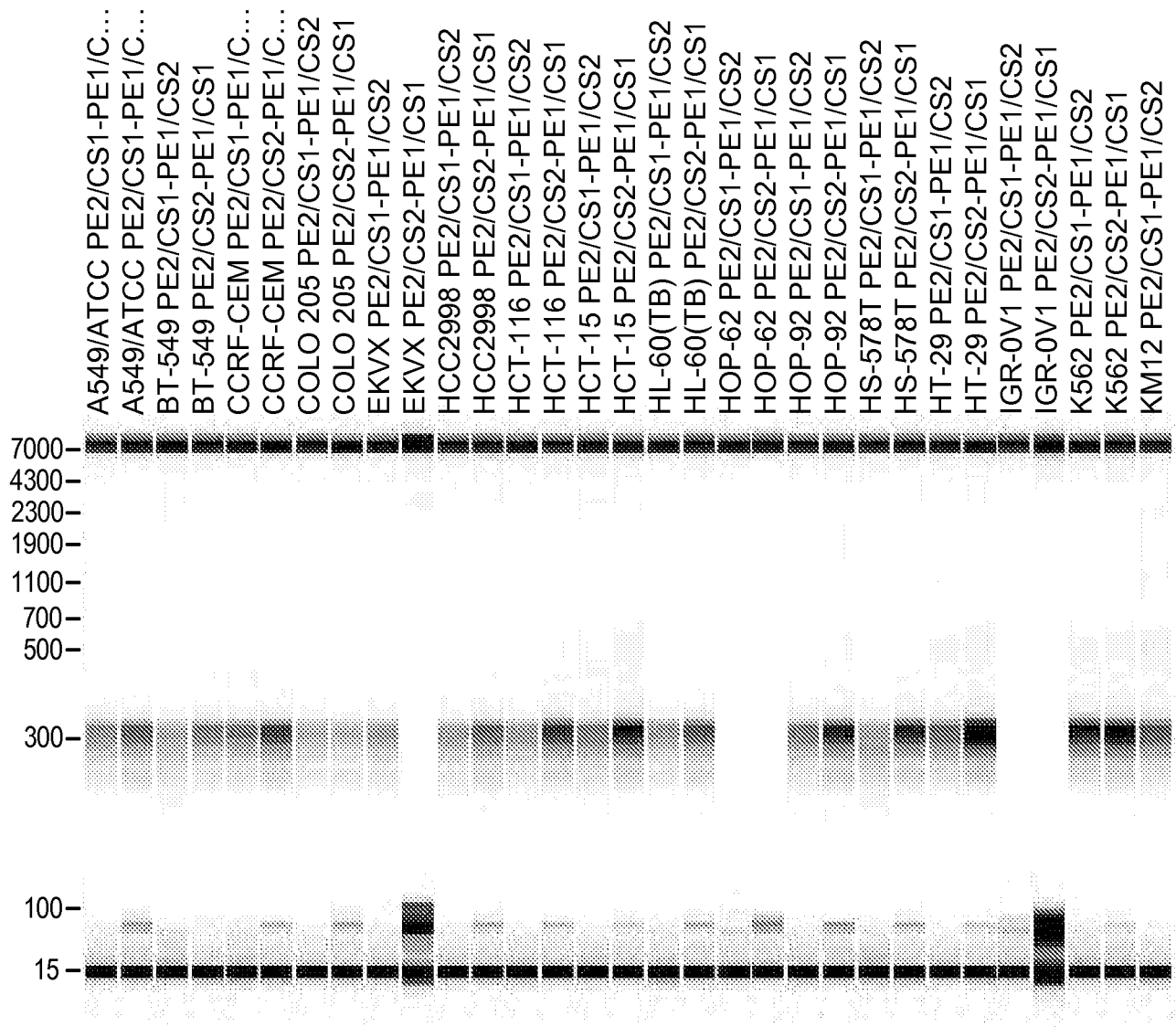


FIG. 28

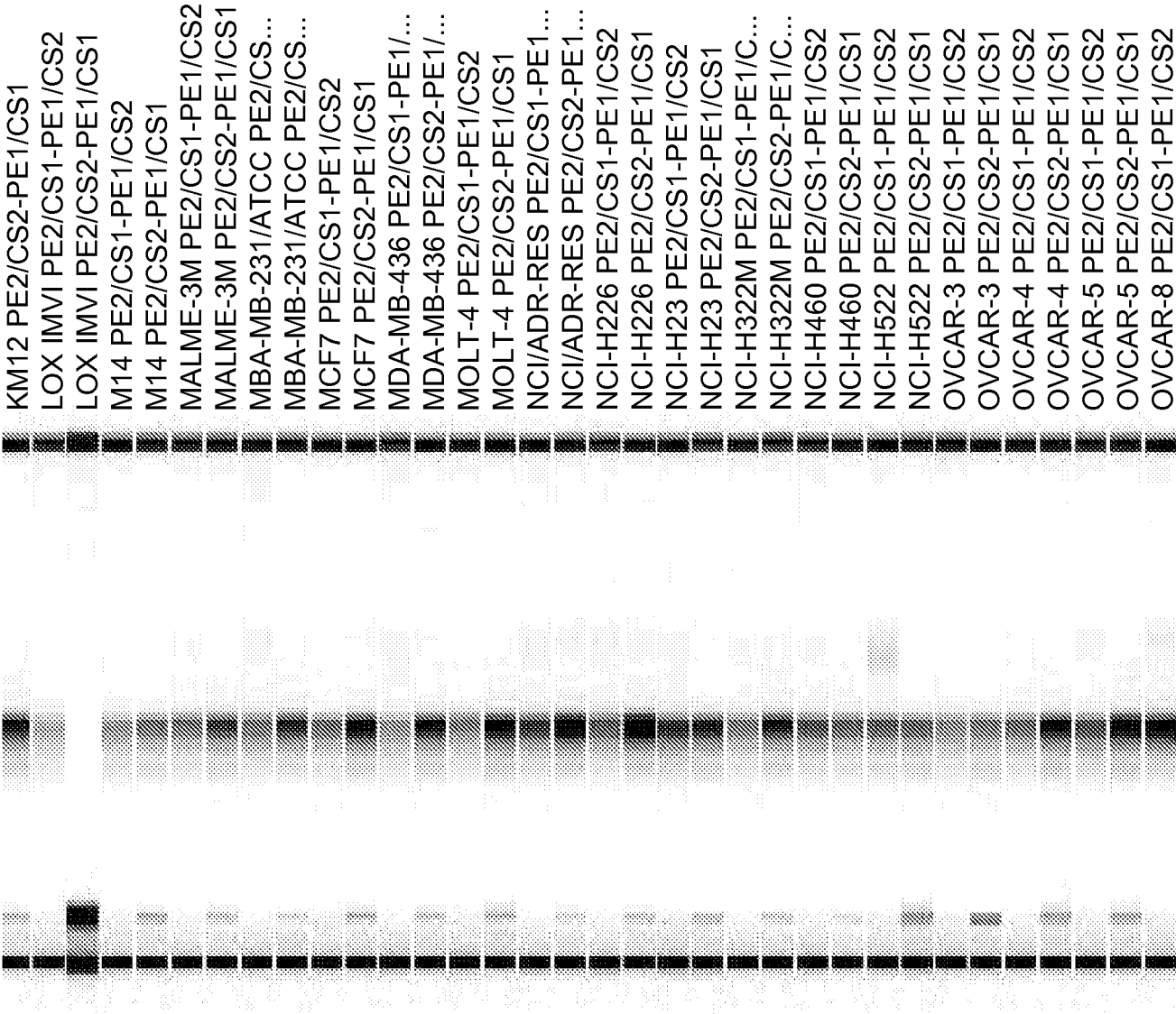


FIG. 28
(Continued)

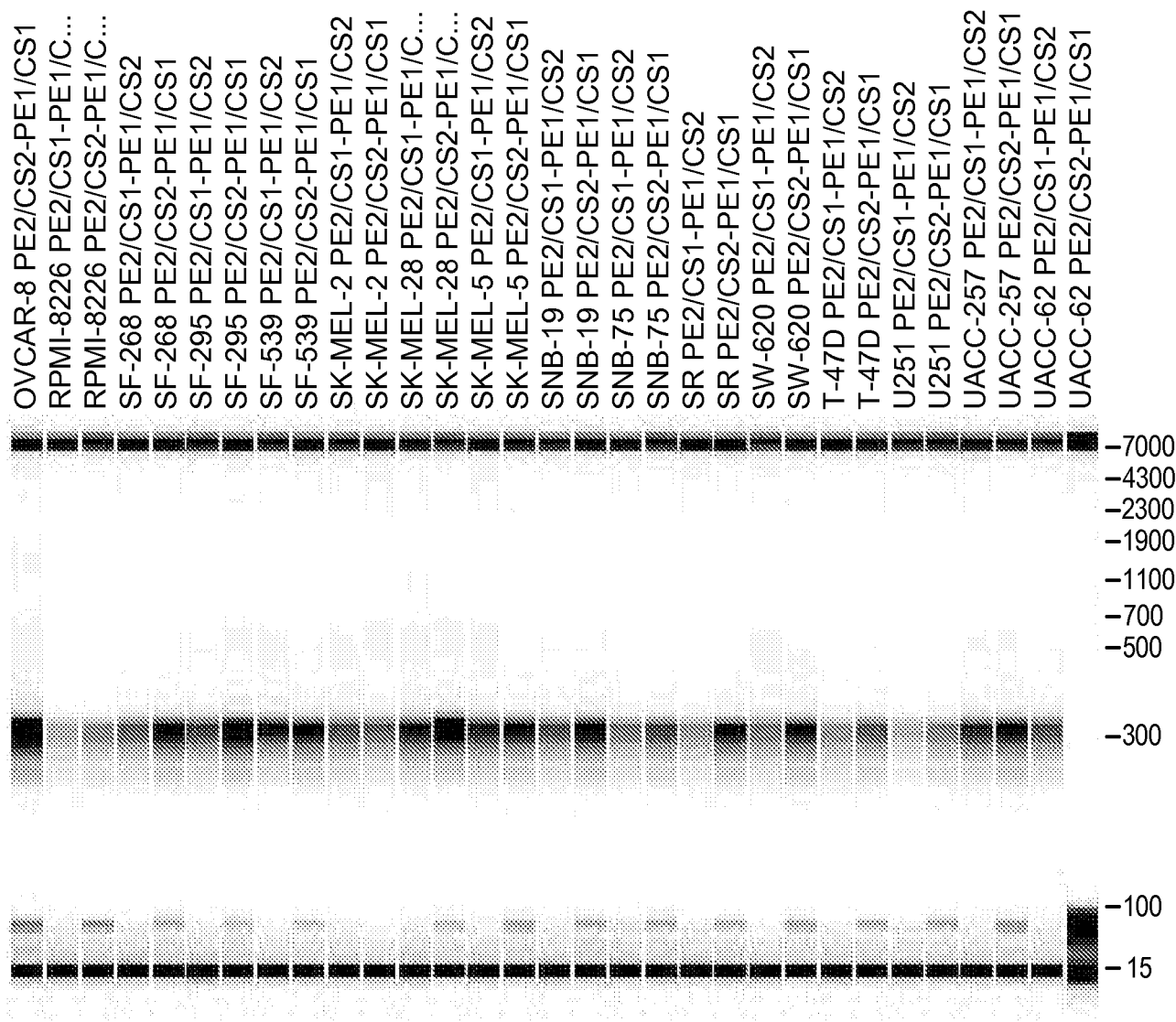


FIG. 28
(Continued)

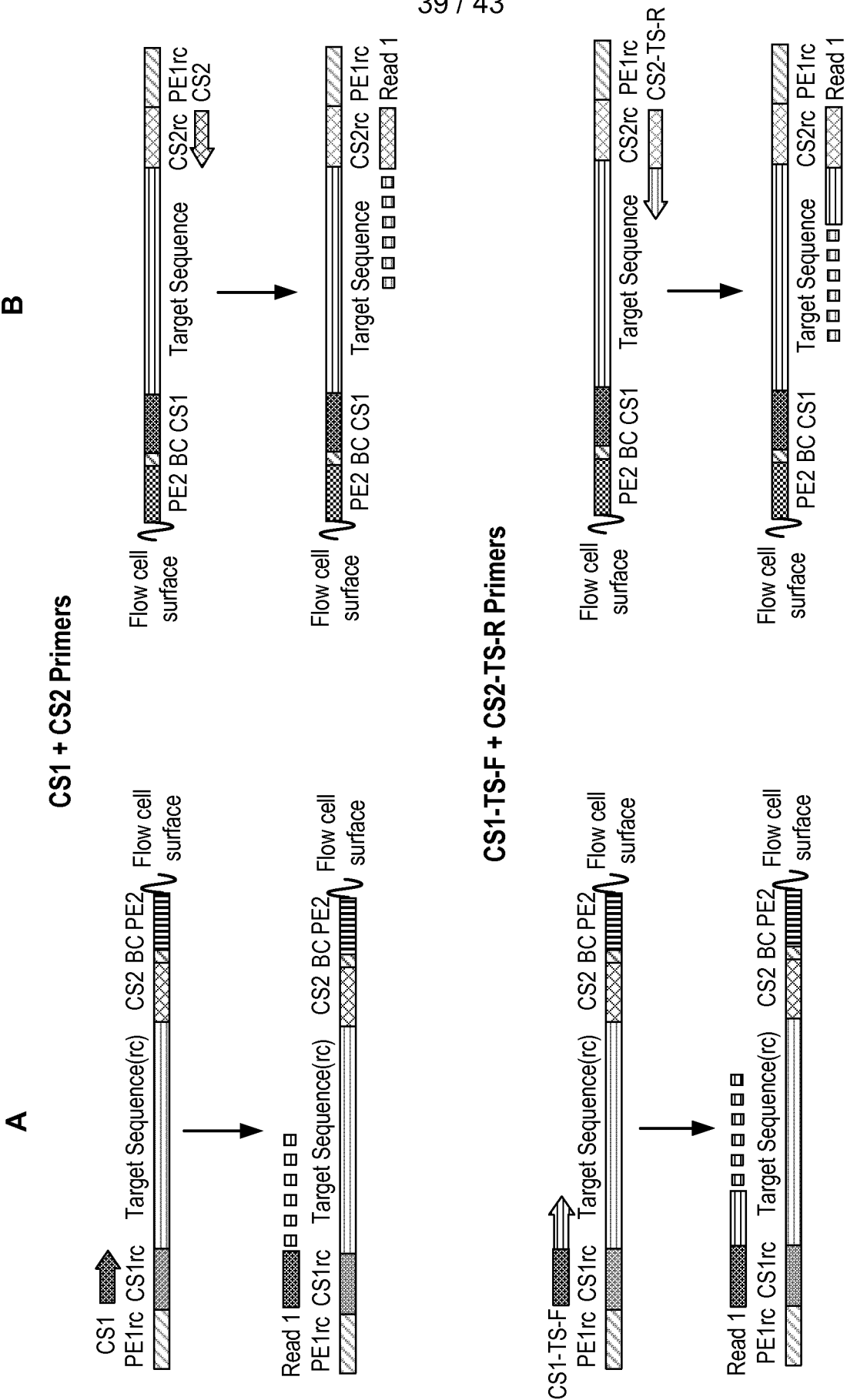


FIG. 29

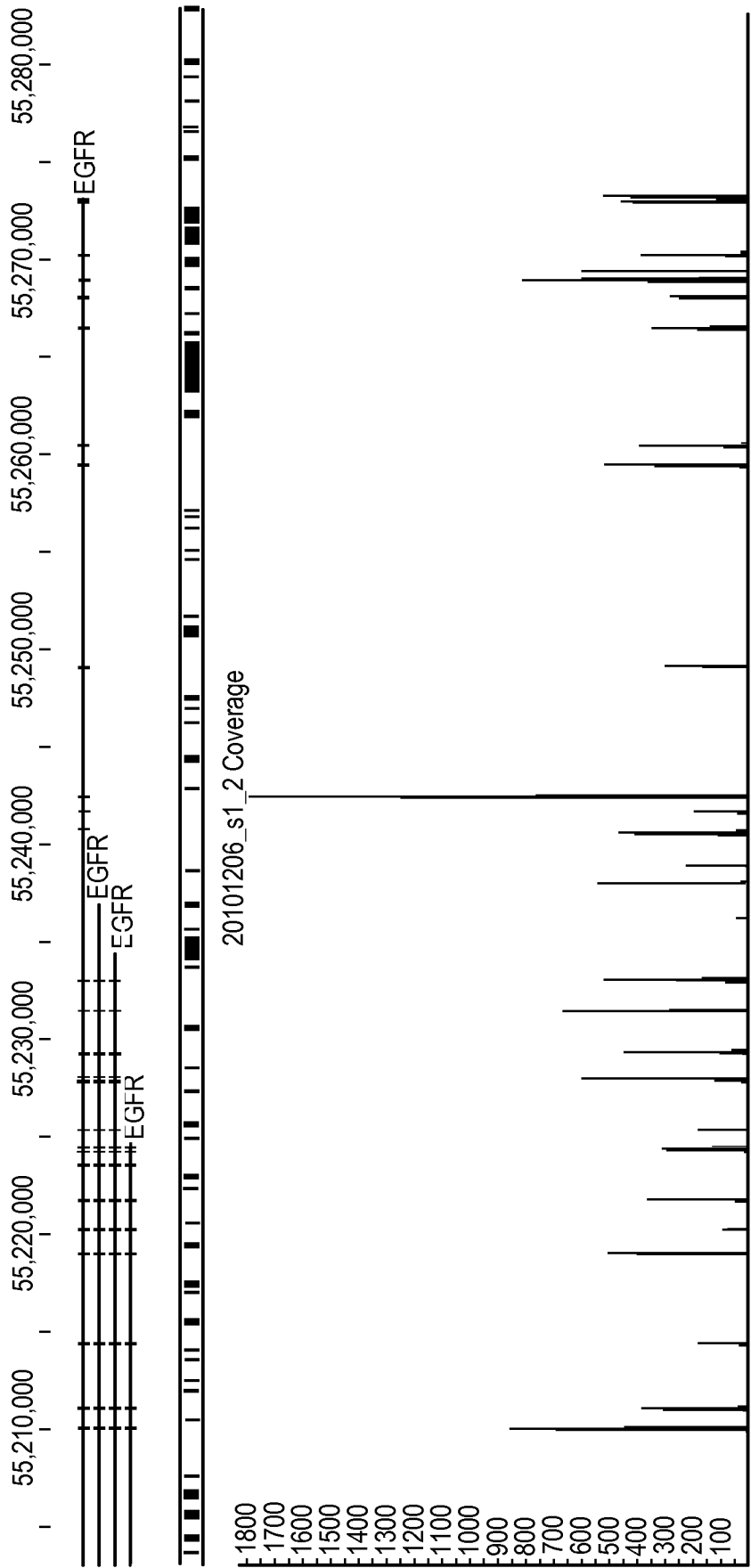
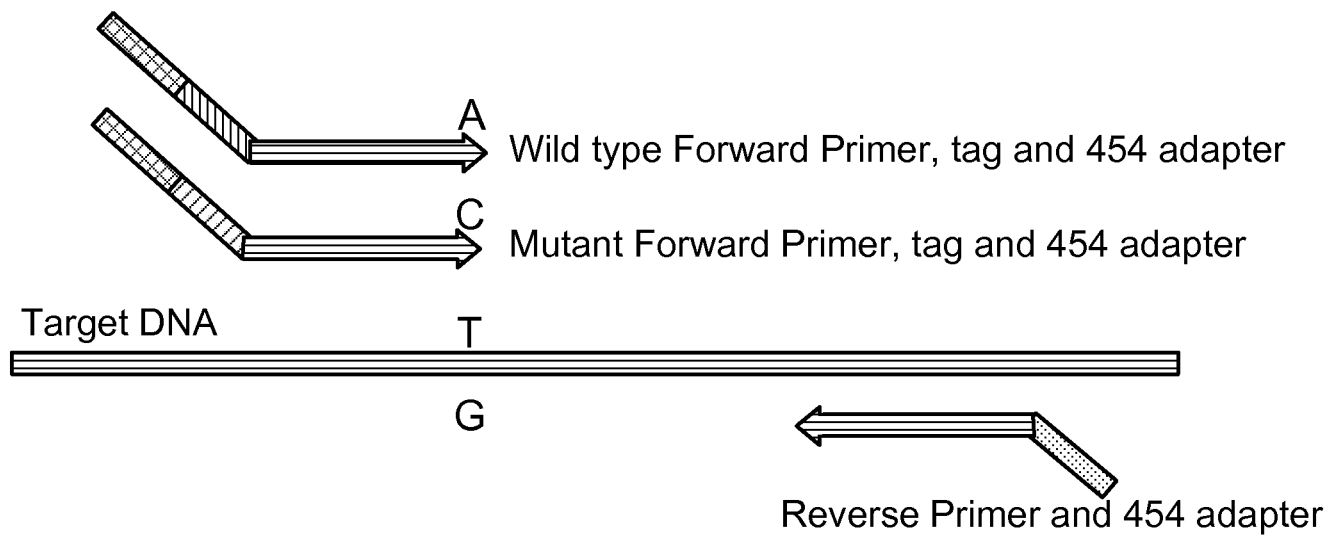


FIG. 30

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**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

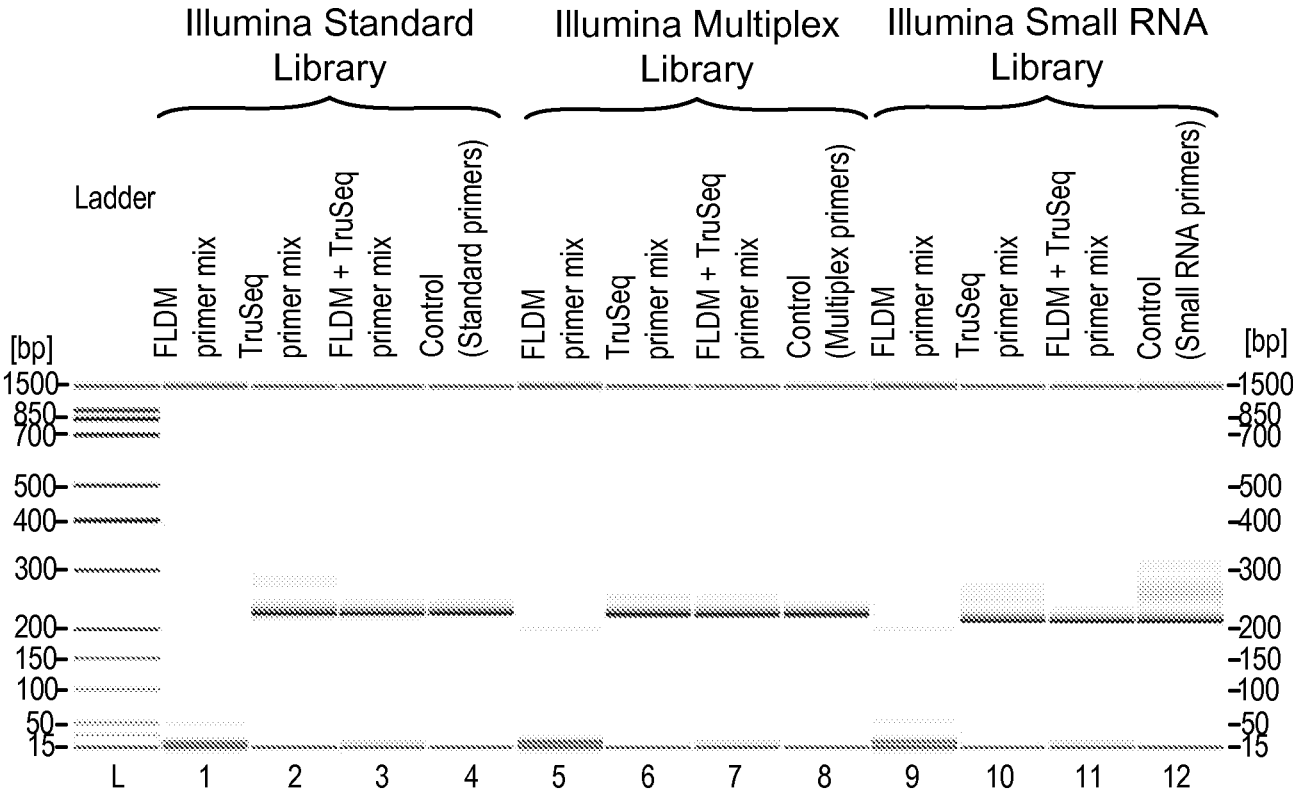


FIG. 32

Access Array Library

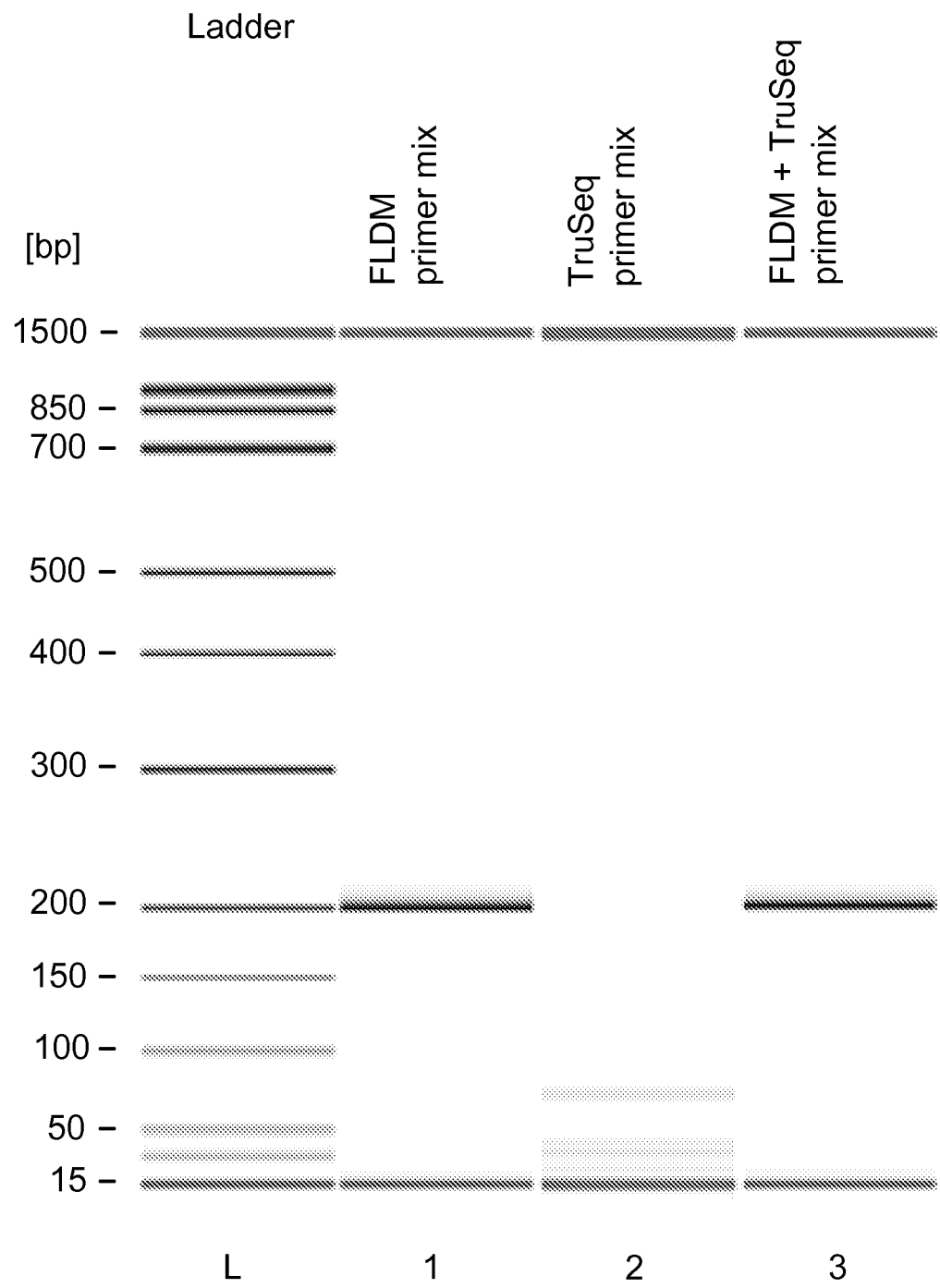


FIG. 33