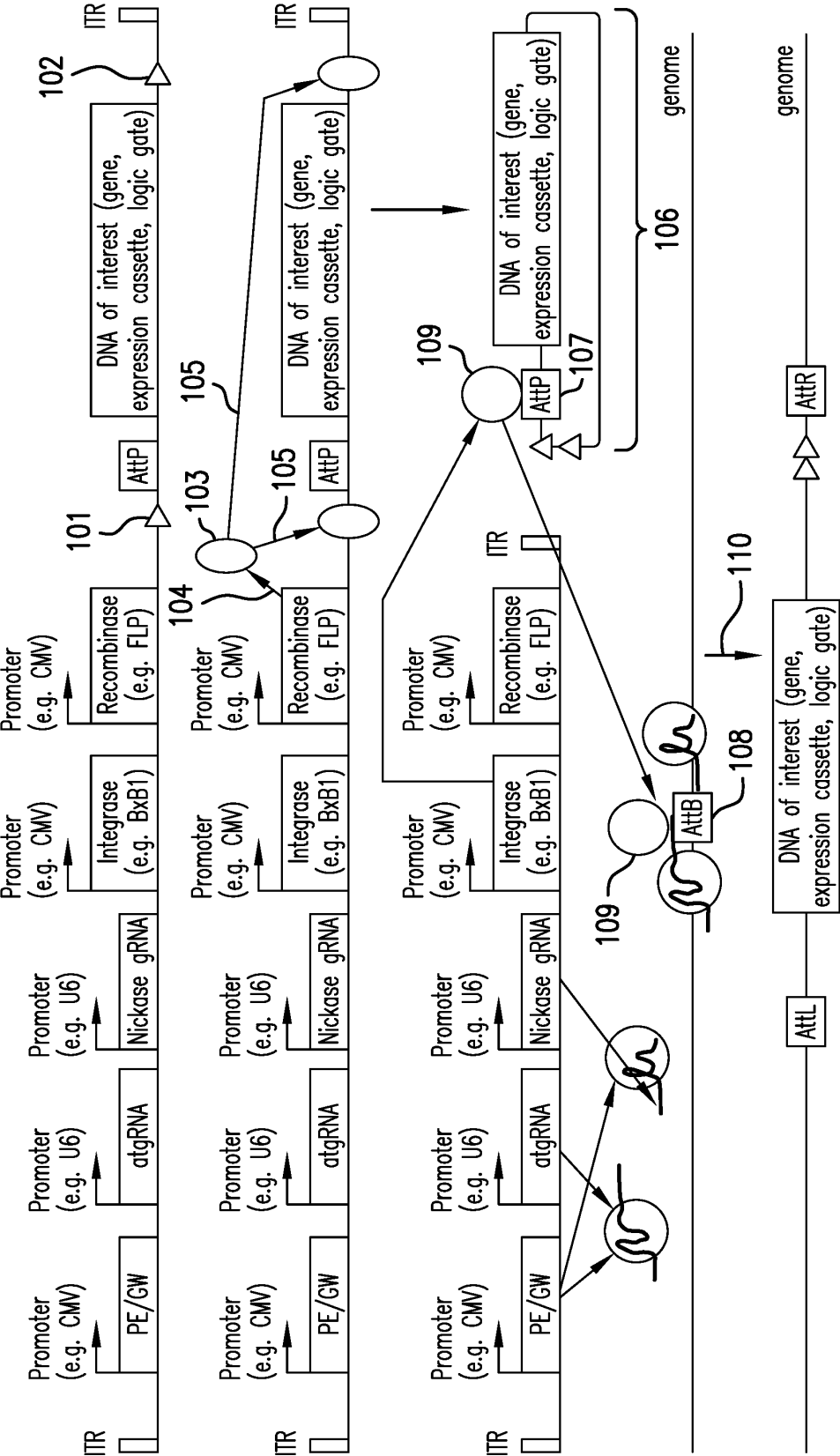




PE/GW = Prime editing or gene writing

FIG.1

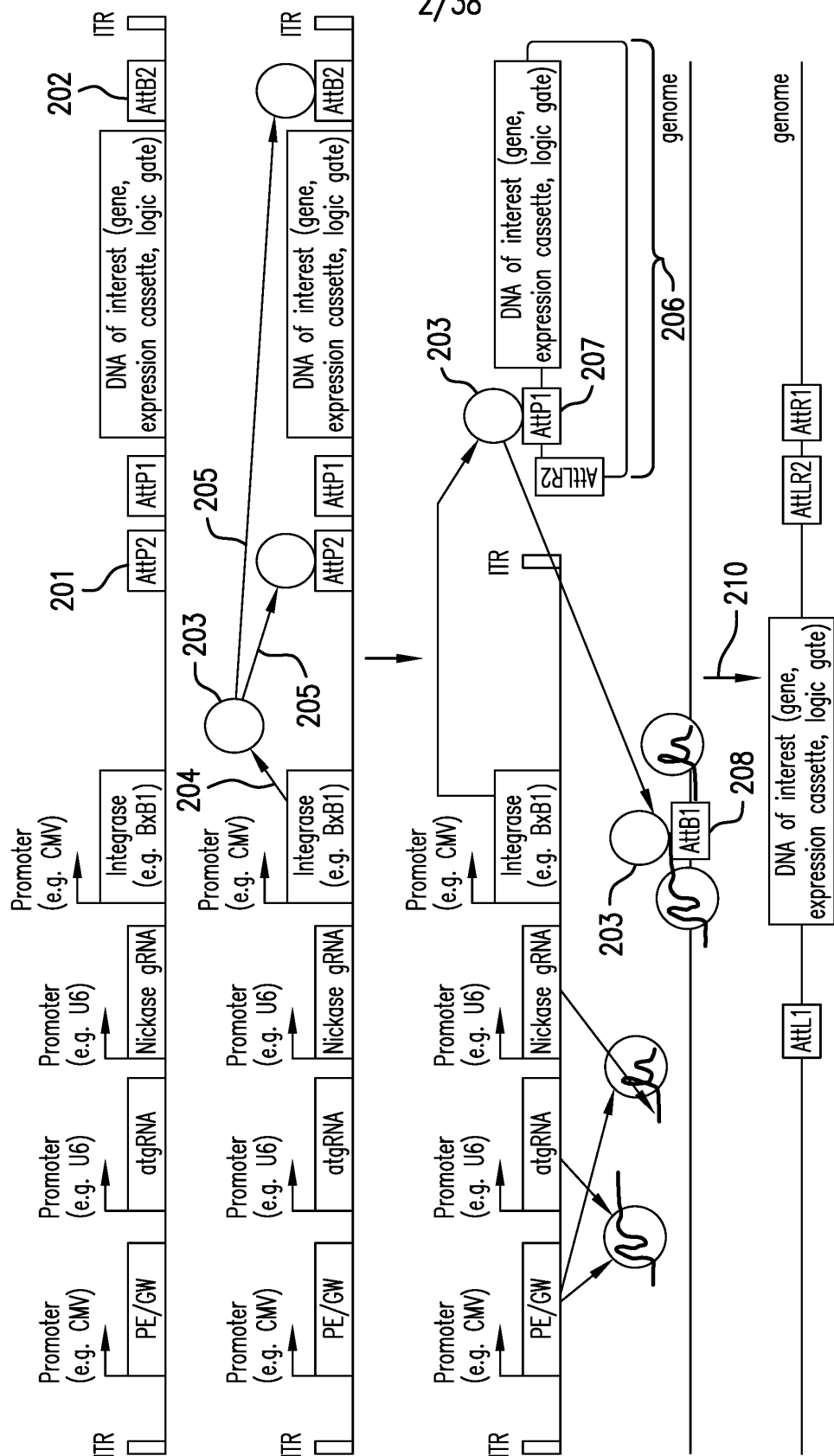


FIG. 2

PE/GW = Prime editing or gene writing

AttP mutants for improved kinetics

WT AttP

GTGGTTTGTCTGGTCAACCACCGCGTCTCAGTGGTGTACGGTACAAACCCA

AttP 1

GTGGTTTGT**TTGATGAACA**ACTGCAGTCTCAGTGGTGTACGGTACAAACCCA

AttP 2

GTGGTTTGT**TTGGTTA**ACCAC**AG**CGGTCTCAGTGGTGTACGGTACAAACCCA

AttP 3

GTGGTTTGT**TTGATGA**ACCAC**TGA**AGTCTCAGTGGTGTACGGTACAAACCCA

FIG.3A

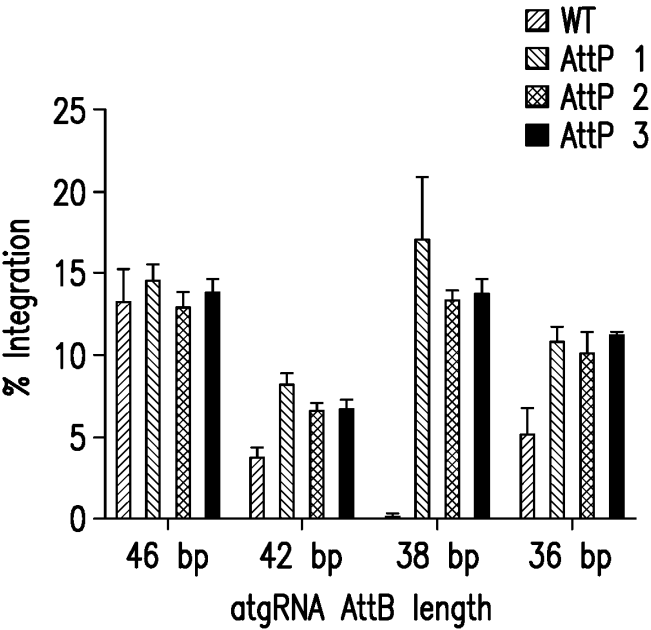


FIG.3B

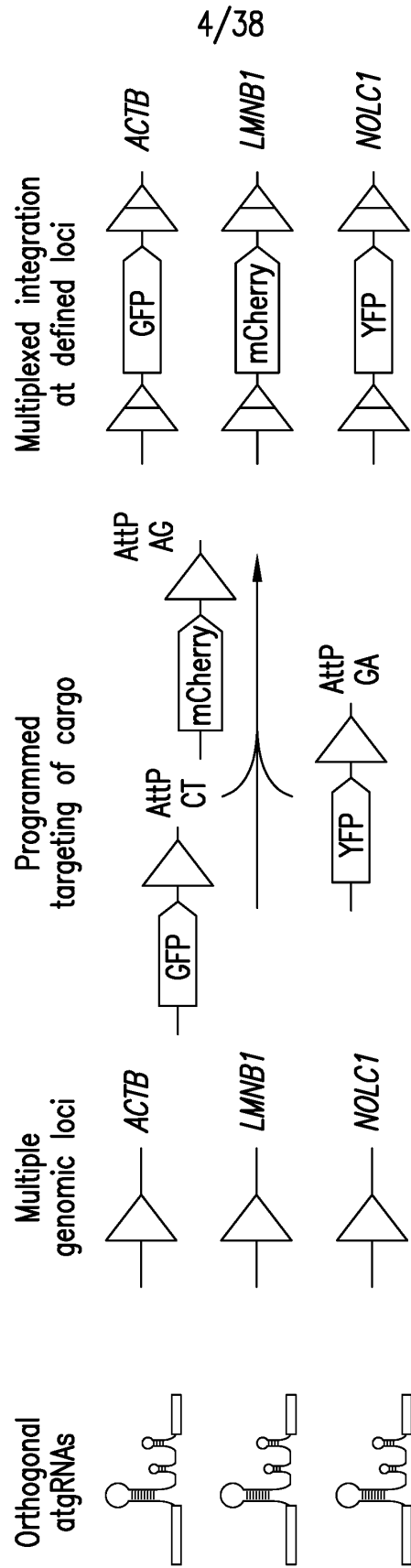


FIG.3C

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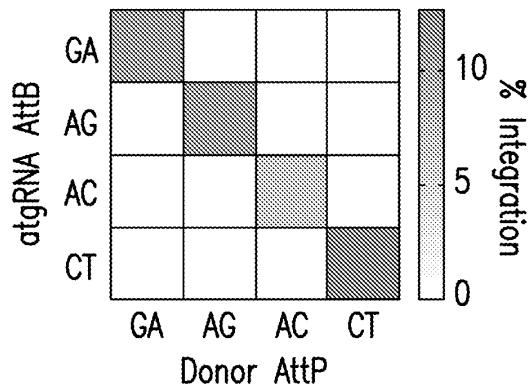


FIG.3D

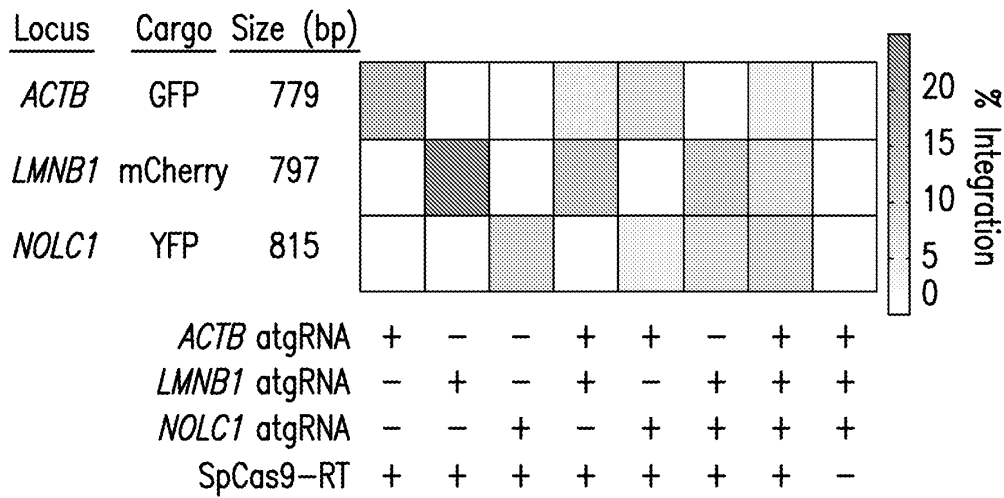


FIG.3E

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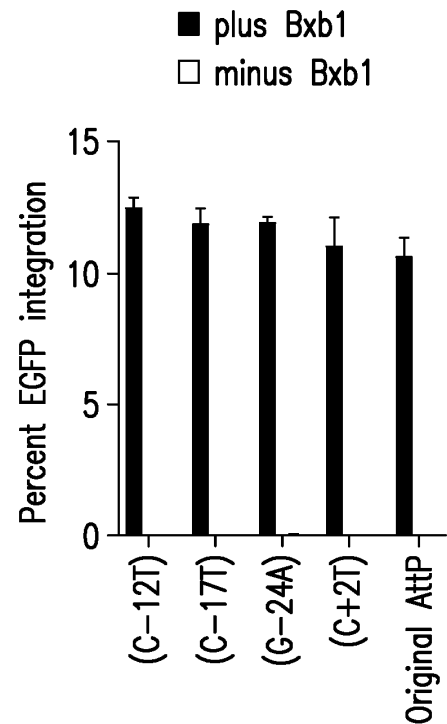


FIG.4A

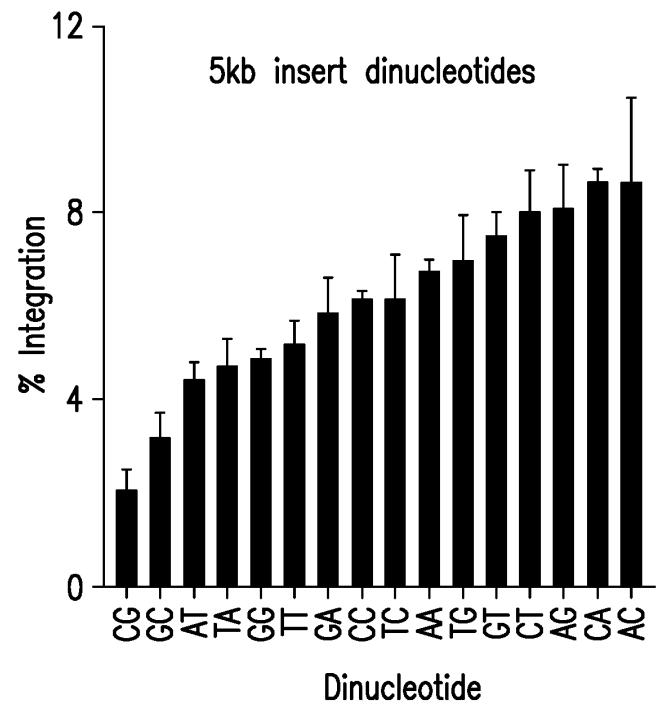


FIG.4B

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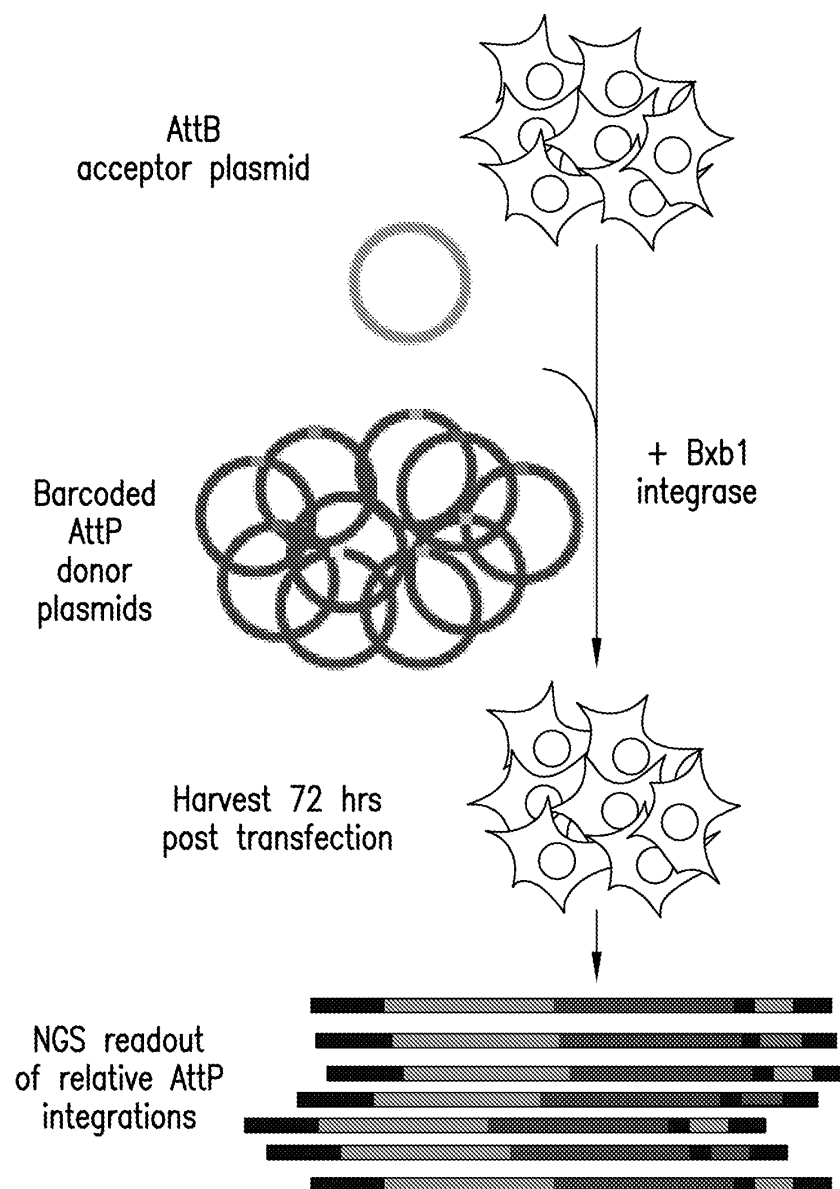


FIG.4C

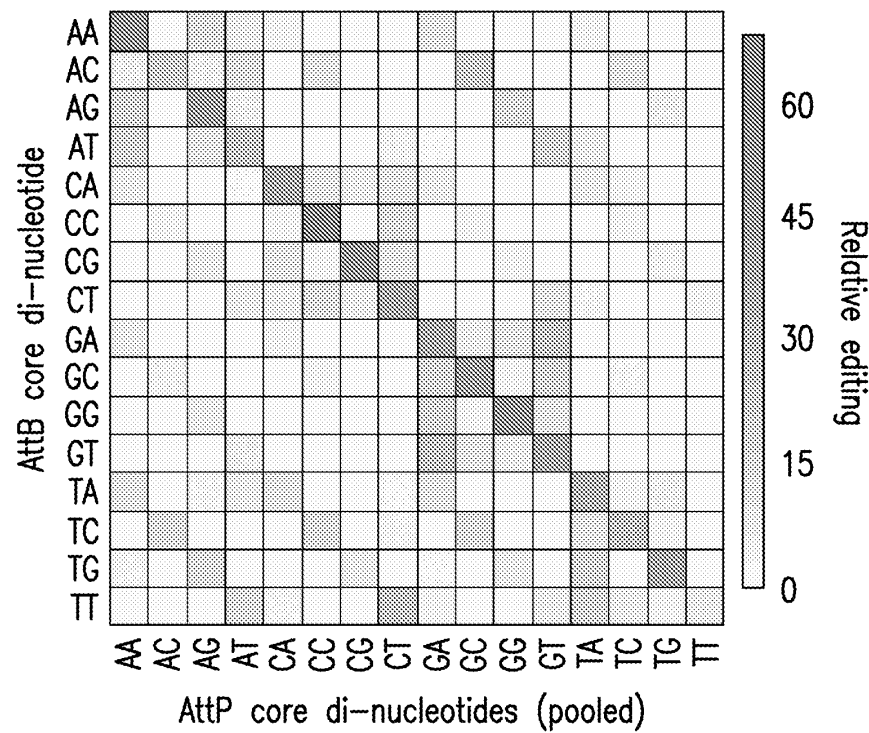
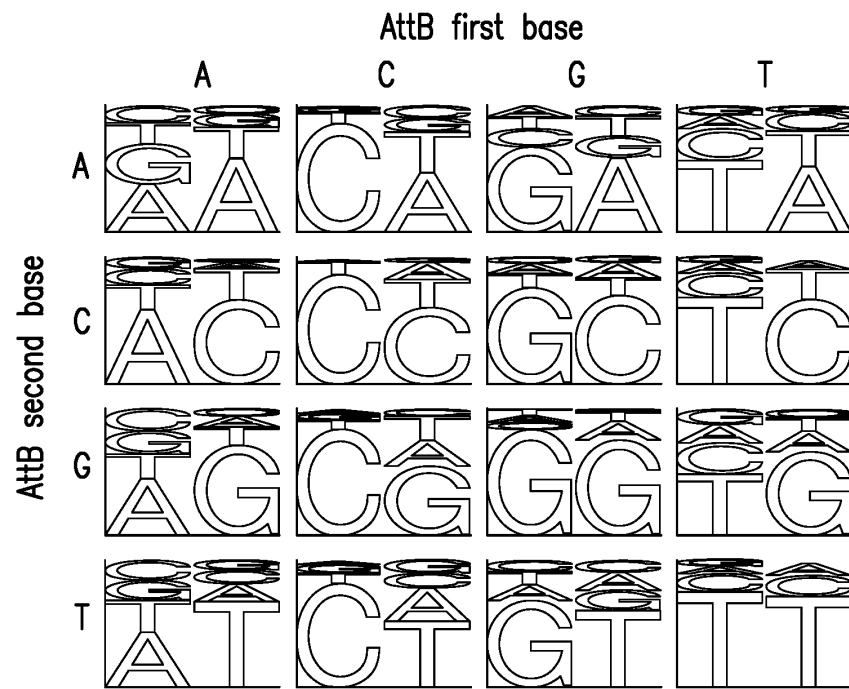


FIG.4D

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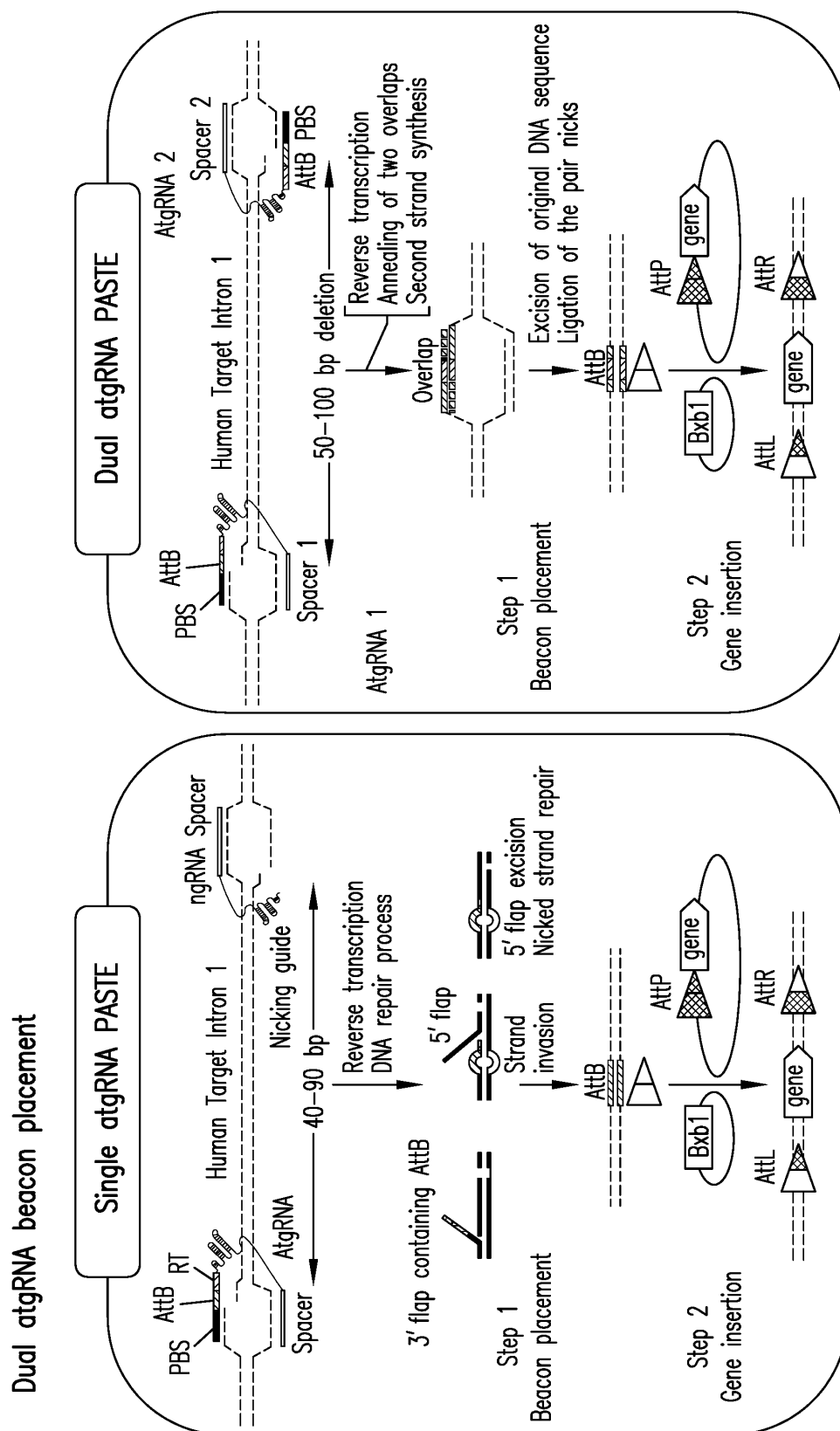
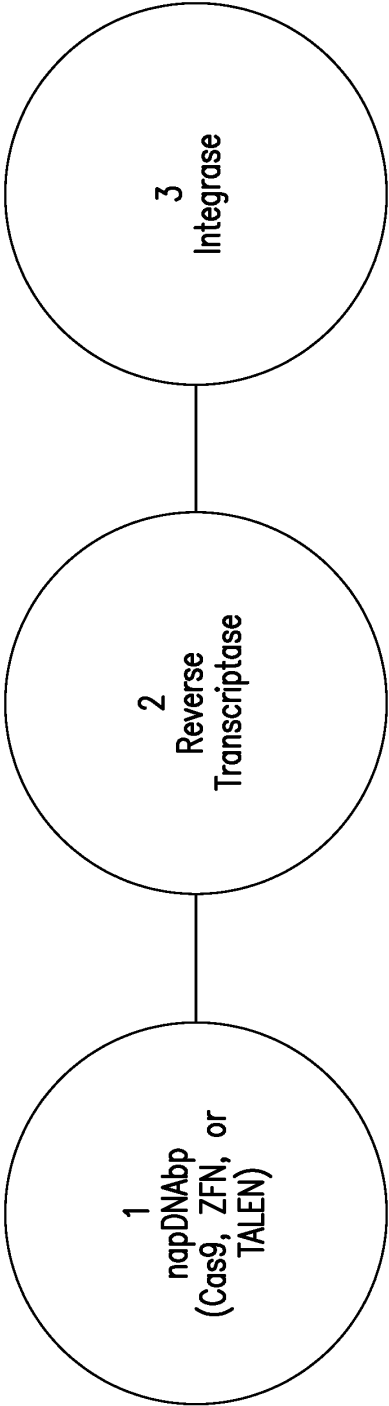


FIG. 5



Linked Order Options:
1-2-3
2-3-1
3-2-1
1-3-2
2-1-3
3-1-2

FIG.6

- AttP (GT) for genome insertion
- AttB _AG and AttP _AG at both ends for circularization in presence of Bxb1
- EF1 α promoter will drive NanoLuc and GFP expression
- Screen for efficient di-nucleotides and configuration
- Based on FG- and HD- AdV vector, can test in plasmid and virus format

Extrachromosomal circular DNA (EccDNA) sensor

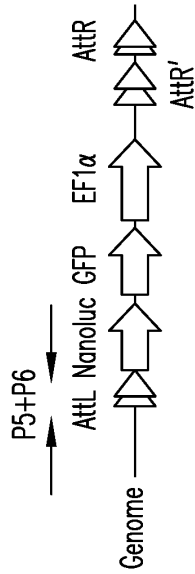
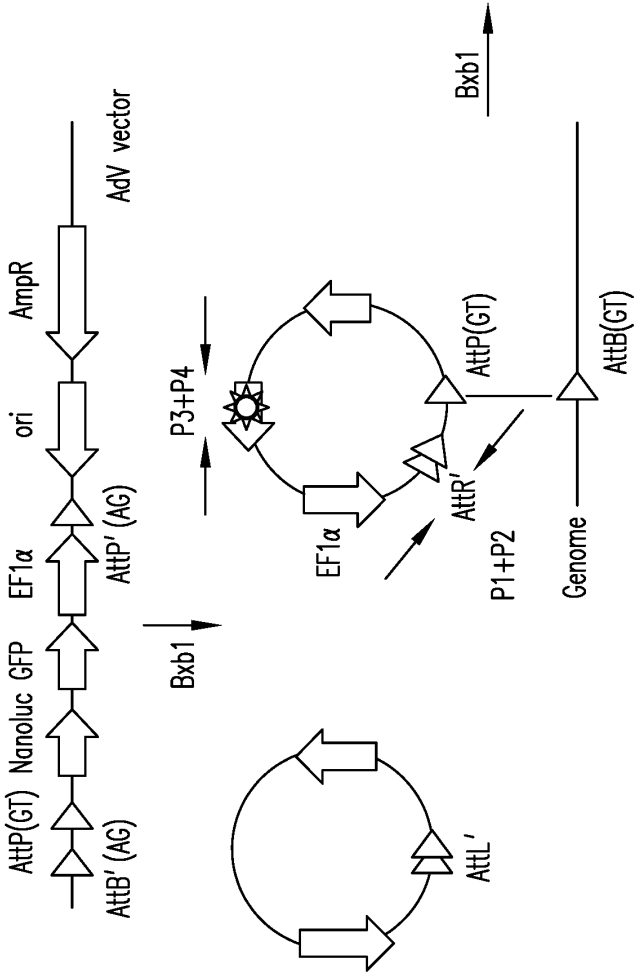


FIG.7

- 200k HEK293T cells, 12–well plate, Lipo3000 transfection, all plasmid form
- Harvest sample 72 hrs post transfection

#	Effector 1 (800ng)	Effector 2 (800ng)	atgRNA (270ng)	Nicking (90ng)	Cargo (500ng)	Total weight (ng)	p3000 (μ l)
1	–	–	–	–	EccSen–1	500	1
2	–	Bxb1 (pDY0191)	–	–	EccSen–1	1300	1.6
3	PE2 (pDY0129)	–	ACTB atgRNA (pDY0207)	ACTB Nicking (pDY0209)	EccSen–1	1660	3.32
4	PE2 (pDY0129)	Bxb1 (pDY0191)	ACTB atgRNA (pDY0207)	ACTB Nicking (pDY0209)	EccSen–1	2460	4.92
5	–	–	–	–	EccSen–2	500	1
6	–	Bxb1 (pDY0191)	–	–	EccSen–2	1300	1.6
7	PE2 (pDY0129)	–	ACTB atgRNA (pDY0207)	ACTB Nicking (pDY0209)	EccSen–2	1660	3.32
8	PE2 (pDY0129)	Bxb1 (pDY0191)	ACTB atgRNA (pDY0207)	ACTB Nicking (pDY0209)	EccSen–2	2460	4.92

FIG.8

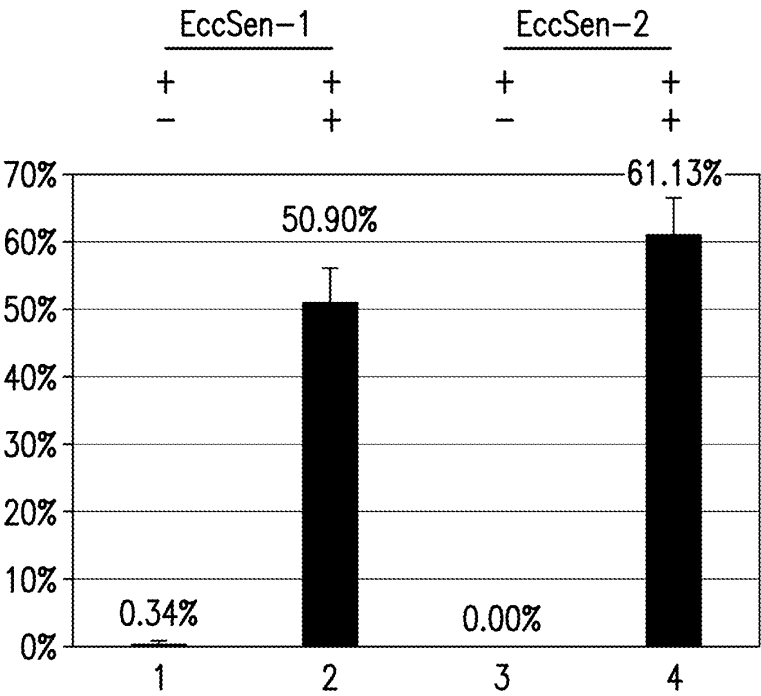
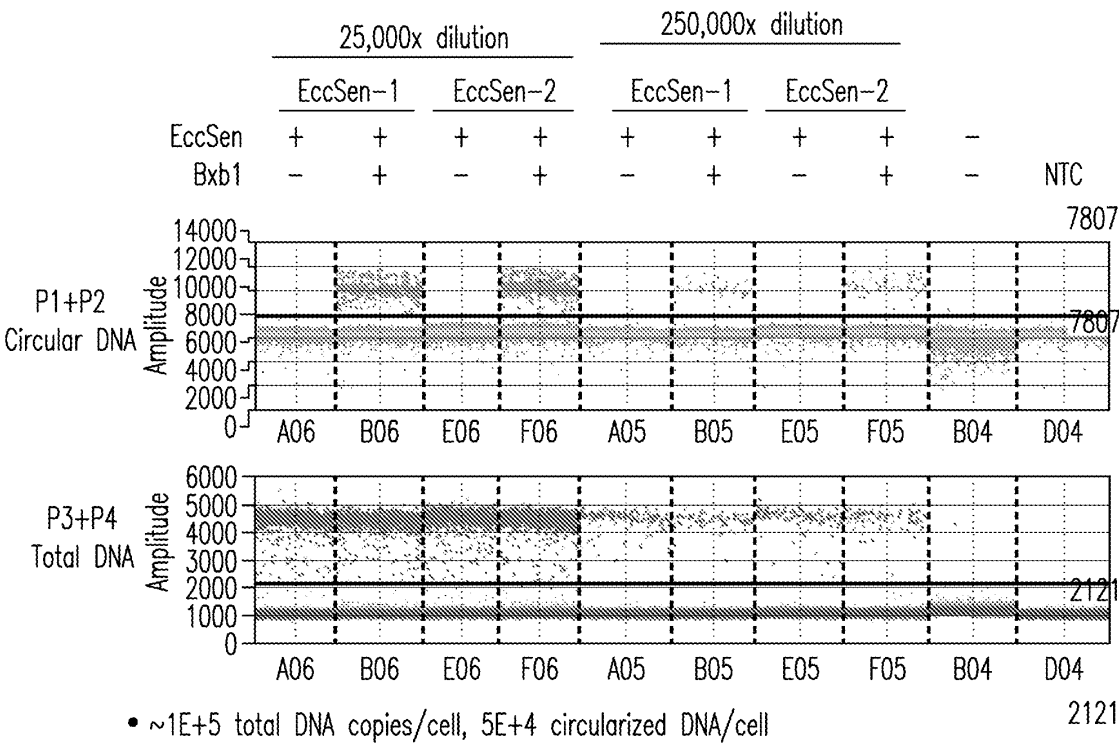


FIG.9

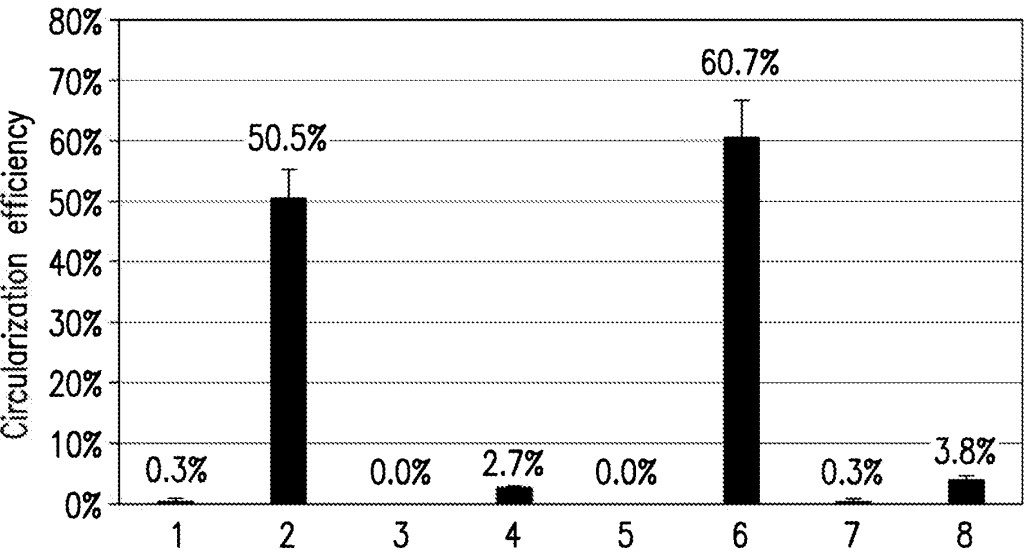
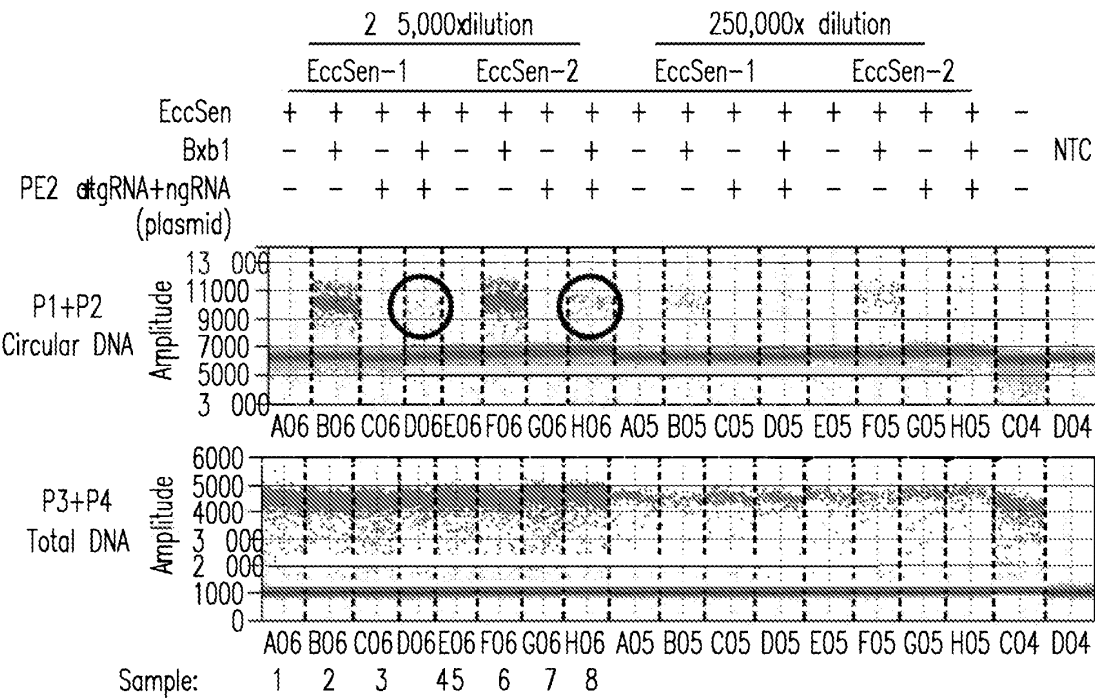


FIG.10

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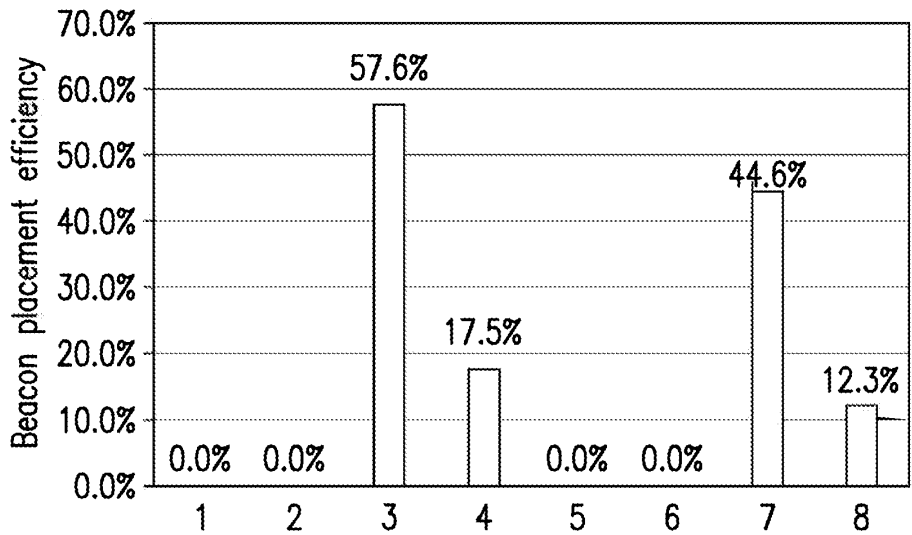
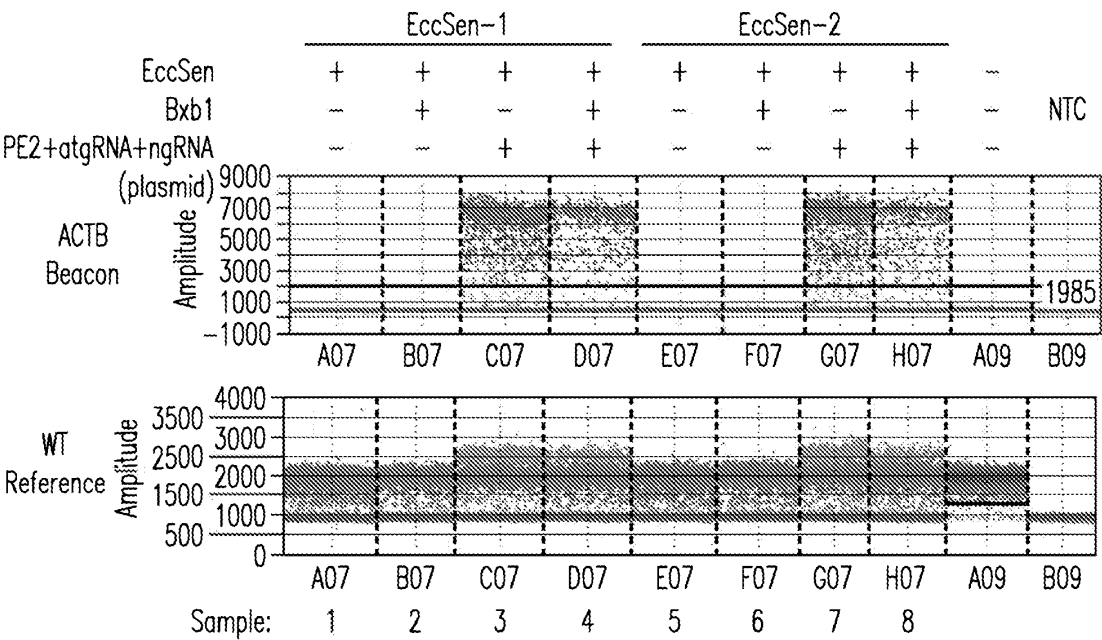


FIG.11

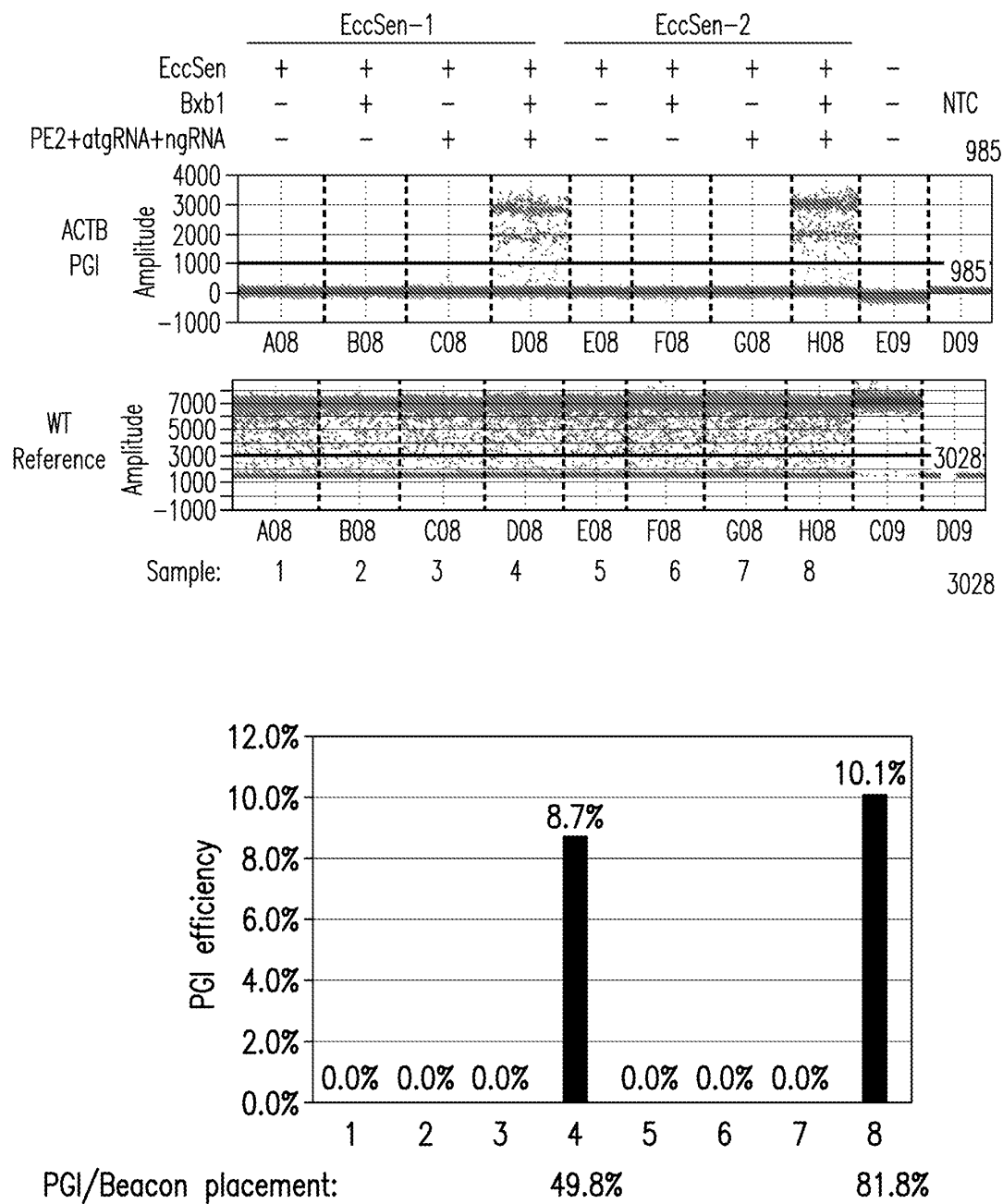


FIG.12

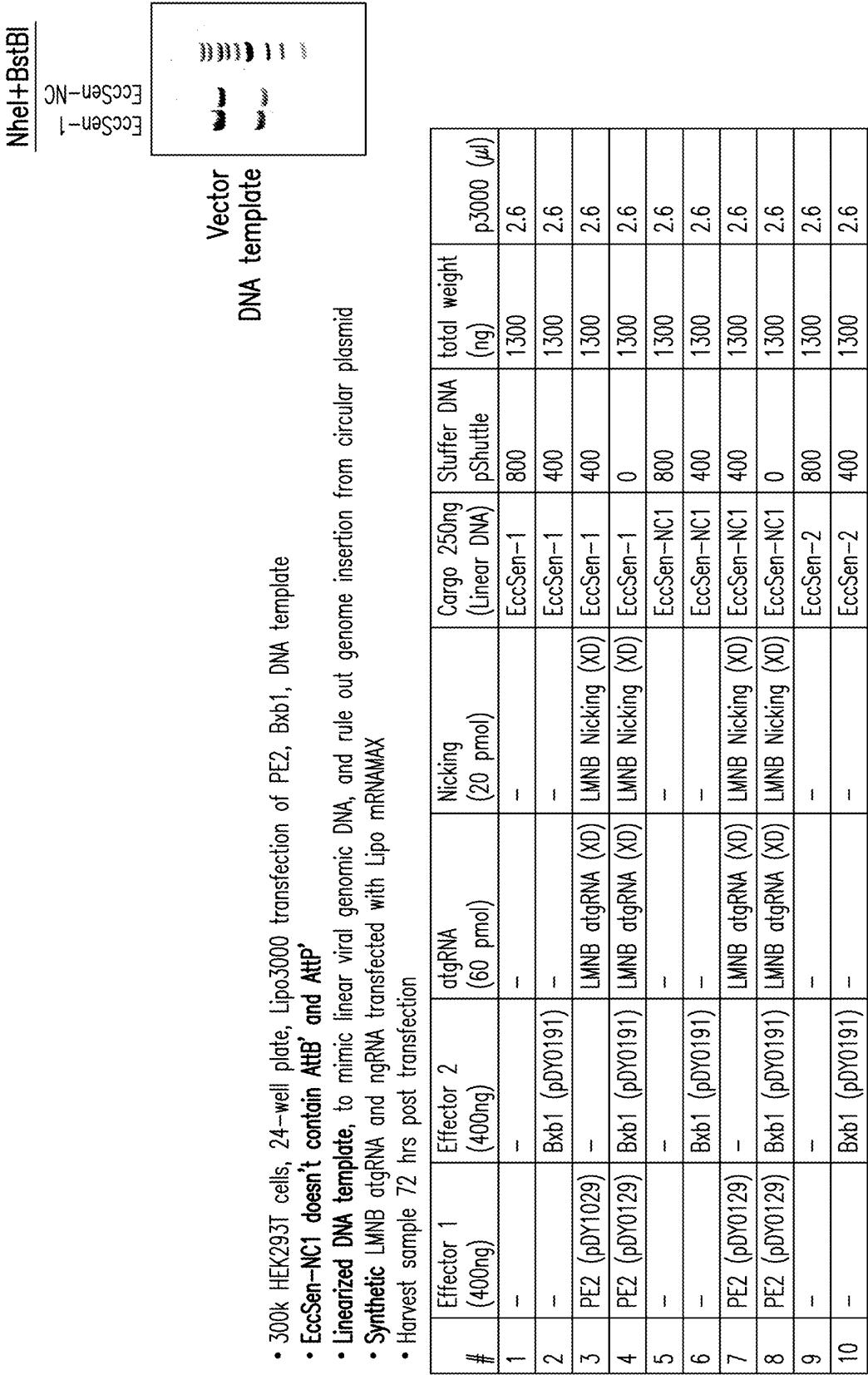


FIG.13

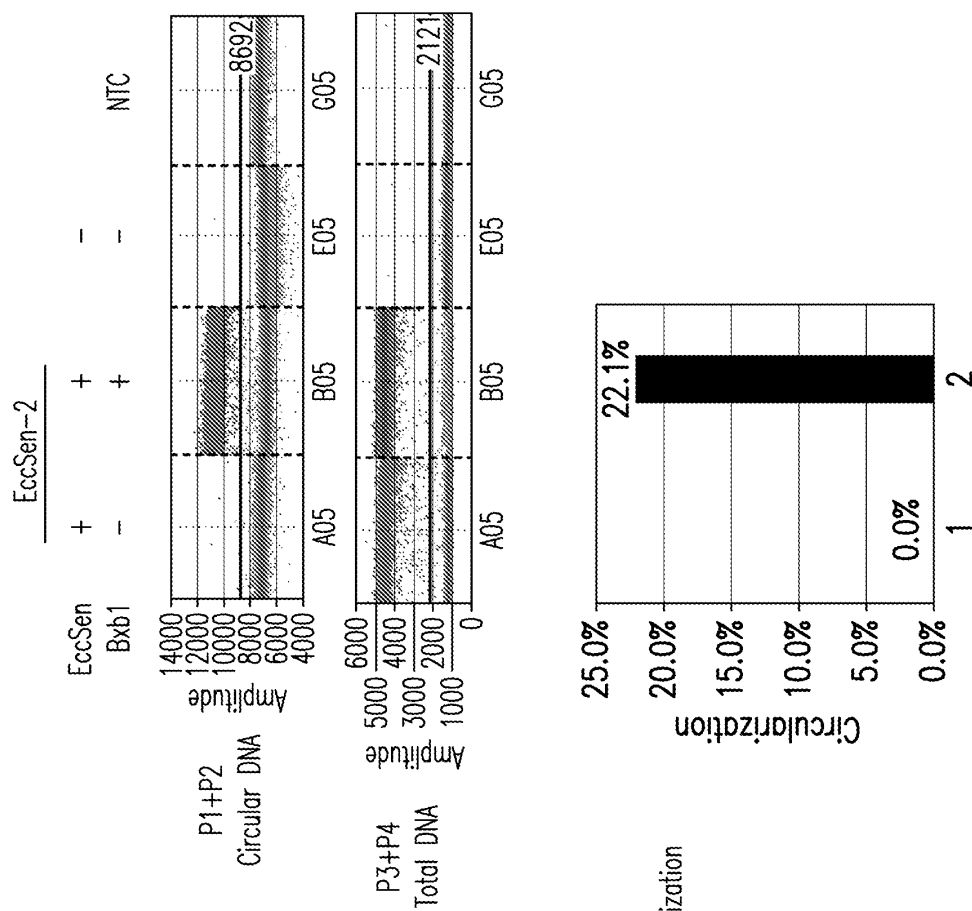
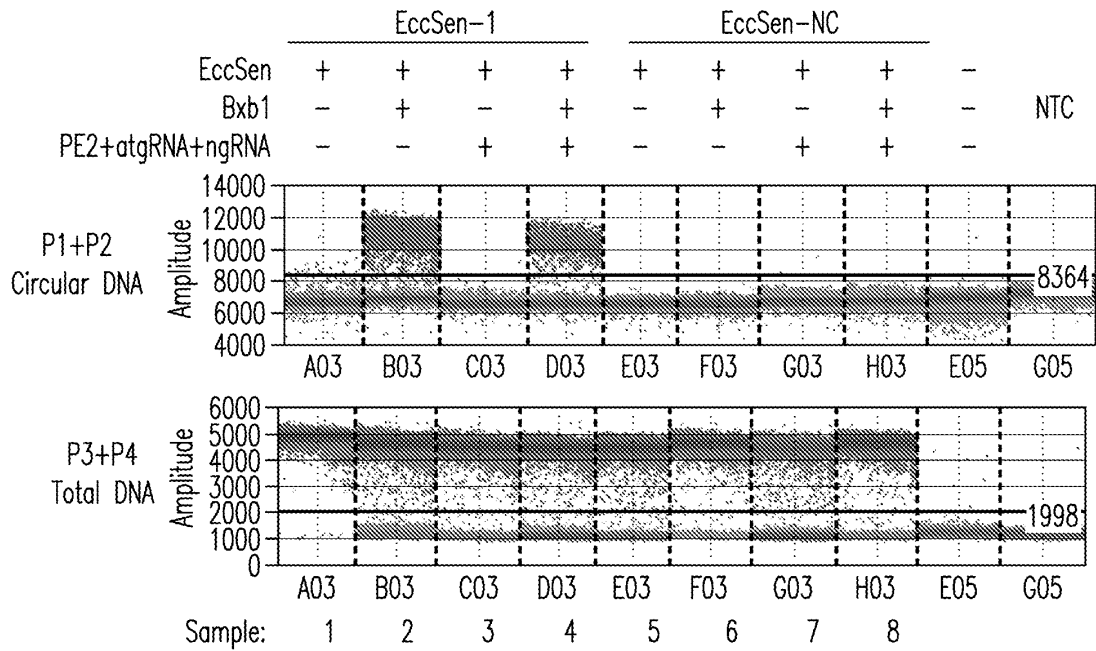


FIG. 14

- GFP expression was activated by EF1a promoter after circularization



- Circularization efficiency of linear DNA template is lower than circular plasmid format
- In the presence of PE2+atgRNA, circularization efficiency decreased by 50%, less significant than plasmid form atgRNA

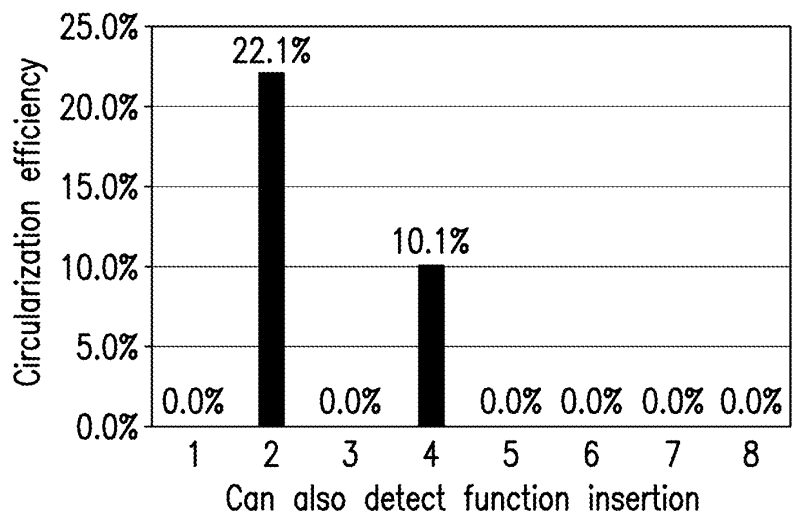
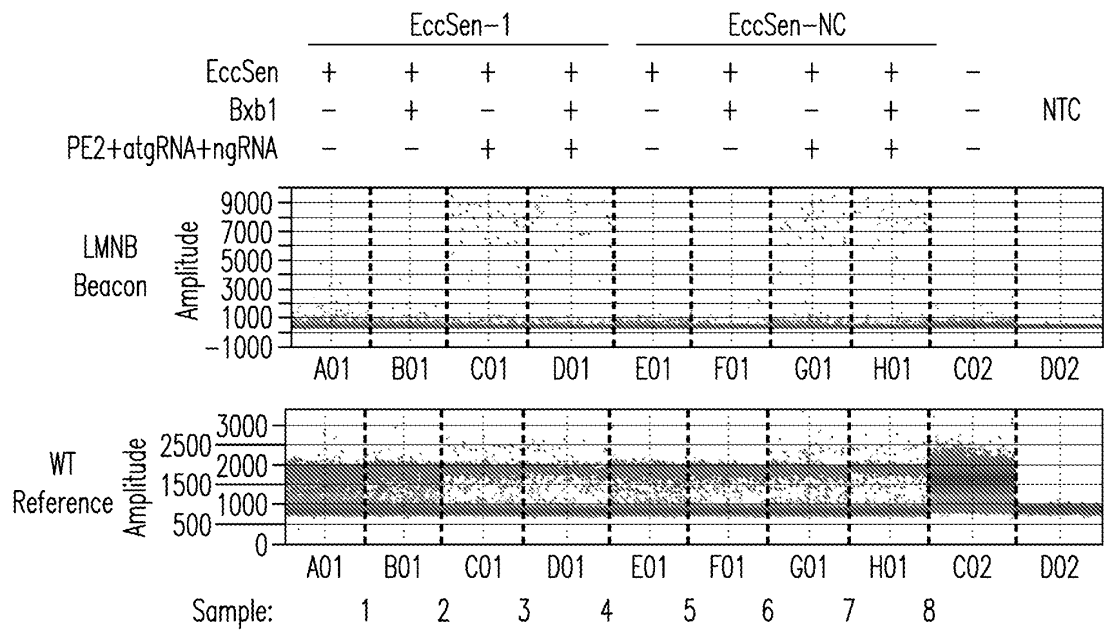


FIG.15

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- Low beacon placement efficiency from synthetic atgRNA transfection
- No significant interactions between atgRNA AttB and DNA donor AttP

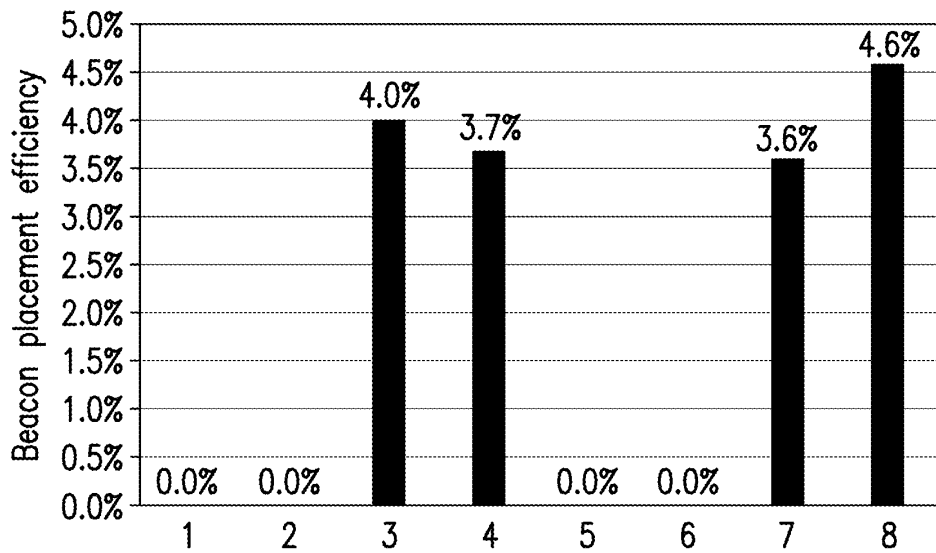
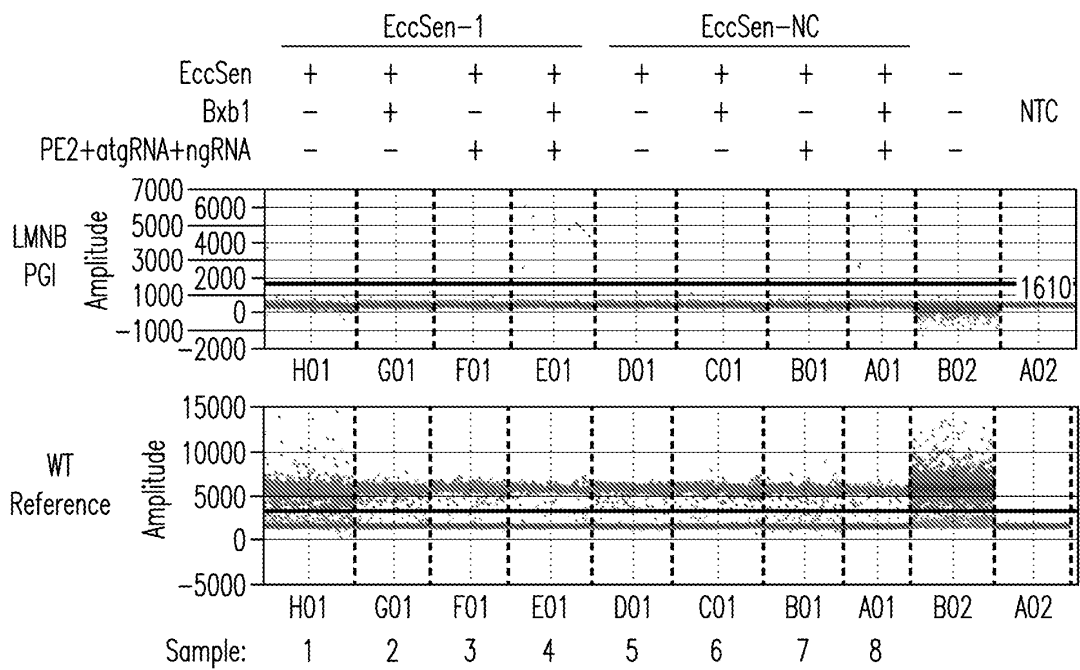


FIG.16

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- Genome insertion with circular DNA donor is ~1 fold higher than linear one

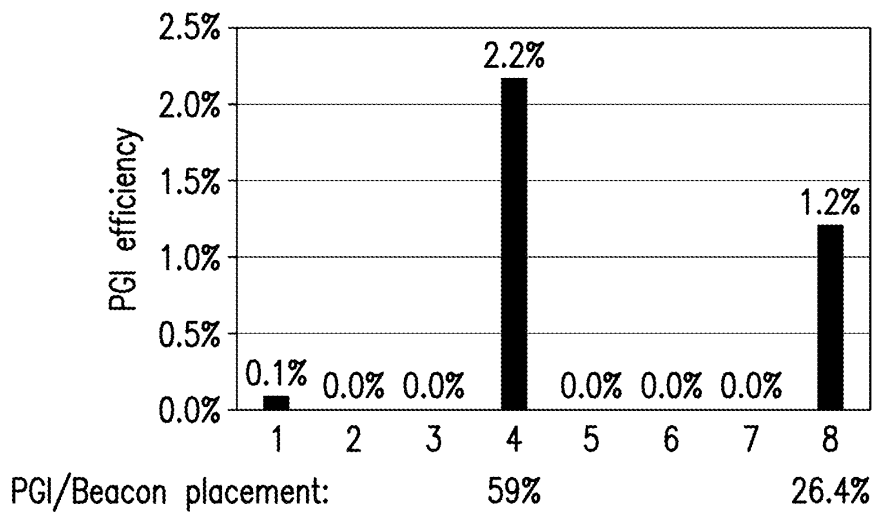


FIG.17

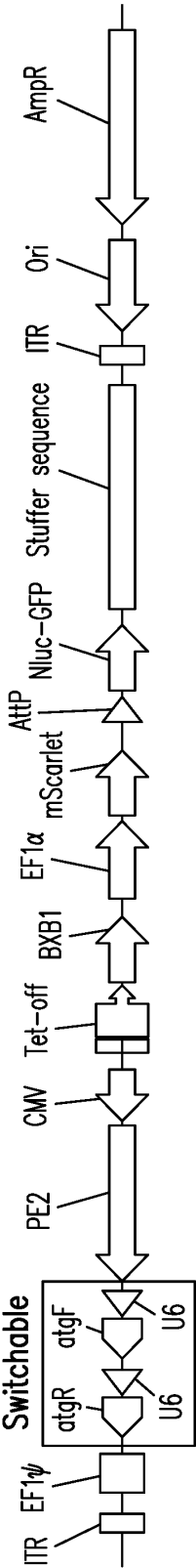


FIG.18

RFP expression on Day 2
post transfection

A10 C5

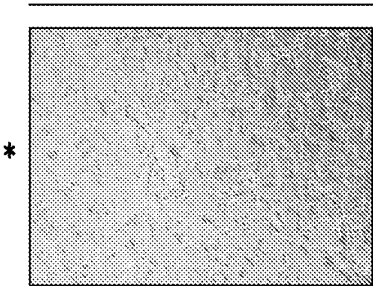


FIG.19A

RFP expression on Day 2
post transfection

A10 C5

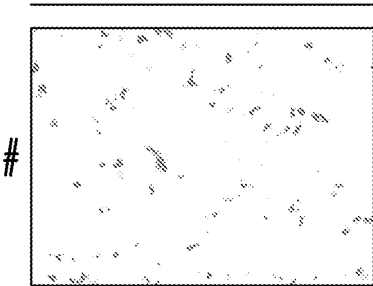


FIG.19B

RFP expression on Day 2
post transfection

A10 C5



FIG.19C

RFP expression on Day 2
post transfection

A10 C5

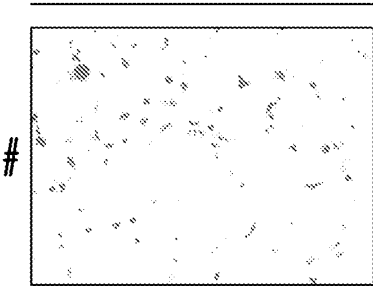


FIG.19D

RFP expression on Day 2
post transfection

AIO C8

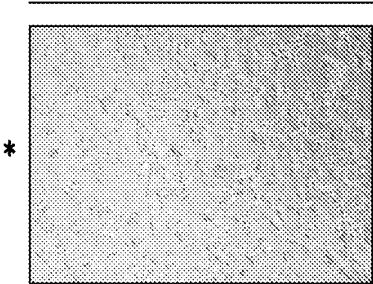


FIG.19E

RFP expression on Day 2
post transfection

AIO C8

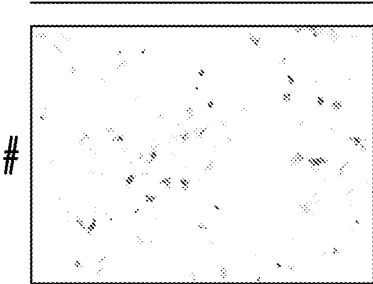


FIG.19F

RFP expression on Day 2
post transfection

AIO C8

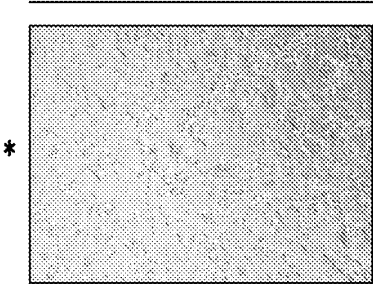


FIG.19G

RFP expression on Day 2
post transfection

AIO C8

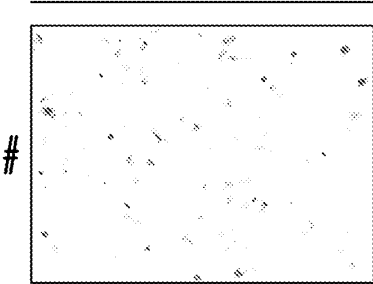


FIG.19H

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RFP expression on Day 2
post transfection

NC

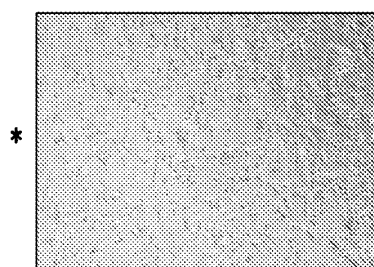


FIG. 19I

RFP expression on Day 2
post transfection

NC



FIG. 19J

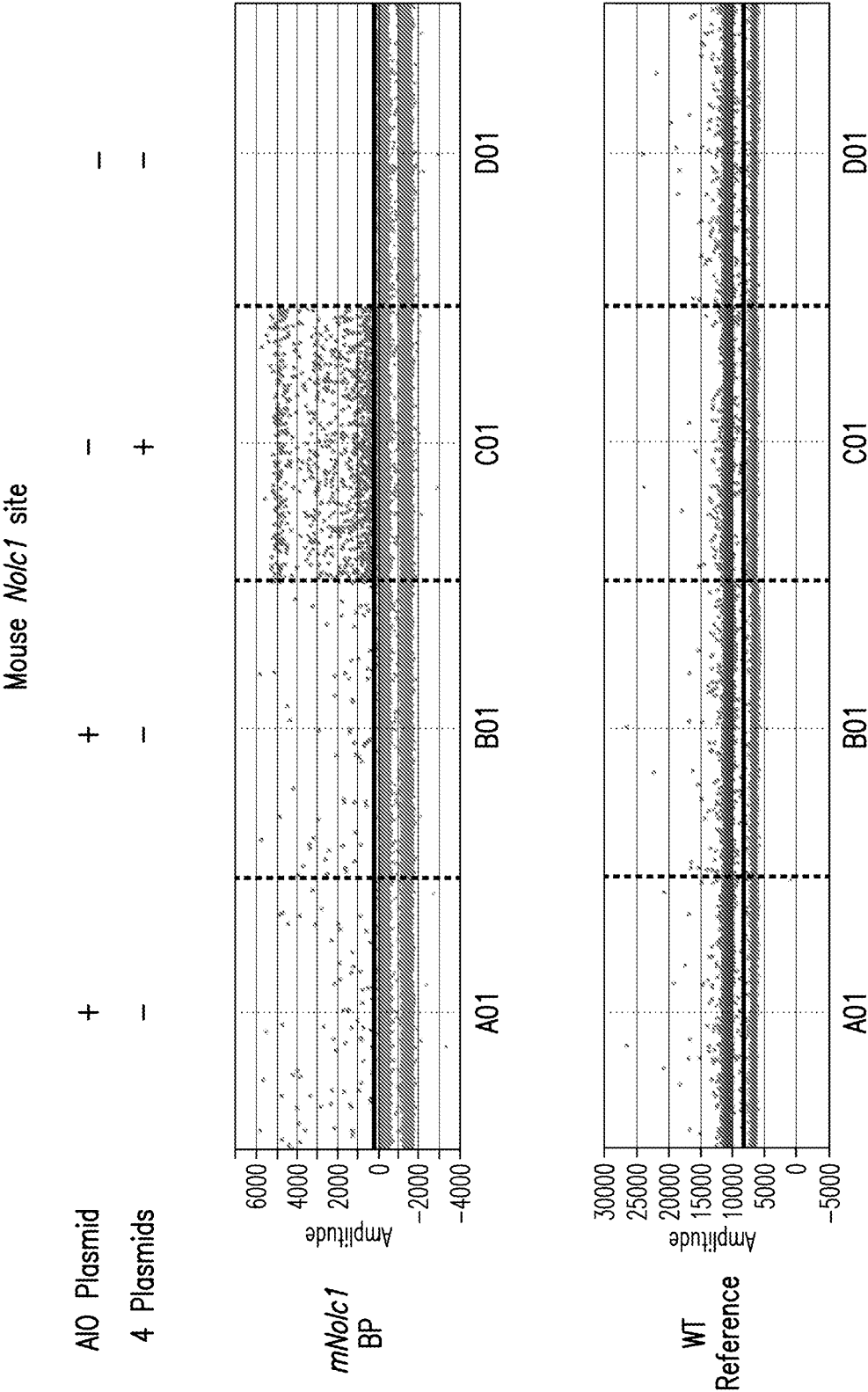


FIG.20A

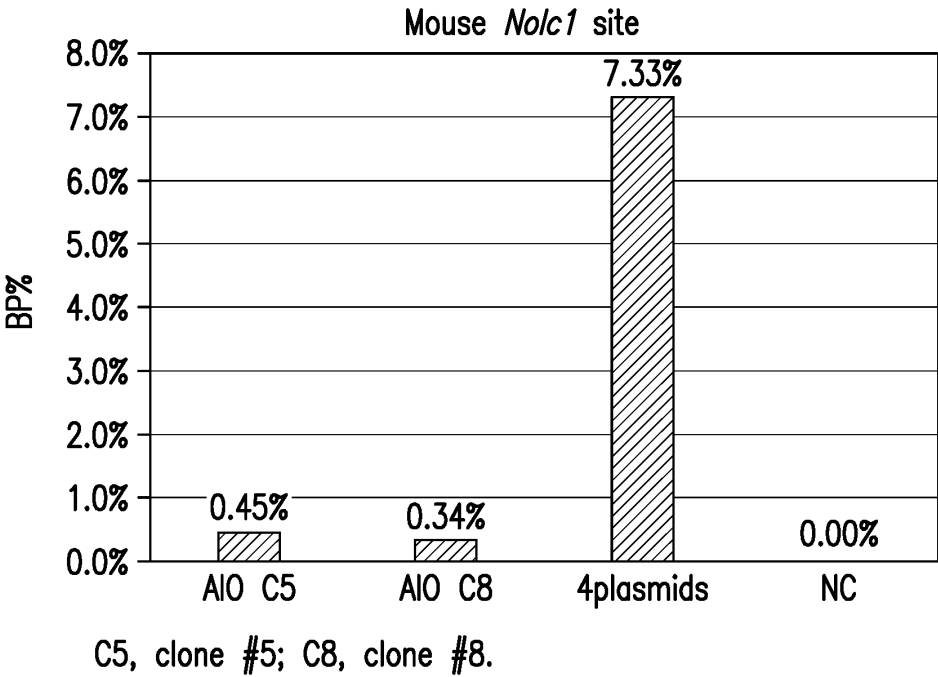


FIG.20B

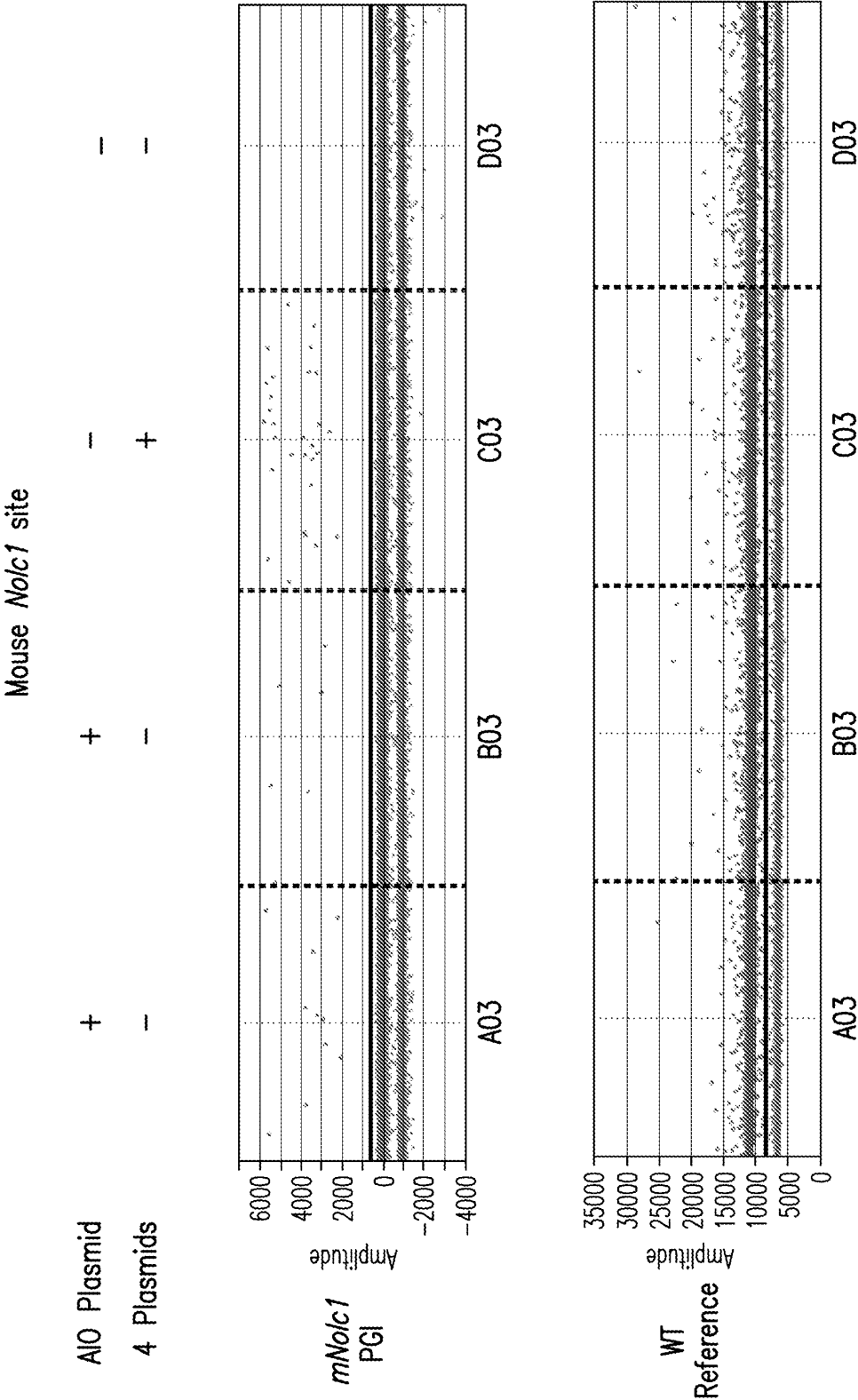


FIG.21A

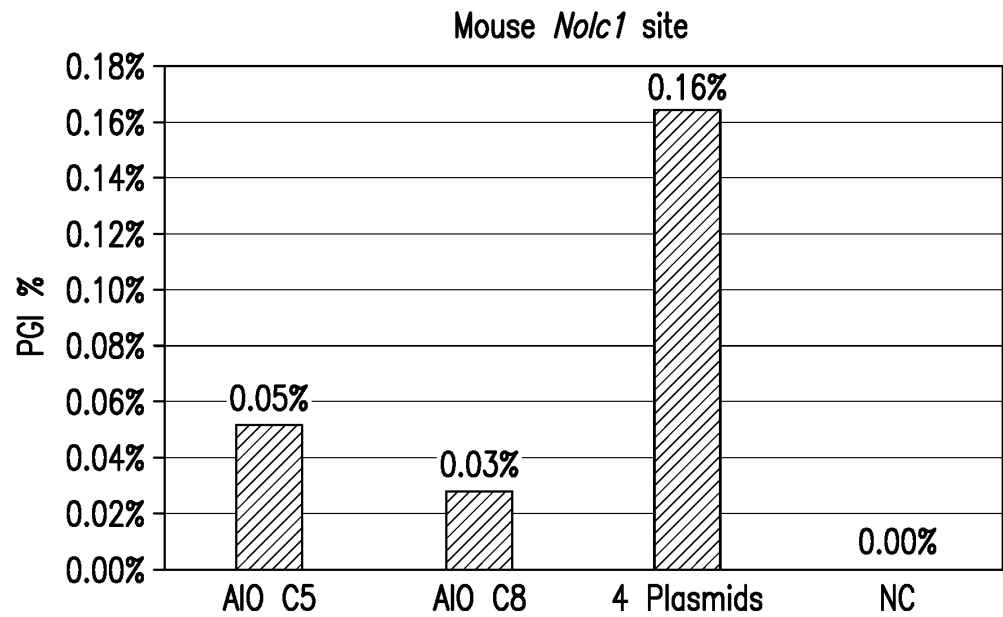


FIG.21B

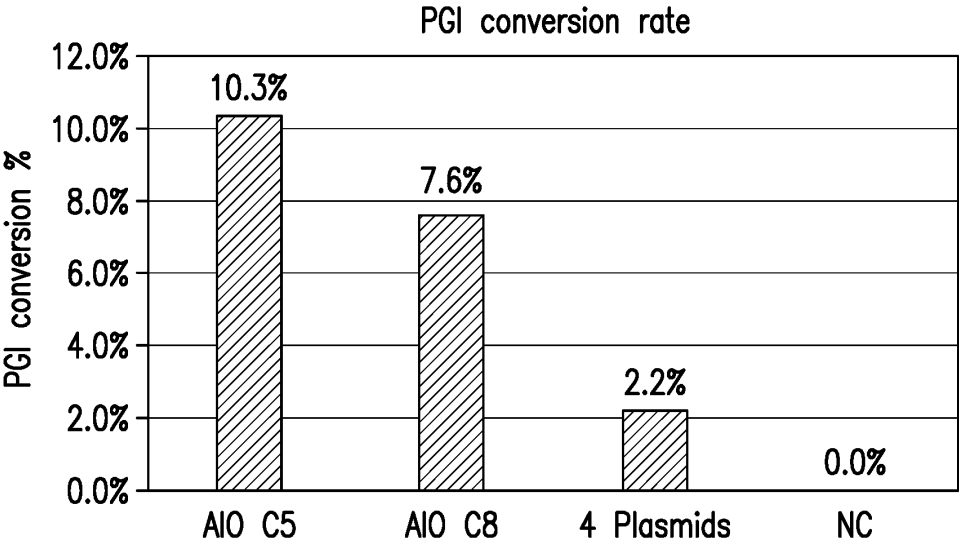


FIG.22

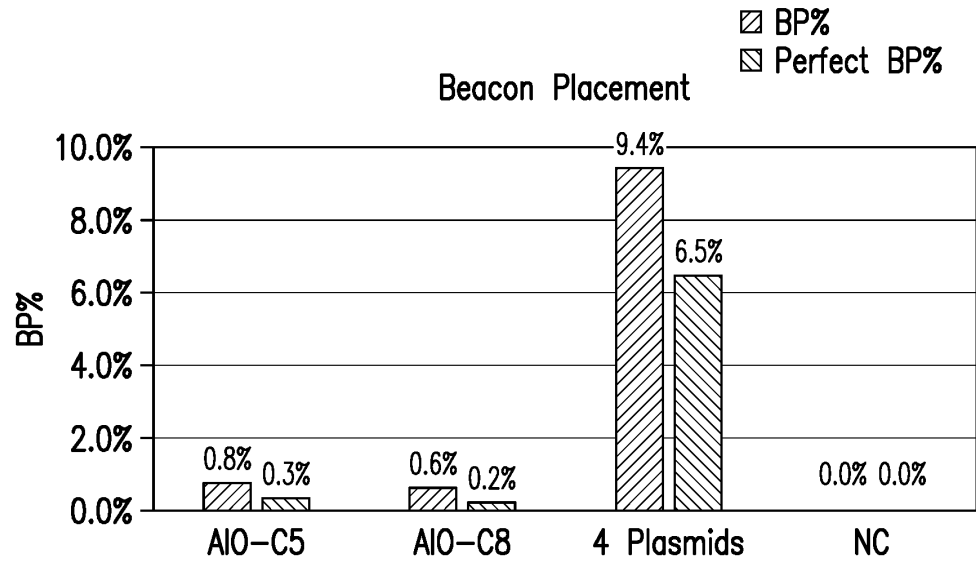


FIG.23A

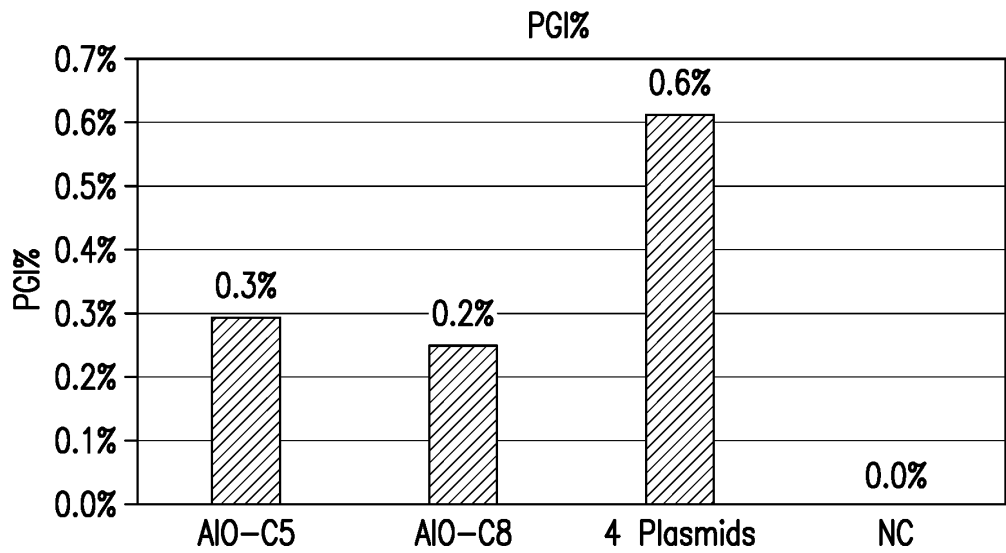


FIG.23B

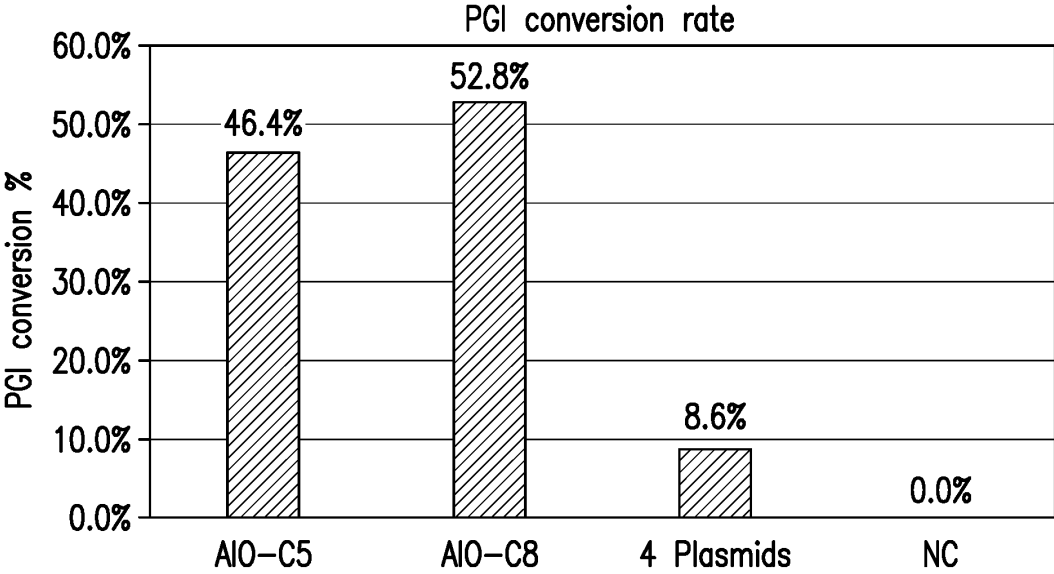


FIG.24

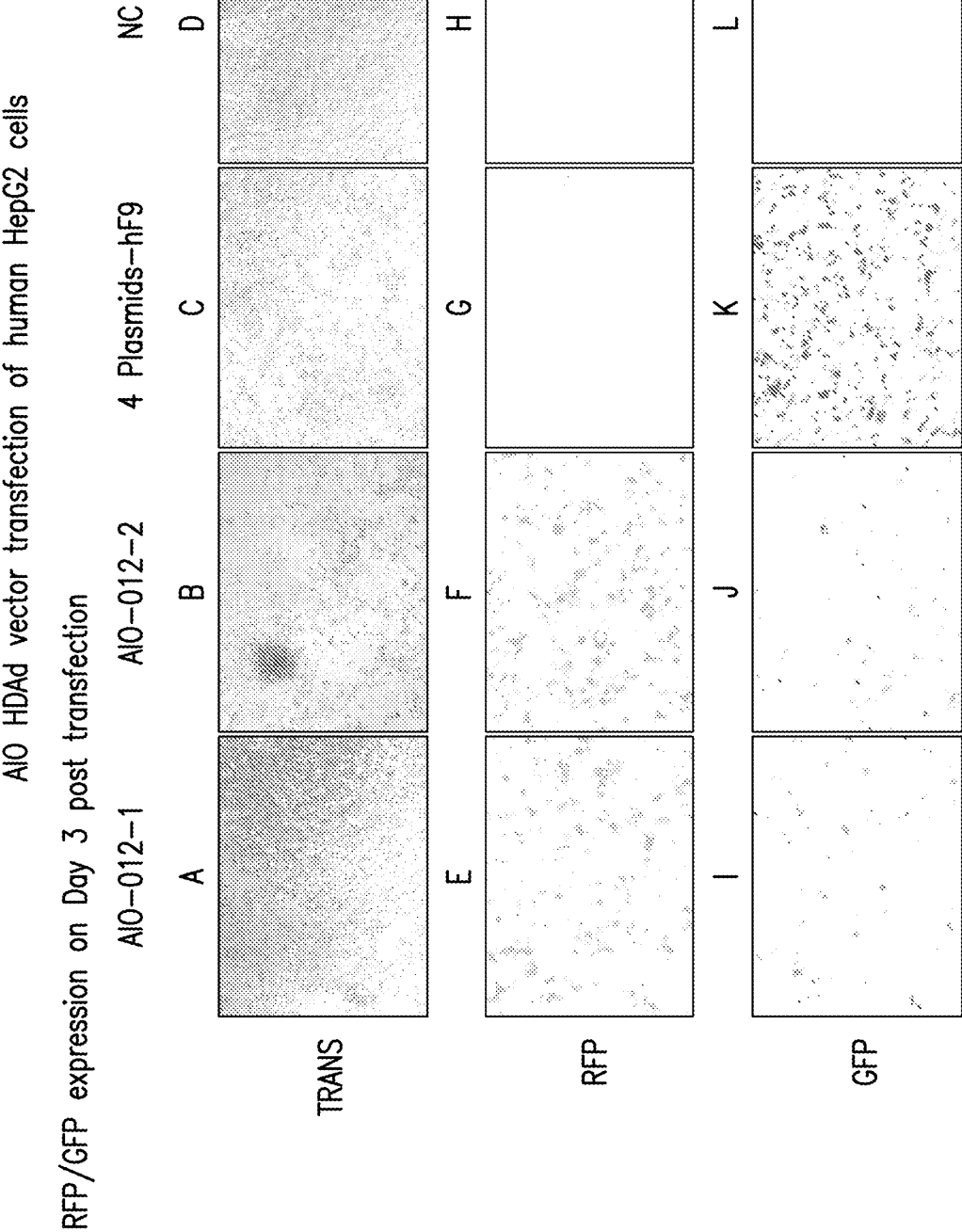


FIG.25

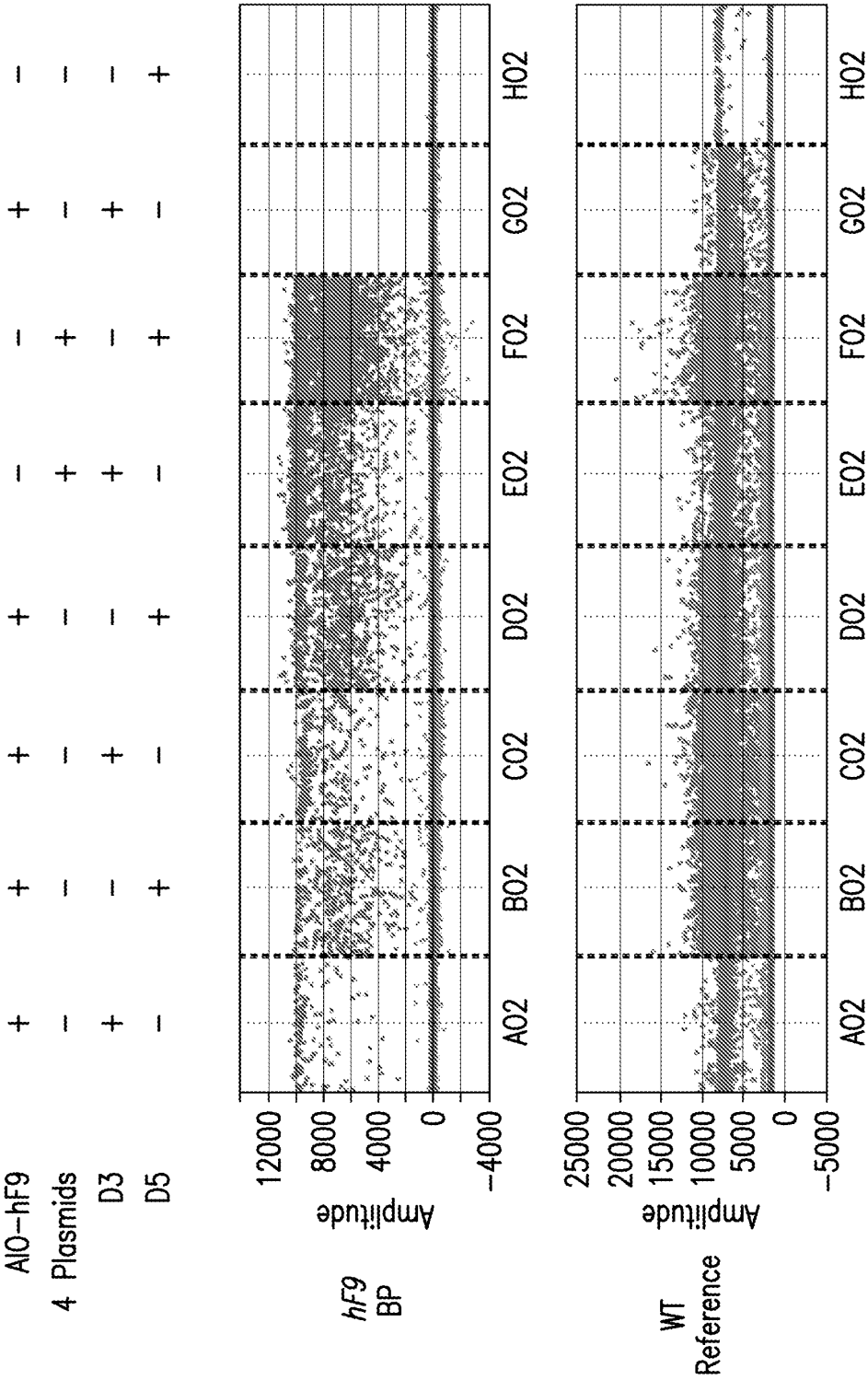


FIG.26A

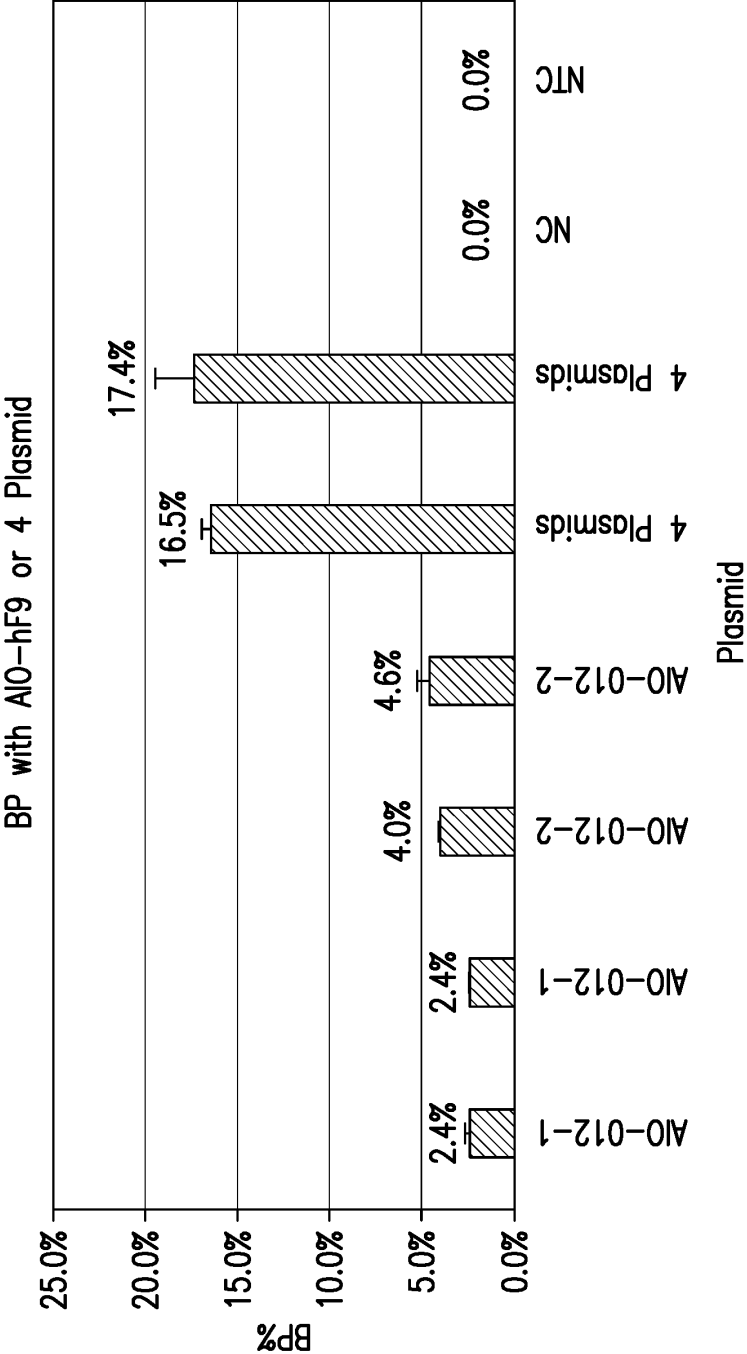


FIG.26B

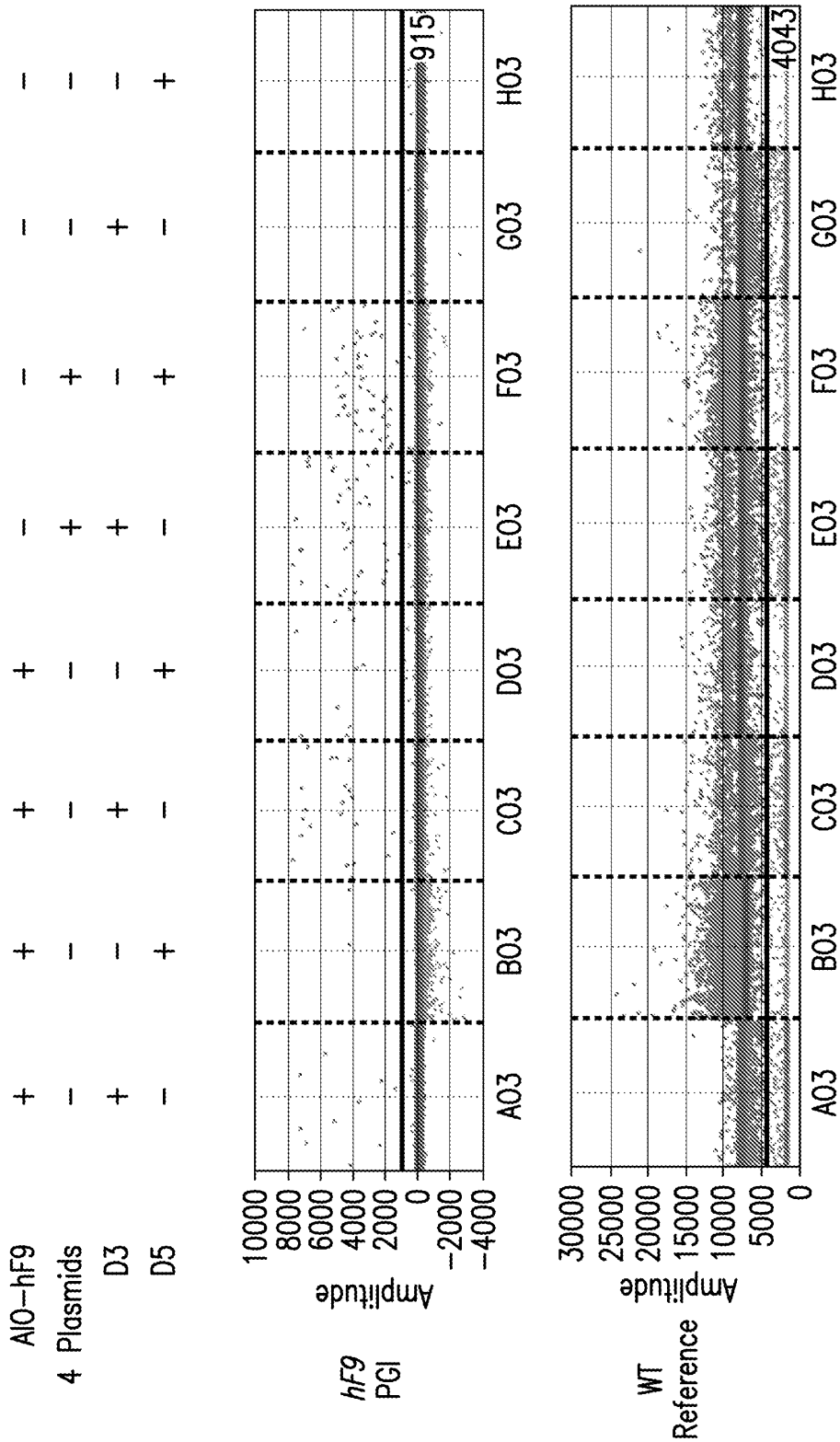


FIG.27A

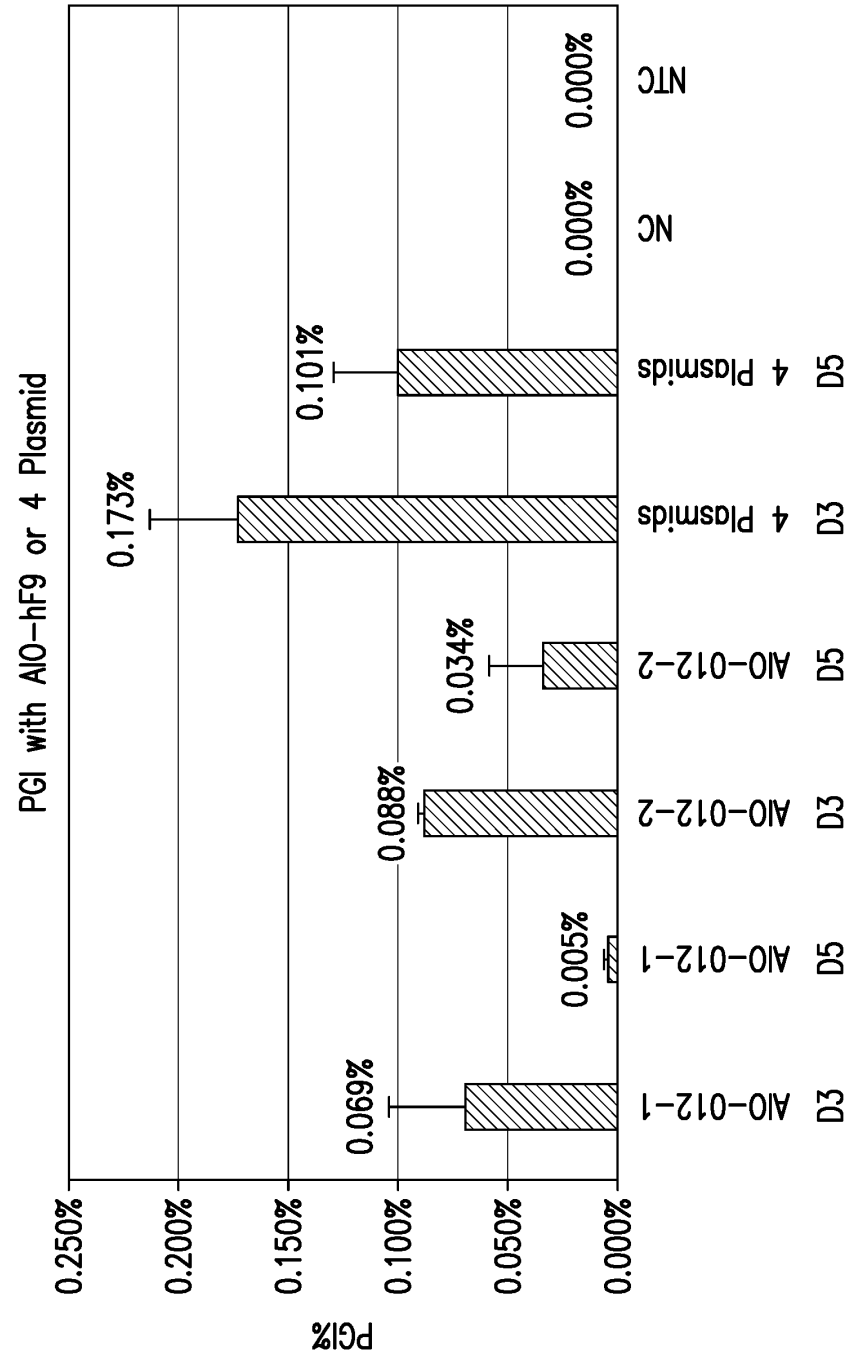


FIG.27B