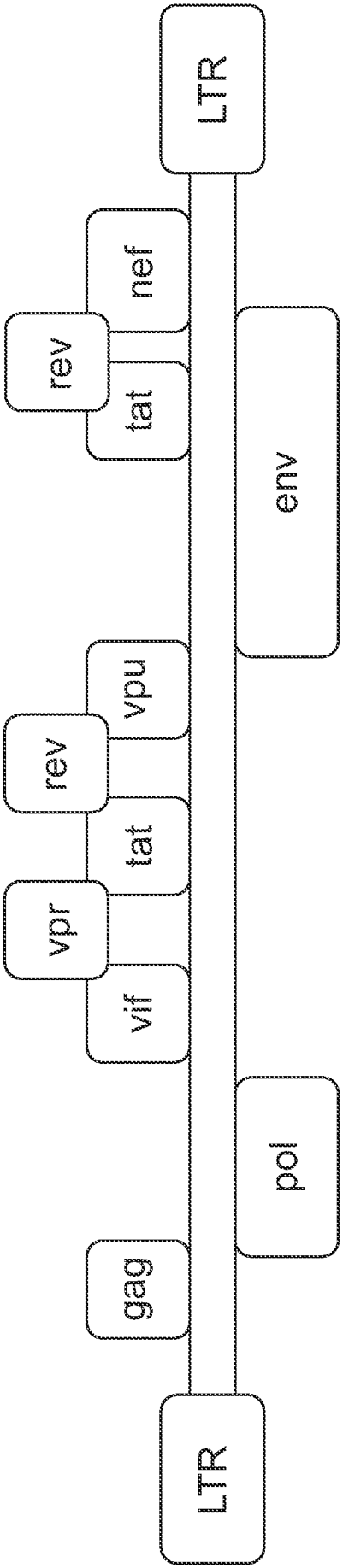


FIG. 1

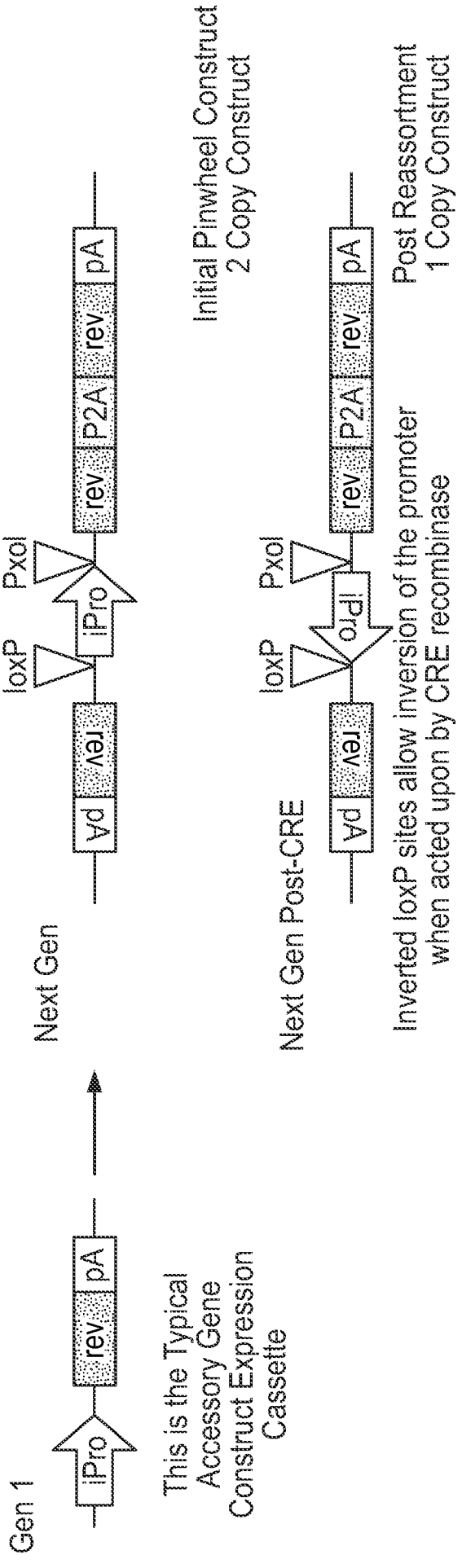
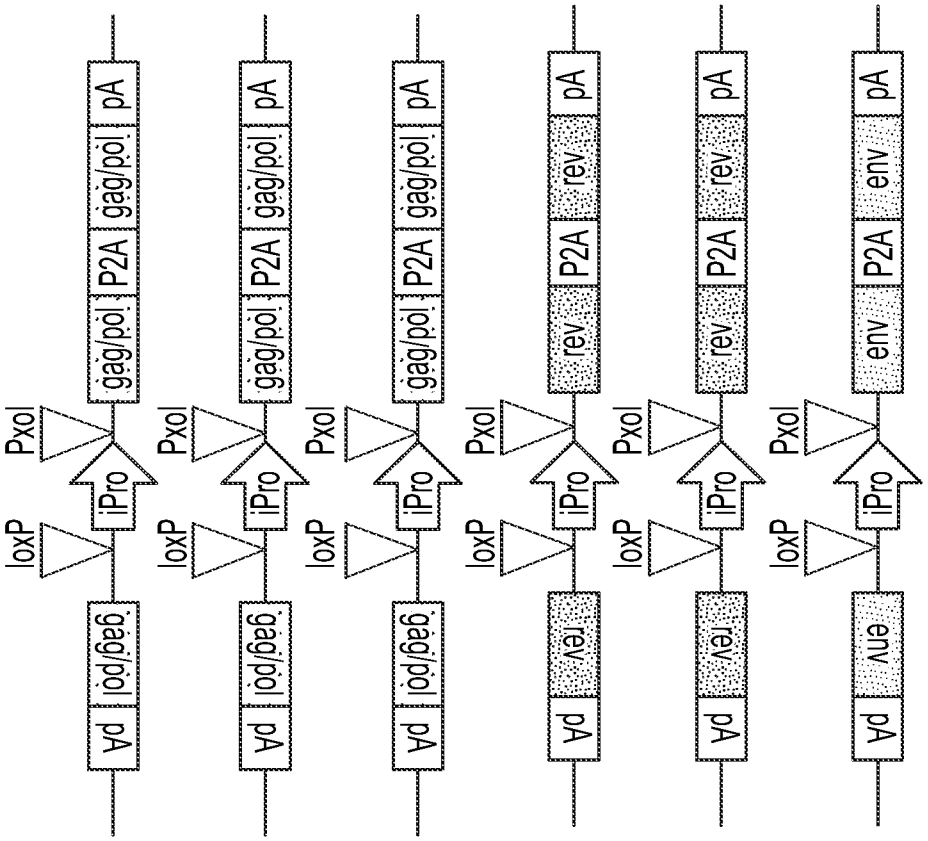

Gen 1
iPro
rev
pA
This is the Typical Accessory Gene Construct Expression Cassette
Next Gen
loxP
iPro
Pxol
pA
rev
rev
P2A
rev
pA
Initial Pinwheel Construct 2 Copy Construct
Next Gen Post-CRE
loxP
iPro
Pxol
pA
rev
rev
P2A
rev
pA
Inverted loxP sites allow inversion of the promoter when acted upon by CRE recombinase
Post Reassortment 1 Copy Construct

FIG. 2



Generation	genome	gag/pol	rev	env	titer	
1	fixed	6	4	2	1e7	Initial
2	fixed	5	3	2	8e6	
2	fixed	4	2	1	3e7	
2	fixed	3	3	2	5e7	Optimized
2	fixed	3	4	1	4e7	
2	fixed	3	3	1	7e7	Optimized

FIG. 3

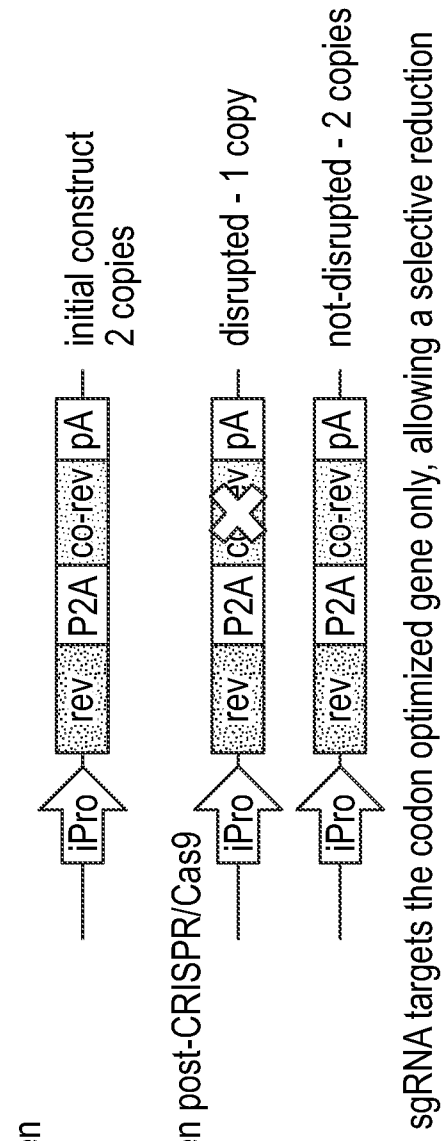

Gen 1
iPro
rev
pA
This is the typical accessory gene construct expression cassette
Next Gen
Next Gen post-CRISPR/Cas9
iPro
rev
P2A
co-rev
pA
initial construct
2 copies
iPro
rev
P2A
co-rev
pA
disrupted - 1 copy
iPro
rev
P2A
co-rev
pA
not-disrupted - 2 copies
sgRNA targets the codon optimized gene only, allowing a selective reduction

FIG. 4

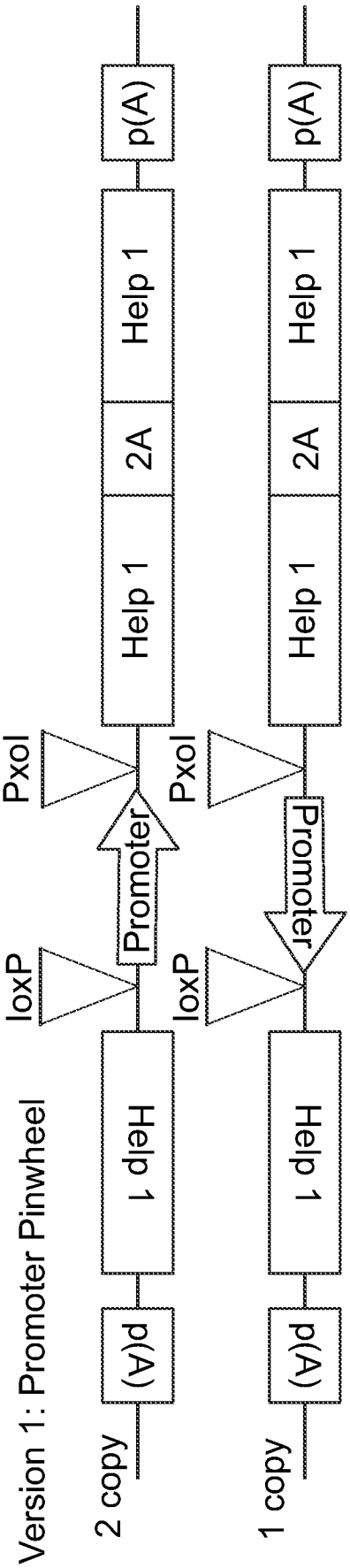

Version 1: Promoter Pinwheel
2 copy
1 copy
p(A)
Help 1
loxP
Promoter
Pxol
2A
Help 1
p(A)
Help 1
loxP
Promoter
Pxol
2A
Help 1
p(A)

FIG. 5

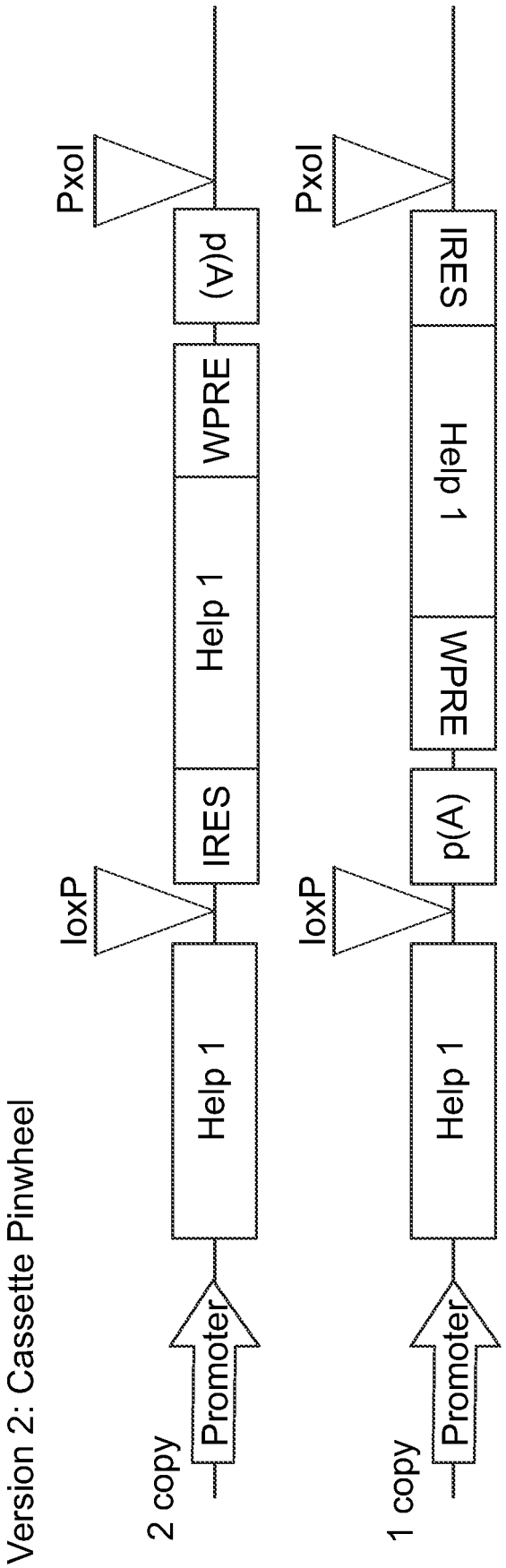

Version 2: Cassette Pinwheel
2 copy
Promoter
Help 1
loxP
IRES
Help 1
WPRE
p(A)
Pxol
1 copy
Promoter
Help 1
loxP
p(A)
WPRE
Help 1
IRES
Pxol

FIG. 6

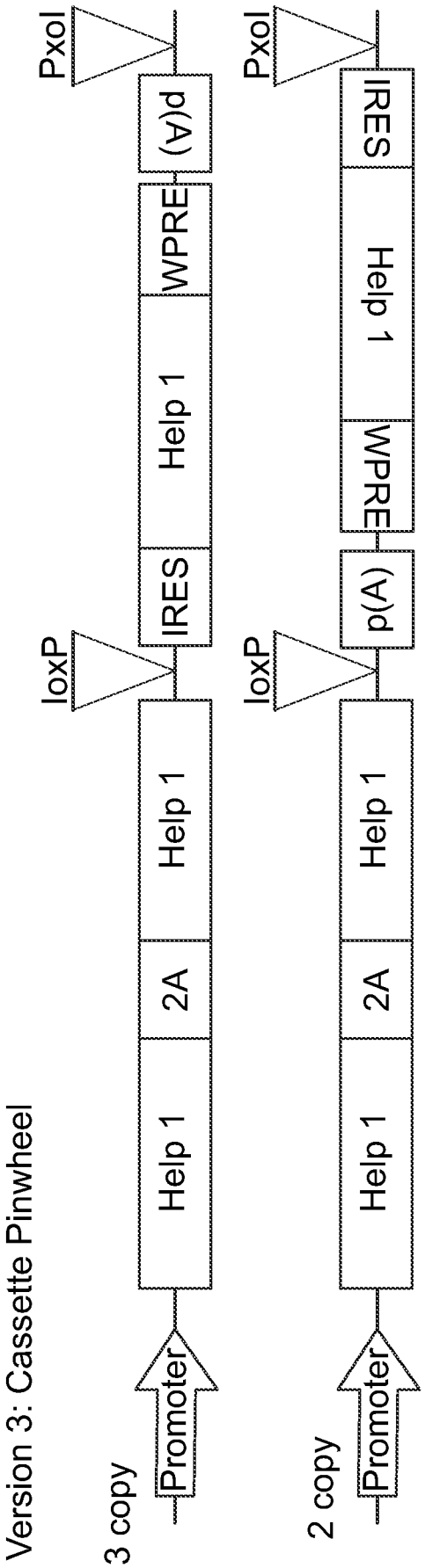

Version 3: Cassette Pinwheel
3 copy
Promoter
Help 1
2A
Help 1
loxP
IRES
Help 1
WPRE
p(A)
PxoI
2 copy
Promoter
Help 1
2A
Help 1
loxP
p(A)
WPRE
Help 1
IRES
PxoI

FIG. 7

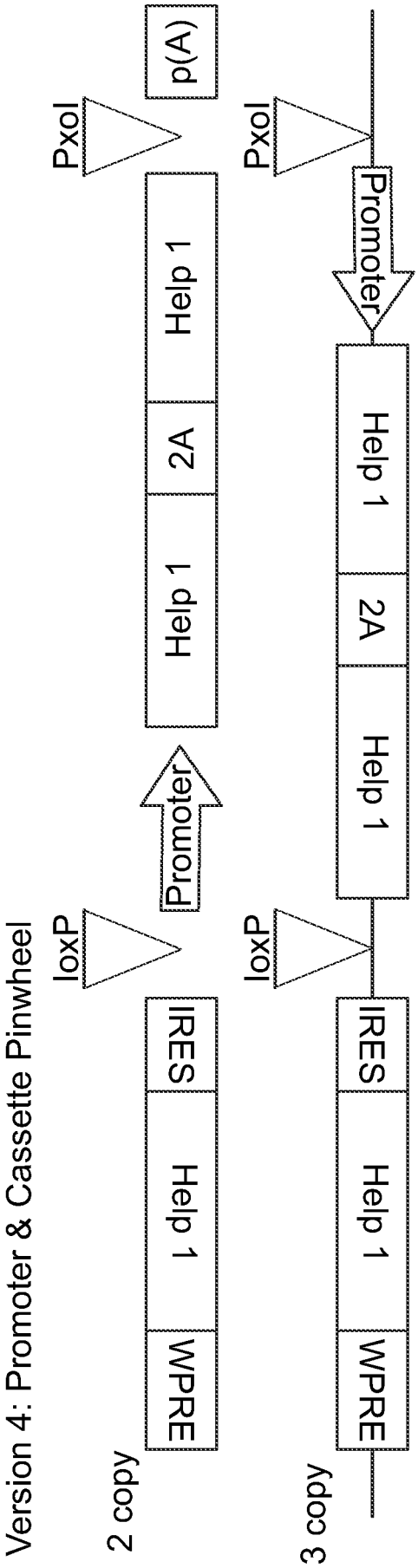

Version 4: Promoter & Cassette Pinwheel
2 copy
WPRE
Help 1
IRES
loxP
Promoter
Help 1
2A
Help 1
Pxol
p(A)
3 copy
WPRE
Help 1
IRES
loxP
Promoter
Help 1
2A
Help 1
Pxol

FIG. 8

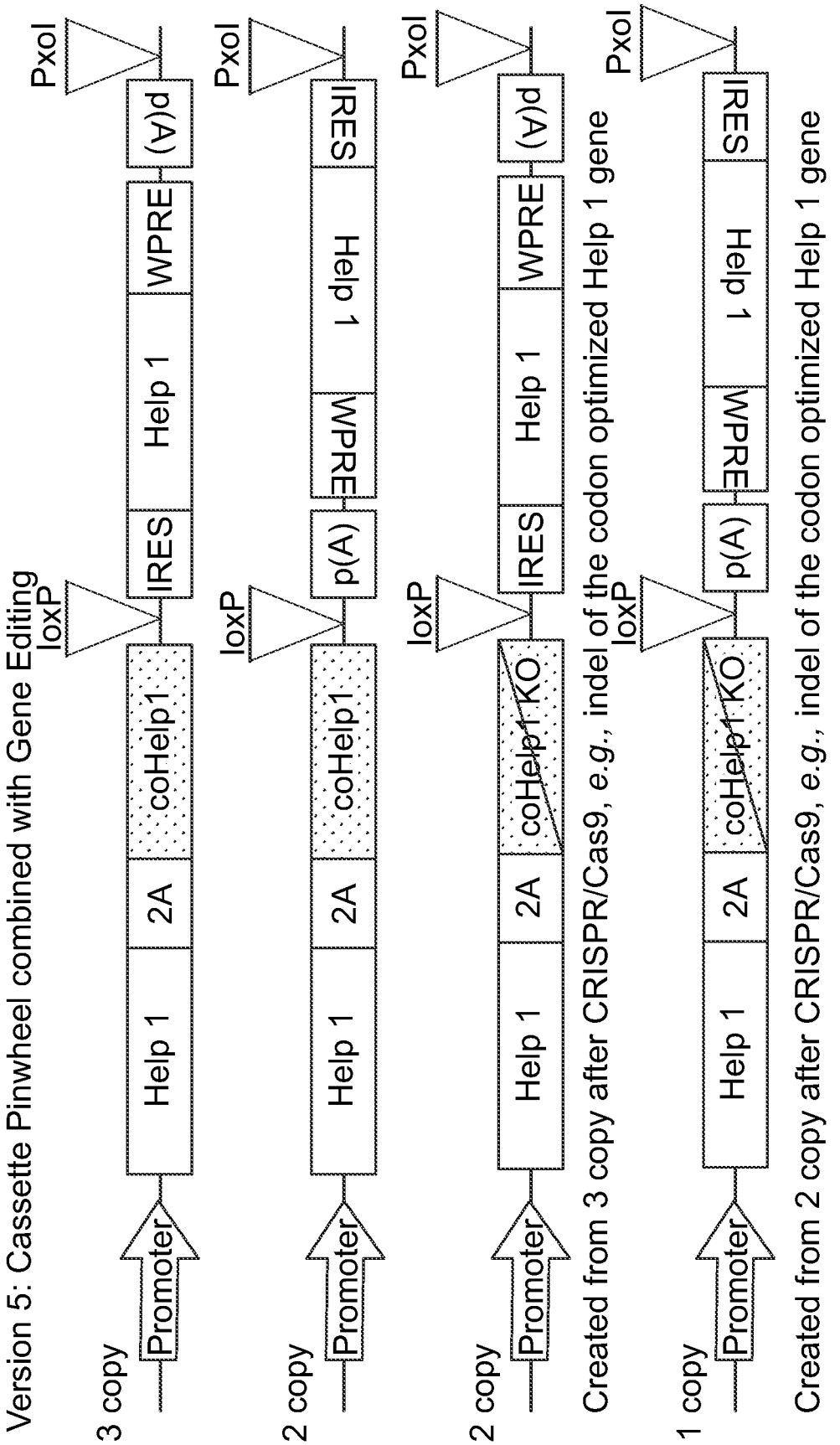

Version 5: Cassette Pinwheel combined with Gene Editing
3 copy
Promoter
Help 1
2A
coHelp1
loxP
IRES
Help 1
WPRE
p(A)
PxoI
2 copy
Promoter
Help 1
2A
coHelp1
loxP
p(A)
WPRE
Help 1
IRES
PxoI
Created from 3 copy after CRISPR/Cas9, e.g., indel of the codon optimized Help 1 gene
2 copy
Promoter
Help 1
2A
coHelp1 KO
loxP
IRES
Help 1
WPRE
p(A)
PxoI
1 copy
Promoter
Help 1
2A
coHelp1 KO
loxP
p(A)
WPRE
Help 1
IRES
PxoI
Created from 2 copy after CRISPR/Cas9, e.g., indel of the codon optimized Help 1 gene

FIG. 9