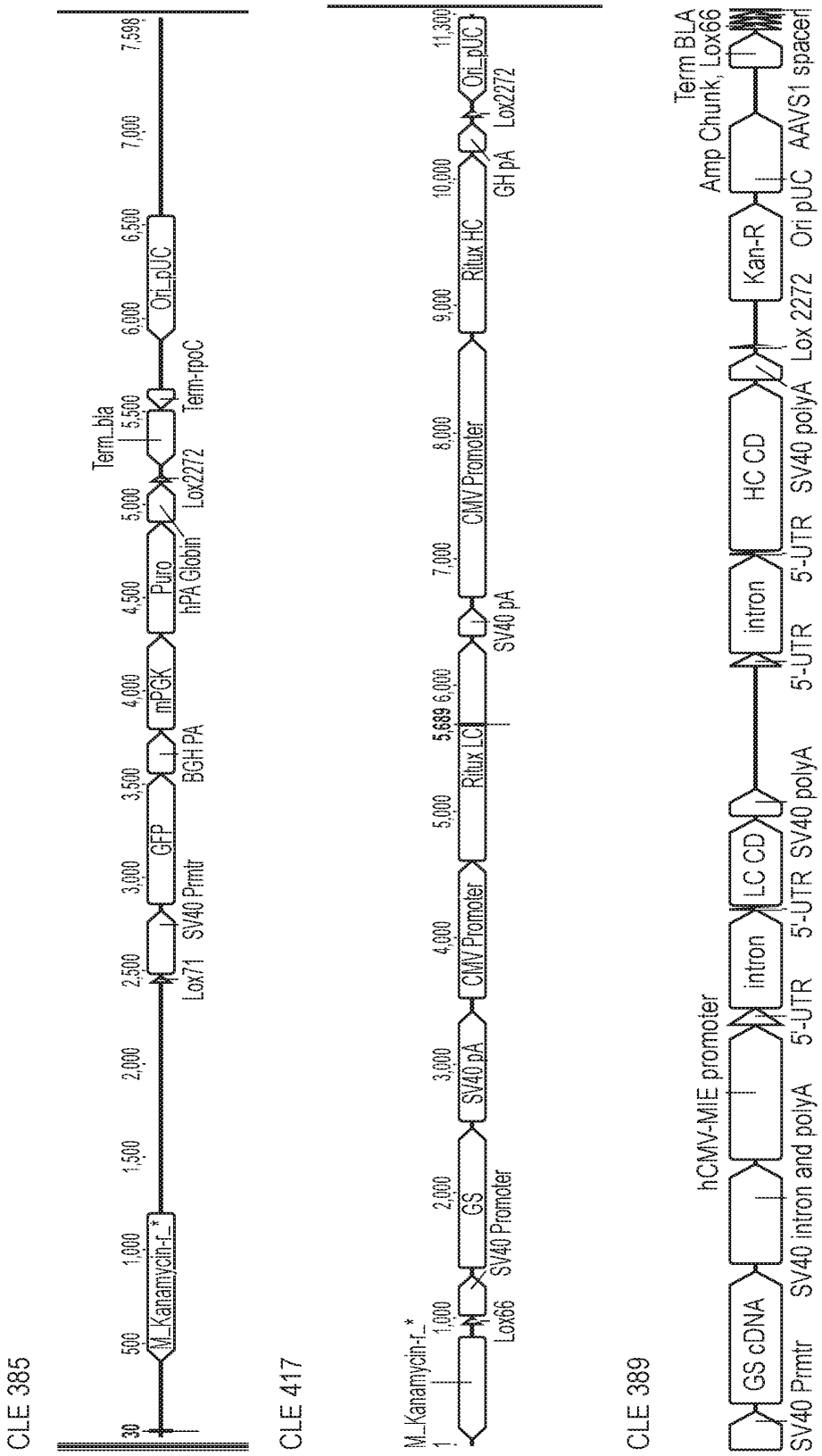

CLE 385
30
500
1,000
1,500
2,000
2,500
3,000
3,500
4,000
4,500
5,000
5,500
6,000
6,500
7,000
7,598
M_Kanamycin-r_*
Lox71
SV40 Prmtr
GFP
BGH PA
mPGK
Puro
hPA Globin
Lox2272
Term_bla
Ori_pUC
Term-rpoC
CLE 417
M_Kanamycin-r_*
1
1,000
2,000
3,000
4,000
5,000
5,689
6,000
7,000
8,000
9,000
10,000
11,300
Loox66
GS
SV40 Promoter
SV40 pA
CMV Promoter
Rilux LC
SV40 pA
Rilux HC
GH pA
Lox2272
Ori_pUC
CLE 389
hCMV-MIE promoter
Term BLA
Amp Chunk, Lox66
SV40 Prmtr
GS cDNA
SV40 intron and polyA
5'-UTR
intron
5'-UTR SV40 polyA
LC CD
5'-UTR
intron
5'-UTR SV40 polyA
HC CD
Kan-R
Lox 2272
Ori pUC
AAVS1 spacer

FIG. 1A

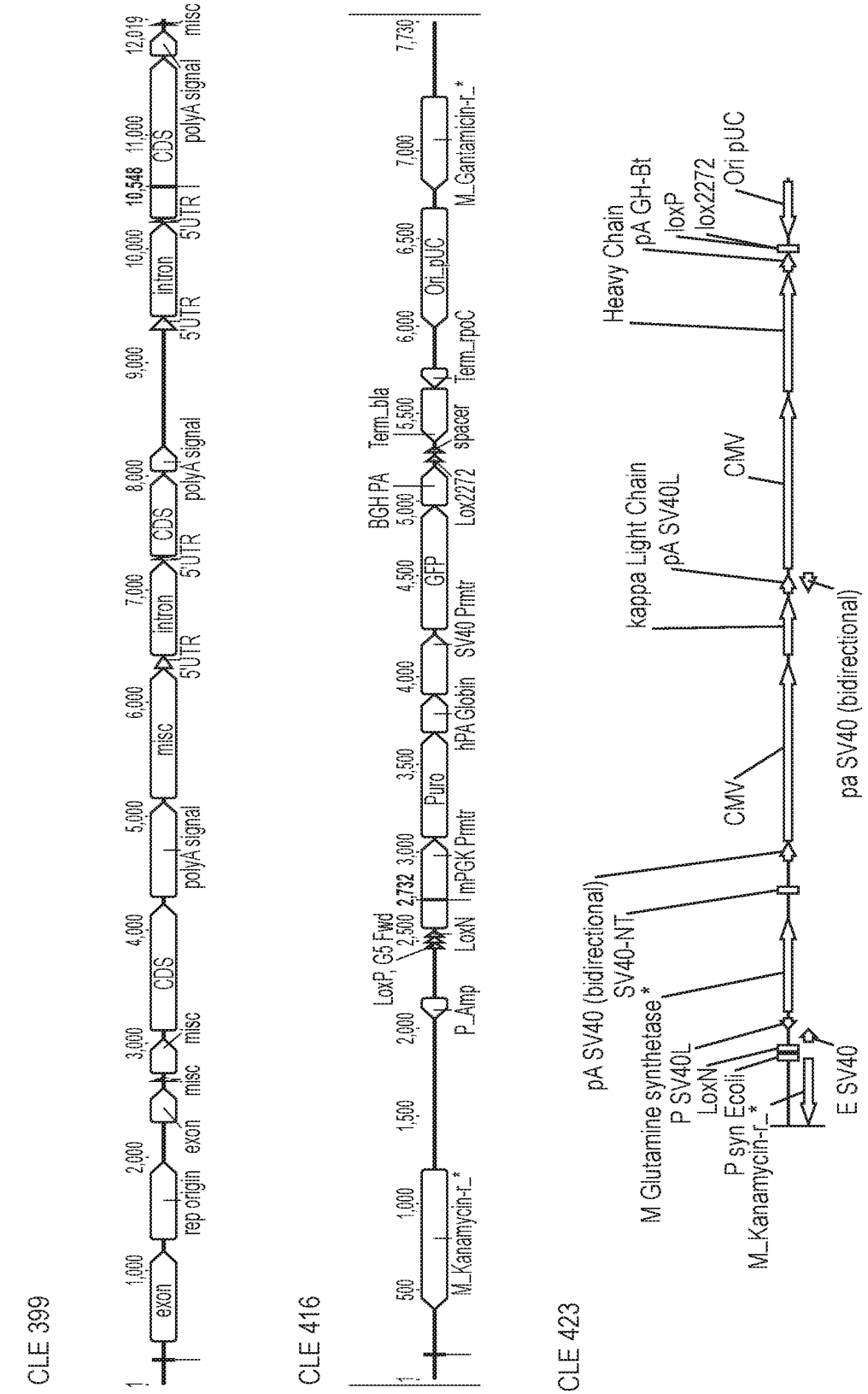
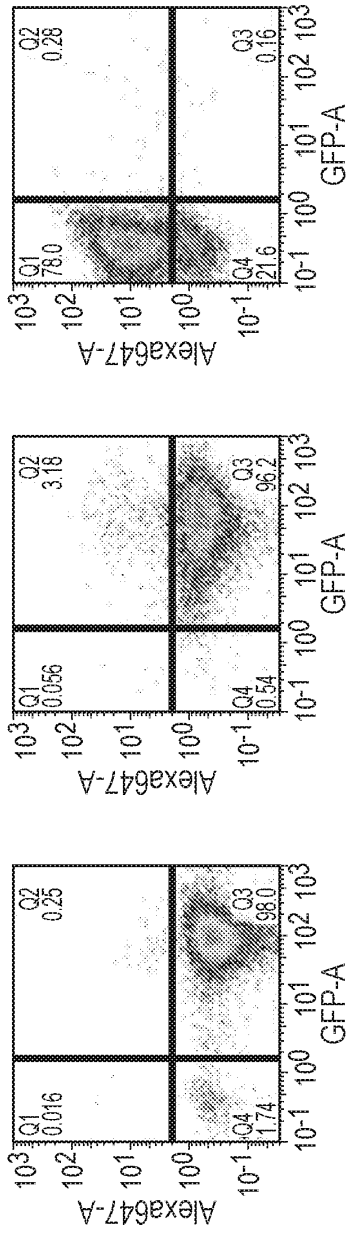

CLE 399
1
1,000
exon
2,000
rep origin
exon
misc
3,000
misc
CDS
4,000
polyA signal
5,000
misc
6,000
5'UTR
intron
7,000
5'UTR
CDS
8,000
polyA signal
9,000
5'UTR
intron
10,000
10,548
5'UTR
CDS
11,000
polyA signal
12,019
misc
CLE 416
1
500
M_Kanamycin-r_*
1,000
1,500
P_Amp
2,000
LoxP, G5 Fwd
2,500
2,732
LoxN
3,000
mPGK Prmtr
3,500
Puro
4,000
hPA Globin
4,500
SV40 Prmtr
GFP
5,000
BGH PA
Lox2272
5,500
Term_bla
spacer
6,000
Ori_pUC
6,500
Term_rpoC
7,000
M_Gantamicin-r_*
7,730
CLE 423
pA SV40 (bidirectional)
SV40-NT
M Glutamine synthetase *
P SV40L
LoxN
P syn Ecoli
M_Kanamycin-r_*
E SV40
CMV
pa SV40 (bidirectional)
kappa Light Chain
pA SV40L
CMV
Heavy Chain
pA GH-Bt
loxP
lox2272
Ori pUC

FIG. 1B



Clone D145 with GFP Landing Pad

Clone D145 with an IgG payload integrated at random. Cells are mix of GFP positive and Some IgG expressing (alexa647)

Clone D145 with same IgG payload and Cre to perform RMCE at landing pad and then selected with no Glutamine (GS-/- line). Cells are mostly expressing IgG and GFP Landing Pad is mostly removed

FIG. 2

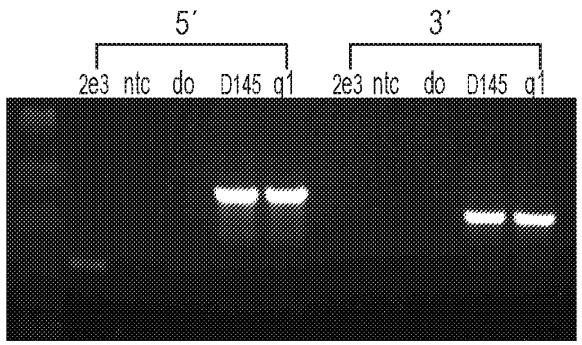
Junction PCR to show RMCE at Landing Pad Site

D145

Junction PCR to show RMCE at Landing Pad Site

Junction PCR primers span the genome at site D145 and the IgG Payload that was introduced by RMCE this product should only exist if the integration of the IgG occurred in a targeted manner at the exact genomic site and in the proper orientation (both 5′ and 3′ loci tested).

Testing numerous primer pairs to find the correct band confirming T1 occurred between the landing pad and the incoming IgG payload - correct band only seen in D145 and q1 and not found in 2e3 clone or donor only (do) pool.



D145 = D145 post RMCE w/ IgG payload and metabolic selection
Q1 = flow-sorted pool containing cells positive for RMCE
Do = pool transfected with IgG payload only (RMCE not possible)
2e3 = negative control - another cell line with neither landing pad or donor
Ntc = non template control for clean PCR reagents

FIG. 3

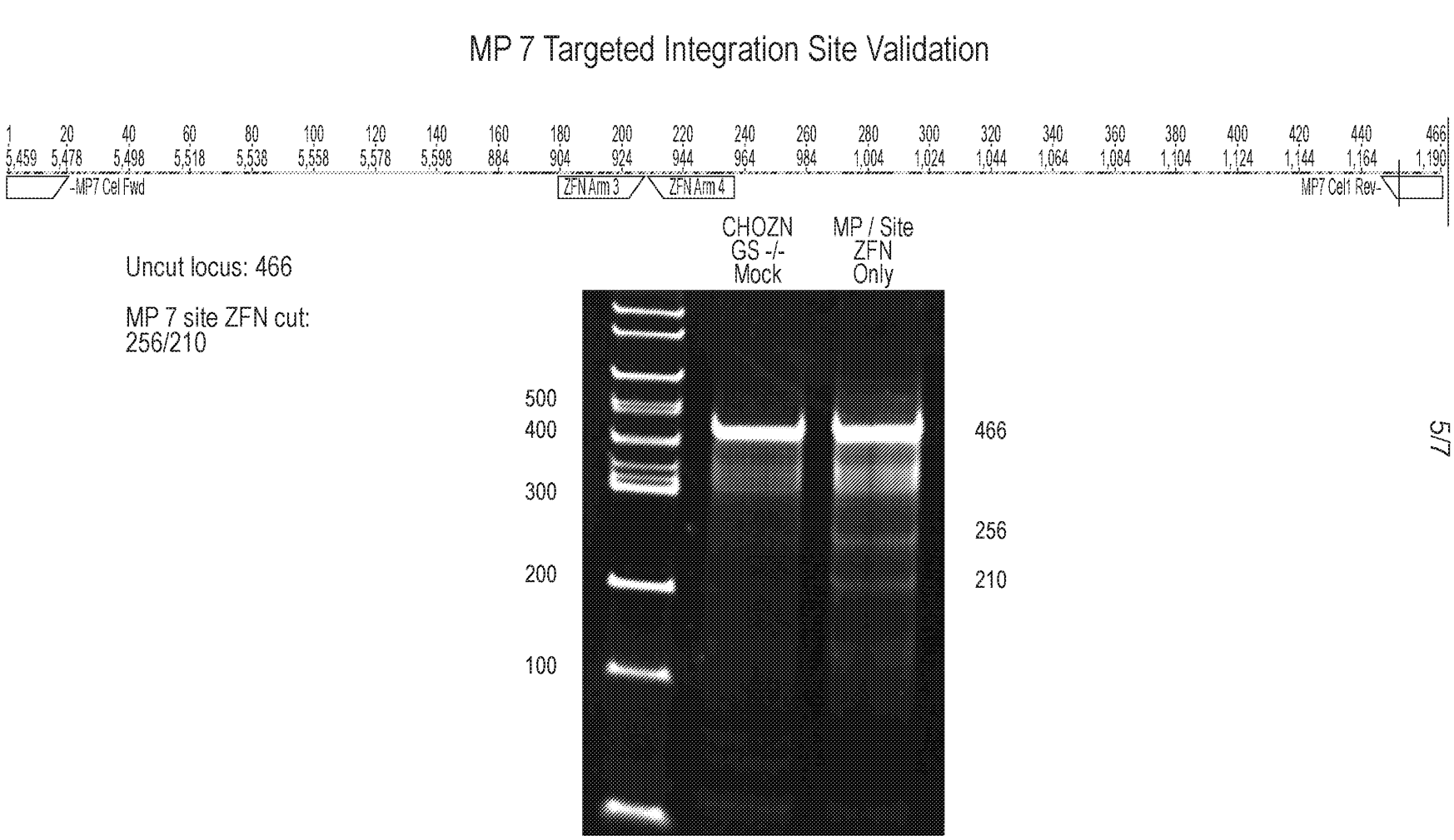

MP 7 Targeted Integration Site Validation
1 20 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 466
5,459 5,478 5,498 5,518 5,538 5,558 5,578 5,598 884 904 924 944 964 984 1,004 1,024 1,044 1,064 1,084 1,104 1,124 1,144 1,164 1,190
-MP7 Cel Fwd
ZFN Arm 3
ZFN Arm 4
MP7 Cel1 Rev-
Uncut locus: 466
MP 7 site ZFN cut:
256/210
CHOZN GS -/- Mock
MP / Site ZFN Only
500
400
300
200
100
466
256
210

FIG. 4

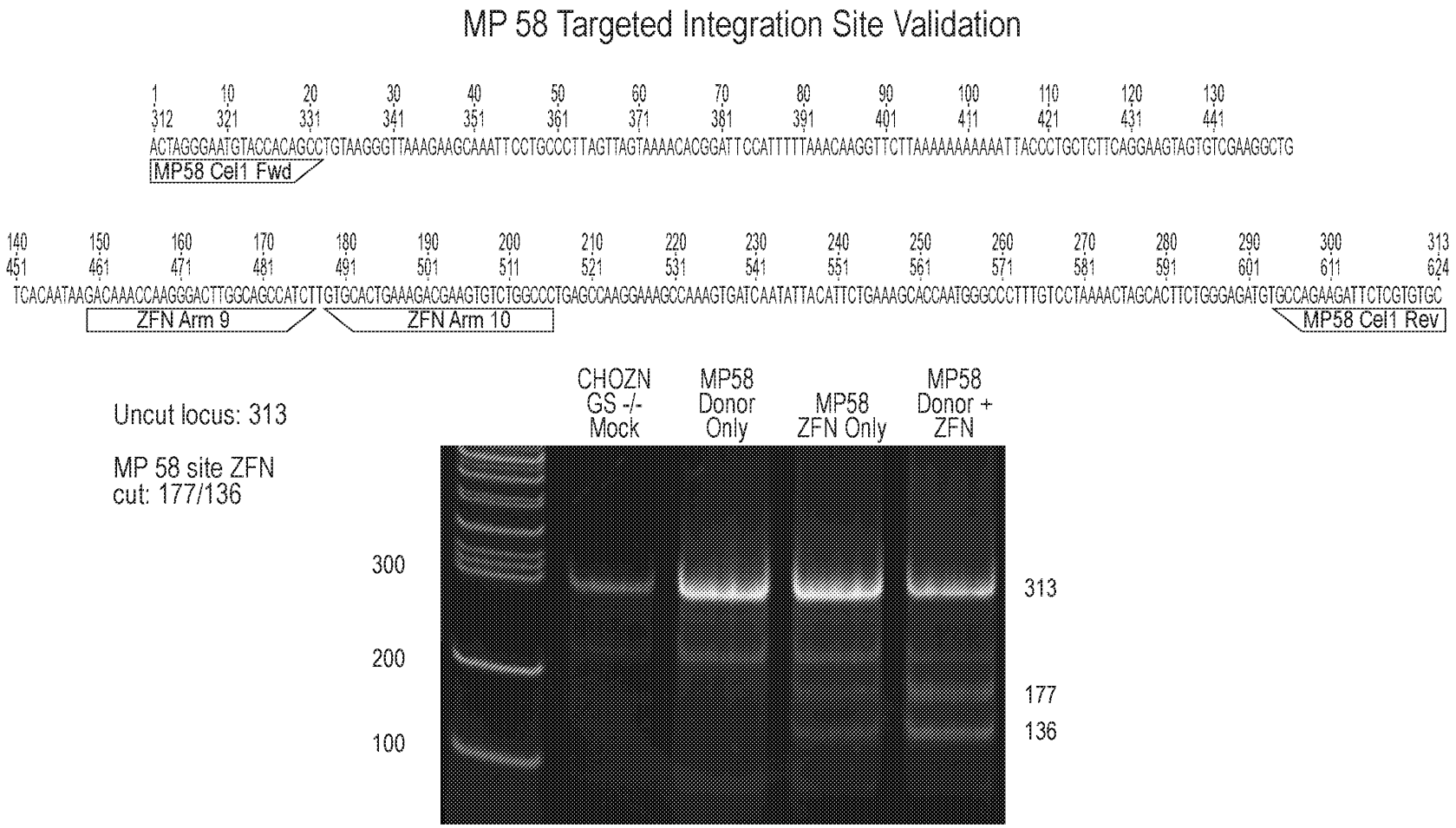
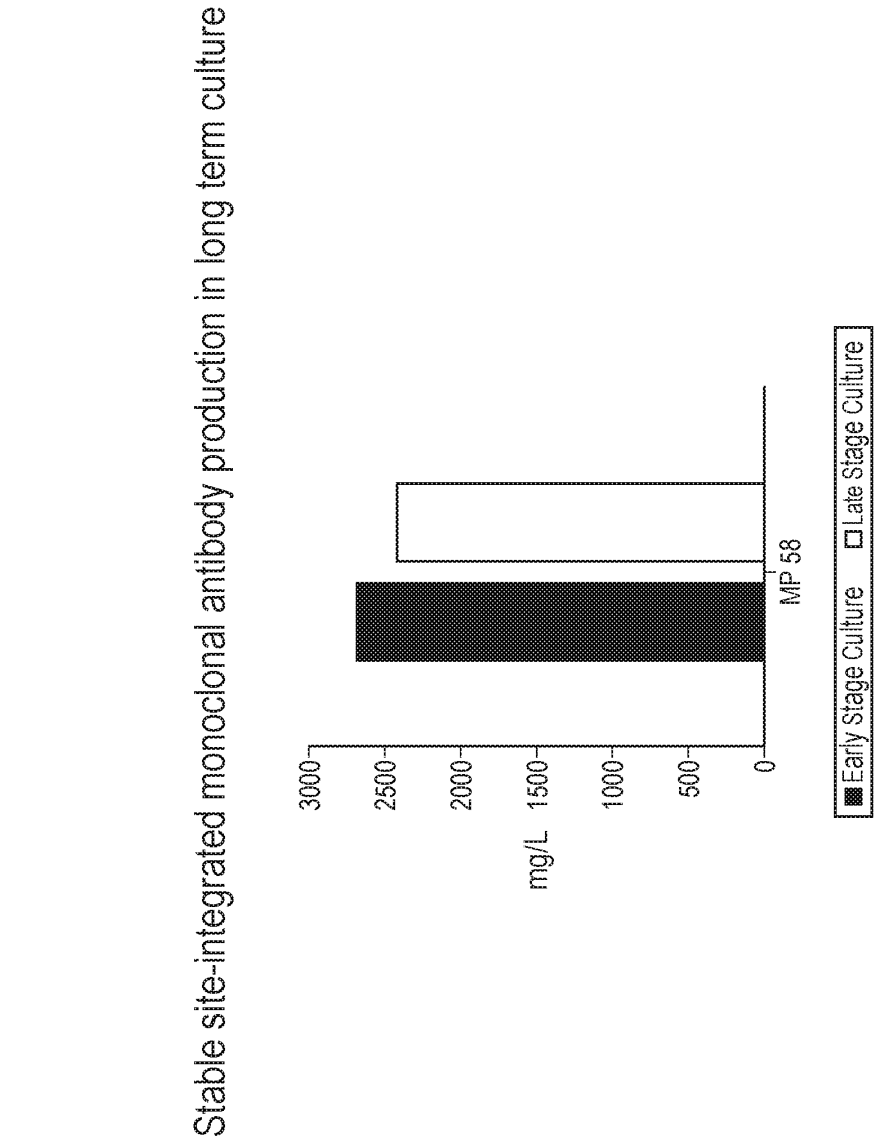

MP 58 Targeted Integration Site Validation
1 10 20 30 40 50 60 70 80 90 100 110 120 130
312 321 331 341 351 361 371 381 391 401 411 421 431 441
ACTAGGGAATGTACCACAGCCTGTAAGGGTTAAAGAAGCAAATTCCTGCCCTTAGTTAGTAAAACACGGATTCCATTTTTAAACAAGGTTCTTAAAAAAAAAAAATTACCCTGCTCTTCAGGAAGTAGTGTCGAAGGCTG
MP58 Cel1 Fwd
140 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300 313
451 461 471 481 491 501 511 521 531 541 551 561 571 581 591 601 611 624
TCACAATAAGACAAACCAAGGGACTTGGCAGCCATCTTGTGCACTGAAAGACGAAGTGTCTGGCCCTGAGCCAAGGAAAGCCAAAGTGATCAATATTACATTCTGAAAGCACCAATGGGCCCTTTGTCCTAAAACTAGCACTTCTGGGAGATGTGCCAGAAGATTCTCGTGTGC
ZFN Arm 9
ZFN Arm 10
MP58 Cel1 Rev
Uncut locus: 313
MP 58 site ZFN cut: 177/136
CHOZN GS -/- Mock
MP58 Donor Only
MP58 ZFN Only
MP58 Donor + ZFN
300
200
100
313
177
136

FIG. 5



Monoclonal antibody production from single integration of CLE399 into site MP 58. Clonal cell lines were assessed for productivity from early (0-7 days) or late stage (>60 days) cultures.

FIG. 6