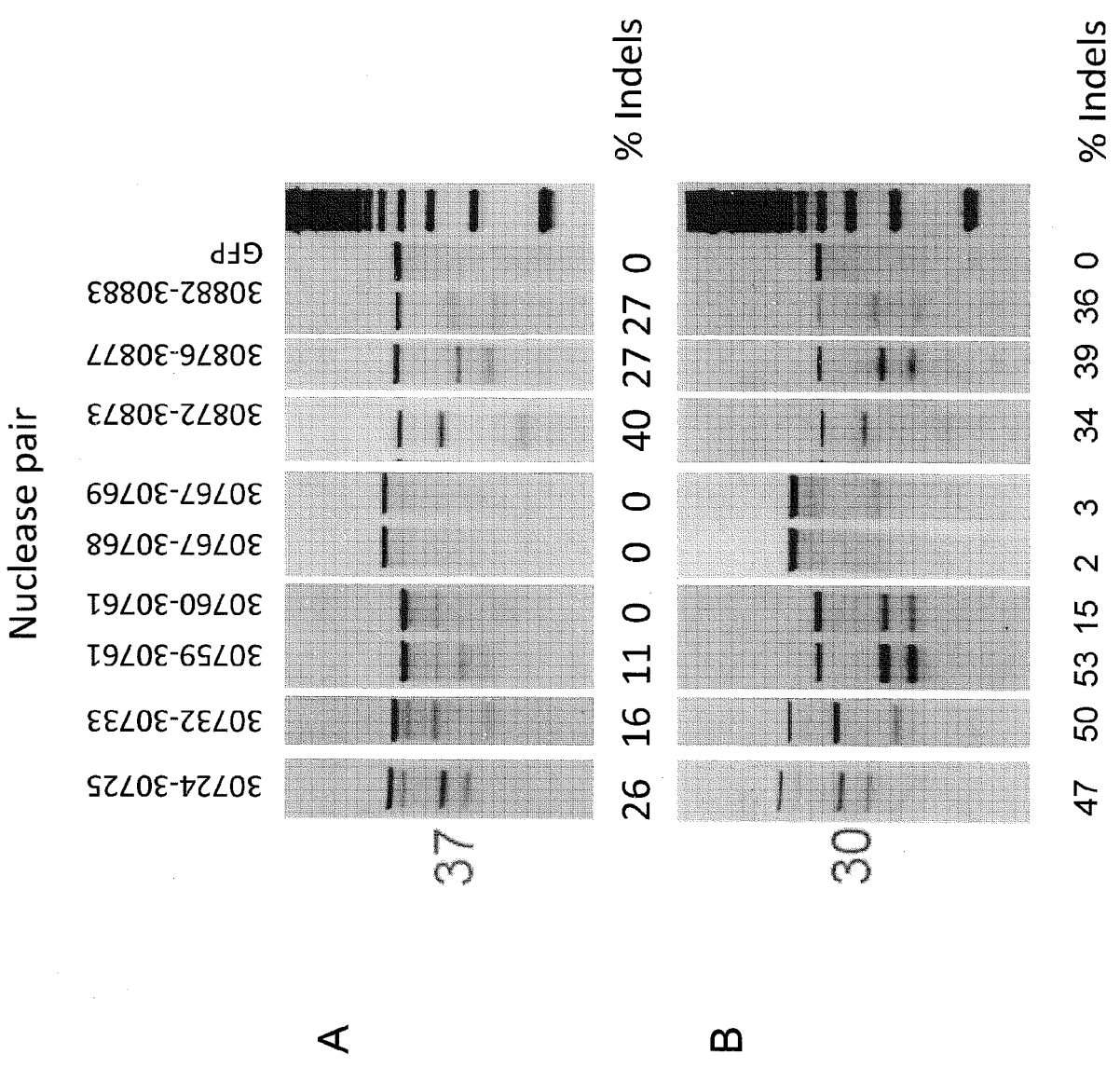

A
B
Nuclease pair
30724-30725
30732-30733
30759-30761
30760-30761
30767-30768
30767-30769
30872-30873
30876-30877
30882-30883
GFP
% Indels
26 16 11 0 0 0 40 27 27 0
% Indels
47 50 53 15 2 3 34 39 36 0
37
30

Figure 1

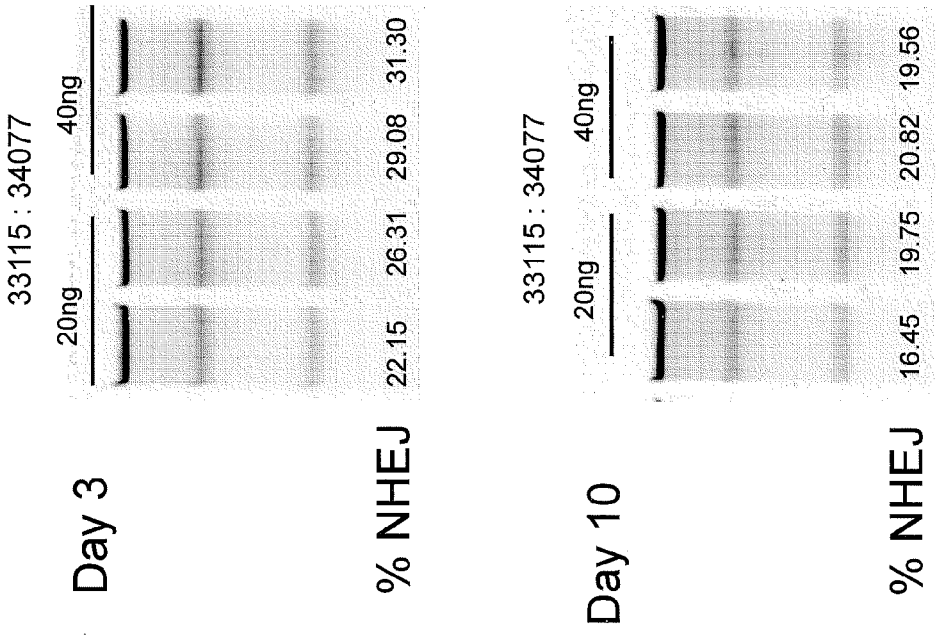

A
Day 3
33115 : 34077
20ng
40ng
% NHEJ
22.15
26.31
29.08
31.30
B
Day 10
33115 : 34077
20ng
40ng
% NHEJ
16.45
19.75
20.82
19.56

Figure 2

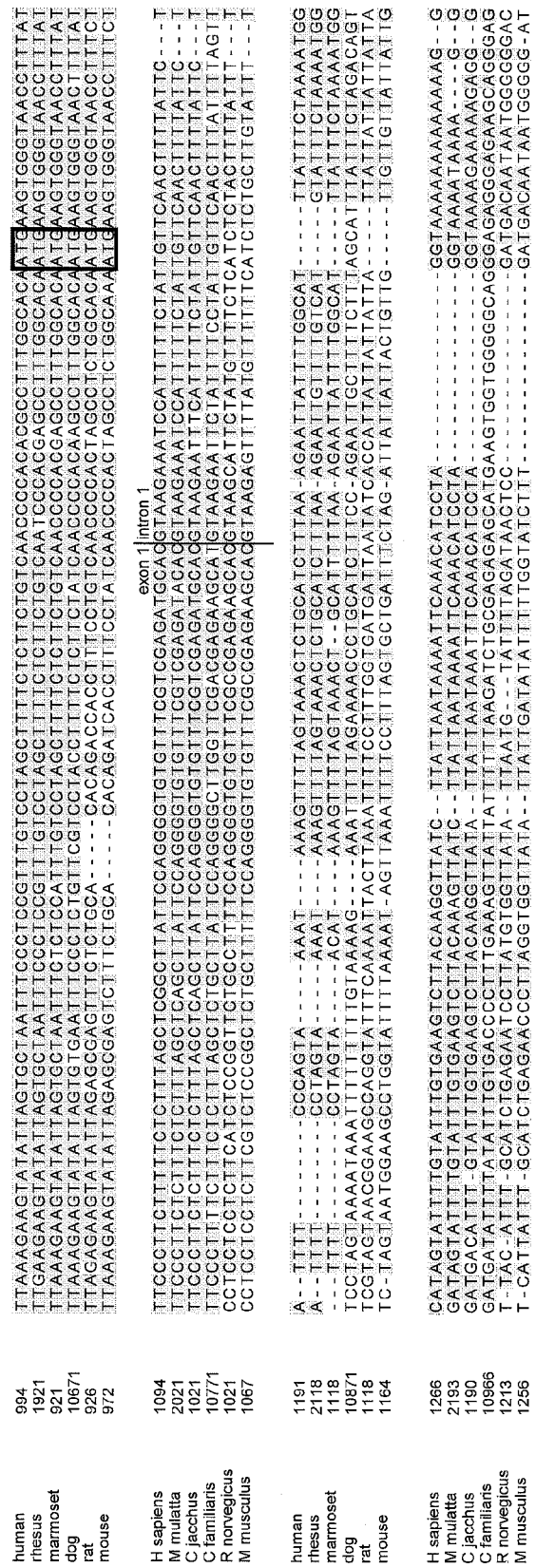


Figure 3A

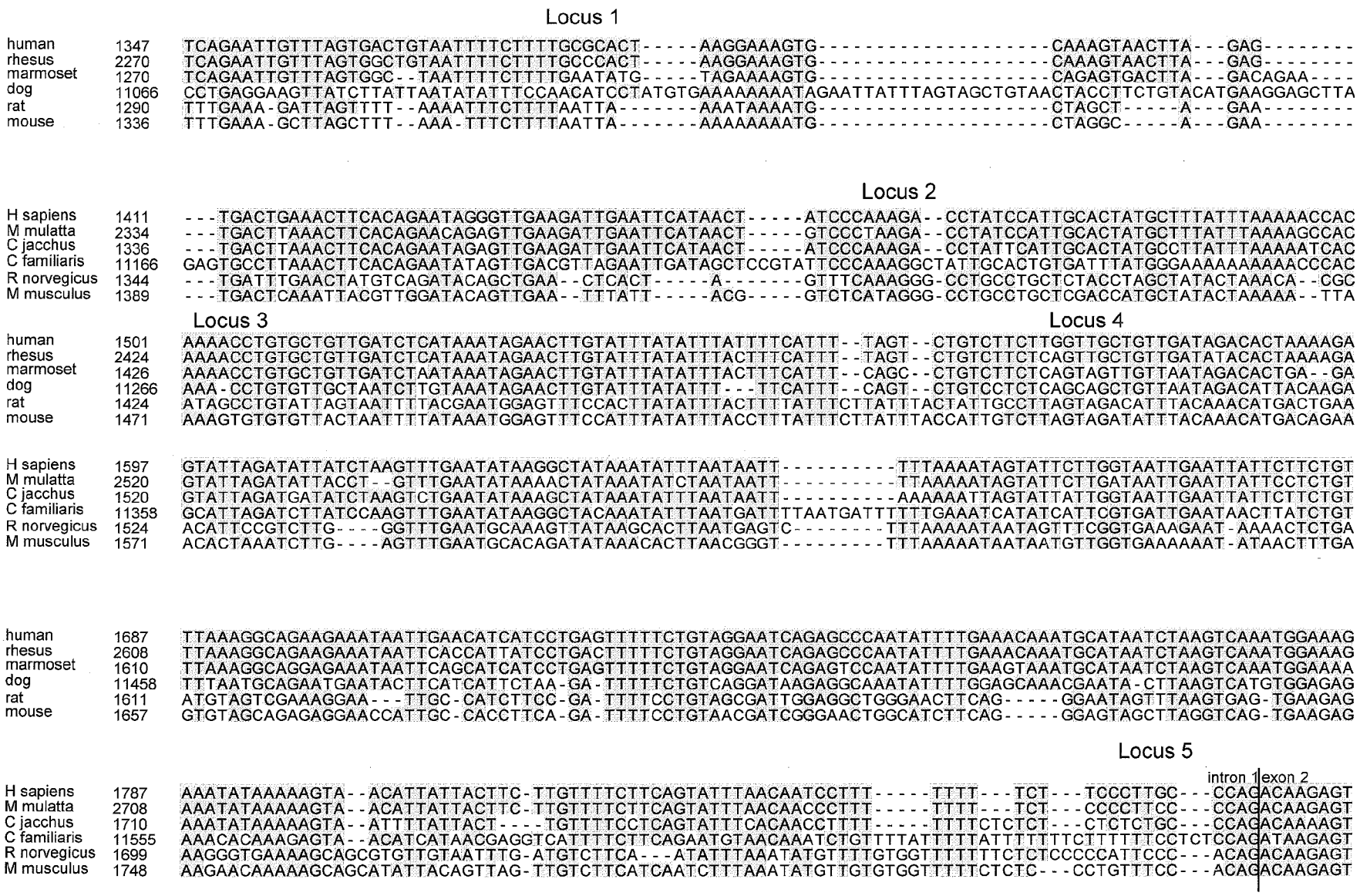

Locus 1
human 1347 TCAGAATTGTTTAGTGACTGTAATTTTCTTTTGCGCACT-----AAGGAAAGTG----------CAAAGTAACTTA---GAG-------
rhesus 2270 TCAGAATTGTTTAGTGGCTGTAATTTTCTTTTGCCCACT-----AAGGAAAGTG----------CAAAGTAACTTA---GAG-------
marmoset 1270 TCAGAATTGTTTAGTGGC--TAATTTTCTTTTGAATATG-----TAGAAAAGTG----------CAGAGTGACTTA---GACAGAA----
dog 11066 CCTGAGGAAGTTATCTTATTAATATATTTCCAACATCCTATGTGAAAAAAAATAGAATTATTTAGTAGCTGTAACTACCTTCTGTACATGAAGGAGCTTA
rat 1290 TTTGAAA-GATTAGTTTT--AAAATTTCTTTTAATTA-------AAATAAAATG----------CTAGCT-----A---GAA-------
mouse 1336 TTTGAAA-GCTTAGCTTT--AAA-TTTCTTTTAATTA-------AAAAAAAATG----------CTAGGC-----A---GAA-------
Locus 2
H sapiens 1411 ---TGACTGAAACTTCACAGAATAGGGTTGAAGATTGAATTCATAACT-----ATCCCAAAGA--CCTATCCATTGCACTATGCTTTATTTAAAAACCAC
M mulatta 2334 ---TGACTTAAACTTCACAGAACAGAGTTGAAGATTGAATTCATAACT-----GTCCCTAAGA--CCTATCCATTGCACTATGCTTTATTTAAAAGCCAC
C jacchus 1336 ---TGACTTAAACTTCACAGAATAGAGTTGAAGATTGAATTCATAACT-----ATCCCAAAGA--CCTATTCATTGCACTATGCCTTATTTAAAAATCAC
C familiaris 11166 GAGTGCCTTAAACTTCACAGAATATAGTTGACGTTAGAATTGATAGCTCCGTATTCCCAAAGGCTATTGCACTGTGATTTATGGGAAAAAAAAAACCCAC
R norvegicus 1344 ---TGATTTGAACTATGTCAGATACAGCTGAA--CTCACT-----A-------GTTTCAAAGGG-CCTGCCTGCTCTACCTAGCTATACTAAACA--CGC
M musculus 1389 ---TGACTCAAATTACGTTGGATACAGTTGAA--TTTATT-----ACG-----GTCTCATAGGG-CCTGCCTGCTCGACCATGCTATACTAAAAA--TTA
Locus 3
Locus 4
human 1501 AAAACCTGTGCTGTTGATCTCATAAATAGAACTTGTATTTATATTTATTTTCATTT--TAGT--CTGTCTTCTTGGTTGCTGTTGATAGACACTAAAAGA
rhesus 2424 AAAACCTGTGCTGTTGATCTCATAAATAGAACTTGTATTTATATTTACTTTCATTT--TAGT--CTGTCTTCTCAGTTGCTGTTGATATACACTAAAAGA
marmoset 1426 AAAACCTGTGCTGTTGATCTAATAAATAGAACTTGTATTTATATTTACTTTCATTT--CAGC--CTGTCTTCTCAGTAGTTGTTAATAGACACTGA--GA
dog 11266 AAA-CCTGTGTTGCTAATCTTGTAAATAGAACTTGTATTTATATTT---TTCATTT--CAGT--CTGTCCTCTCAGCAGCTGTTAATAGACATTACAAGA
rat 1424 ATAGCCTGTATTAGTAATTTTACGAATGGAGTTTCCACTTATATTTACTTTTATTTCTTATTTACTATTGCCTTAGTAGACATTTACAAACATGACTGAA
mouse 1471 AAAGTGTGTGTTACTAATTTTATAAATGGAGTTTCCATTTATATTTACCTTTATTTCTTATTTACCATTGTCTTAGTAGATATTTACAAACATGACAGAA
H sapiens 1597 GTATTAGATATTATCTAAGTTTGAATATAAGGCTATAAATATTTAATAATT----------TTTAAAATAGTATTCTTGGTAATTGAATTATTCTTCTGT
M mulatta 2520 GTATTAGATATTACCT--GTTTGAATATAAAACTATAAATATCTAATAATT----------TTAAAAATAGTATTCTTGATAATTGAATTATTCCTCTGT
C jacchus 1520 GTATTAGATGATATCTAAGTCTGAATATAAAGCTATAAATATTTAATAATT----------AAAAAATTAGTATTATTGGTAATTGAATTATTCTTCTGT
C familiaris 11358 GCATTAGATCTTATCCAAGTTTGAATATAAGGCTACAAATATTTAATGATTTTAATGATTTTTTGAAATCATATCATTCGTGATTGAATAACTTATCTGT
R norvegicus 1524 ACATTCCGTCTTG----GGTTTGAATGCAAAGTTATAAGCACTTAATGAGTC--------TTTAAAAATAATAGTTTCGGTGAAAGAAT-AAAACTCTGA
M musculus 1571 ACACTAAATCTTG----AGTTTGAATGCACAGATATAAACACTTAACGGGT---------TTTAAAAATAATAATGTTGGTGAAAAAAT-ATAACTTTGA
human 1687 TTAAAGGCAGAAGAAATAATTGAACATCATCCTGAGTTTTTCTGTAGGAATCAGAGCCCAATATTTTGAAACAAATGCATAATCTAAGTCAAATGGAAAG
rhesus 2608 TTAAAGGCAGAAGAAATAATTCACCATTATCCTGACTTTTTCTGTAGGAATCAGAGCCCAATATTTTGAAACAAATGCATAATCTAAGTCAAATGGAAAG
marmoset 1610 TTAAAGGCAGGAGAAATAATTCAGCATCATCCTGAGTTTTTCTGTAGGAATCAGAGTCCAATATTTTGAAGTAAATGCATAATCTAAGTCAAATGGAAAA
dog 11458 TTTAATGCAGAATGAATACTTCATCATTCTAA-GA-TTTTTCTGTCAGGATAAGAGGCAAATATTTTGGAGCAAACGAATA-CTTAAGTCATGTGGAGAG
rat 1611 ATGTAGTCGAAAGGAA---TTGC-CATCTTCC-GA-TTTTCCTGTAGCGATTGGAGGCTGGGAACTTCAG-----GGAATAGTTTAAGTGAG-TGAAGAG
mouse 1657 GTGTAGCAGAGAGGAACCATTGC-CACCTTCA-GA-TTTTCCTGTAACGATCGGGAACTGGCATCTTCAG-----GGAGTAGCTTAGGTCAG-TGAAGAG
Locus 5
intron 1
exon 2
H sapiens 1787 AAATATAAAAAGTA--ACATTATTACTTC-TTGTTTTCTTCAGTATTTAACAATCCTTT-----TTTT---TCT---TCCCTTGC---CCAGACAAGAGT
M mulatta 2708 AAATATAAAAAGTA--ACATTATTACTTC-TTGTTTTCTTCAGTATTTAACAACCCTTT-----TTTT---TCT---CCCCTTCC---CCAGACAAGAGT
C jacchus 1710 AAATATAAAAAGTA--ATTTTATTACT----TGTTTTCCTCAGTATTTCACAACCTTTT-----TTTTCTCTCT---CTCTCTGC---CCAGACAAAAGT
C familiaris 11555 AAACACAAAGAGTA--ACATCATAACGAGGTCATTTTCTTCAGAATGTAACAAATCTGTTTTATTTTTATTTTTTTCTTTTTTCCTCTCCAGATAAGAGT
R norvegicus 1699 AAGGGTGAAAAGCAGCGTGTTGTAATTTG-ATGTCTTCA---ATATTTAAATATGTTTTGTGGTTTTTTTCTCTCCCCCATTCCC---ACAGACAAGAGT
M musculus 1748 AAGAACAAAAAGCAGCATATTACAGTTAG-TTGTCTTCATCAATCTTTAAATATGTTGTGTGGTTTTTCTCTC---CCTGTTTCC---ACAGACAAGAGT

Figure 3B

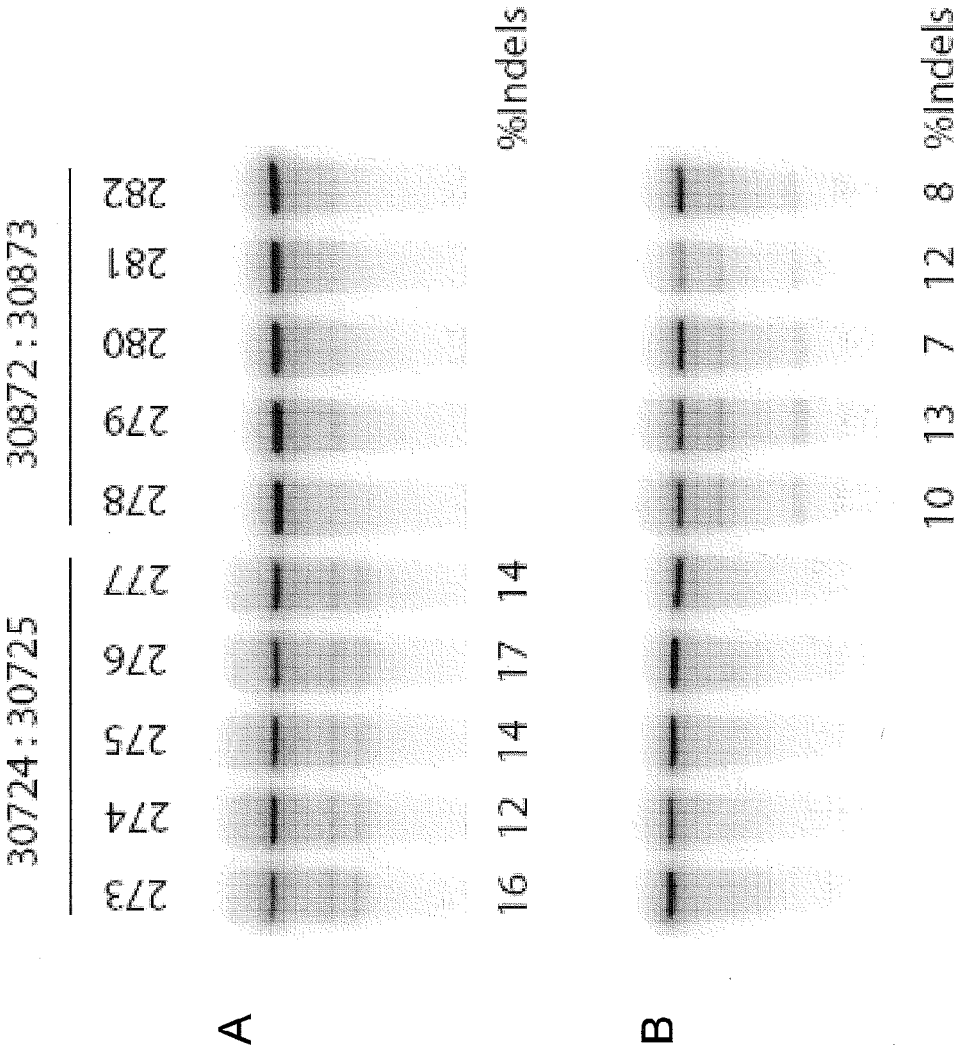

A
B
30724:30725
30872:30873
273
274
275
276
277
278
279
280
281
282
%Indels
16 12 14 17 14
10 13 7 12 8

Figure 4

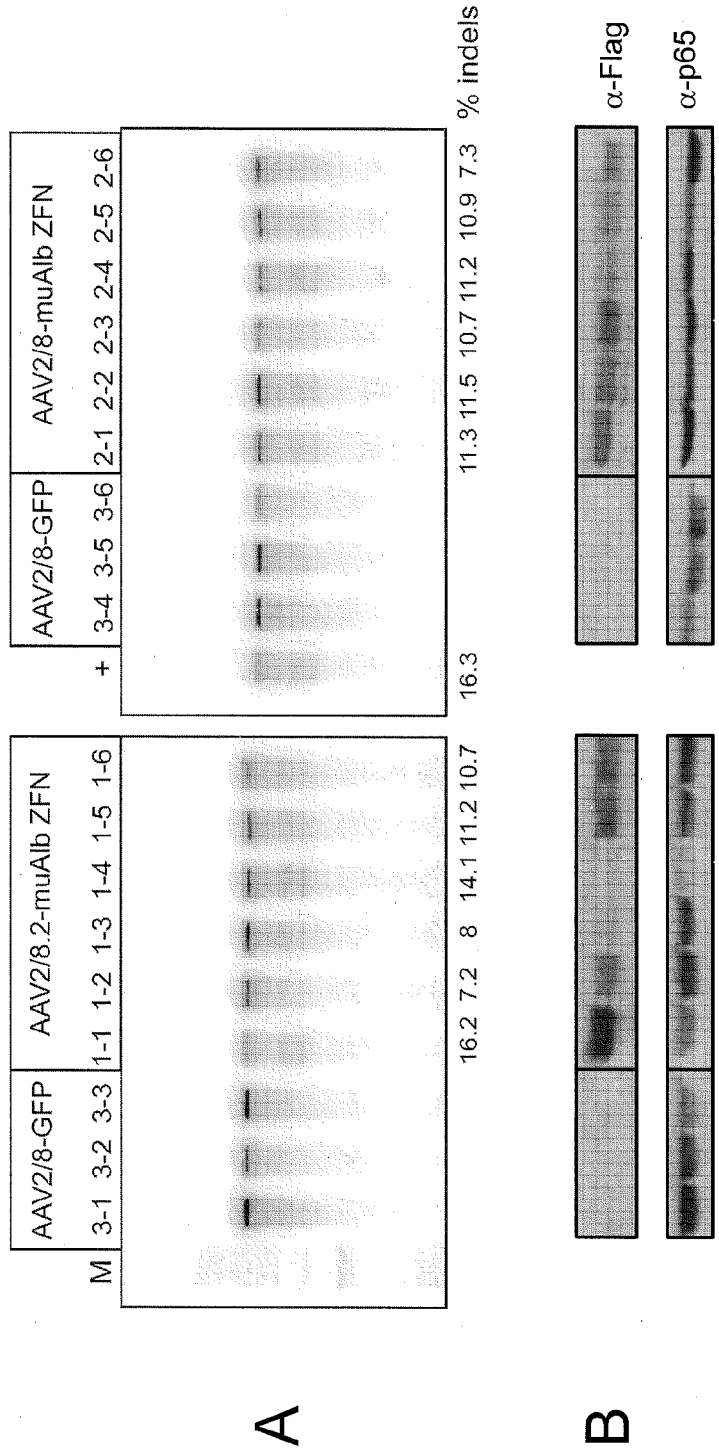

A
M
AAV2/8-GFP
3-1 3-2 3-3
AAV2/8.2-muAlb ZFN
1-1 1-2 1-3 1-4 1-5 1-6
16.2 7.2 8 14.1 11.2 10.7
+
AAV2/8-GFP
3-4 3-5 3-6
AAV2/8-muAlb ZFN
2-1 2-2 2-3 2-4 2-5 2-6
16.3 11.3 11.5 10.7 11.2 10.9 7.3
% indels
B
α-Flag
α-p65

Figure 5

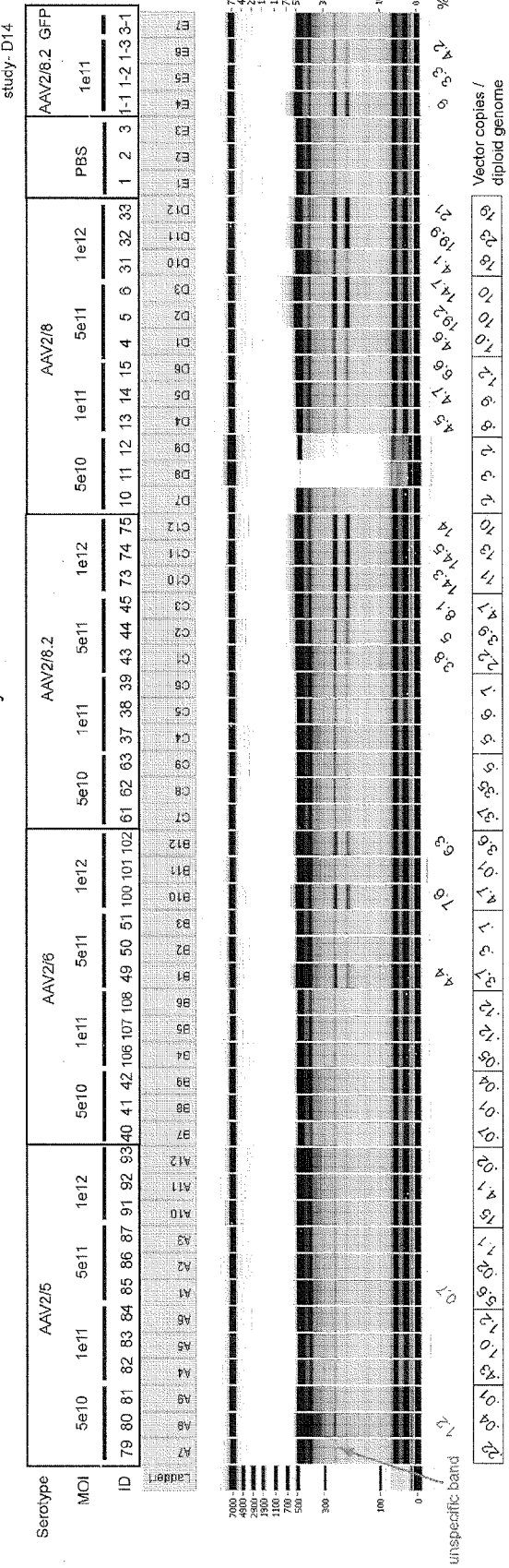

IVS19321 - mouse study - D14
Mini-mouse study- D14
Serotype
MOI
ID
AAV2/5
AAV2/6
AAV2/8.2
AAV2/8
PBS
AAV2/8.2 GFP
5e10
1e11
5e11
1e12
Ladder
unspecific band
% indels
Vector copies / diploid genome

Figure 6

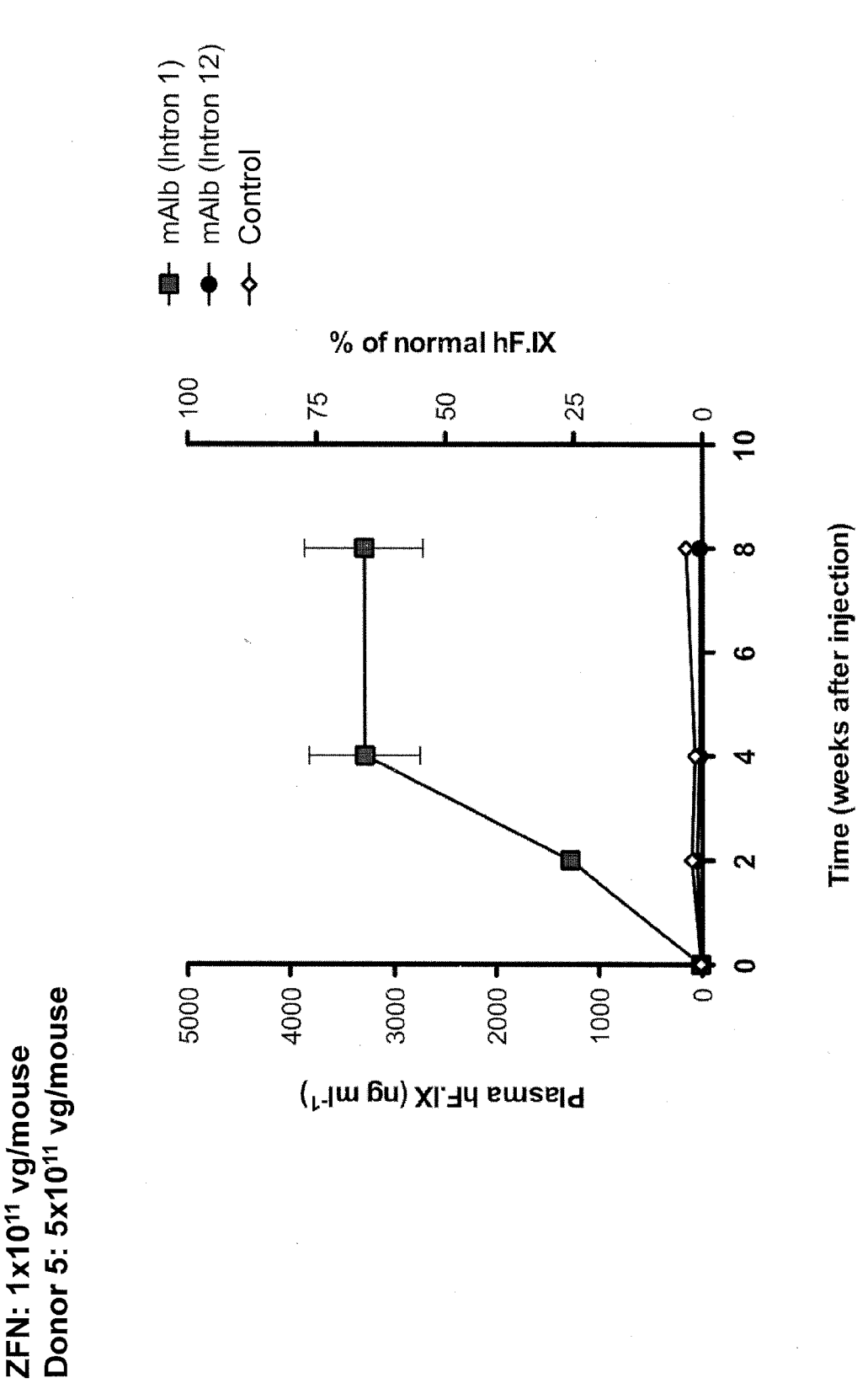
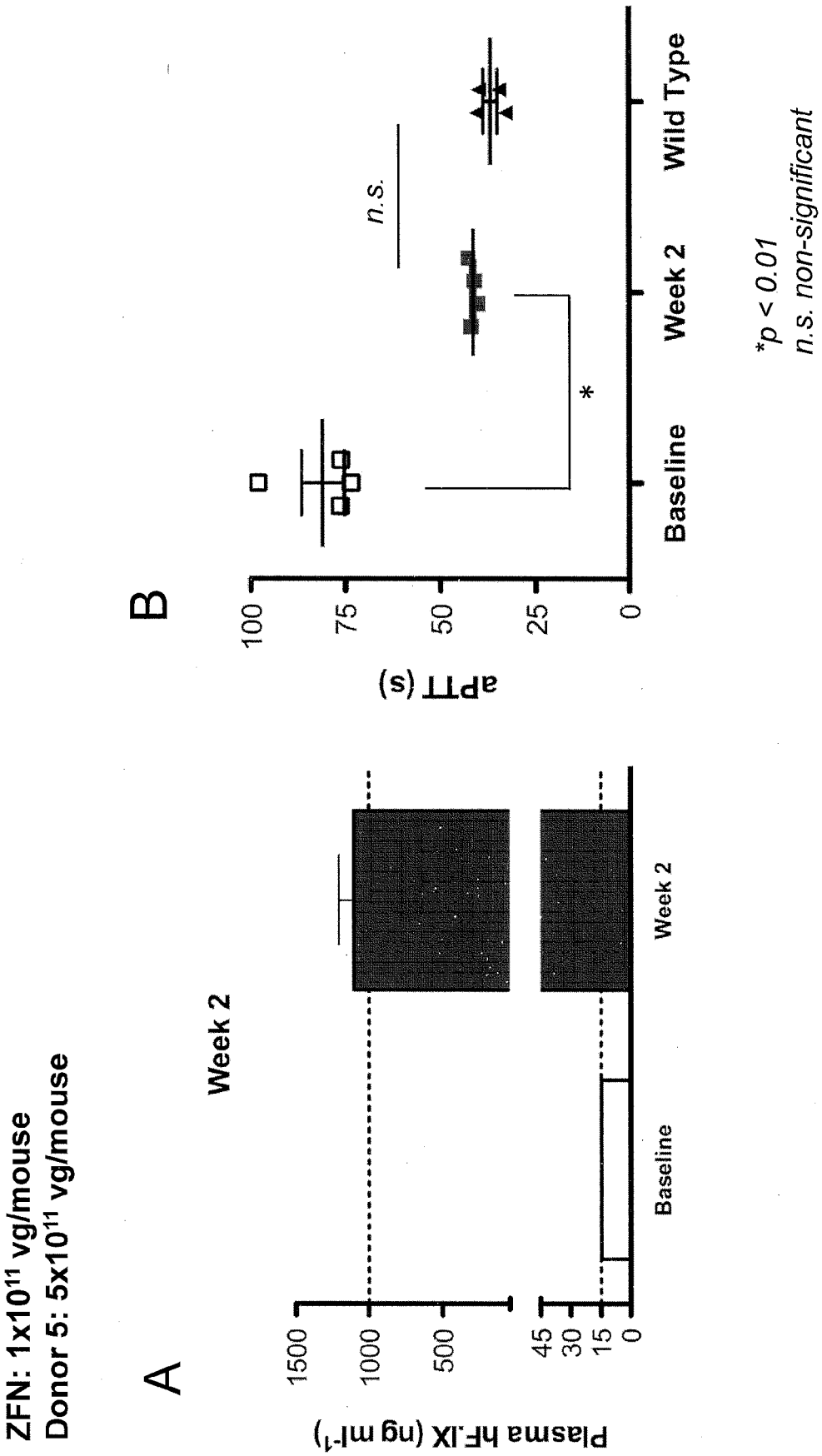

ZFN: 1x10^11 vg/mouse
Donor 5: 5x10^11 vg/mouse
mAlb (Intron 1)
mAlb (Intron 12)
Control
% of normal hF.IX
100
75
50
25
0
Plasma hF.IX (ng ml-1)
5000
4000
3000
2000
1000
0
0
2
4
6
8
10
Time (weeks after injection)

Figure 7



*p < 0.01
n.s. non-significant

Figure 8

HUMAN ALBUMIN, EXON 1/INTRON 1 BORDER REGION

EXON 1 | INTRON 1

1141 GTCGAGATGCAC|GTAAGAAA TCCATTTTTC TATTGTTCAA CTTTTATTCT ATTTTCCCAG

1201 TAAAATAAAG TTTTAGTAAA CTCTGCATCT TTAAAGAATT ATTTTGGCAT TTATTTCTAA

1261 AATGGCATAG TATTTTGTAT TTGTGAAGTC TTACAAGGTT ATCTTATTAA TAAAATTCAA

1321 ACATCCTAGG TAAAAAAAAA AAAAGGTCAG AATTGTTTAG TGACTGTAAT TTTCTTTTGC

1381 GCACTAAGGA AAGTGCAAAG TAACTTAGAG TGACTGAAAC TTCACAGAAT AGGGTTGAAG

35361, 35364, 35370, 35379 35426, 35428

35393, 35394, 35396, 35398, 35399, 35405

1441 ATTGAATTCA TAACTATCCC AAAGACCTAT CCATTGCACT ATGCTTTATT TAAAAACCAC

35458, 35480

1501 AAAACCTGTG CTGTTGATCT CATAAATAGA ACTTGTATTT ATATTTATTT TCATTTTAGT

2101 ATGGCTATTG AGTACTTCAA ATATGACAAG TGCAACTGAG AAACAAAAAC TTAAATTGTA

Figure 9

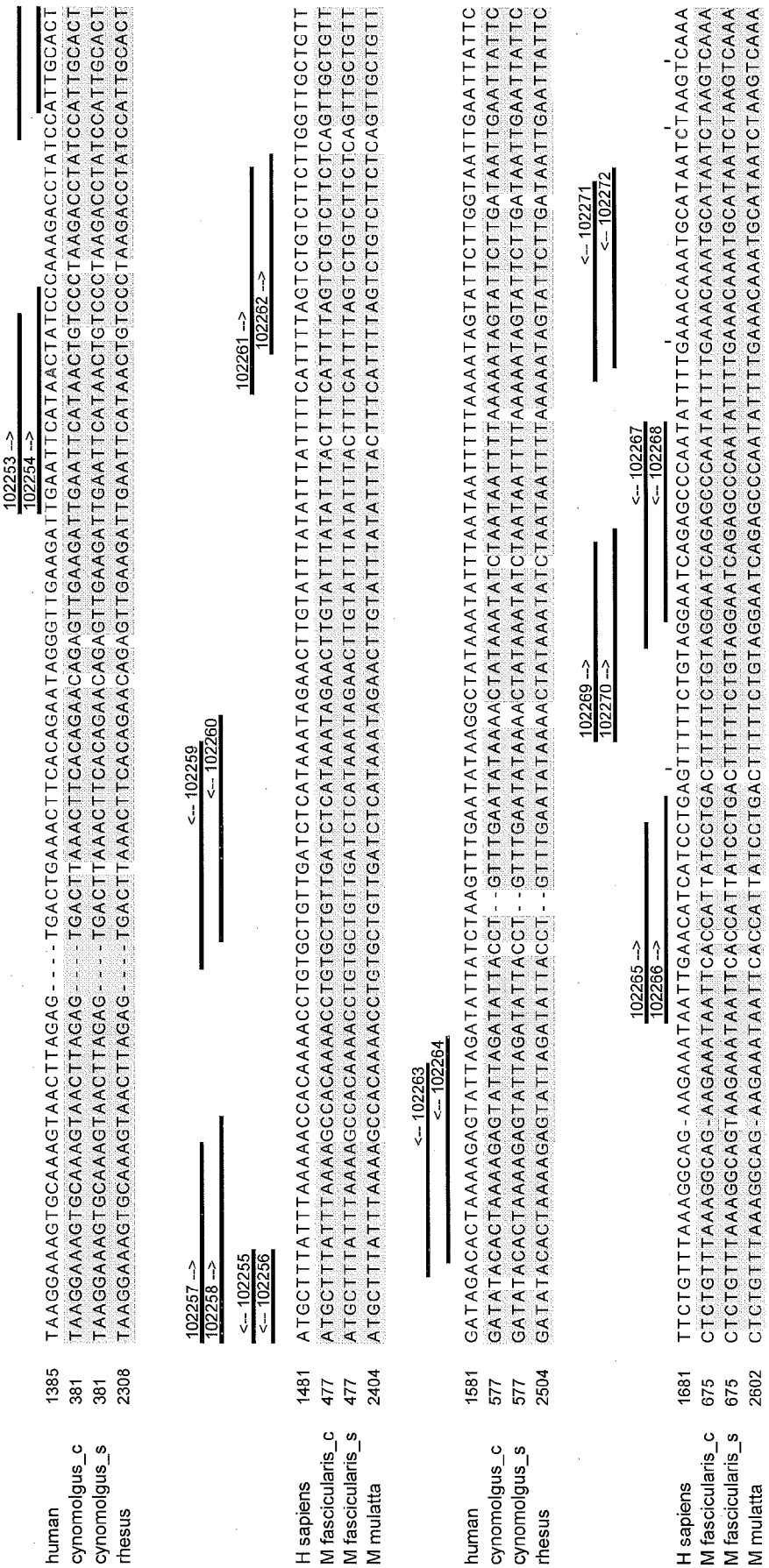

102249 -->
102250 -->
<-- 102251
<-- 102252
102253 -->
102254 -->
human 1385 TAAGGAAAGTGCAAAGTAACTTAGAG----TGACTGAAACTTCACAGAATAGGGTTGAAGATTGAATTCATAACTATCCCAAAGACCTATCCATTGCACT
cynomolgus_c 381 TAAGGAAAGTGCAAAGTAACTTAGAG----TGACTTAAACTTCACAGAACAGAGTTGAAGATTGAATTCATAACTGTCCCTAAGACCTATCCATTGCACT
cynomolgus_s 381 TAAGGAAAGTGCAAAGTAACTTAGAG----TGACTTAAACTTCACAGAACAGAGTTGAAGATTGAATTCATAACTGTCCCTAAGACCTATCCATTGCACT
rhesus 2308 TAAGGAAAGTGCAAAGTAACTTAGAG----TGACTTAAACTTCACAGAACAGAGTTGAAGATTGAATTCATAACTGTCCCTAAGACCTATCCATTGCACT
102257 -->
102258 -->
<-- 102259
<-- 102260
<-- 102255
<-- 102256
102261 -->
102262 -->
H sapiens 1481 ATGCTTTATTTAAAAACCACAAAACCTGTGCTGTTGATCTCATAAATAGAACTTGTATTTATATTTTCATTTTAGTCTGTCTTCTTGGTTGCTGTT
M fascicularis_c 477 ATGCTTTATTTAAAAGGCACAAAACCTGTGCTGTTGATCTCATAAATAGAACTTGTATTTATATTTTACTTTCATTTTAGTCTGTCTTCTCAGTTGCTGTT
M fascicularis_s 477 ATGCTTTATTTAAAAGCCACAAAACCTGTGCTGTTGATCTCATAAATAGAACTTGTATTTATATTTTACTTTCATTTTAGTCTGTCTTCTCAGTTGCTGTT
M mulatta 2404 ATGCTTTATTTAAAAGCCACAAAACCTGTGCTGTTGATCTCATAAATAGAACTTGTATTTATATTTTACTTTCATTTTAGTCTGTCTTCTCAGTTGCTGTT
<-- 102263
<-- 102264
human 1581 GATAGACACTAAAAGAGTATTATCTAAGTTTGAATATAAGGCTATAAATATTTAATAATTTTTAAAATAGTATTCTTGGTAATTGAATTATTC
cynomolgus_c 577 GATATACACTAAAAGAGTATTAGATATTACCT--GTTTGAATATAAAACTATAAATATCTAATAATTTTTAAAAATAGTATTCTTGATAATTGAATTATTC
cynomolgus_s 577 GATATACACTAAAAGAGTATTAGATATTACCT--GTTTGAATATAAAACTATAAATATCTAATAATTTTTAAAAATAGTATTCTTGATAATTGAATTATTC
rhesus 2504 GATATACACTAAAAGAGTATTAGATATTACCT--GTTTGAATATAAAACTATAAATATCTAATAATTTTTAAAAATAGTATTCTTGATAATTGAATTATTC
102269 -->
102270 -->
<-- 102271
<-- 102272
102265 -->
102266 -->
<-- 102267
<-- 102268
H sapiens 1681 TTCTGTTTAAAGGCAG-AAGAAATAATTGAACATCATCCTGAGTTTTTCTGTAGGAATCAGAGCCCAATATTTTGAAACAAATGCATAATCTAAGTCAAA
M fascicularis_c 675 CTCTGTTTAAAGGCAG-AAGAAATAATTCACCATTATCCTGACTTTTTCTGTAGGAATCAGAGCCCAATATTTTGAAACAAATGCATAATCTAAGTCAAA
M fascicularis_s 675 CTCTGTTTAAAGGCAGTAAGAAATAATTCACCATTATCCTGACTTTTTCTGTAGGAATCAGAGCCCAATATTTTGAAACAAATGCATAATCTAAGTCAAA
M mulatta 2602 CTCTGTTTAAAGGCAG-AAGAAATAATTCACCATTATCCTGACTTTTTCTGTAGGAATCAGAGCCCAATATTTTGAAACAAATGCATAATCTAAGTCAAA

Figure 10

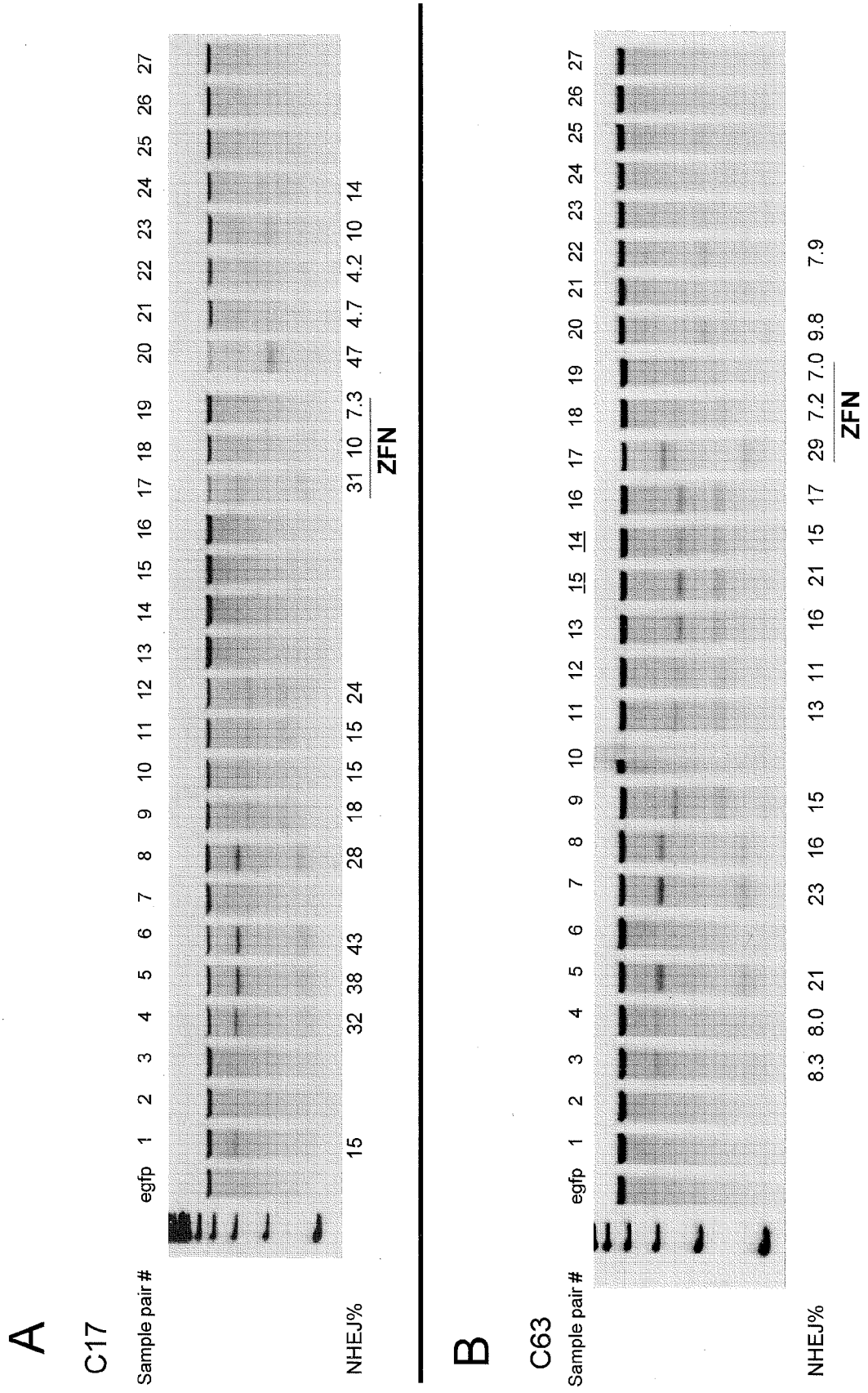

A
C17
Sample pair #
egfp 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
NHEJ%
15 32 38 43 28 18 15 15 24 31 10 7.3 47 4.7 4.2 10 14
ZFN
B
C63
Sample pair #
egfp 1 2 3 4 5 6 7 8 9 10 11 12 13 15 14 16 17 18 19 20 21 22 23 24 25 26 27
NHEJ%
8.3 8.0 21 23 16 15 13 11 16 21 15 17 29 7.2 7.0 9.8 7.9
ZFN

Figure 11

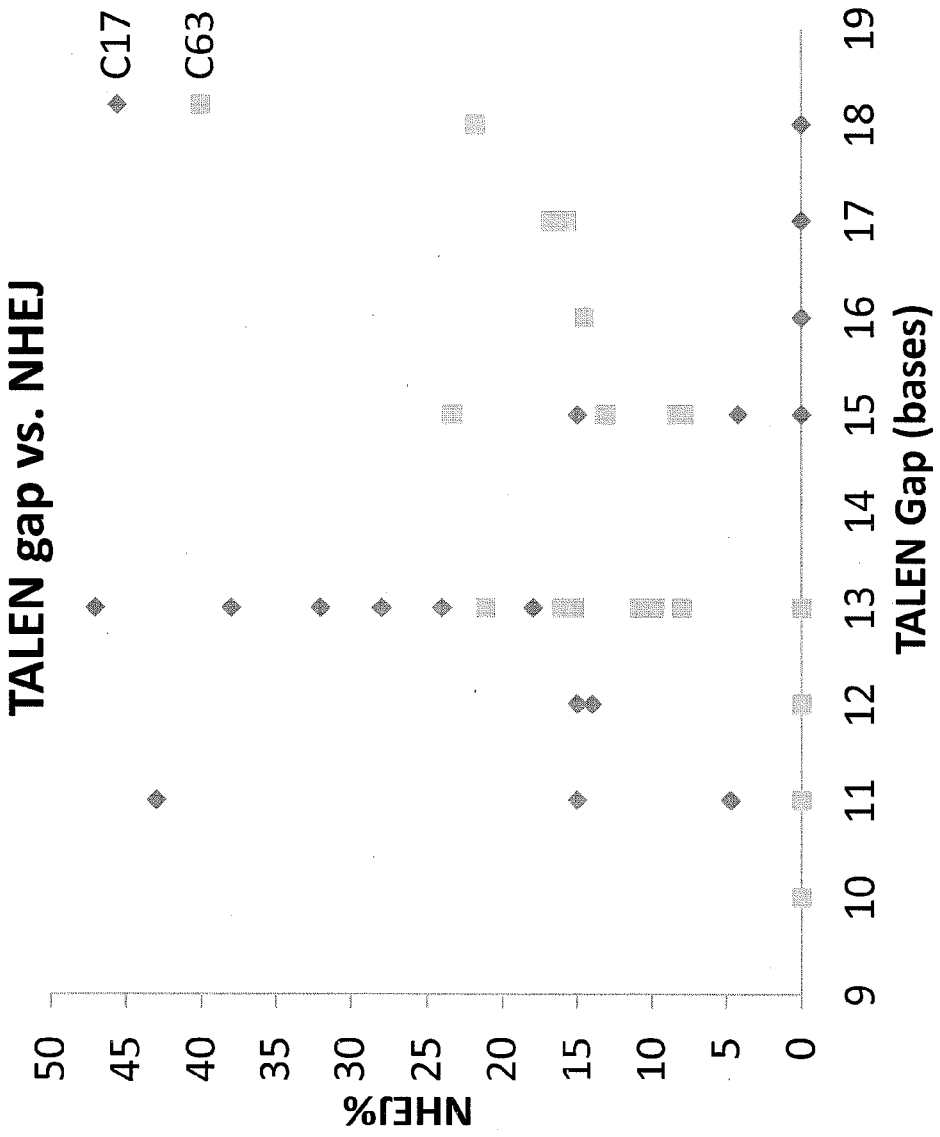

TALEN gap vs. NHEJ
C17
C63
NHEJ%
0
5
10
15
20
25
30
35
40
45
50
9
10
11
12
13
14
15
16
17
18
19
TALEN Gap (bases)

Figure 11C

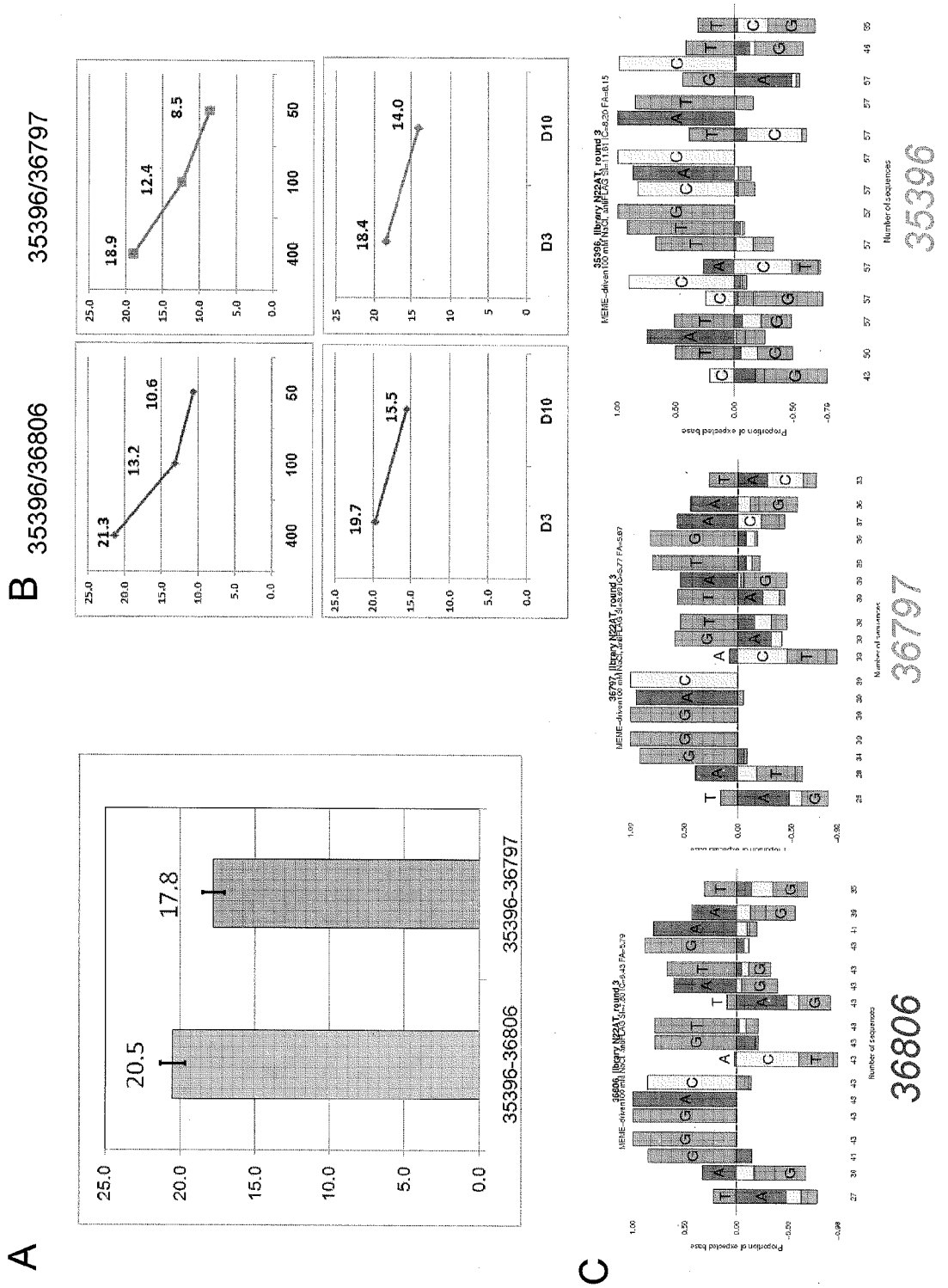
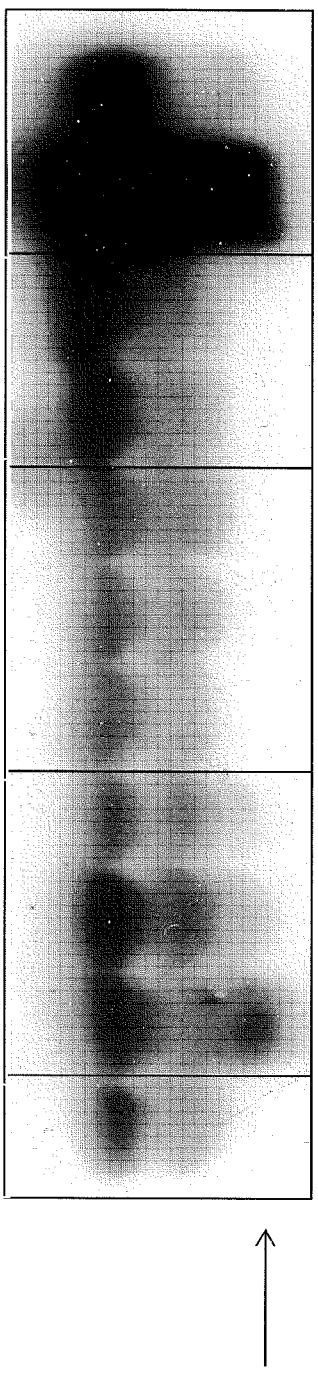
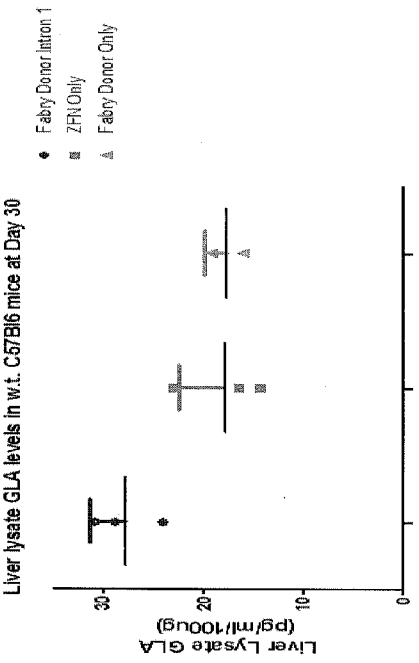

A
20.5
17.8
35396-36806
35396-36797
B
35396/36806
35396/36797
21.3
13.2
10.6
18.9
12.4
8.5
19.7
15.5
18.4
14.0
D3
D10
C
36806
36797
35396

Figure 12


A
Naive
mu alb ZFN + hu Fabry donor
1-1
1-2
1-3
4-1
mu alb ZFN
4-2
4-3
hu Fabry donor
5-1
5-2
human liver lysate


Liver lysate GLA levels in w.t. C57Bl6 mice at Day 30
Fabry Donor Intron 1
ZFN Only
Fabry Donor Only
Liver Lysate GLA
(pg/ml/100ug)
30
20
10
0

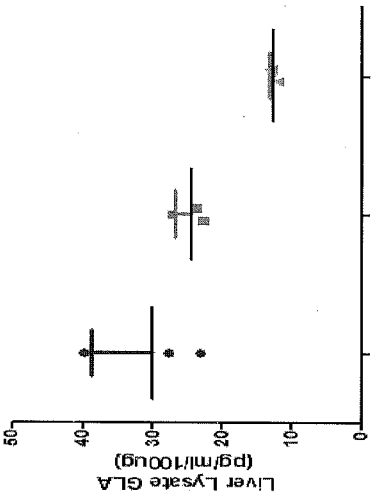

B
Liver lysate GLA levels in w.t. C57Bl6 mice at Day 14
Fabry Donor Intron 1
ZFN Only
Fabry Donor Only
Liver Lysate GLA
(pg/ml/100ug)
50
40
30
20
10
0

Figure 13