Fig. 1

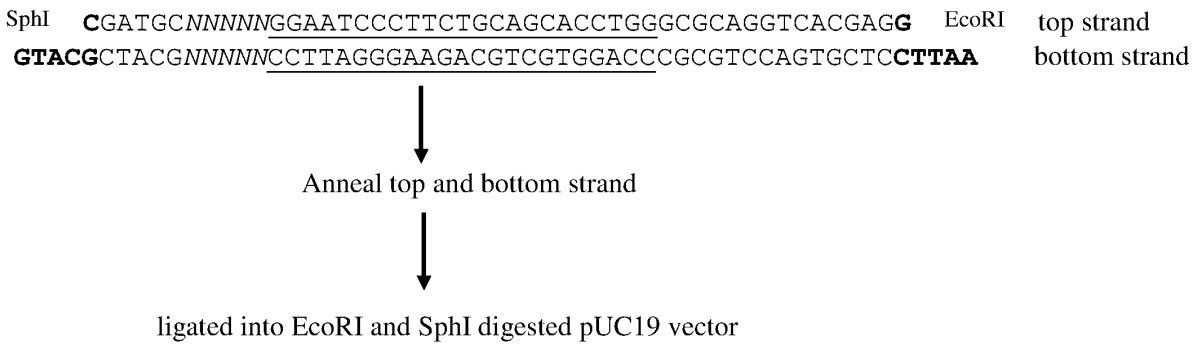

SphI
CGATGCNNNNNGGAATCCCTTCTGCAGCACCTGGGCGCAGGTCACGAGG
EcoRI
top strand
GTACGCTACGNNNNNCCTTAGGGAAGACGTCGTGGACCCGCGTCCAGTGCTCCTTAA
bottom strand
Anneal top and bottom strand
ligated into EcoRI and SphI digested pUC19 vector

Fig. 2

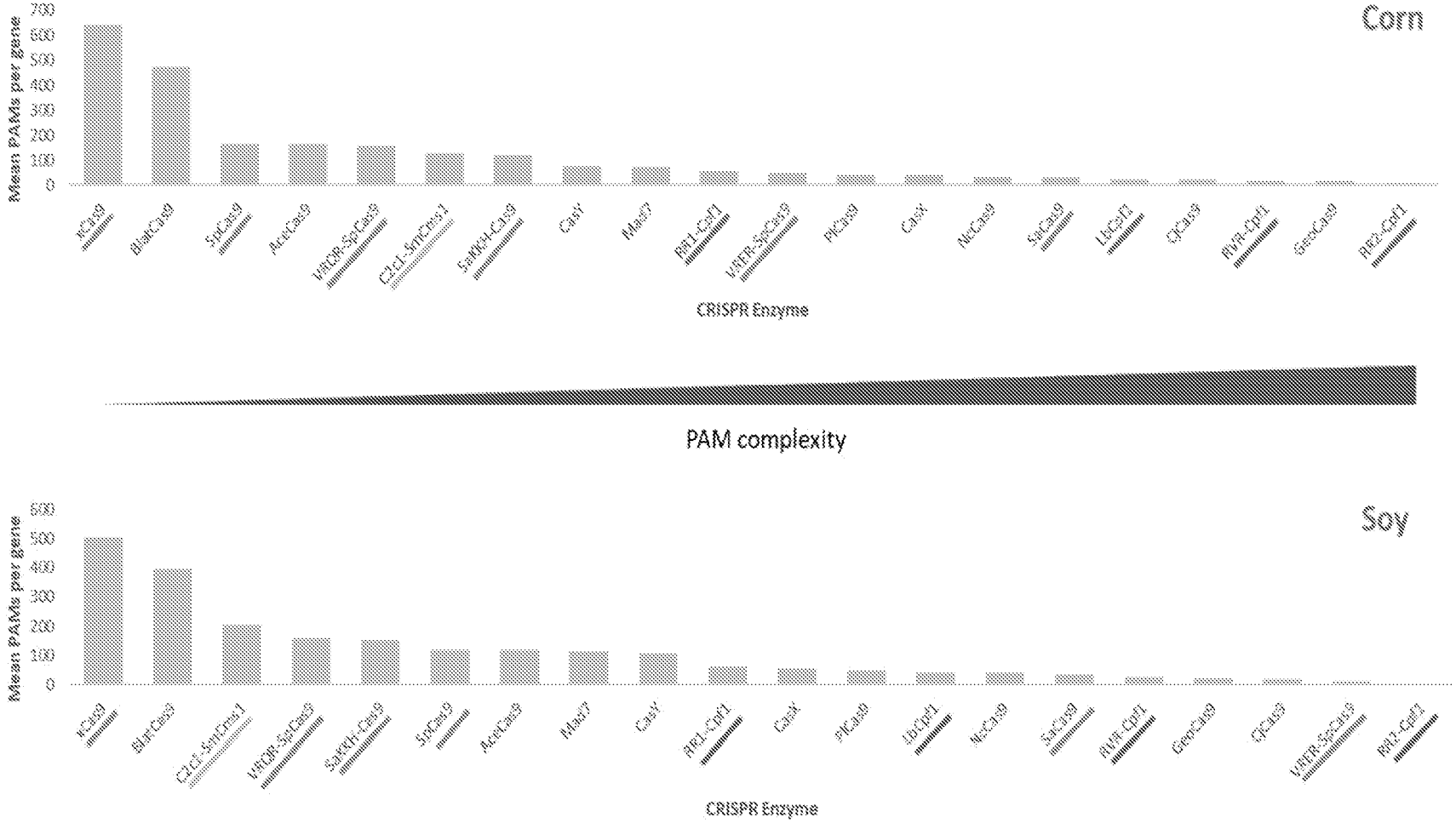
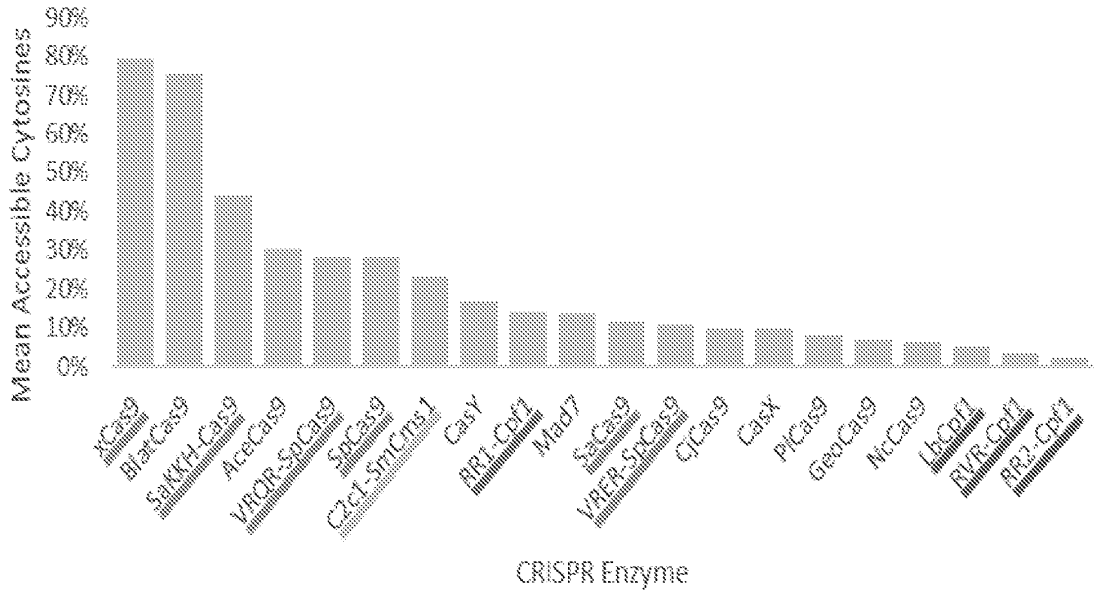

Corn
Mean PAMs per gene
700
600
500
400
300
200
100
0
xCas9
BlatCas9
SpCas9
AceCas9
VRQR-SpCas9
C2c1-SmCms1
SaKKH-Cas9
CasY
Mad7
RR1-Cpf1
VRER-SpCas9
PlCas9
CasX
NcCas9
SaCas9
LbCpf1
CjCas9
RVR-Cpf1
GeoCas9
RR2-Cpf1
CRISPR Enzyme
PAM complexity
Soy
Mean PAMs per gene
600
500
400
300
200
100
0
xCas9
BlatCas9
C2c1-SmCms1
VRQR-SpCas9
SaKKH-Cas9
SpCas9
AceCas9
Mad7
CasY
RR1-Cpf1
CasX
PlCas9
LbCpf1
NcCas9
SaCas9
RVR-Cpf1
GeoCas9
CjCas9
VRER-SpCas9
RR2-Cpf1
CRISPR Enzyme

Fig. 3

A


Mean Accessible Cytosines
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
xCas9
BlatCas9
SaKKH-Cas9
AceCas9
VRQR-SpCas9
SpCas9
C2c1-SmCms1
CasY
RR1-Cpf1
Mad7
SaCas9
VRER-SpCas9
CjCas9
CasX
PlCas9
GeoCas9
NcCas9
LbCpf1
RVR-Cpf1
RR2-Cpf1
CRISPR Enzyme

B

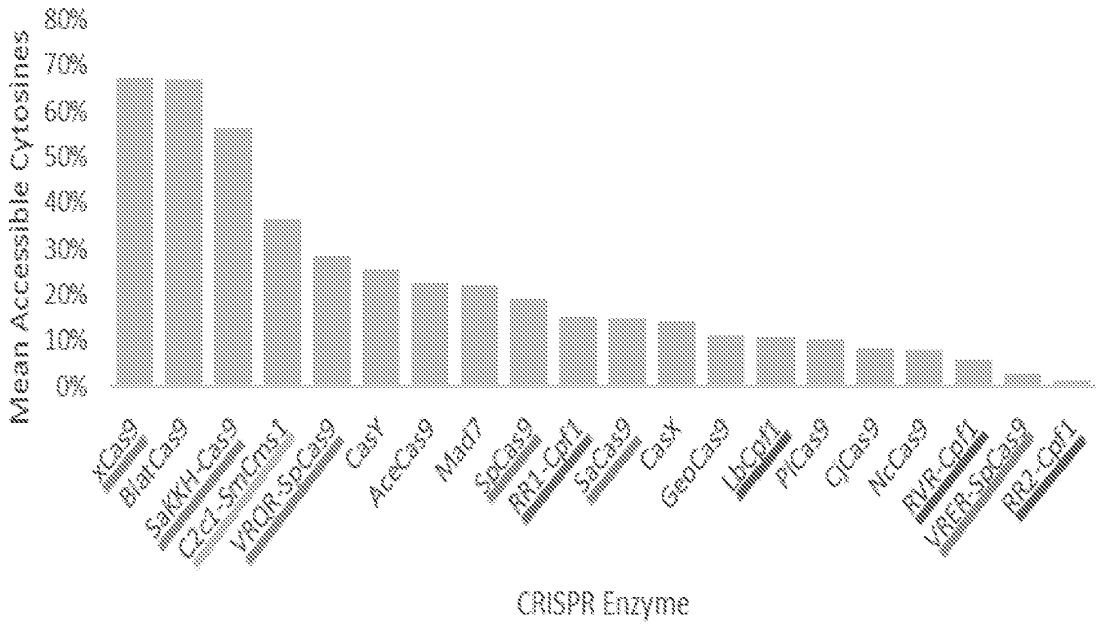
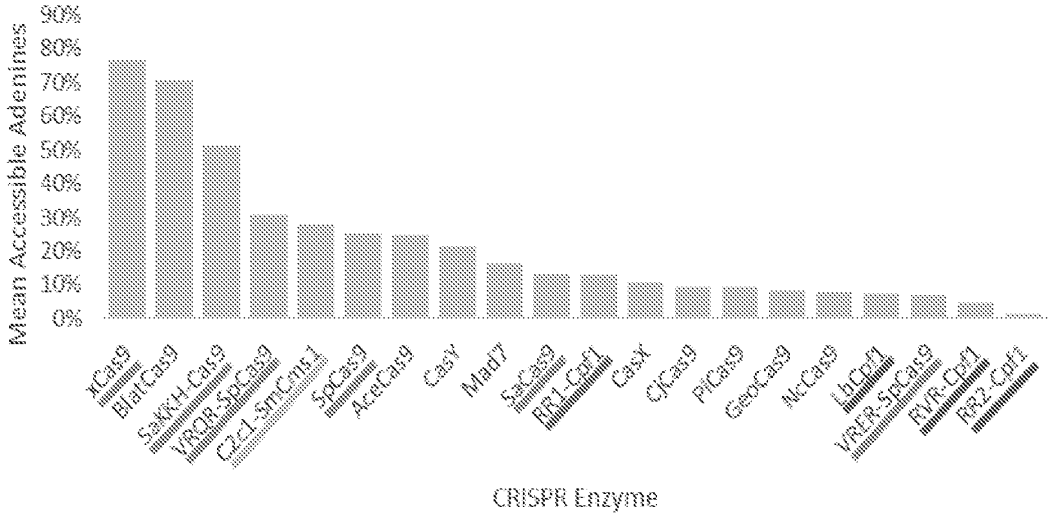

Mean Accessible Cytosines
80%
70%
60%
50%
40%
30%
20%
10%
0%
xCas9
BlatCas9
SaKKH-Cas9
C2c1-SmCms1
VRQR-SpCas9
CasY
AceCas9
Mad7
SpCas9
RR1-Cpf1
SaCas9
CasX
GeoCas9
LbCpf1
PlCas9
CjCas9
NcCas9
RVR-Cpf1
VRER-SpCas9
RR2-Cpf1
CRISPR Enzyme

Fig. 3 (con't)

C


Mean Accessible Adenines
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
xCas9
BlatCas9
SaKKH-Cas9
VRQR-SpCas9
CjCd-SmCms1
SpCas9
AceCas9
CasY
MadZ
SaCas9
RR1-Cdf1
CasX
CjCas9
PlCas9
GeoCas9
NcCas9
LbCpf1
VREF-SpCas9
RVR-Cpf1
RR2-Cpf1
CRISPR Enzyme

D

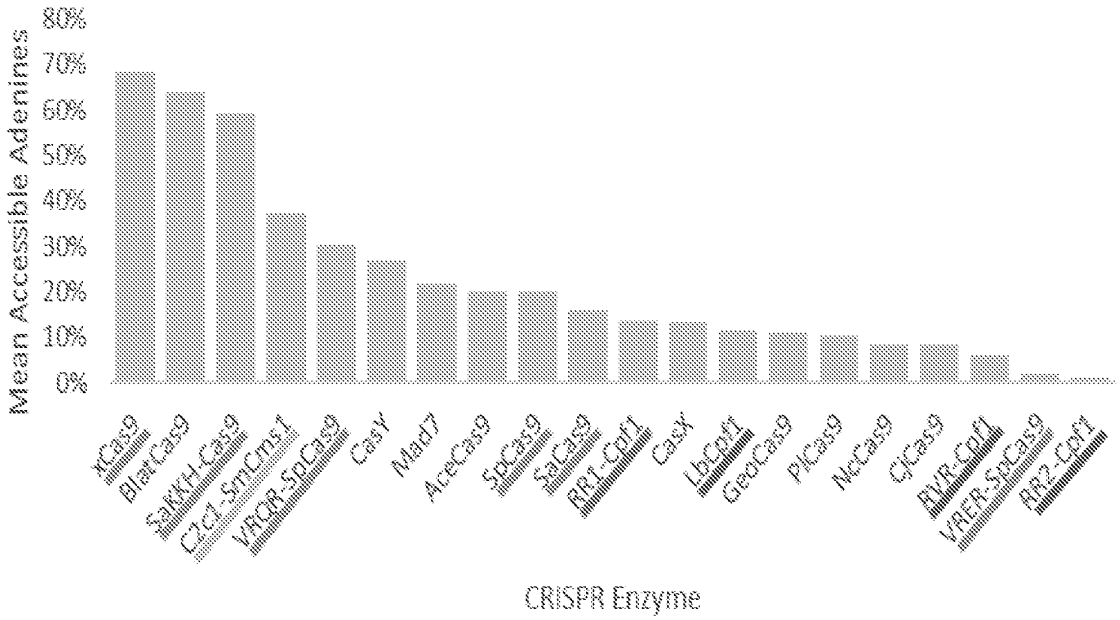

Mean Accessible Adenines
80%
70%
60%
50%
40%
30%
20%
10%
0%
xCas9
BlatCas9
SaKKH-Cas9
CjCd-SmCms1
VRQR-SpCas9
CasY
MadZ
AceCas9
SpCas9
SaCas9
RR1-Cdf1
CasX
LbCpf1
GeoCas9
PlCas9
NcCas9
CjCas9
RVR-Cpf1
VREF-SpCas9
RR2-Cpf1
CRISPR Enzyme

Fig. 4

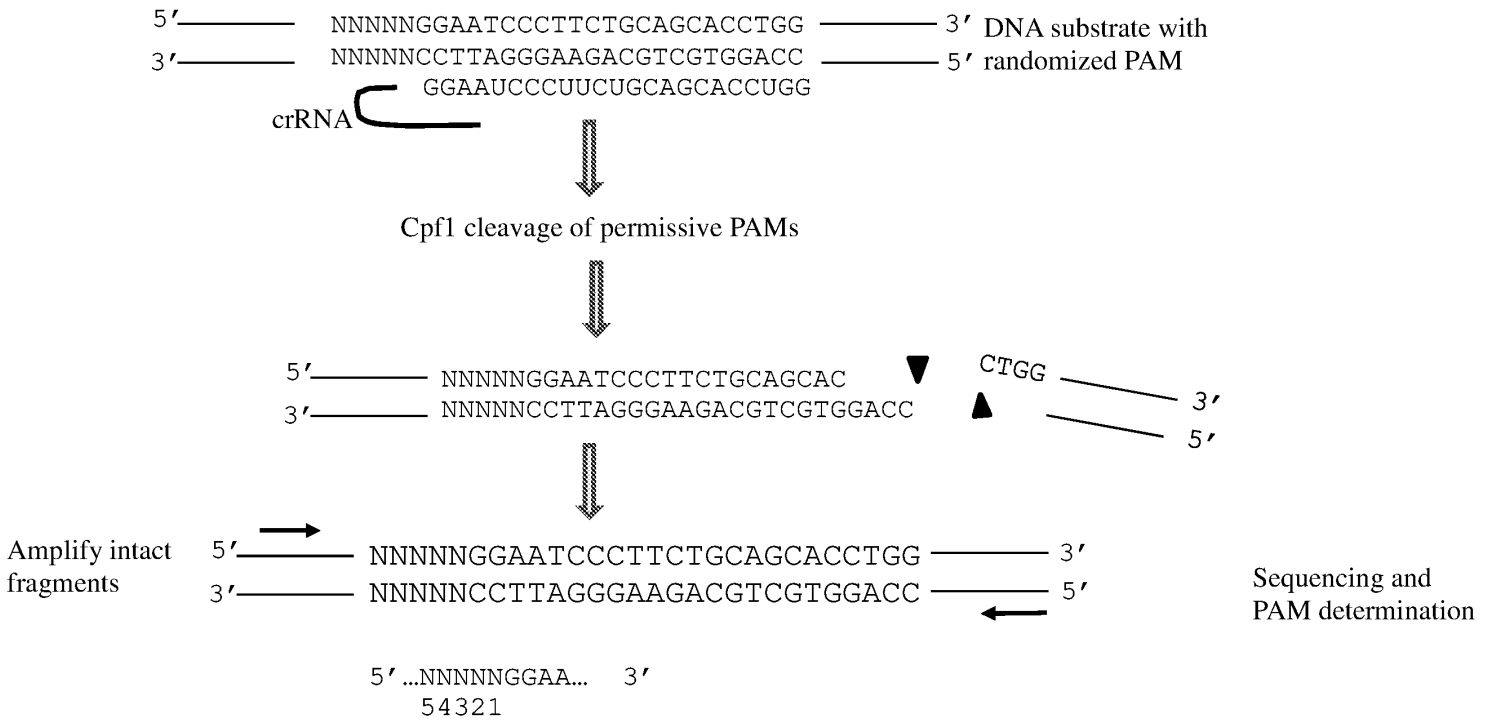

5′ NNNNNGGAATCCCTTCTGCAGCACCTGG 3′ DNA substrate with
3′ NNNNNCCTTAGGGAAGACGTCGTGGACC 5′ randomized PAM
GGAAUCCCUUCUGCAGCACCUGG
crRNA
Cpf1 cleavage of permissive PAMs
5′ NNNNNGGAATCCCTTCTGCAGCAC
3′ NNNNNCCTTAGGGAAGACGTCGTGGACC
CTGG
3′
5′
Amplify intact fragments
5′ NNNNNGGAATCCCTTCTGCAGCACCTGG 3′
3′ NNNNNCCTTAGGGAAGACGTCGTGGACC 5′
Sequencing and PAM determination
5′...NNNNNGGAA... 3′
54321

Fig. 5

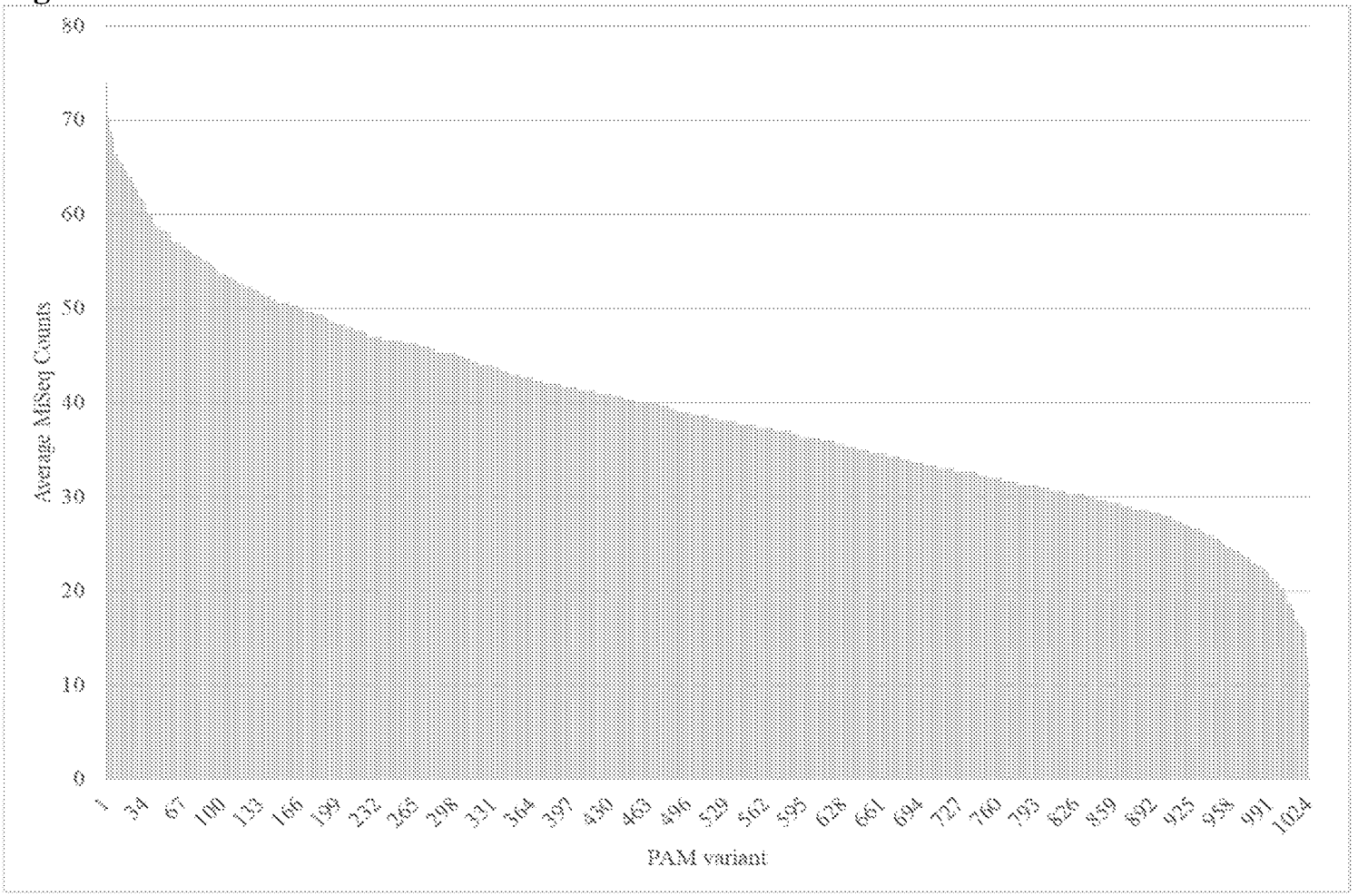
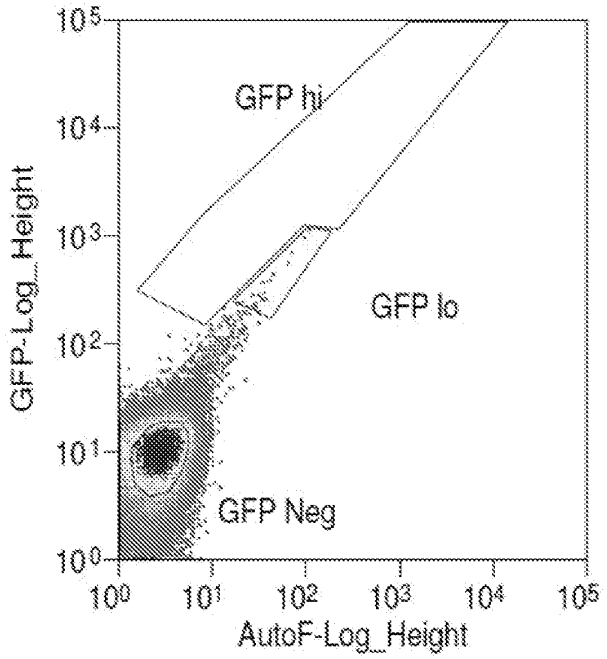
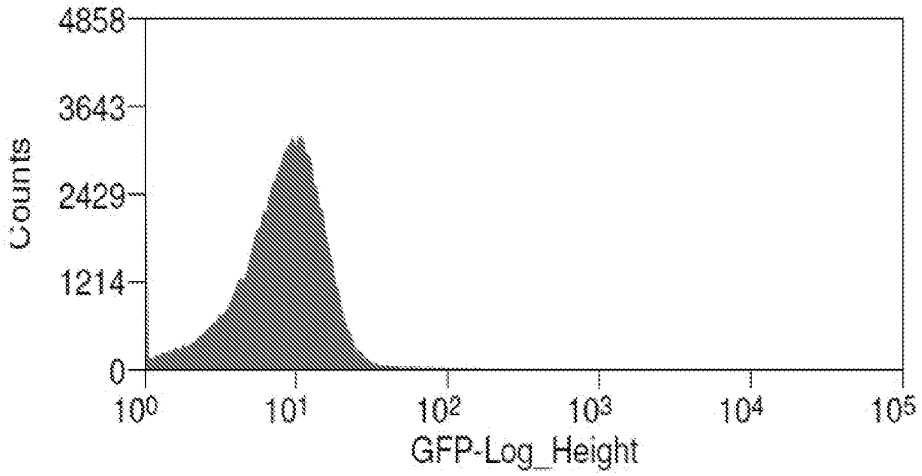

80
70
60
50
40
30
20
10
0
Average MiSeq Counts
1
34
67
100
133
166
199
232
265
298
331
364
397
430
463
496
529
562
595
628
661
694
727
760
793
826
859
892
925
958
991
1024
PAM variant

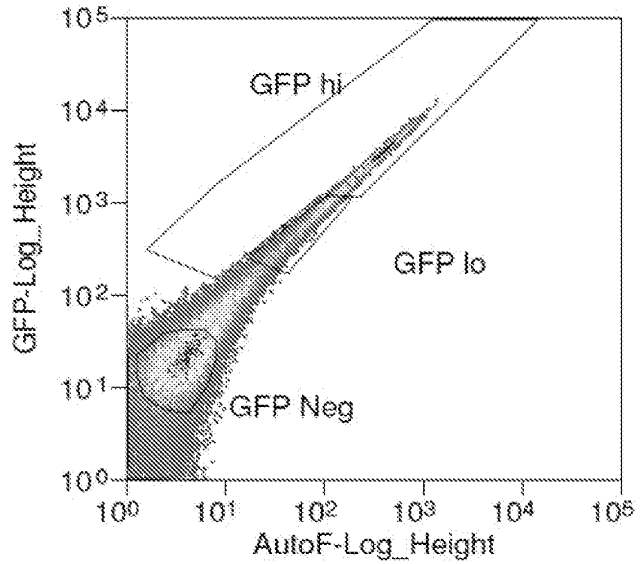
Fig. 6



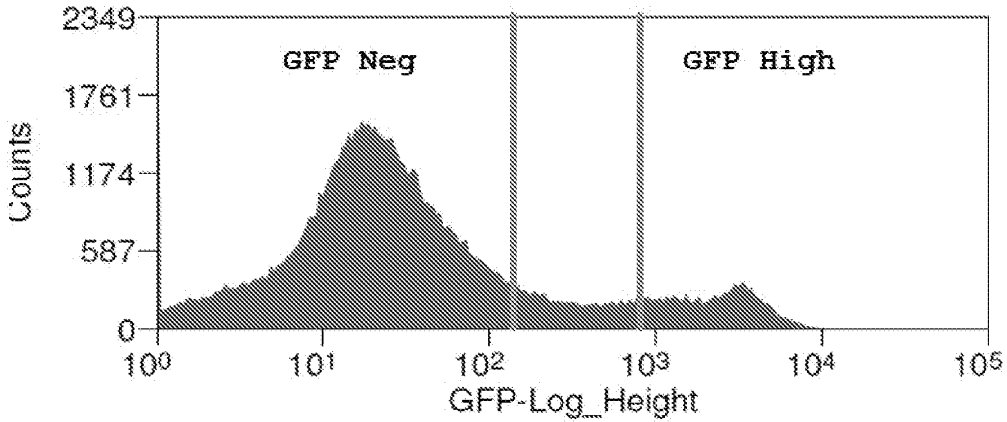


Region	Count	% Hist	% All	Bounds
Total	100000	100.00	100.00	(1.00,100000....

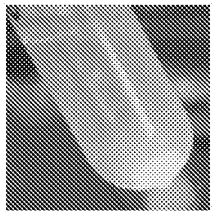
Fig. 7



Region	Count	% Hist	% All
Total	67983	100.00	67.98
GFP hi	6065	8.92	6.07
GFP lo	4883	7.18	4.88
FP Neg	28305	41.64	28.31



Region	Count	% Hist	% All	Bounds
Total	100000	100.00	100.00	(1.00,100000....



High GFP

Fig. 8

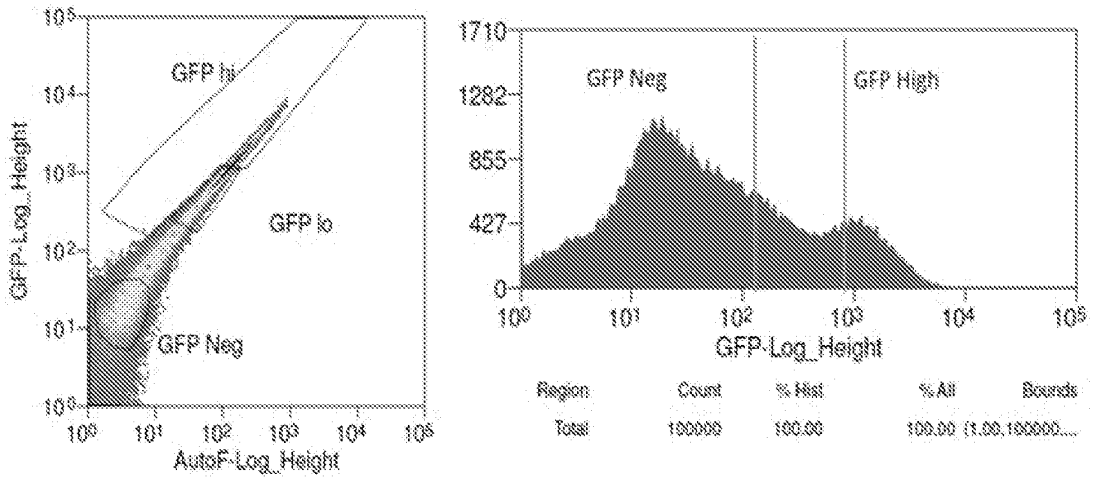

GFP hi
GFP lo
GFP Neg
GFP-Log_Height
AutoF-Log_Height
GFP Neg
GFP High
GFP-Log_Height
Region
Count
% Hist
% All
Bounds
Total
100000
100.00
100.00

Fig. 9

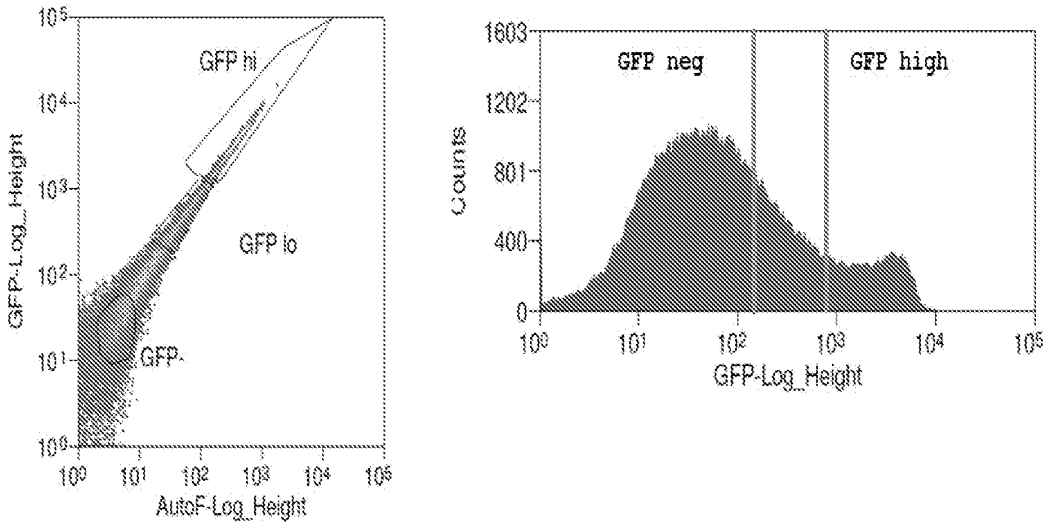

GFP hi
GFP lo
GFP-
GFP-Log_Height
AutoF-Log_Height
GFP neg
GFP high
Counts
GFP-Log_Height

Fig. 10

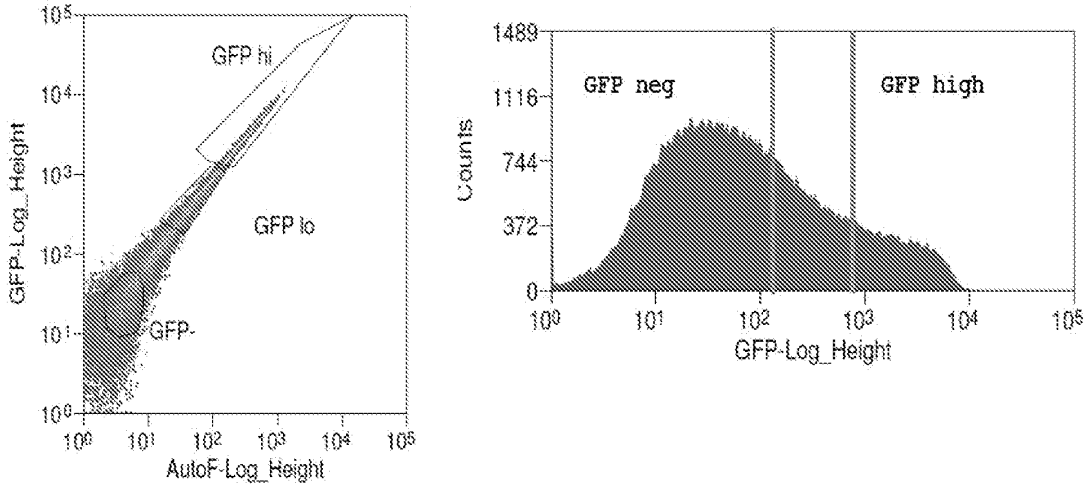

GFP hi
GFP lo
GFP-
GFP-Log_Height
AutoF-Log_Height
GFP neg
GFP high
Counts
GFP-Log_Height

Fig. 11

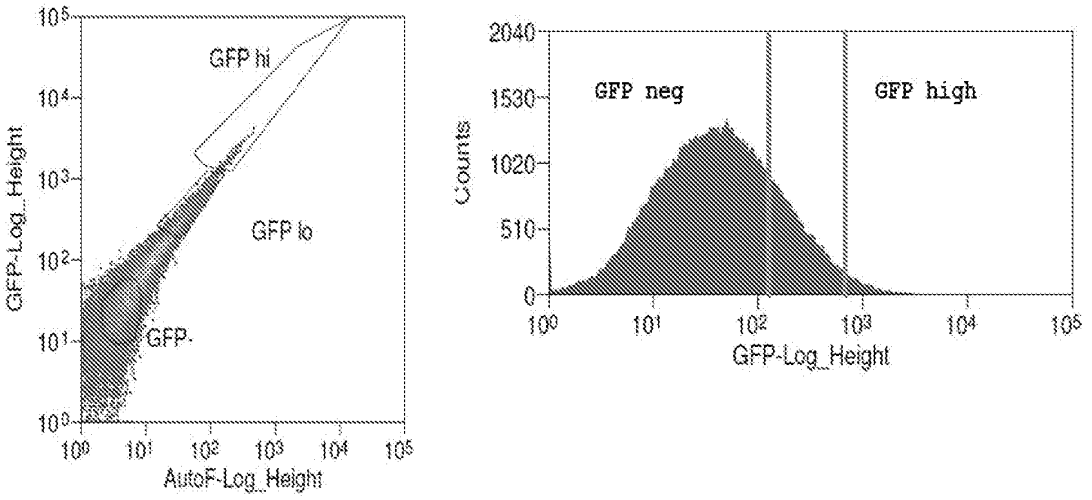

GFP hi
GFP lo
GFP-
GFP-Log_Height
AutoF-Log_Height
GFP neg
GFP high
Counts
GFP-Log_Height

Fig 12

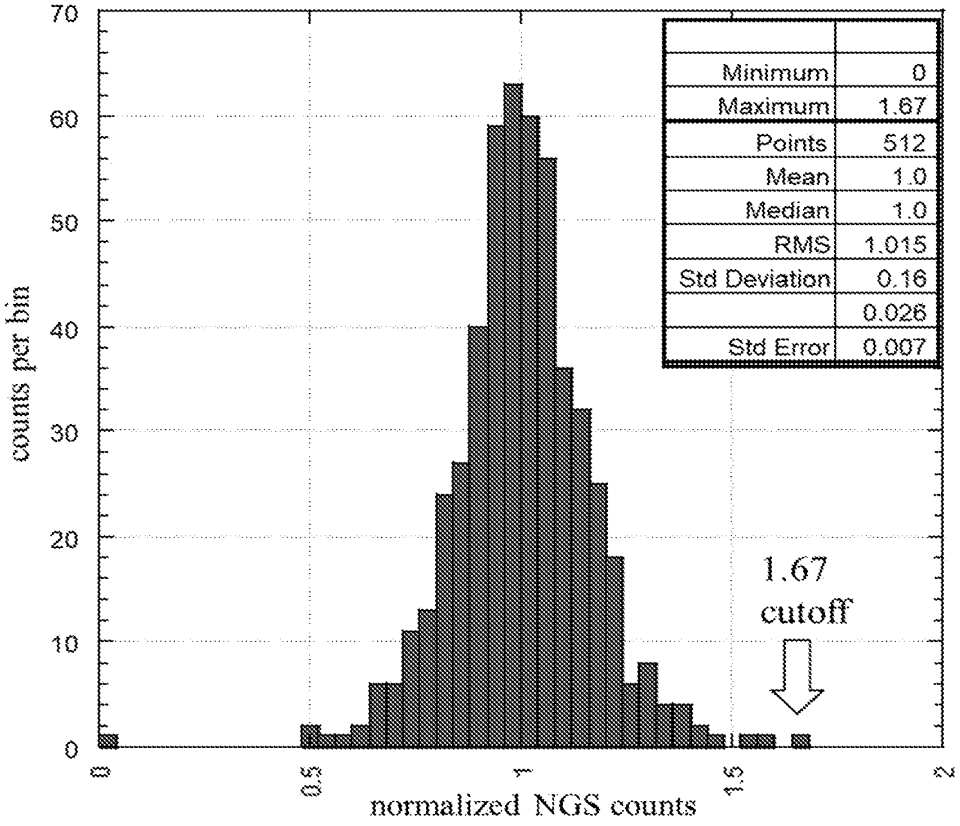

70
60
50
40
30
20
10
0
counts per bin
0
0.5
1
1.5
2
normalized NGS counts
1.67
cutoff
Minimum 0
Maximum 1.67
Points 512
Mean 1.0
Median 1.0
RMS 1.015
Std Deviation 0.16
0.026
Std Error 0.007

Fig. 13

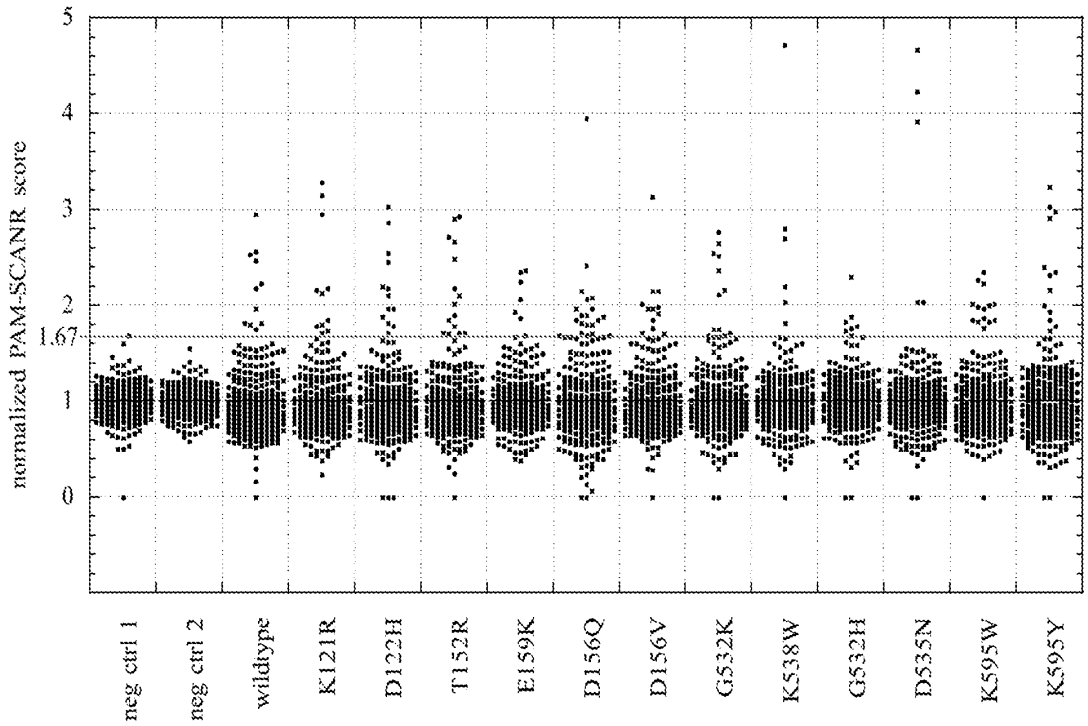

normalized PAM-SCANR score
5
4
3
2
1.67
1
0
neg ctrl 1
neg ctrl 2
wildtype
K121R
D122H
T152R
E159K
D156Q
D156V
G532K
K538W
G532H
D535N
K595W
K595Y

Fig. 14

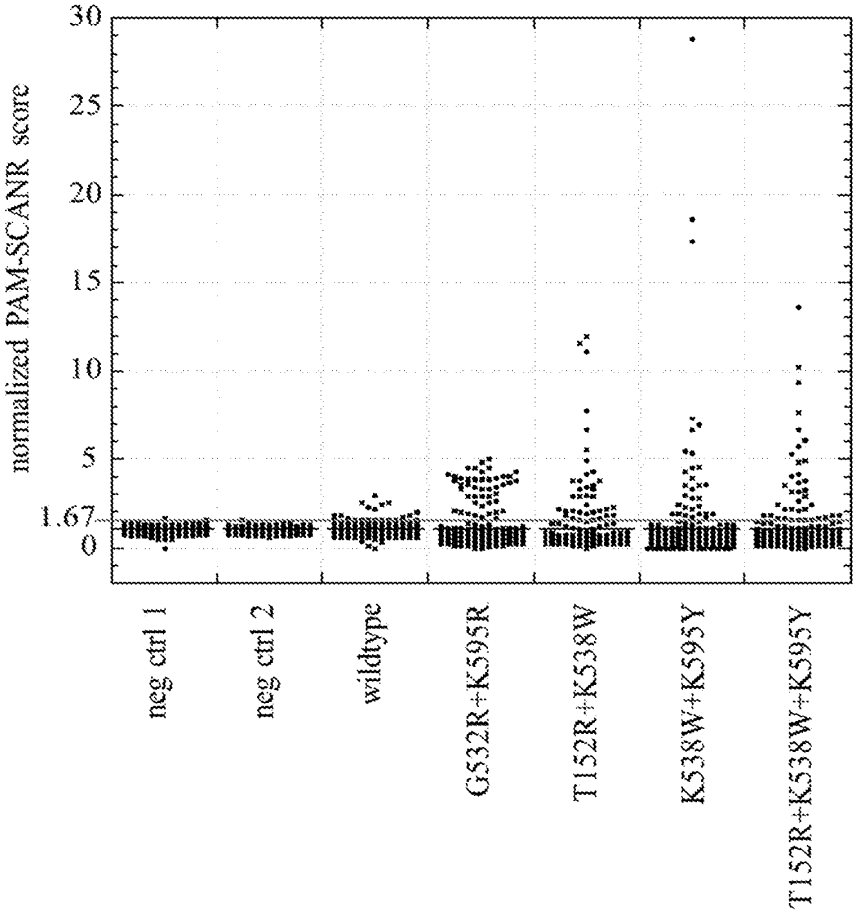

normalized PAM-SCANR score
30
25
20
15
10
5
1.67
0
neg ctrl 1
neg ctrl 2
wildtype
G532R+K595R
T152R+K538W
K538W+K595Y
T152R+K538W+K595Y

Fig. 15

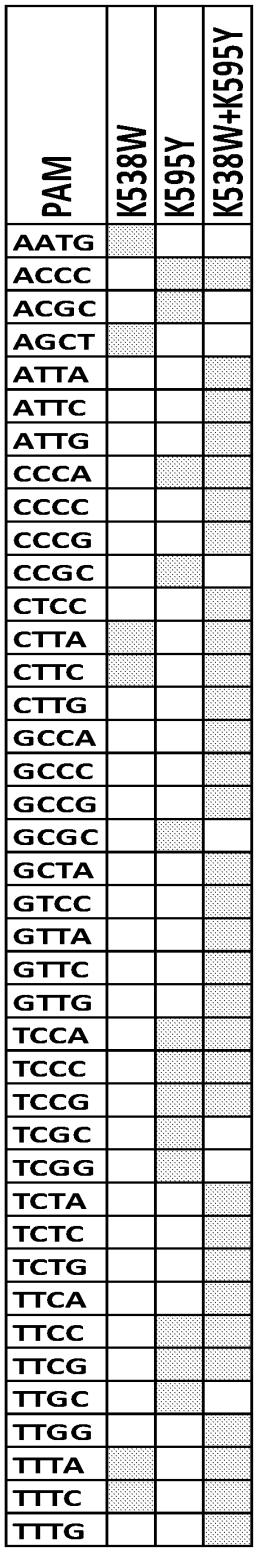

PAM
K538W
K595Y
K538W+K595Y
AATG
ACCC
ACGC
AGCT
ATTA
ATTC
ATTG
CCCA
CCCC
CCCG
CCGC
CTCC
CTTA
CTTC
CTTG
GCCA
GCCC
GCCG
GCGC
GCTA
GTCC
GTTA
GTTC
GTTG
TCCA
TCCC
TCCG
TCGC
TCGG
TCTA
TCTC
TCTG
TTCA
TTCC
TTCG
TTGC
TTGG
TTTA
TTTC
TTTG

Fig. 16

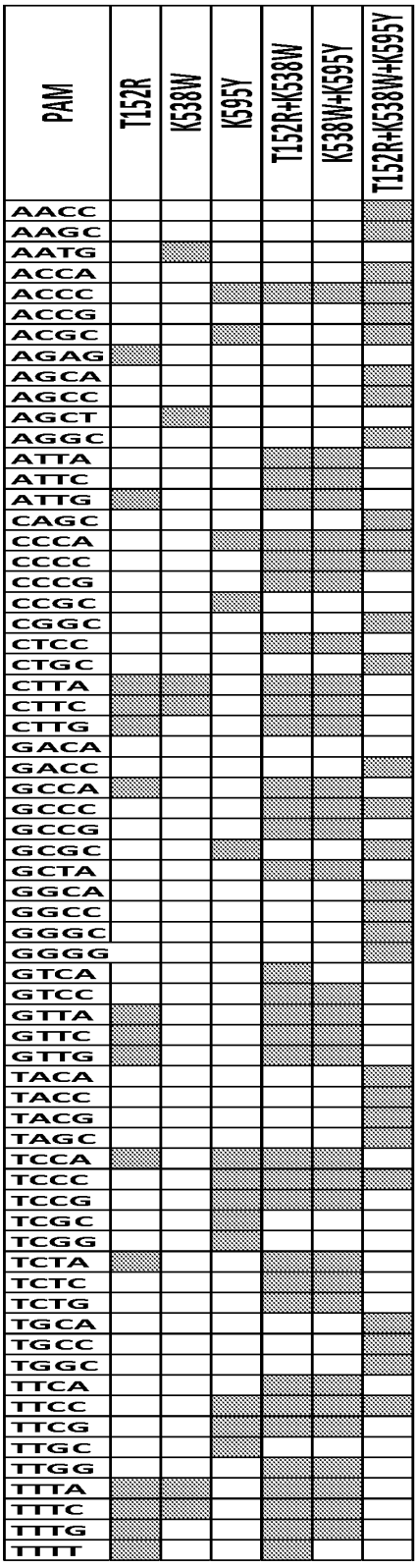

PAM
T152R
K538W
K595Y
T152R+K538W
K538W+K595Y
T152R+K538W+K595Y
AACC
AAGC
AATG
ACCA
ACCC
ACCG
ACGC
AGAG
AGCA
AGCC
AGCT
AGGC
ATTA
ATTC
ATTG
CAGC
CCCA
CCCC
CCCG
CCGC
CGGC
CTCC
CTGC
CTTA
CTTC
CTTG
GACA
GACC
GCCA
GCCC
GCCG
GCGC
GCTA
GGCA
GGCC
GGGC
GGGG
GTCA
GTCC
GTTA
GTTC
GTTG
TACA
TACC
TACG
TAGC
TCCA
TCCC
TCCG
TCGC
TCGG
TCTA
TCTC
TCTG
TGCA
TGCC
TGGC
TTCA
TTCC
TTCG
TTGC
TTGG
TTTA
TTTC
TTTG
TTTT

Fig. 17

PAM	K595Y	PAM Depletion Score
ACCC		18.7
ACGC		13.8
CCCA		11.5
CCGC		16.7
GCGC		17.5
TCCA		16.9
TCCC		18.6
TCCG		16.2
TCGC		13.3
TCGG		14.7
TTCC		11.3
TTCG		17.8
TTGC		11.5

PAM	T152R	PAM Depletion Score
AGAG		
ATTG		10.8
CTTA		10.9
CTTC		16.1
CTTG		9.5
GCCA		10.4
GTTA		10.3
GTTC		9.9
GTTG		12.7
TCCA		10.7
TCTA		10.4
TTTT		10.4

Fig. 18

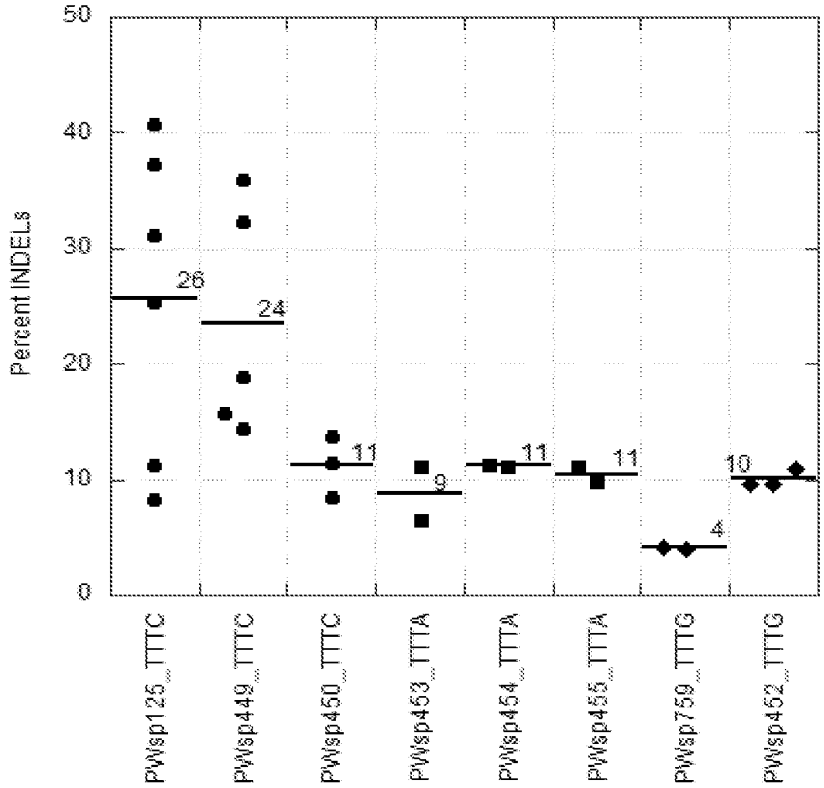


Fig. 19

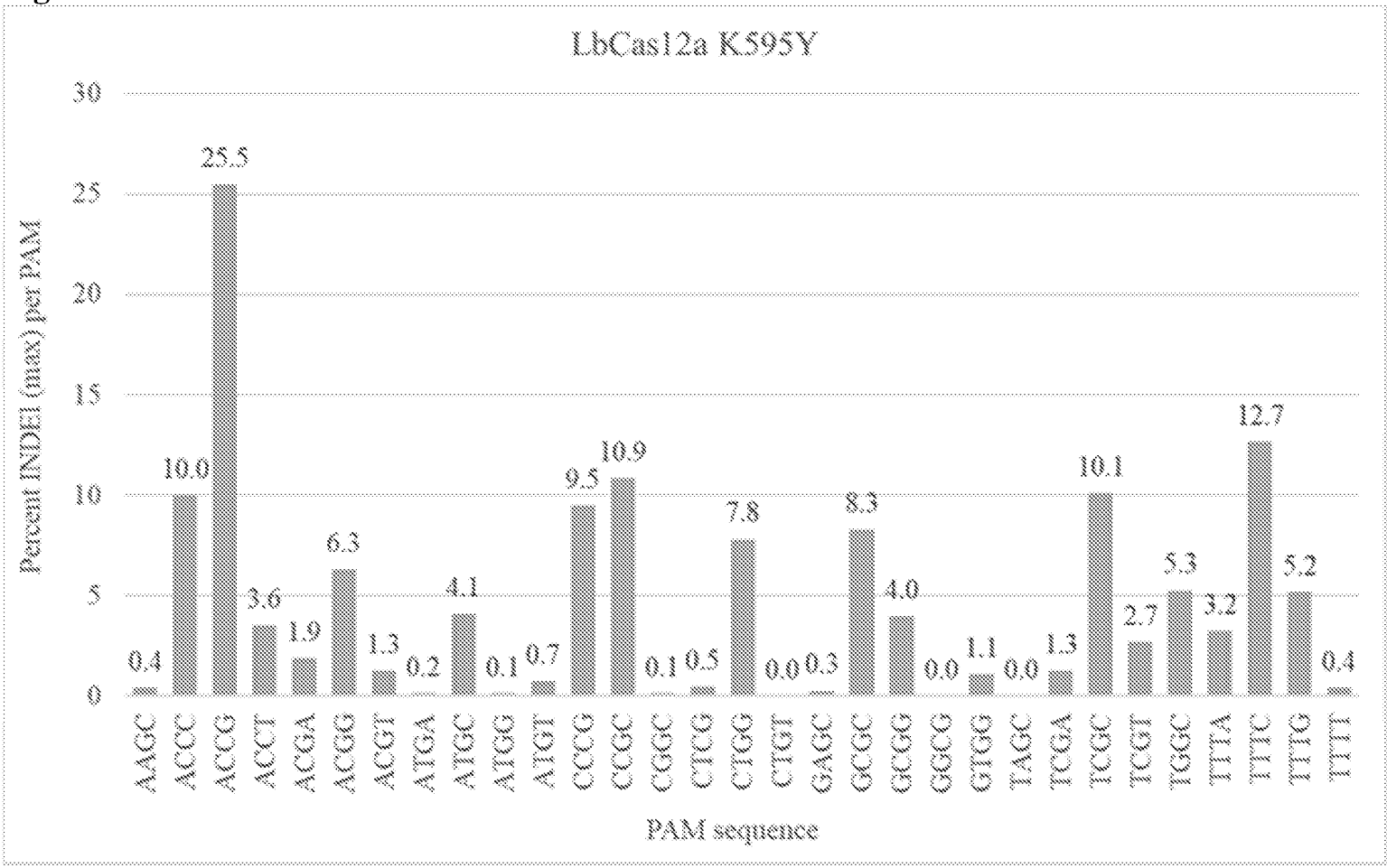

LbCas12a K595Y
Percent INDEI (max) per PAM
30
25
20
15
10
5
0
0.4
10.0
25.5
3.6
1.9
6.3
1.3
0.2
4.1
0.1
0.7
9.5
10.9
0.1
0.5
7.8
0.0
0.3
8.3
4.0
0.0
1.1
0.0
1.3
10.1
2.7
5.3
3.2
12.7
5.2
0.4
AAGC
ACCC
ACCG
ACCT
ACGA
ACGG
ACGT
ATGA
ATGC
ATGG
ATGT
CCCG
CCGC
CGGC
CTCG
CTGG
CTGT
GAGC
GCGC
GCGG
GGCG
GTGG
TAGC
TCGA
TCGC
TCGT
TGGC
TTTA
TTTC
TTTG
TTTT
PAM sequence

Fig. 20

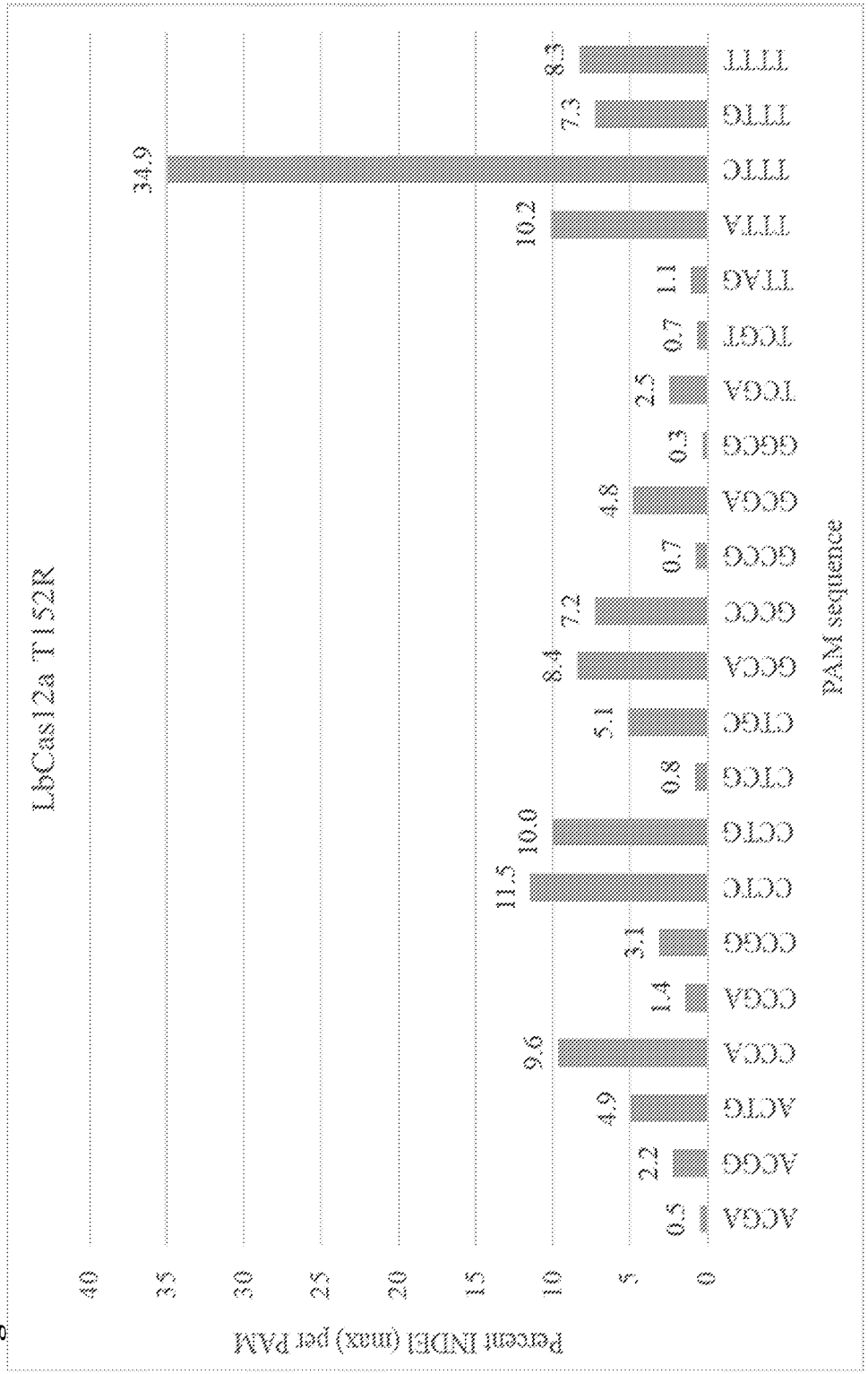

LbCas12a T152R
Percent INDEL (max) per PAM
PAM sequence
0
5
10
15
20
25
30
35
40
ACGA 0.5
ACGG 2.2
ACTG 4.9
CCCA 9.6
CCGA 1.4
CCGG 3.1
CCTC 11.5
CCTG 10.0
CTGA 0.8
CTGC 5.1
GCCA 8.4
GCCC 7.2
GCCG 0.7
GCGA 4.8
GGCG 0.3
TCGA 2.5
TCGT 0.7
TTAG 1.1
TTTA 10.2
TTTC 34.9
TTTG 7.3
TTTT 8.3

Fig. 21

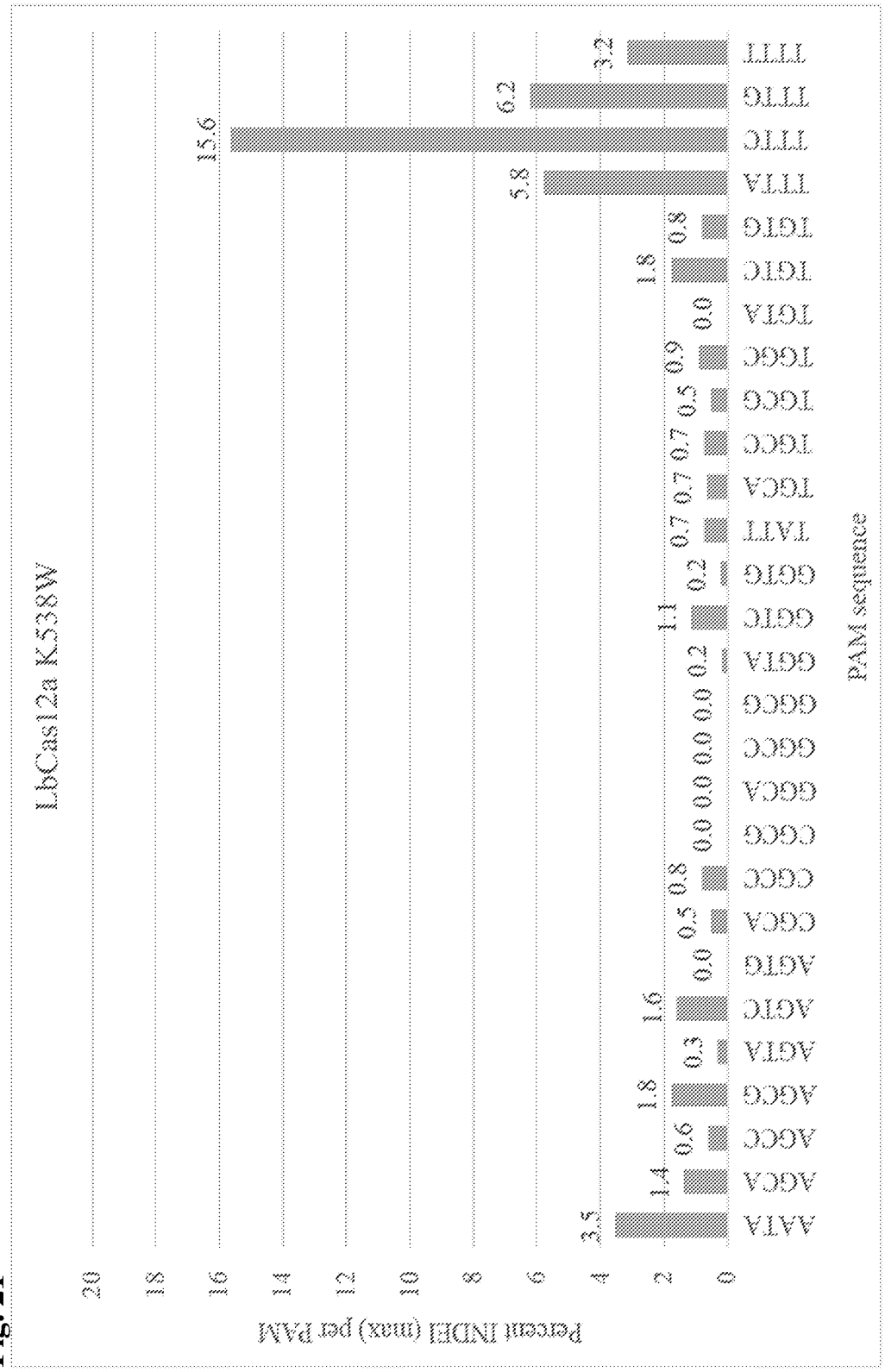

LbCas12a K538W
Percent INDEL (max) per PAM
PAM sequence
0
2
4
6
8
10
12
14
16
18
20
AATA
AGCA
AGCC
AGCG
AGTA
AGTC
AGTG
CGCA
CGCC
CGCG
GGCA
GGCC
GGCG
GGTA
GGTC
GGTG
TATT
TGCA
TGCC
TGCG
TGGC
TGTA
TGTC
TGTG
TTTA
TTTC
TTTG
TTTT
3.5
1.4
0.6
1.8
0.3
1.6
0.0
0.5
0.8
0.0
0.0
0.0
0.2
1.1
0.2
0.7
0.7
0.7
0.5
0.9
0.0
1.8
0.8
5.8
15.6
6.2
3.2

Fig. 22A

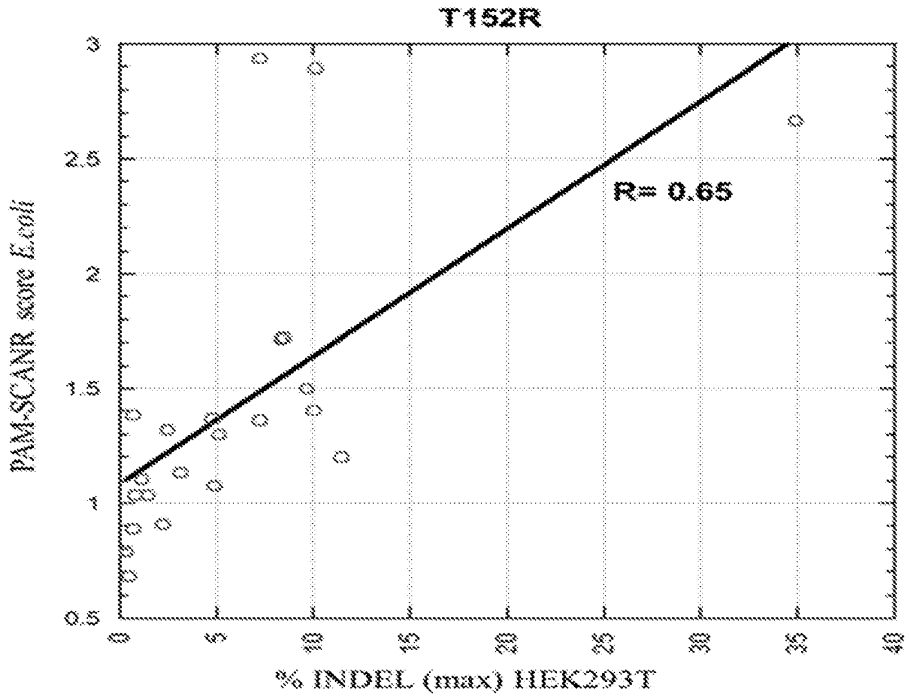


Fig. 22B

