Figure 1

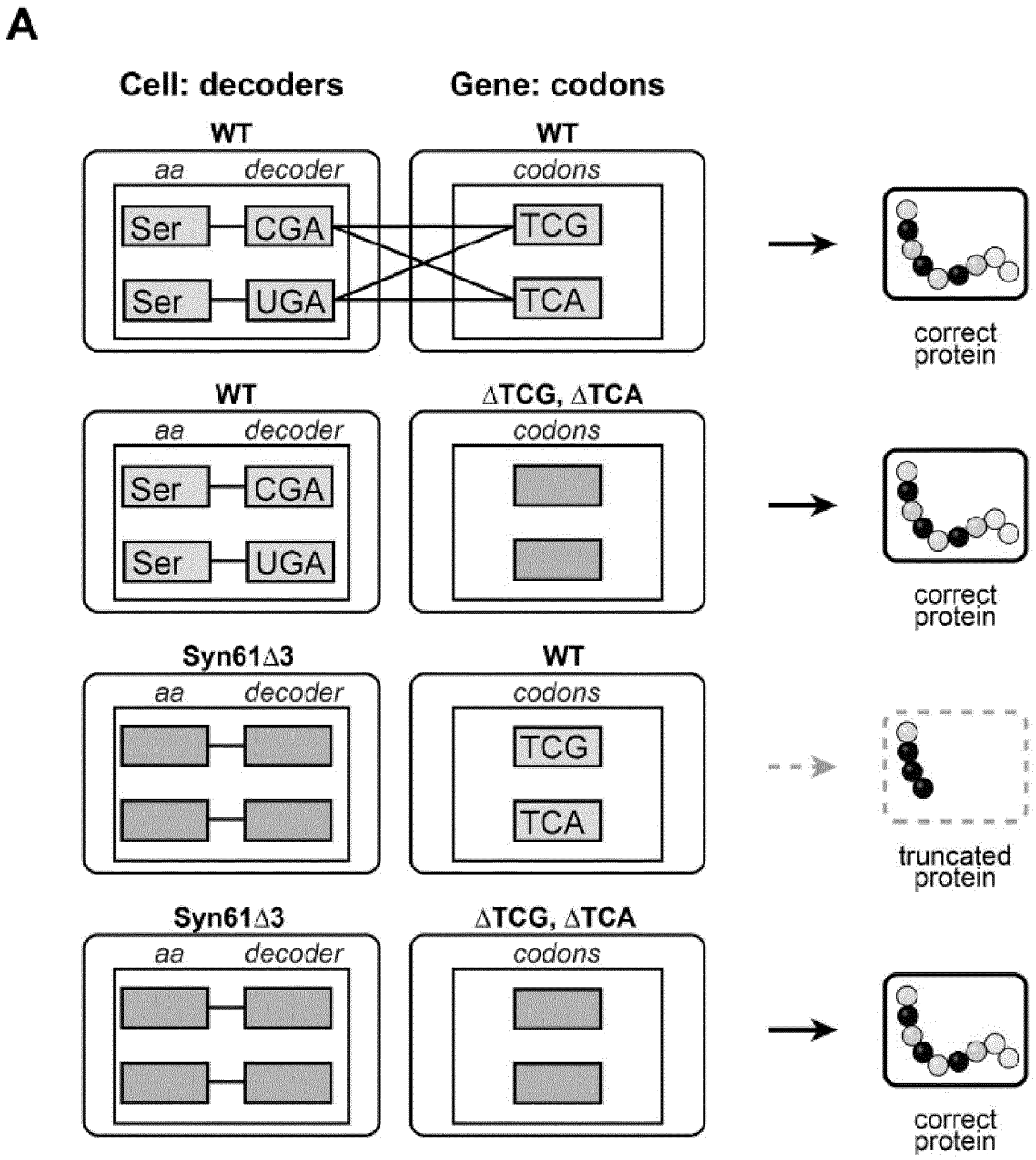

A
Cell: decoders
Gene: codons
WT
aa
decoder
Ser
CGA
Ser
UGA
WT
codons
TCG
TCA
correct protein
WT
aa
decoder
Ser
CGA
Ser
UGA
ΔTCG, ΔTCA
codons
correct protein
Syn61Δ3
aa
decoder
WT
codons
TCG
TCA
truncated protein
Syn61Δ3
aa
decoder
ΔTCG, ΔTCA
codons
correct protein

Figure 1 (cont)

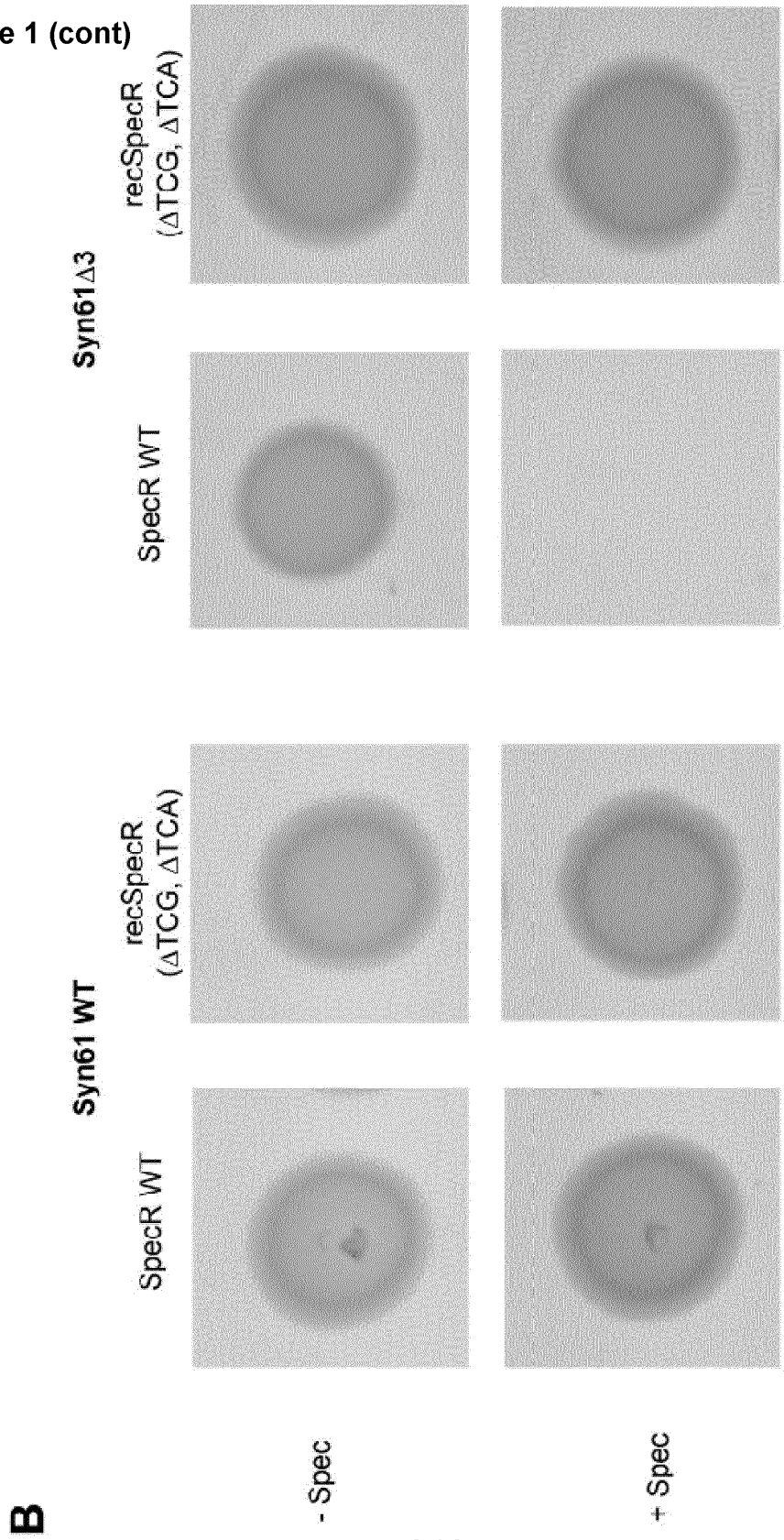


Figure 1 (cont)

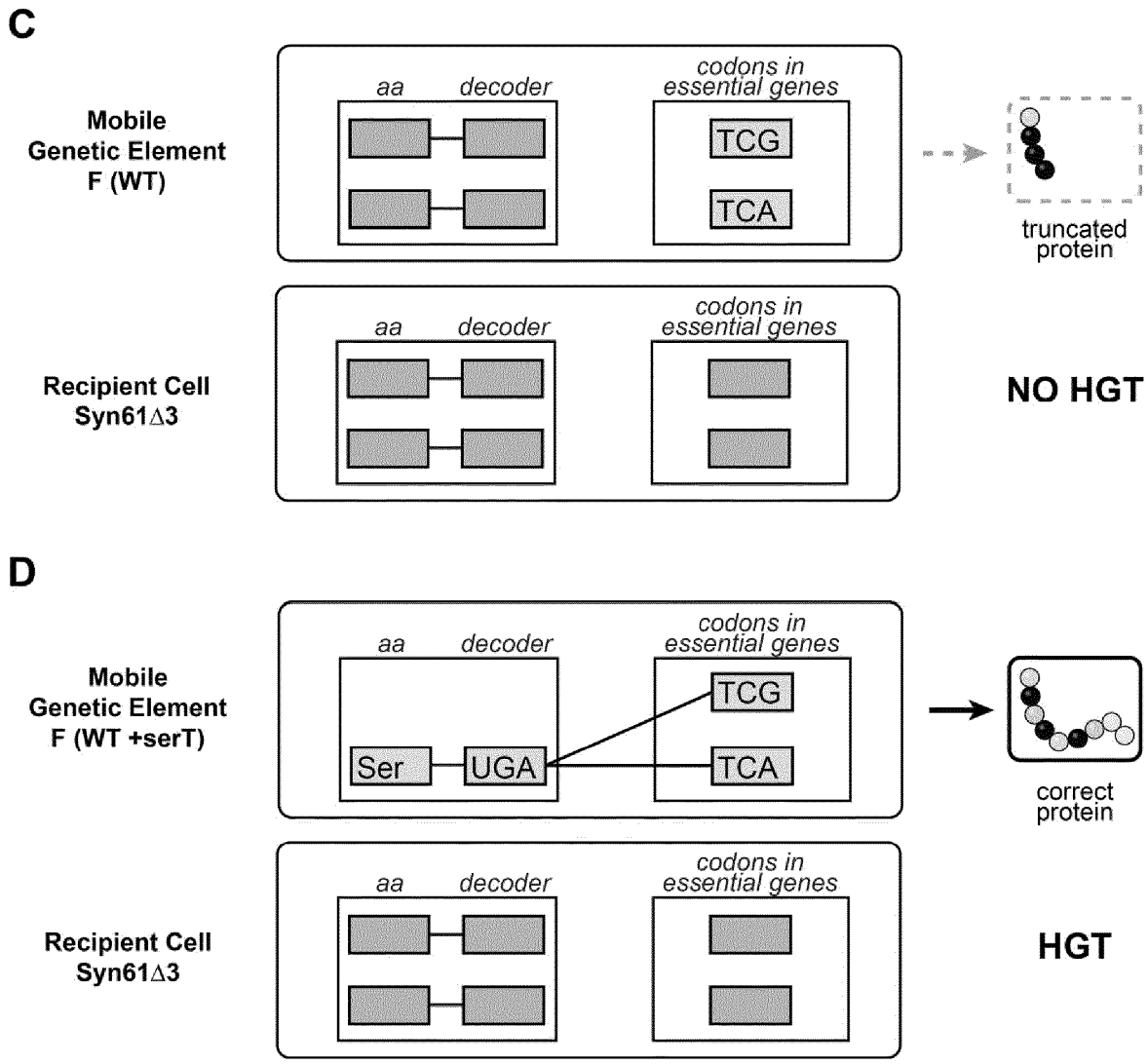


Figure 1 (cont)

E

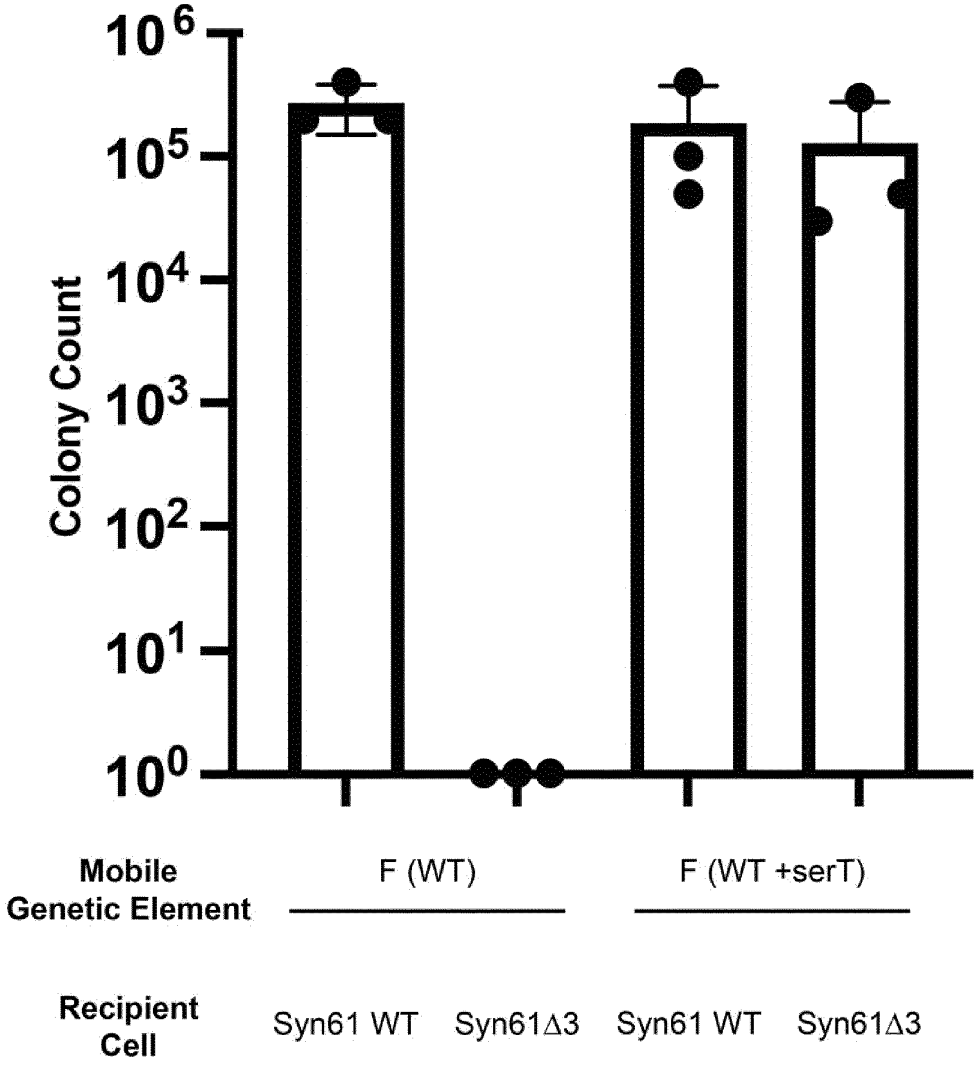


Figure 2

A

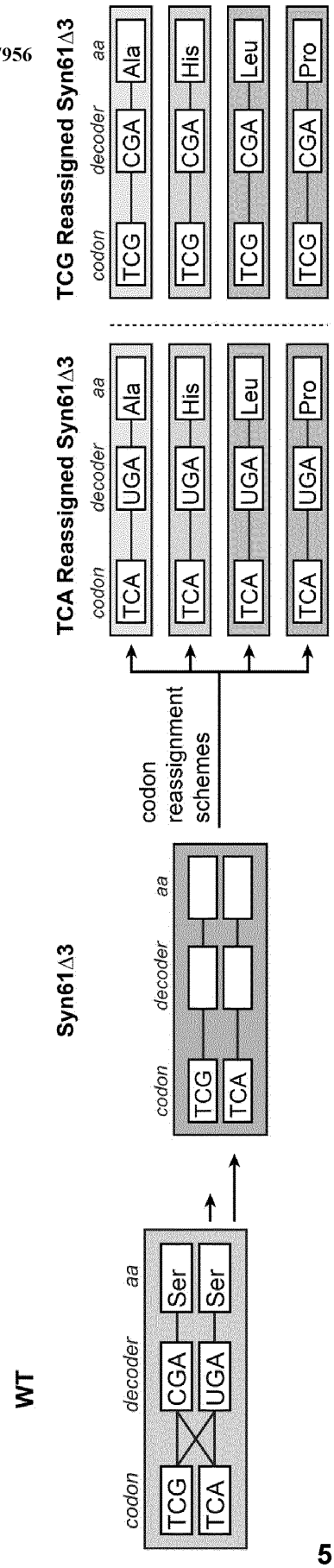

WT
codon
decoder
aa
TCG
TCA
CGA
UGA
Ser
Ser
Syn61Δ3
codon
decoder
aa
TCG
TCA
codon reassignment schemes
TCA Reassigned Syn61Δ3
codon
decoder
aa
TCA
TCA
TCA
TCA
UGA
UGA
UGA
UGA
Ala
His
Leu
Pro
TCG Reassigned Syn61Δ3
codon
decoder
aa
TCG
TCG
TCG
TCG
CGA
CGA
CGA
CGA
Ala
His
Leu
Pro

Figure 2 (cont)

B

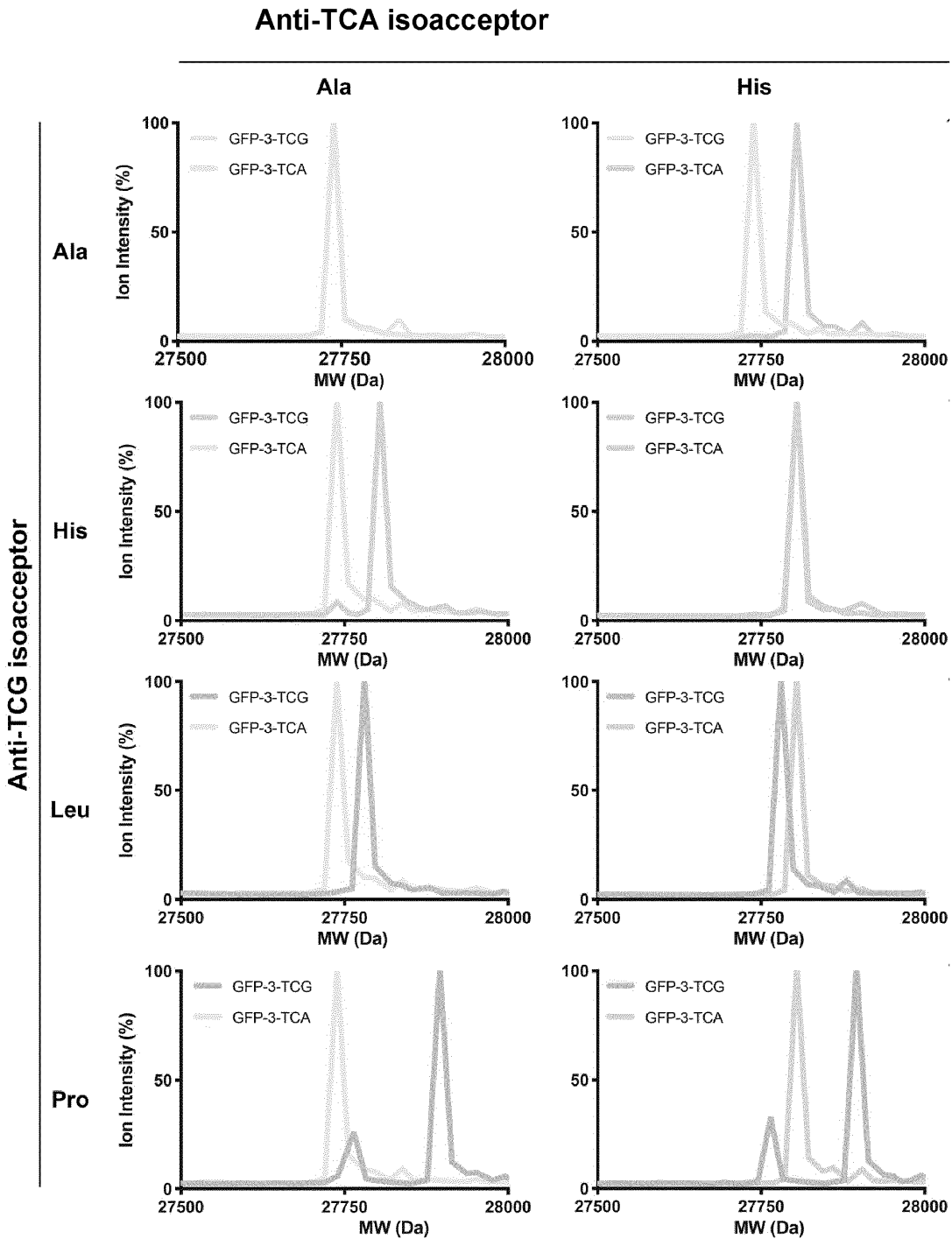


Figure 2 (cont)

B (cont)

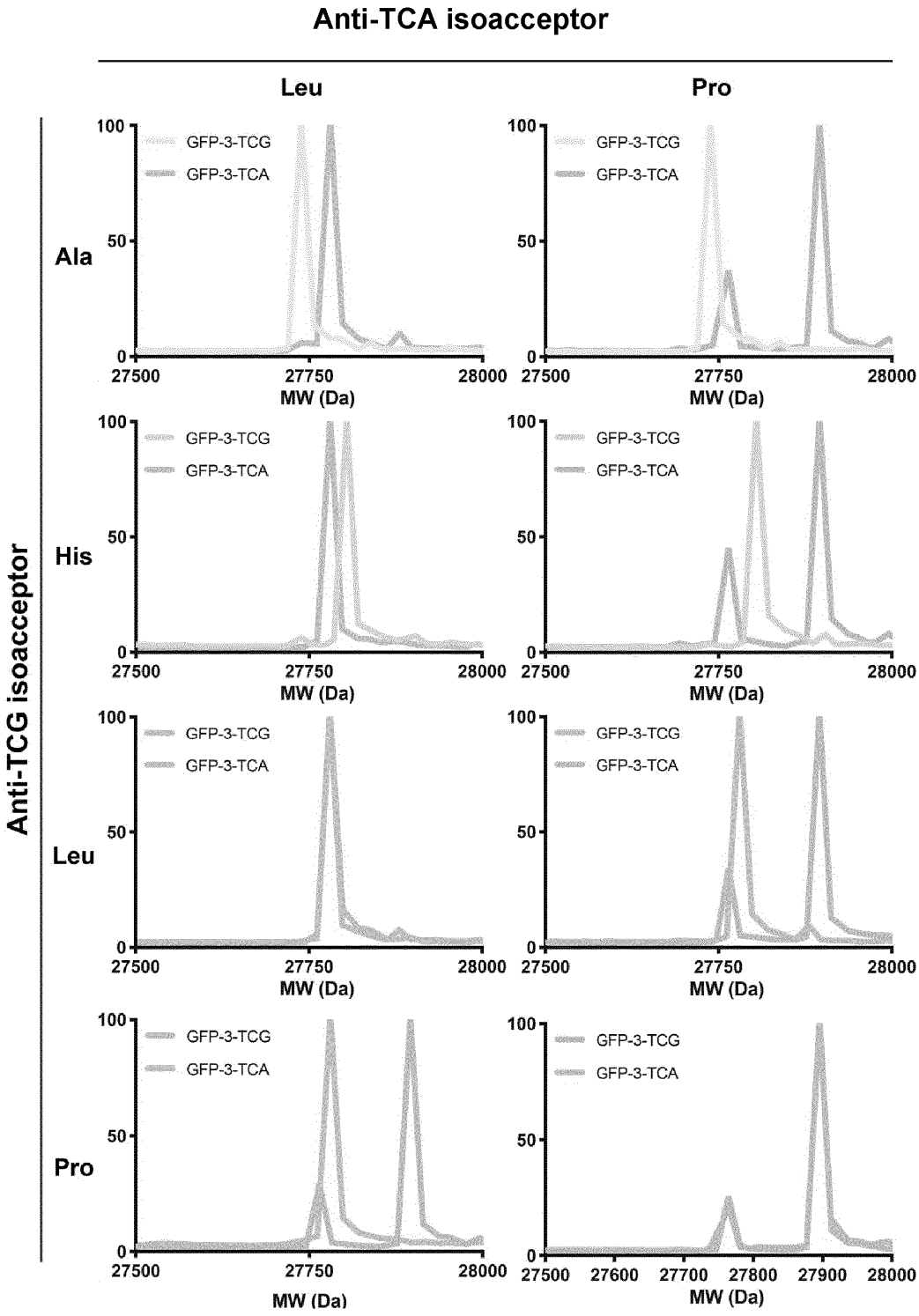


Figure 2 (cont)

C

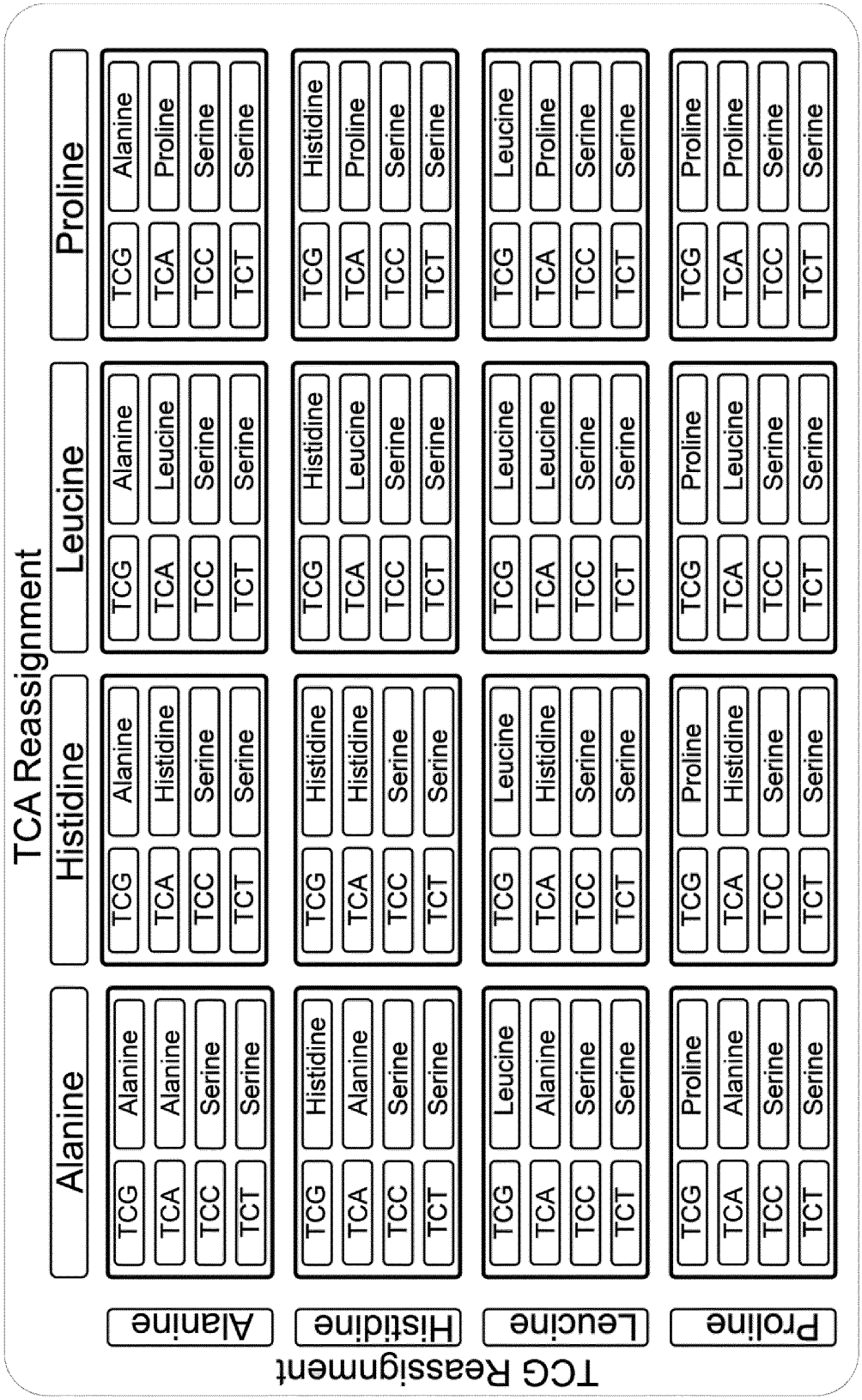


Figure 3

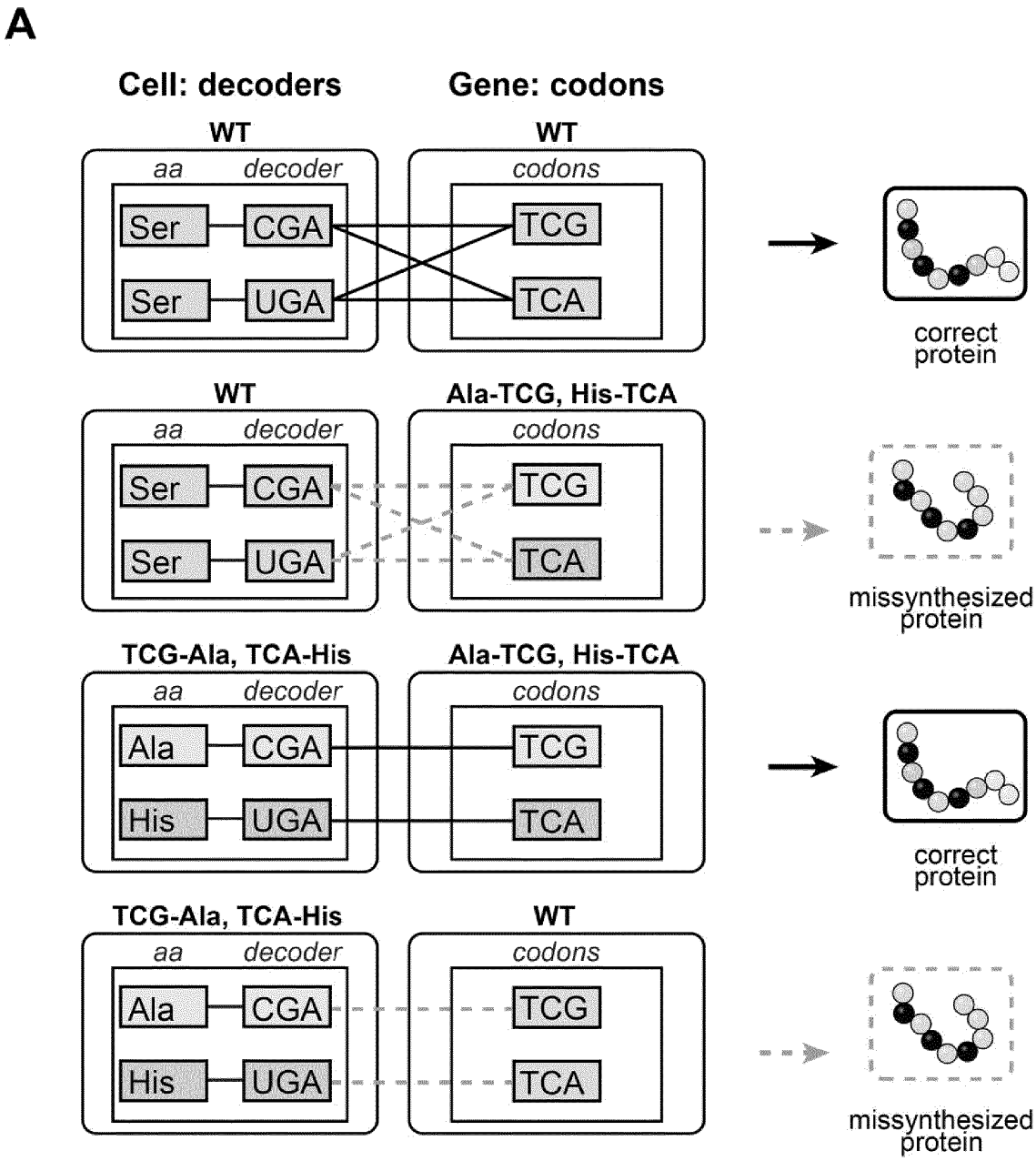

A
Cell: decoders
Gene: codons
WT
aa
decoder
Ser
CGA
Ser
UGA
WT
codons
TCG
TCA
correct protein
WT
Ala-TCG, His-TCA
aa
decoder
Ser
CGA
Ser
UGA
codons
TCG
TCA
missynthesized protein
TCG-Ala, TCA-His
Ala-TCG, His-TCA
aa
decoder
Ala
CGA
His
UGA
codons
TCG
TCA
correct protein
TCG-Ala, TCA-His
WT
aa
decoder
Ala
CGA
His
UGA
codons
TCG
TCA
missynthesized protein

Figure 3 (cont)

B

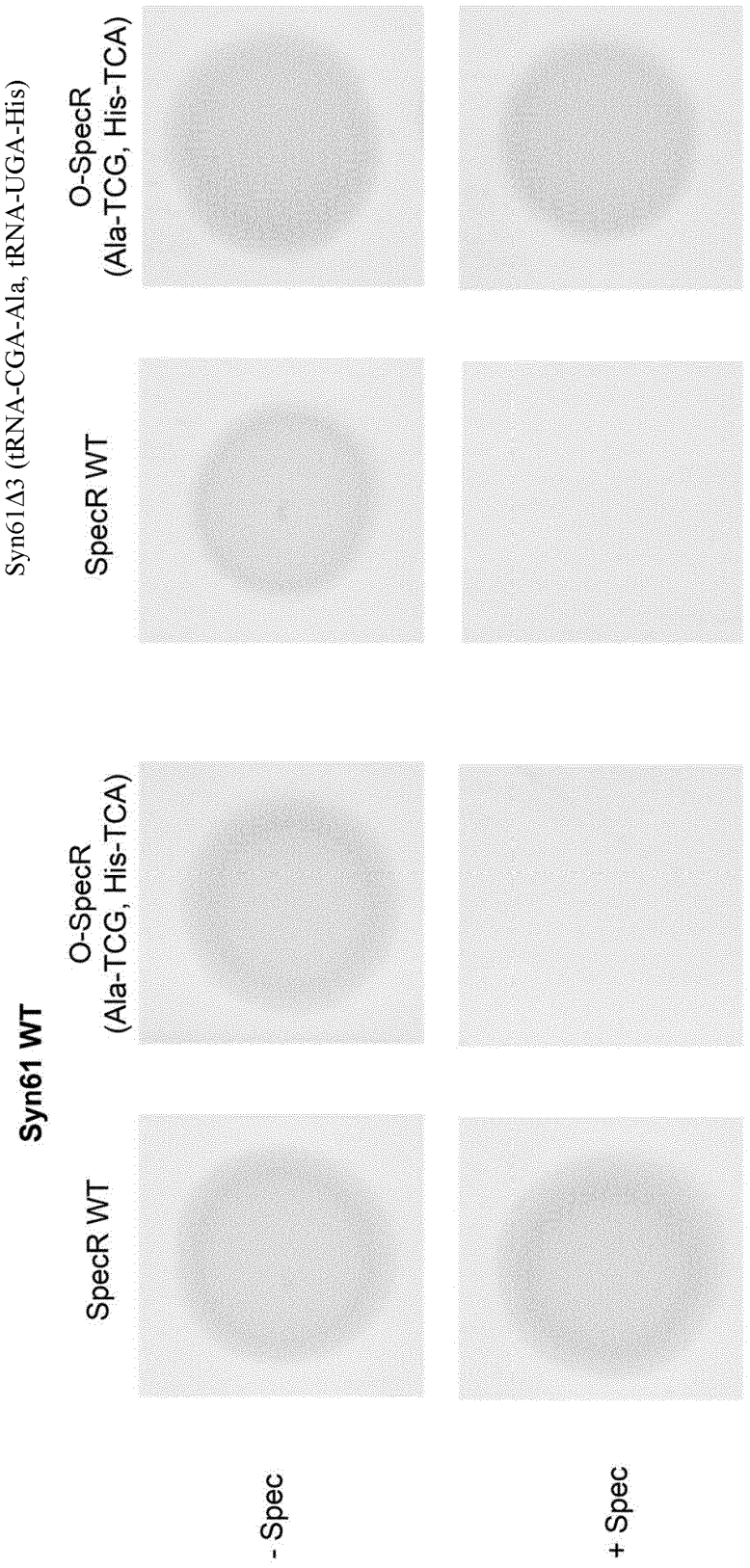


Figure 4

A

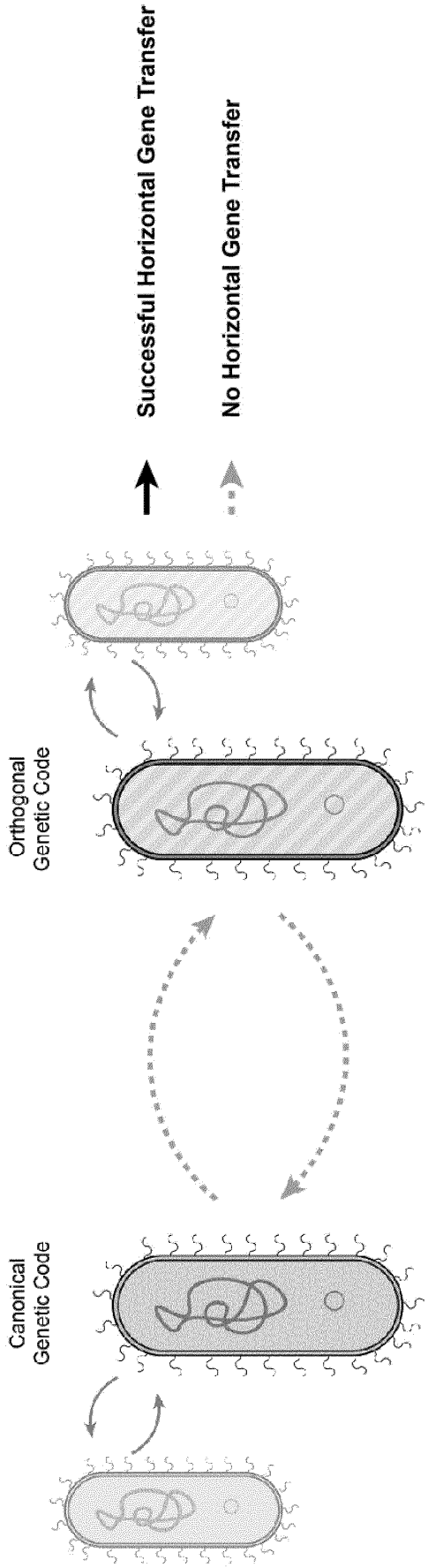
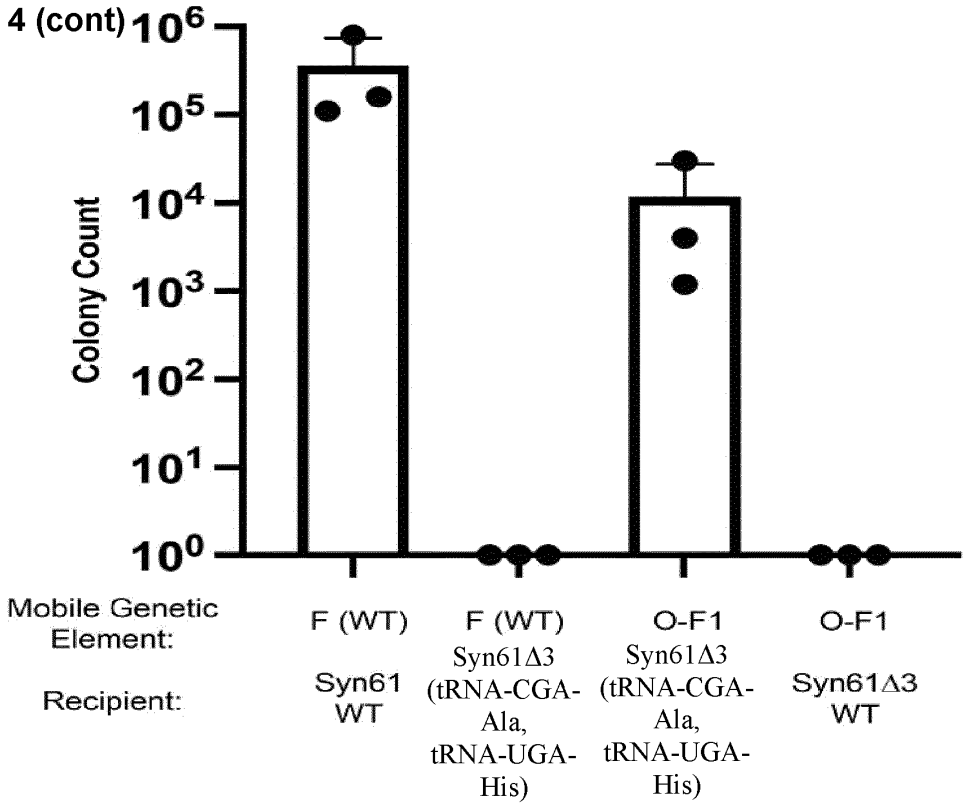

Successful Horizontal Gene Transfer
No Horizontal Gene Transfer
Orthogonal Genetic Code
Canonical Genetic Code

Figure 4 (cont)

B



C

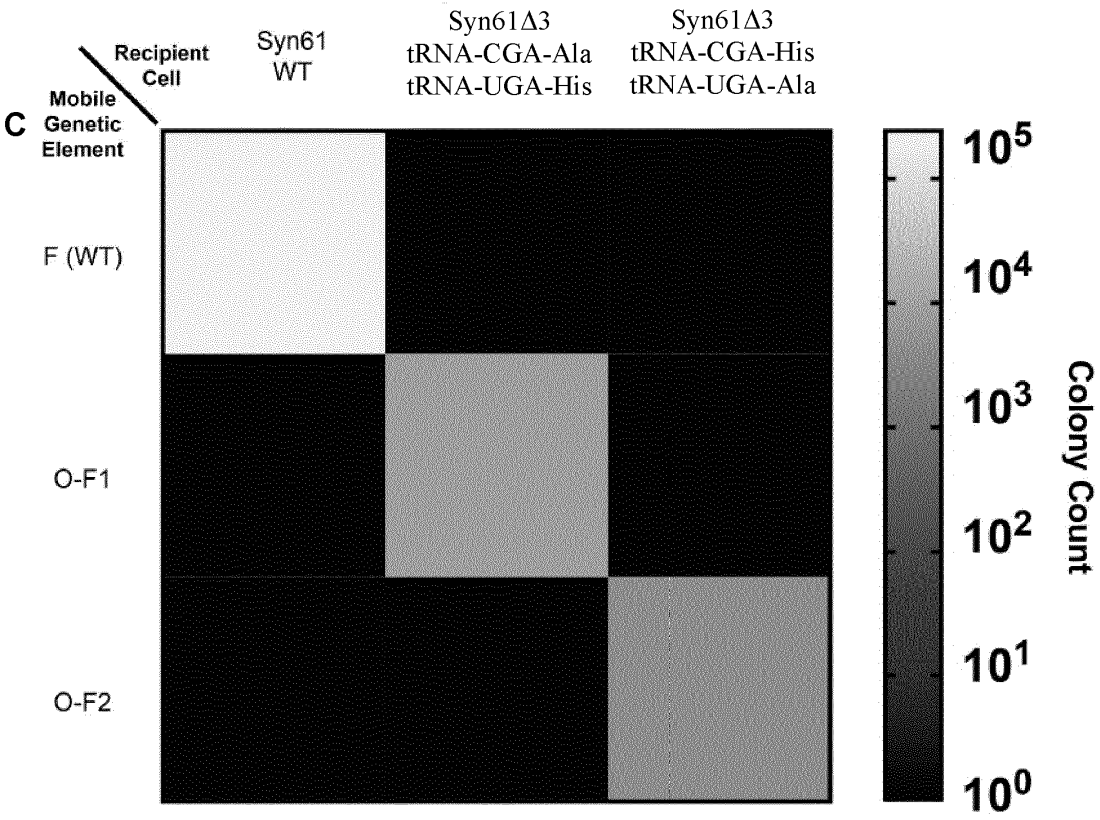


Figure 4 (cont)

D

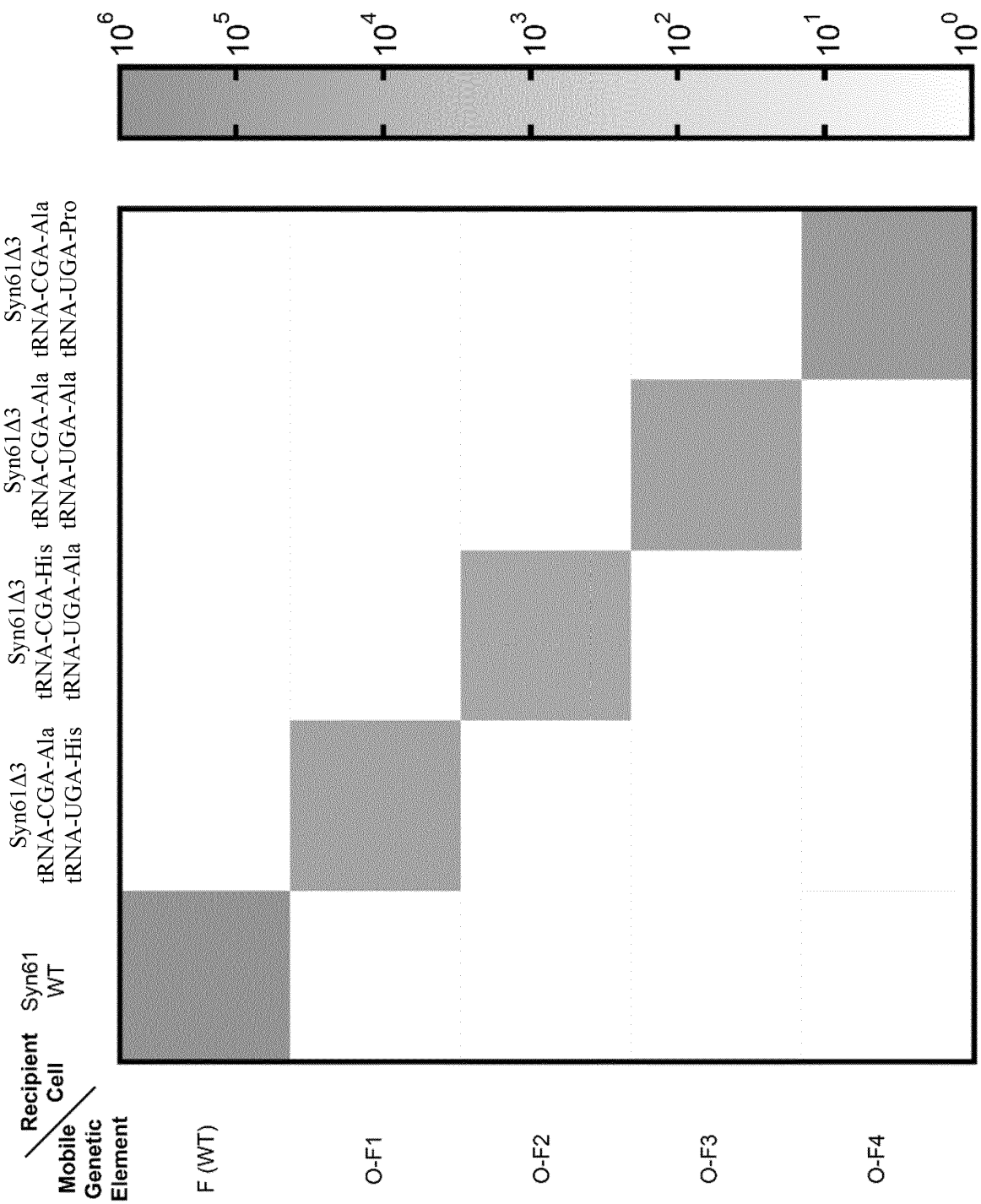


Figure 5

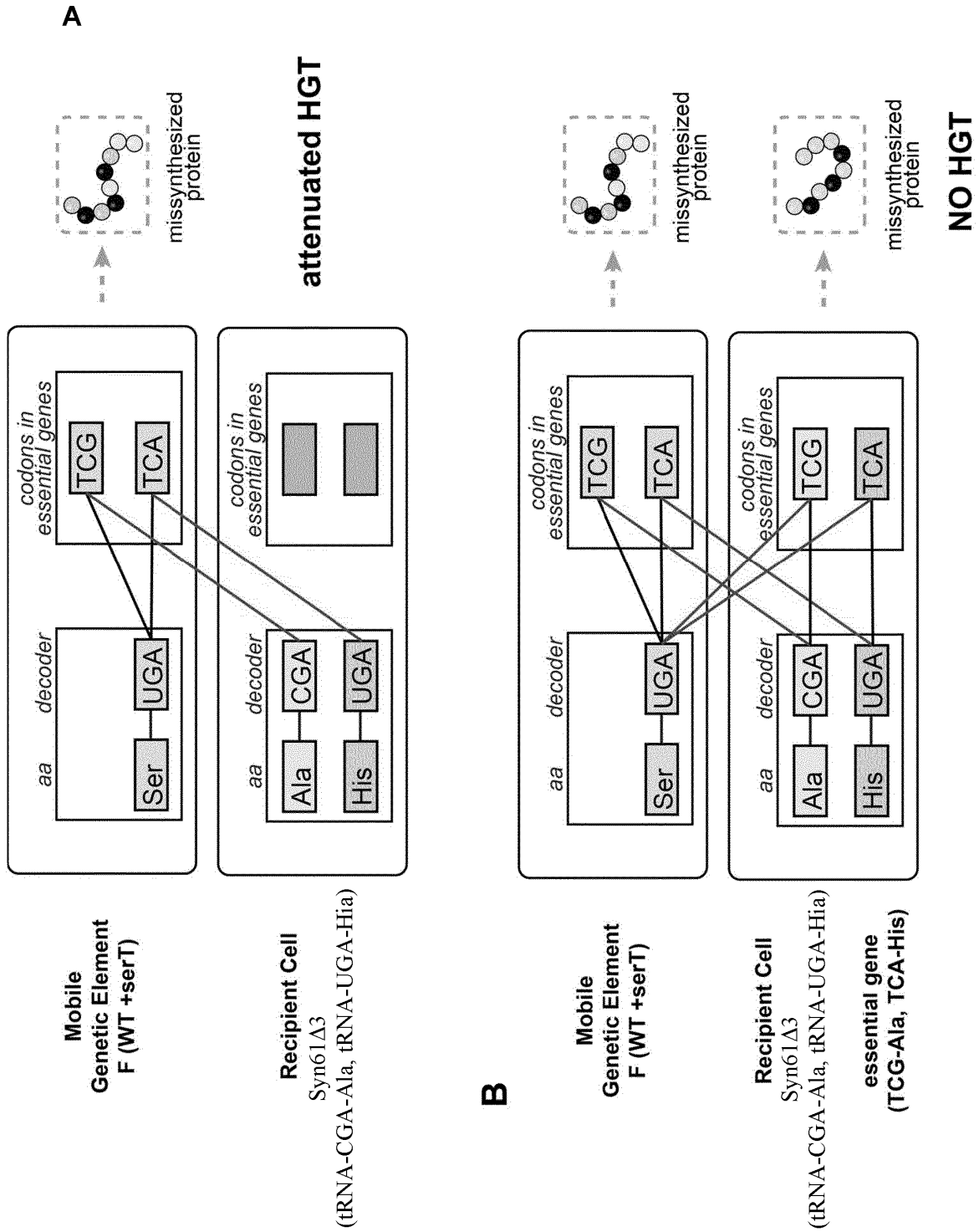

A
attenuated HGT
missynthesized protein
codons in essential genes
TCG
TCA
aa decoder
Ser
UGA
Ala
CGA
His
UGA
Mobile Genetic Element F (WT +serT)
Recipient Cell Syn61Δ3 (tRNA-CGA-Ala, tRNA-UGA-Hia)
B
NO HGT
missynthesized protein
codons in essential genes
TCG
TCA
aa decoder
Ser
UGA
Ala
CGA
His
UGA
Mobile Genetic Element F (WT +serT)
Recipient Cell Syn61Δ3 (tRNA-CGA-Ala, tRNA-UGA-Hia)
essential gene (TCG-Ala, TCA-His)

Figure 5 (cont)

C

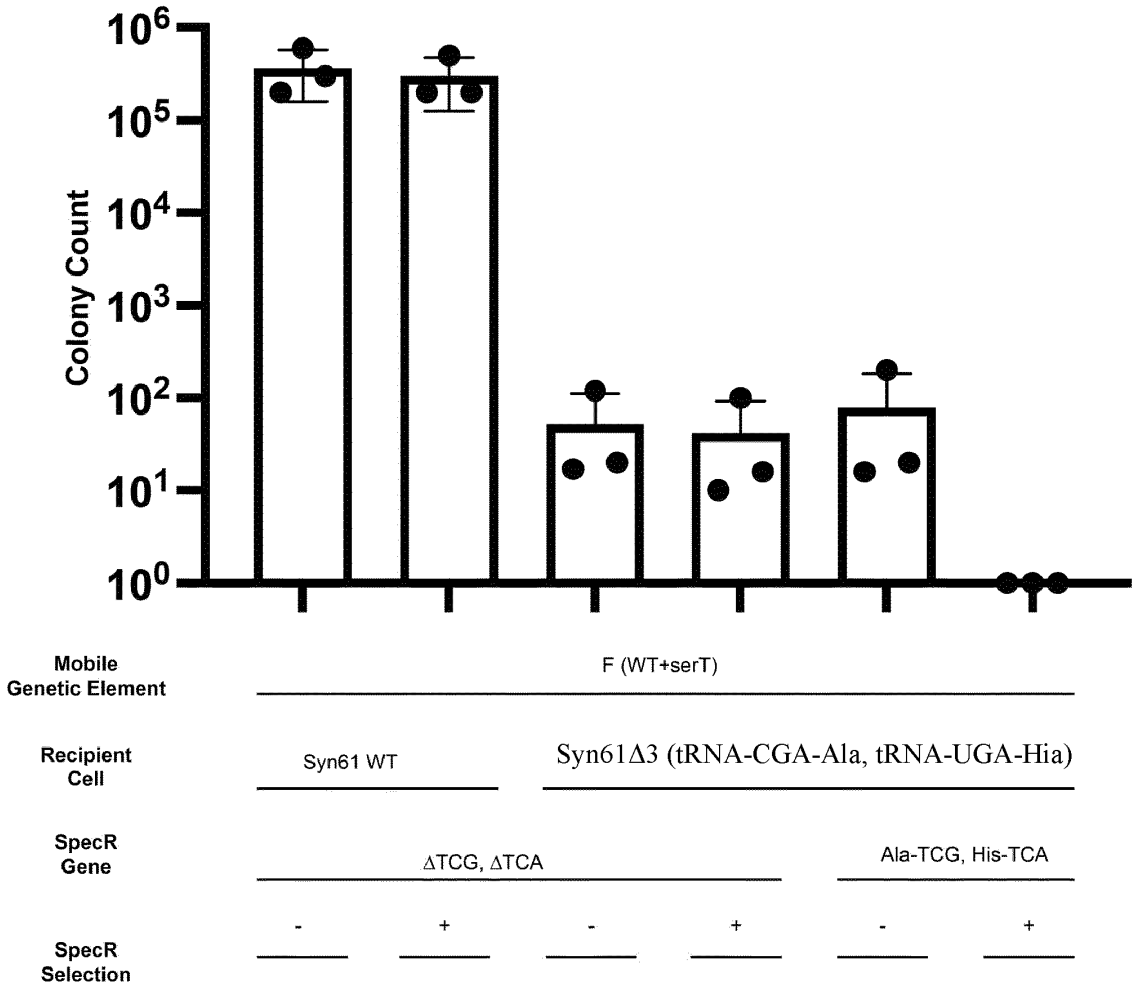


Figure 5 (cont)

D

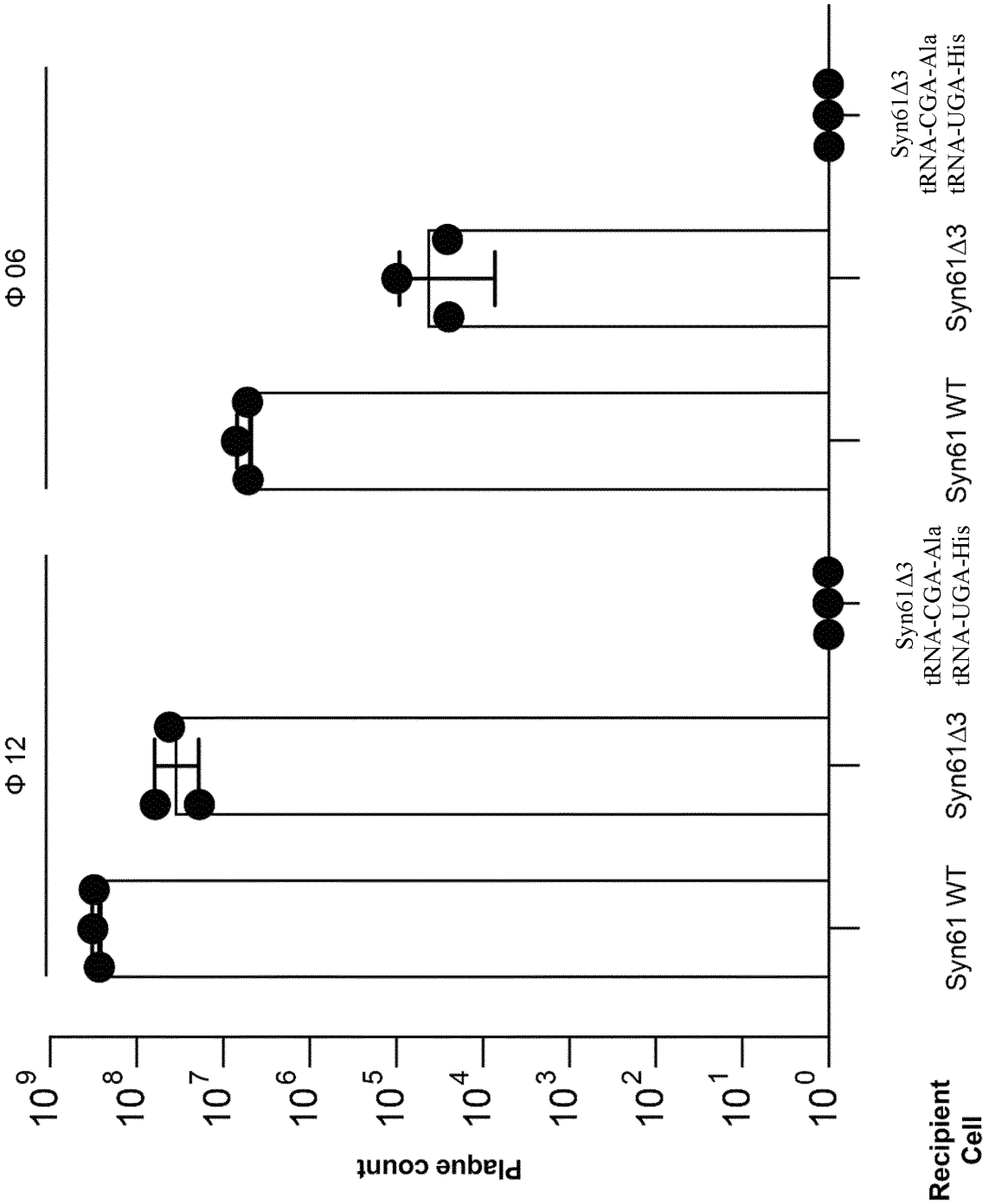


Figure 6 (Fig. S1)

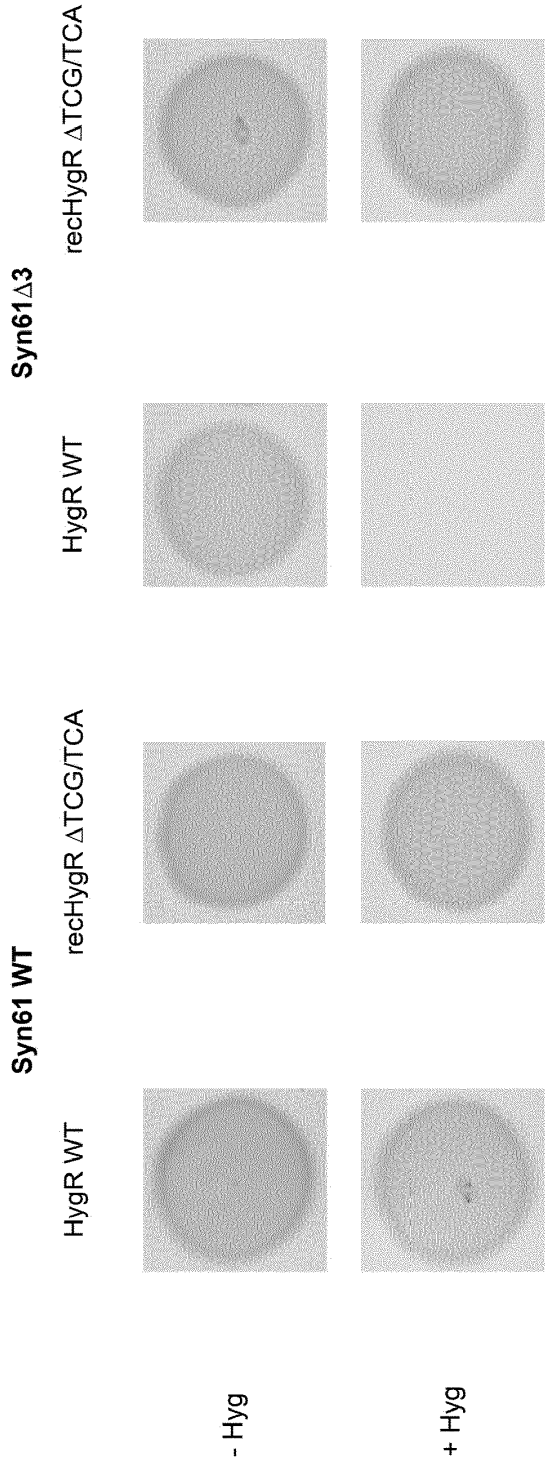
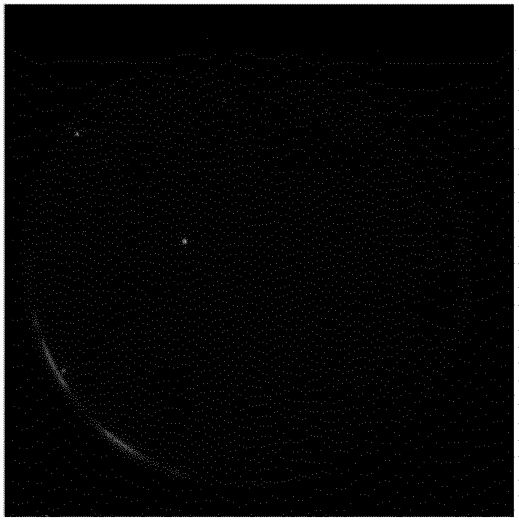


Figure 7 (Fig. S2) A



B

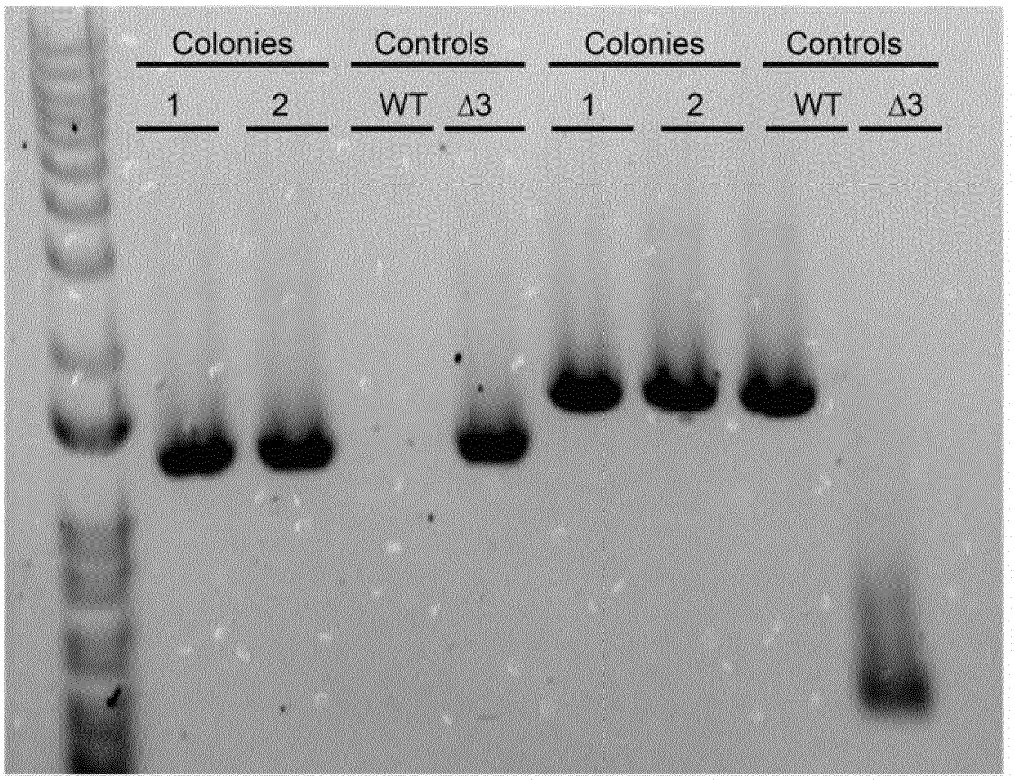


Figure 7 (Fig. S2) (cont)

B (cont)

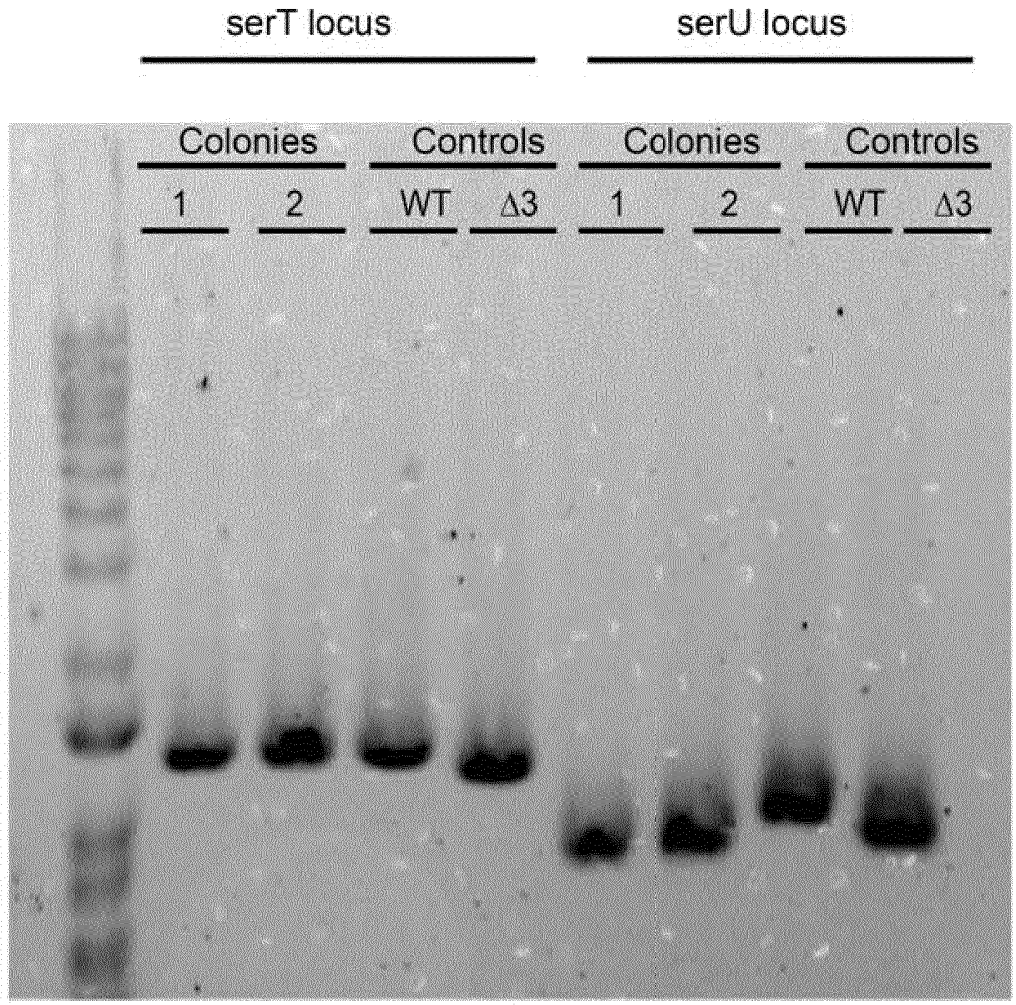


Figure 7 (Fig. S2) (cont)

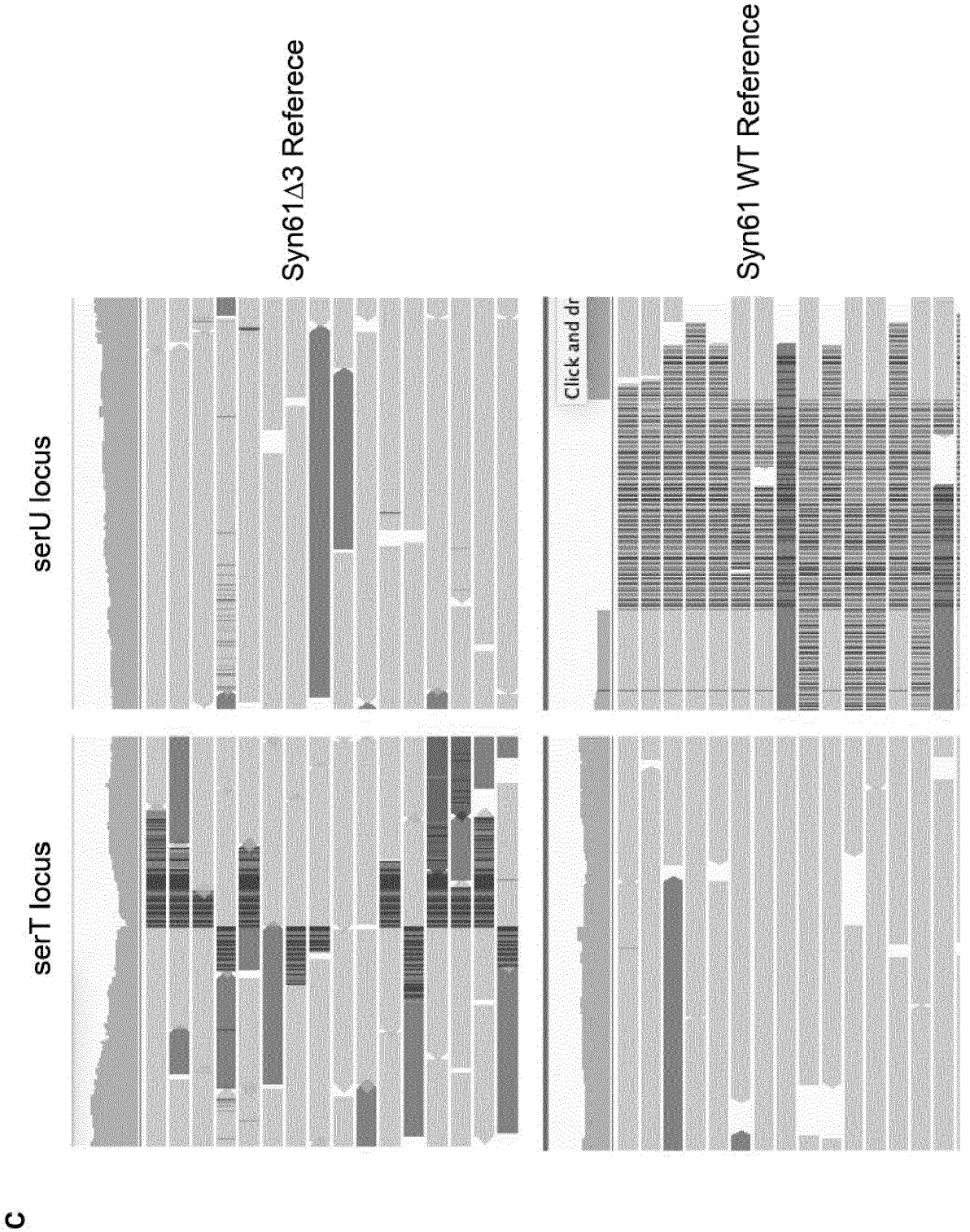


Figure 7 (Fig. S2) (cont)

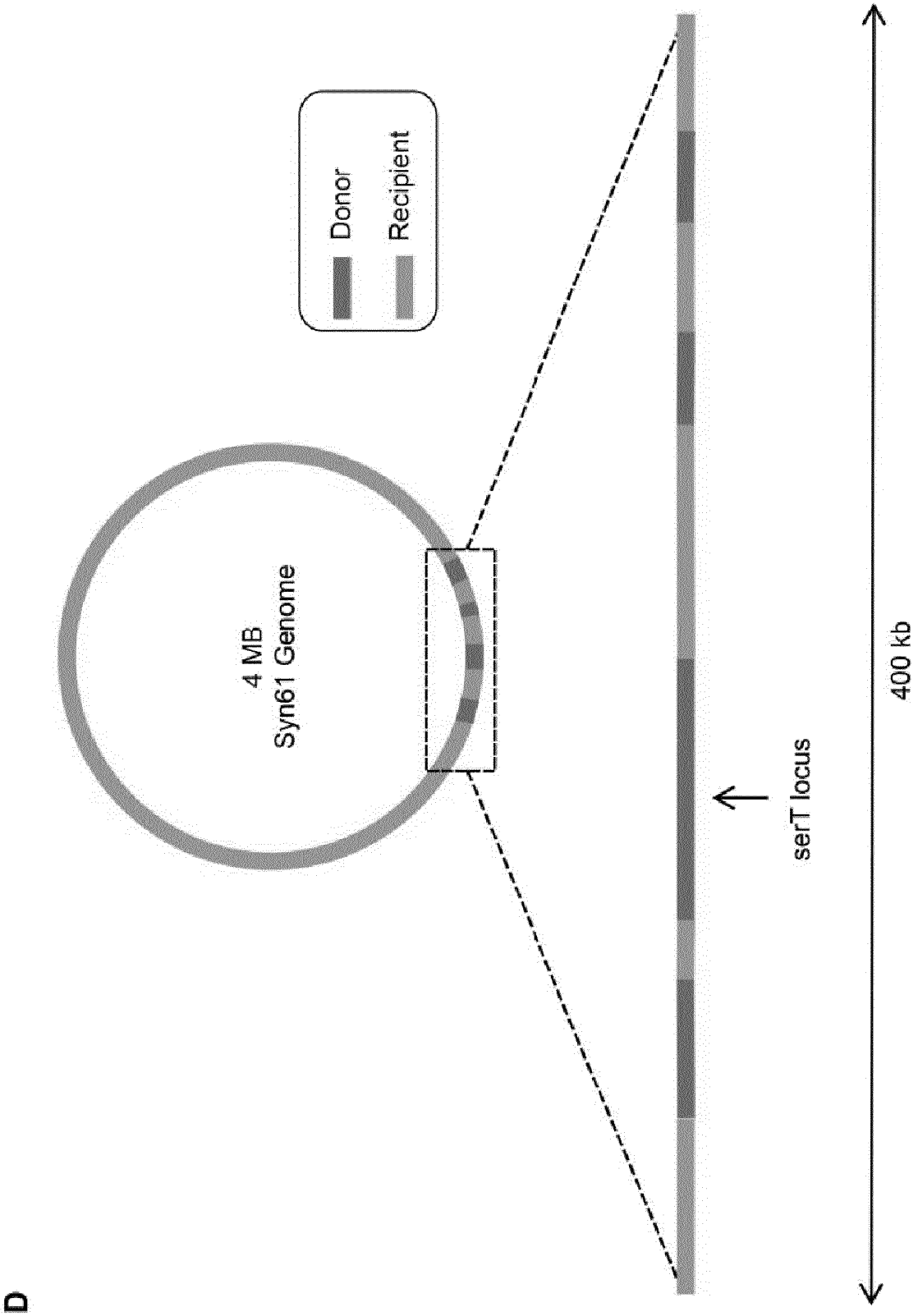
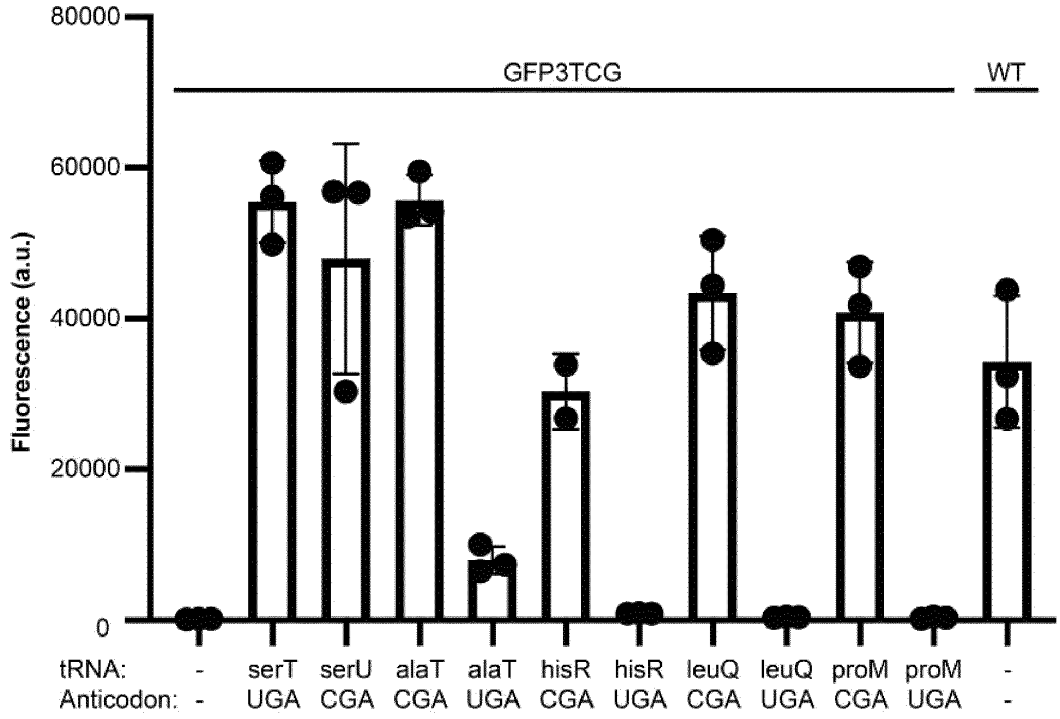


Figure 8 (Fig. S3)

A



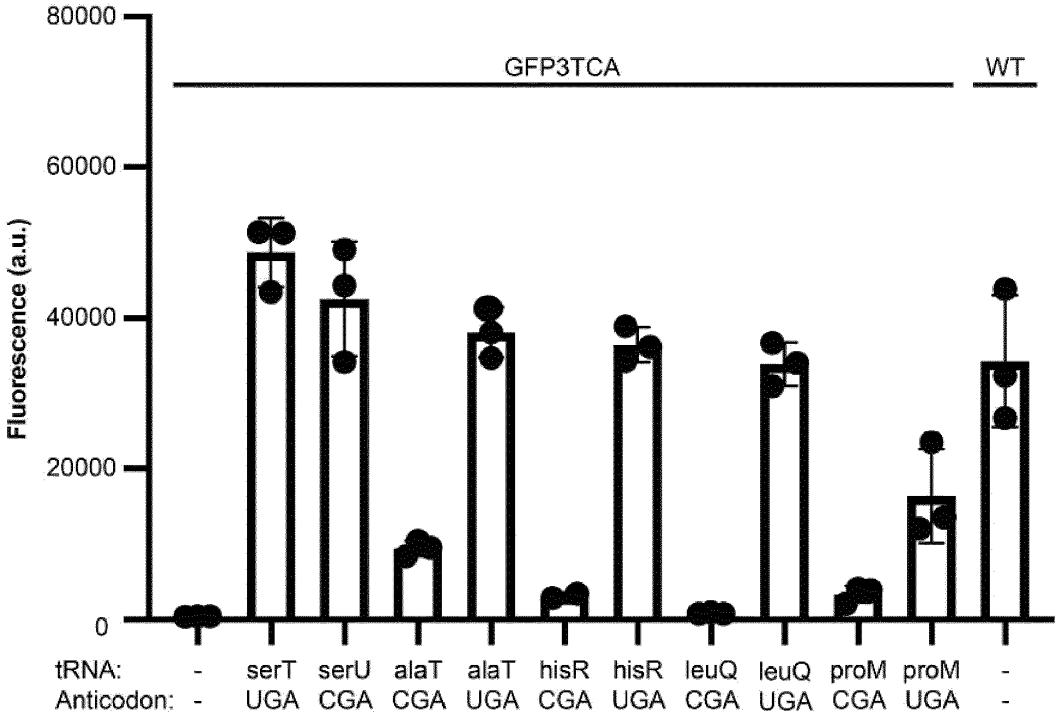


Figure 8 (Fig. S3) (cont)

B

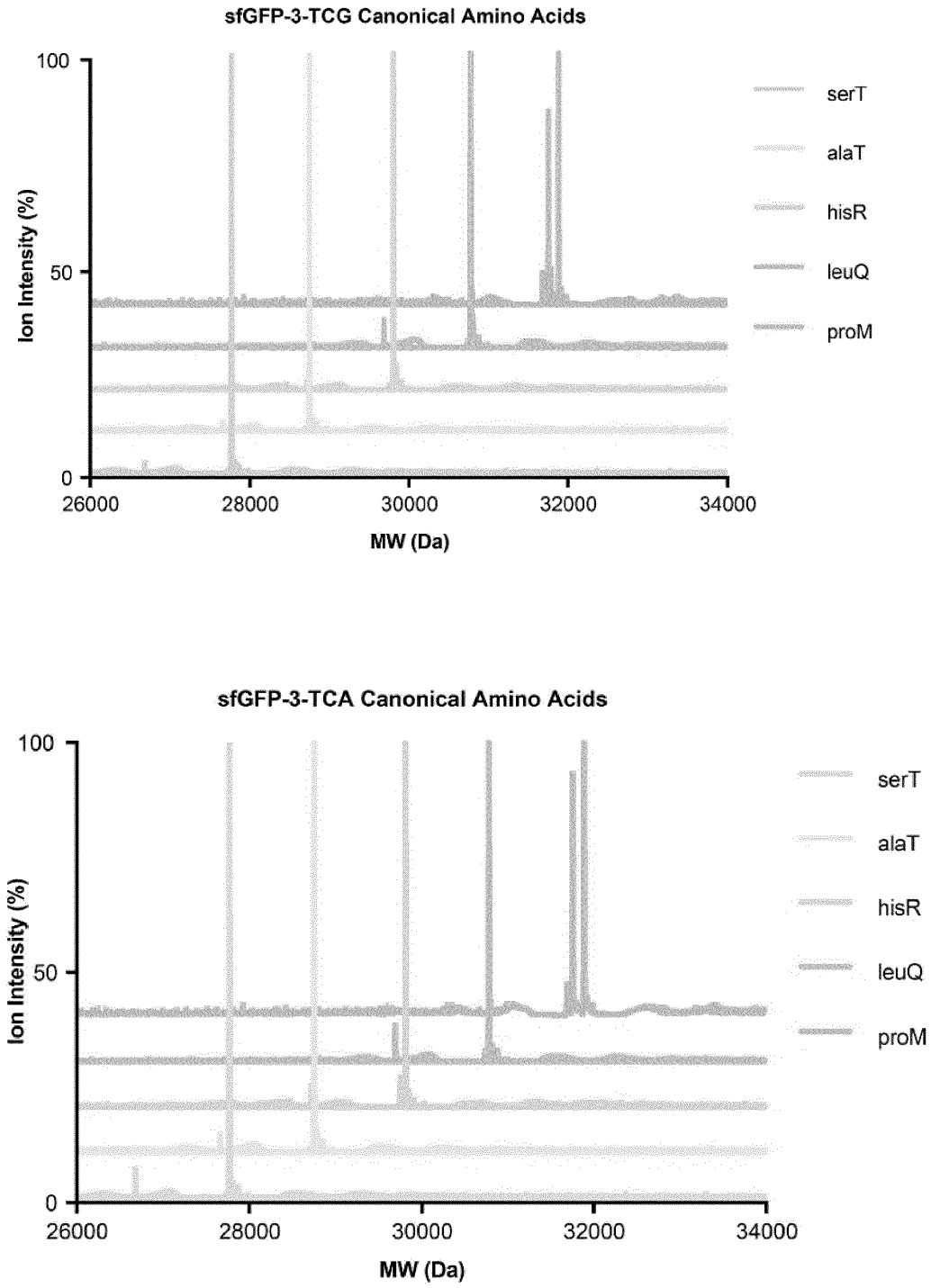


Figure 9 (Fig. S4)



Figure 9 (Fig. S4) (cont)

A (cont)

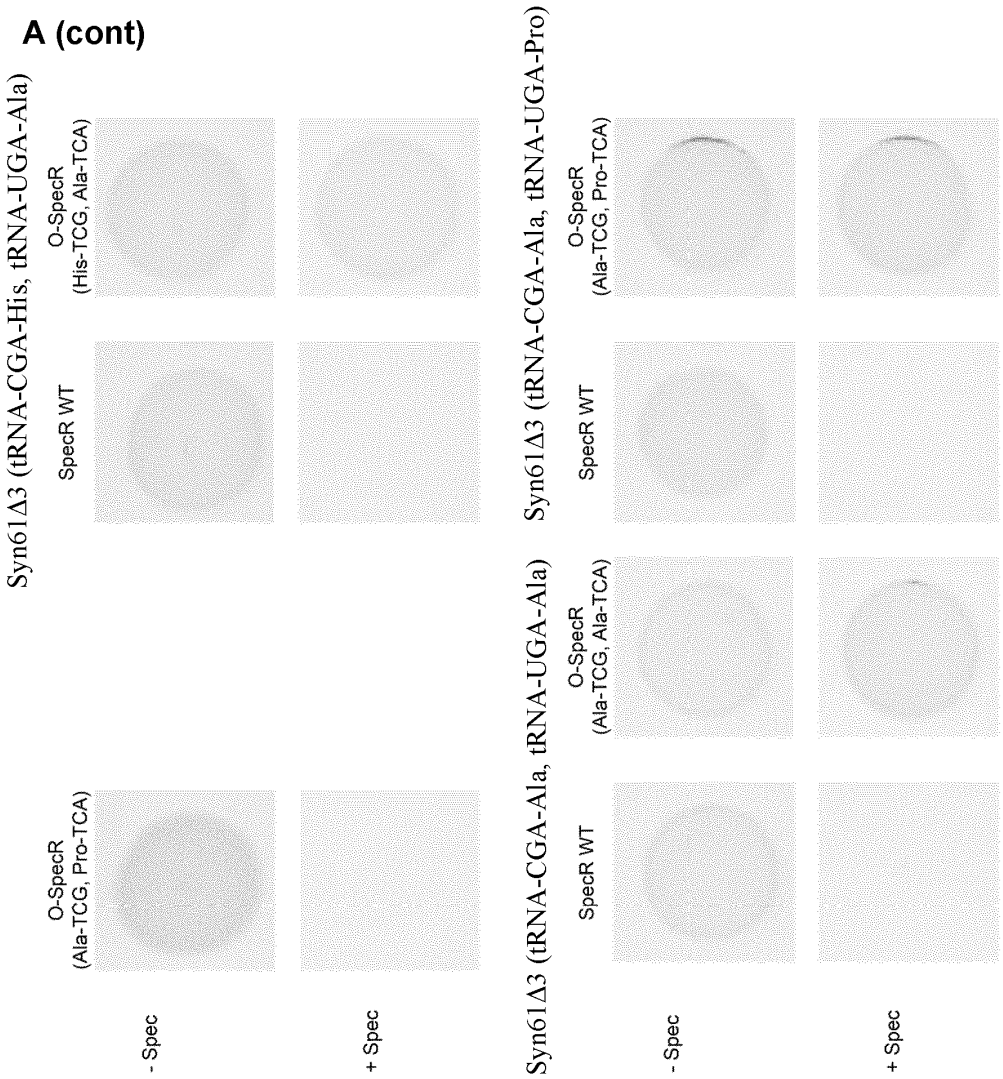


Figure 9 (Fig. S4) (cont)

B

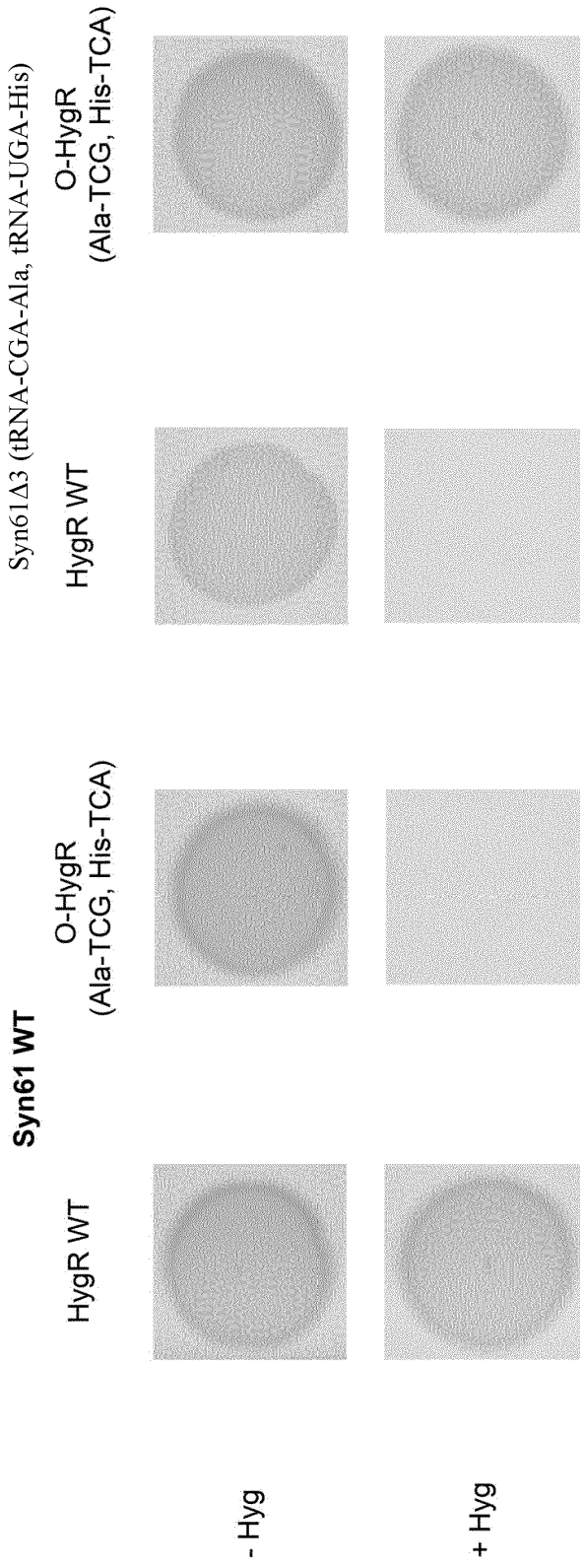


Figure 10 (Fig. S5)

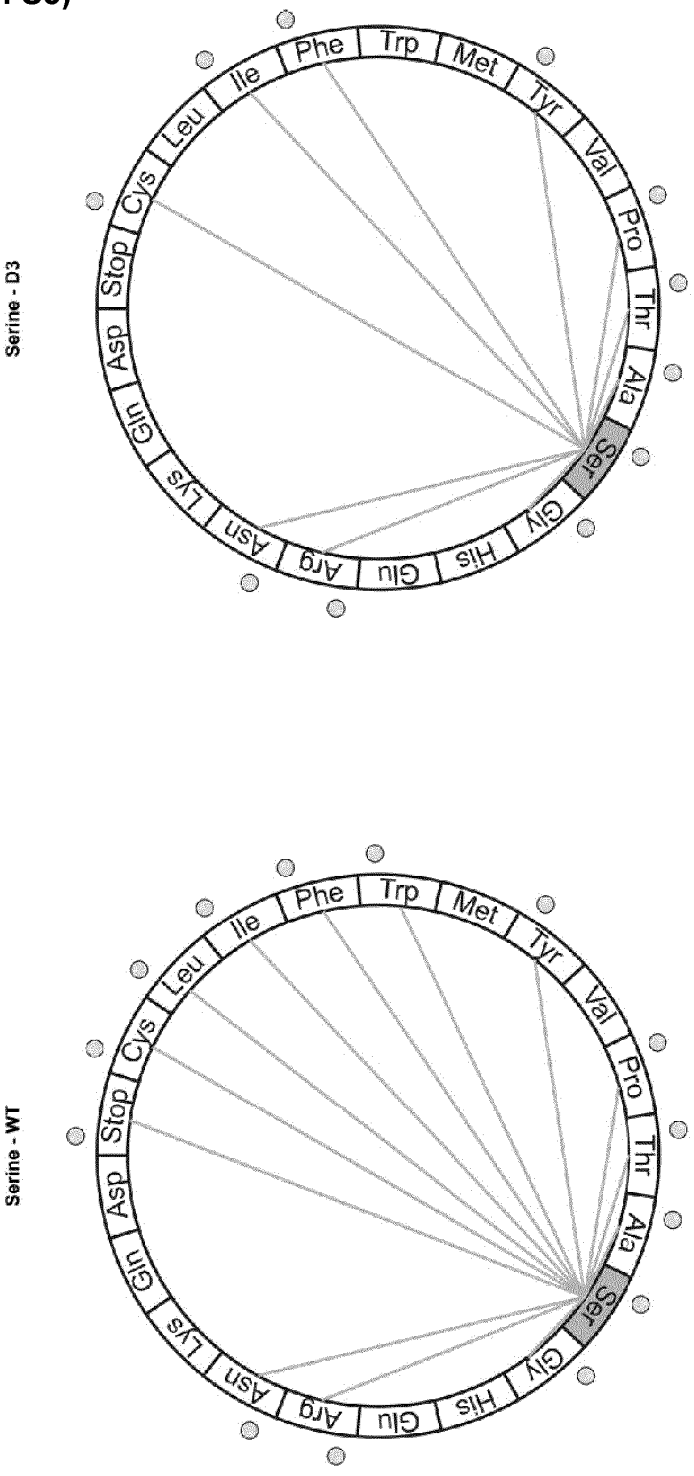


Figure 10 (Fig. S5) (cont)

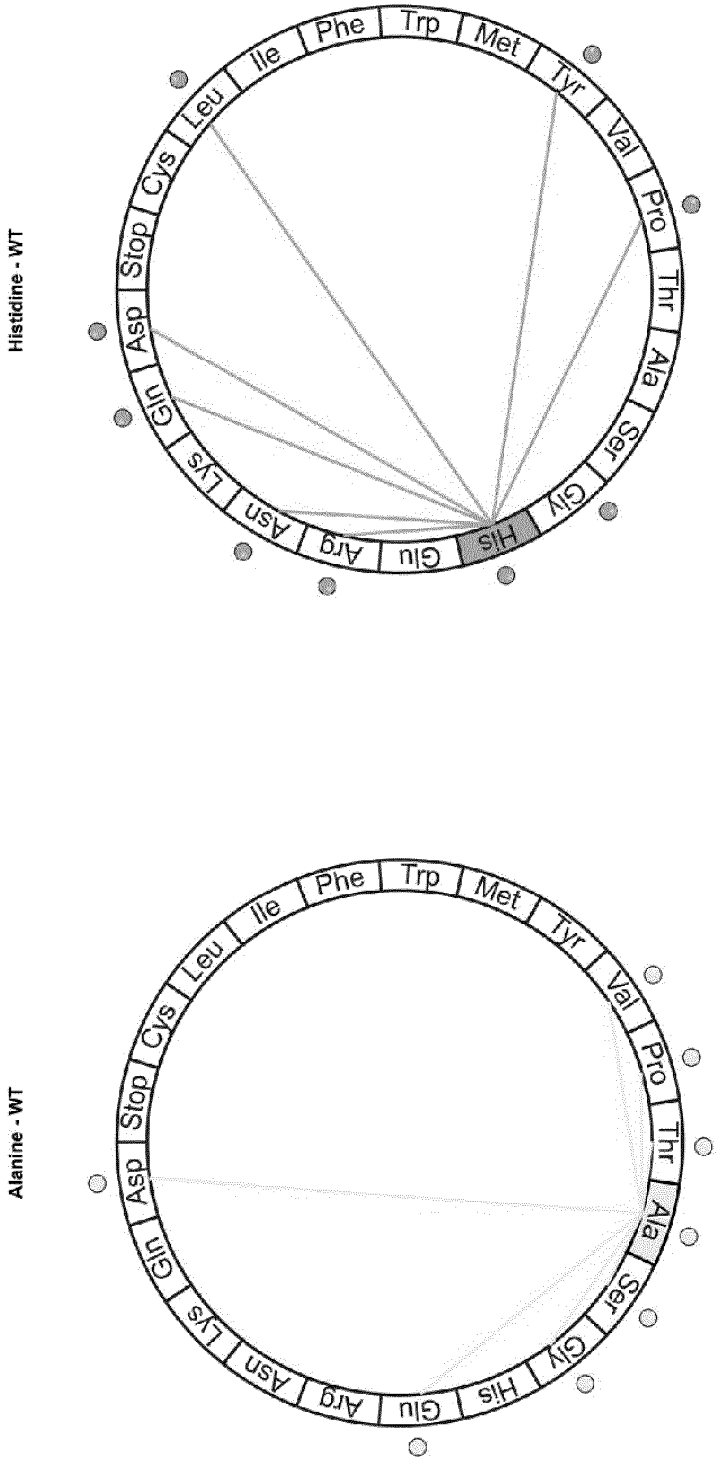
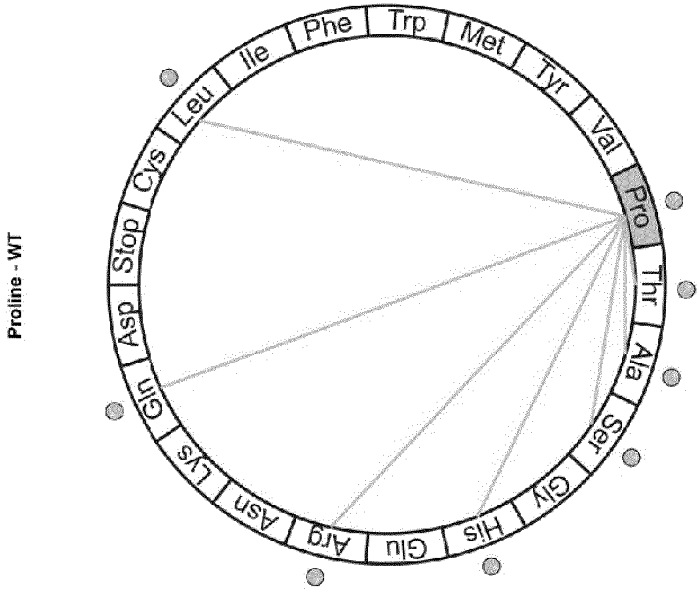


Figure 10 (Fig. S5) (cont)



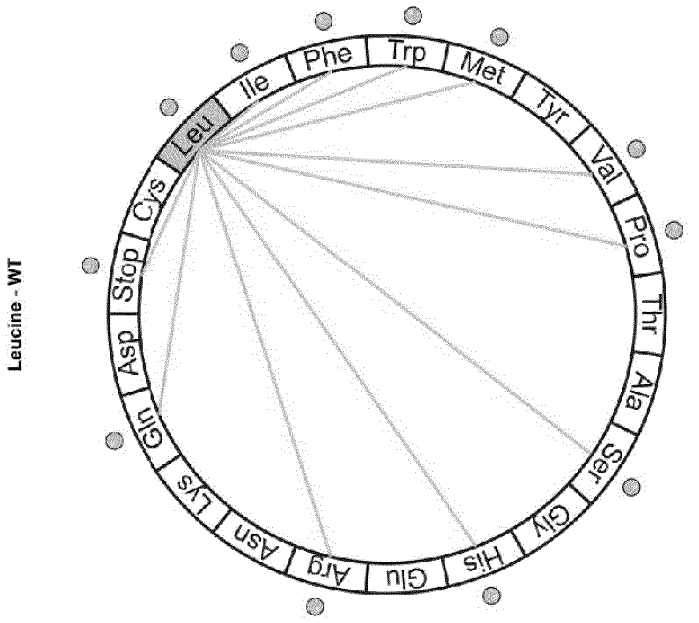


Figure 11 (Fig. S6)

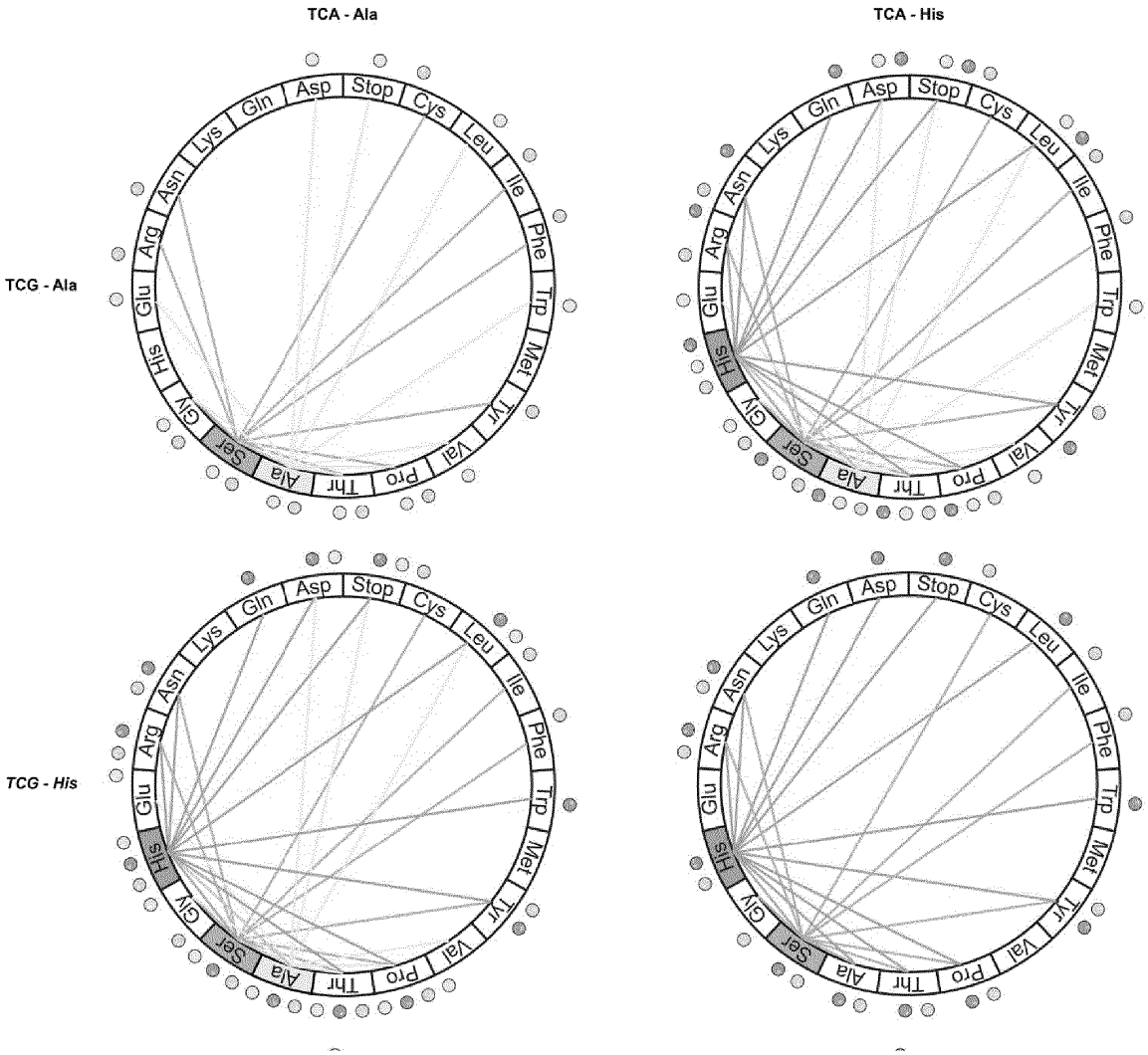


Figure 11 (Fig. S6) (cont)

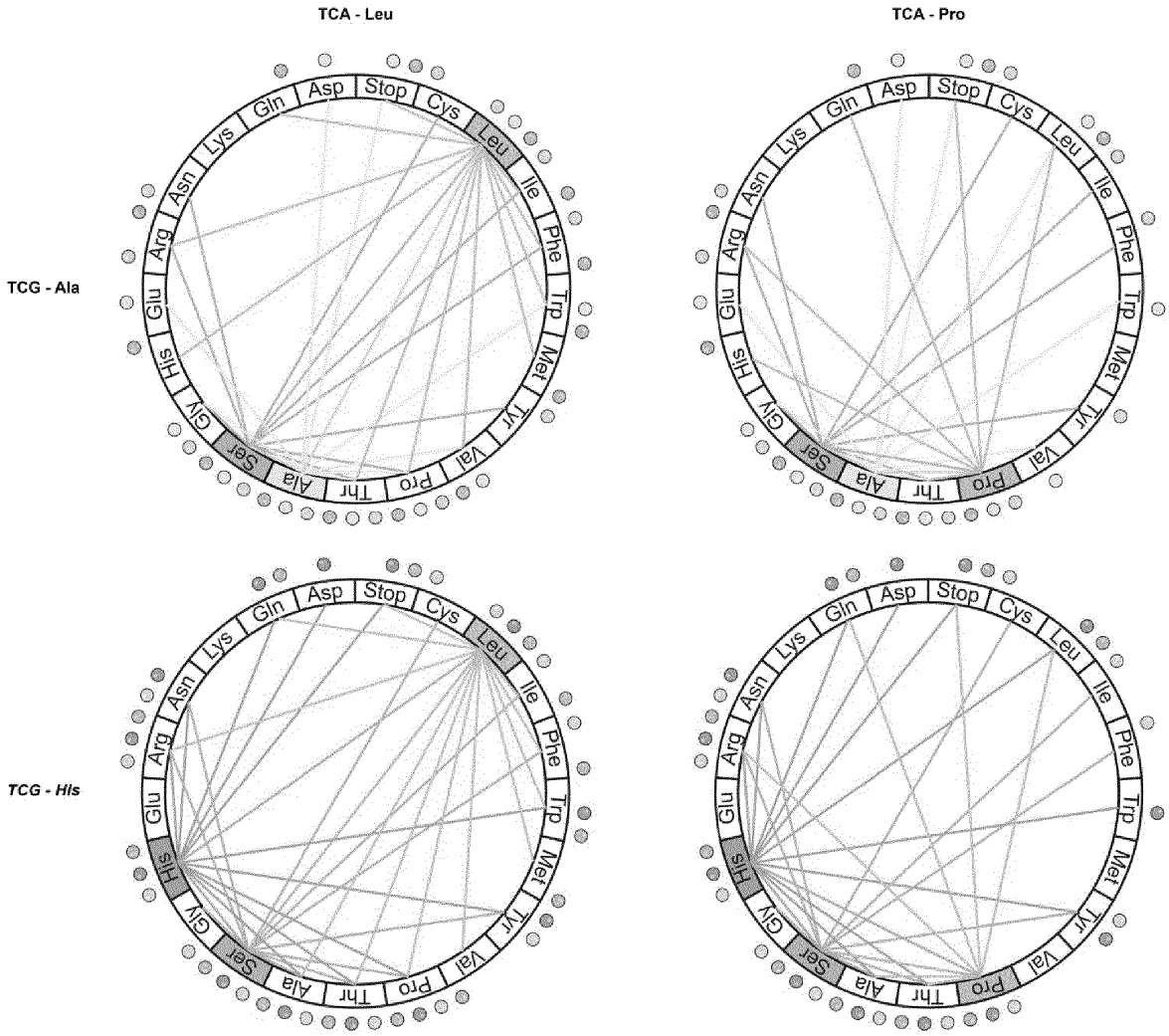


Figure 11 (Fig. S6) (cont)

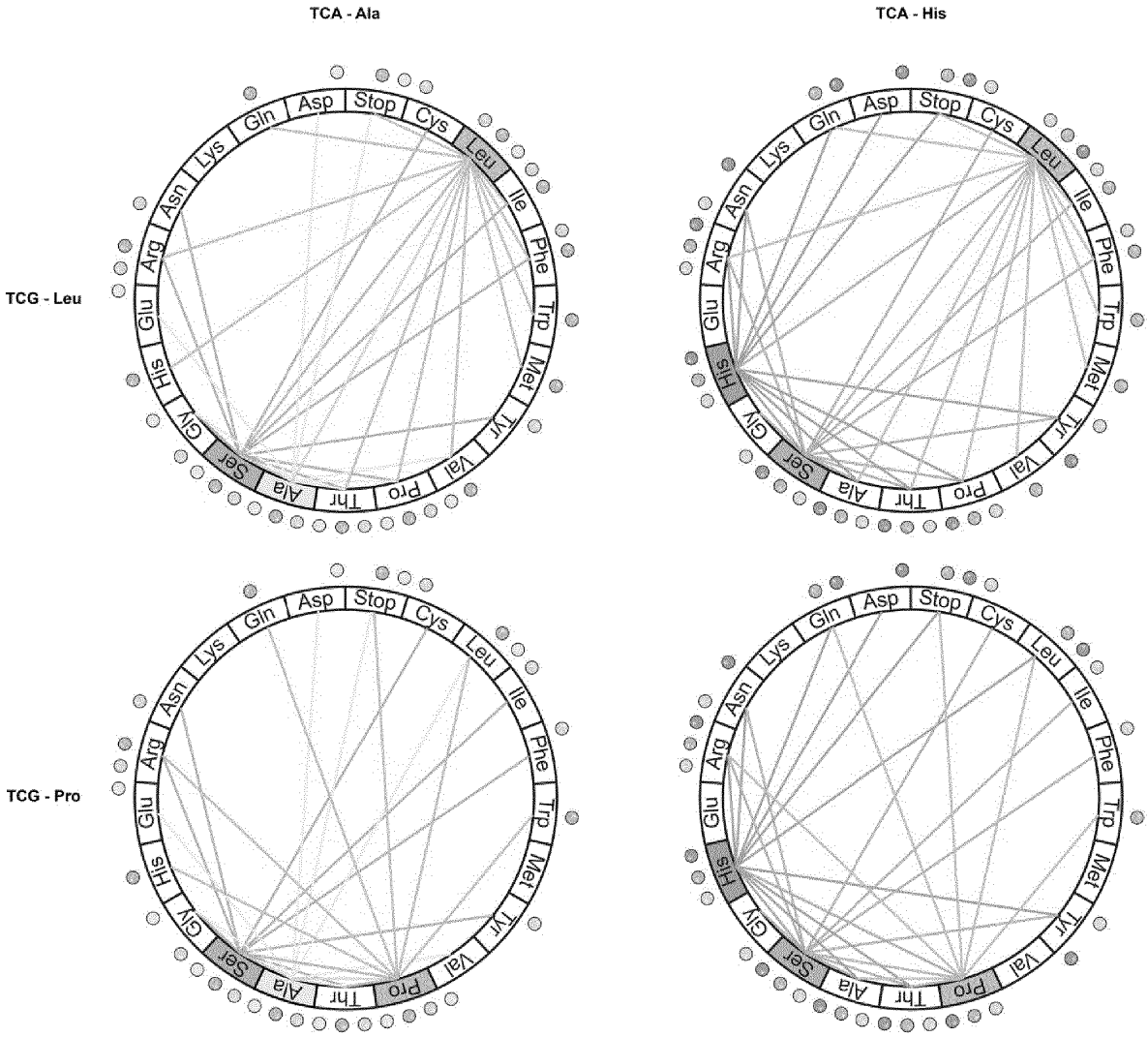


Figure 11 (Fig. S6) (cont)

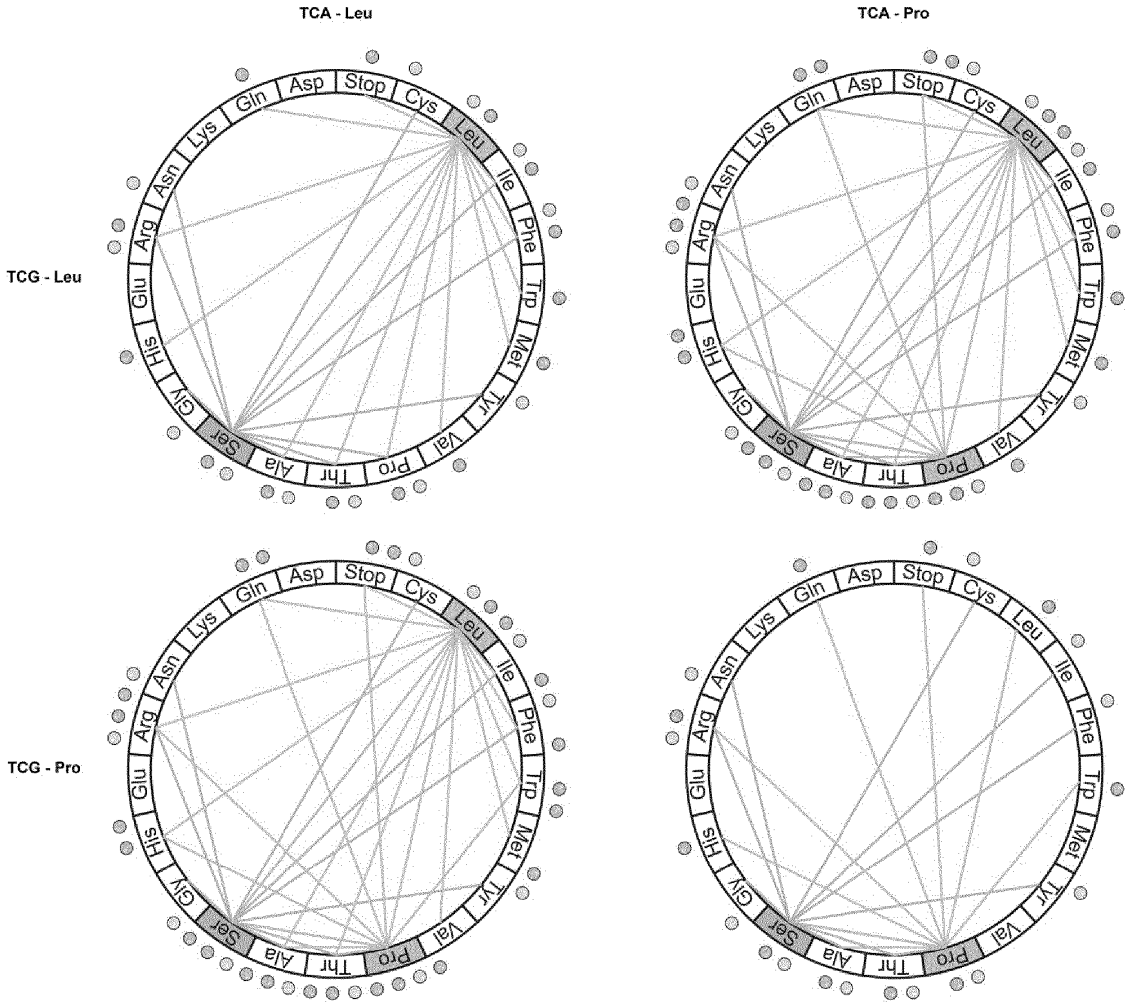


Figure 12

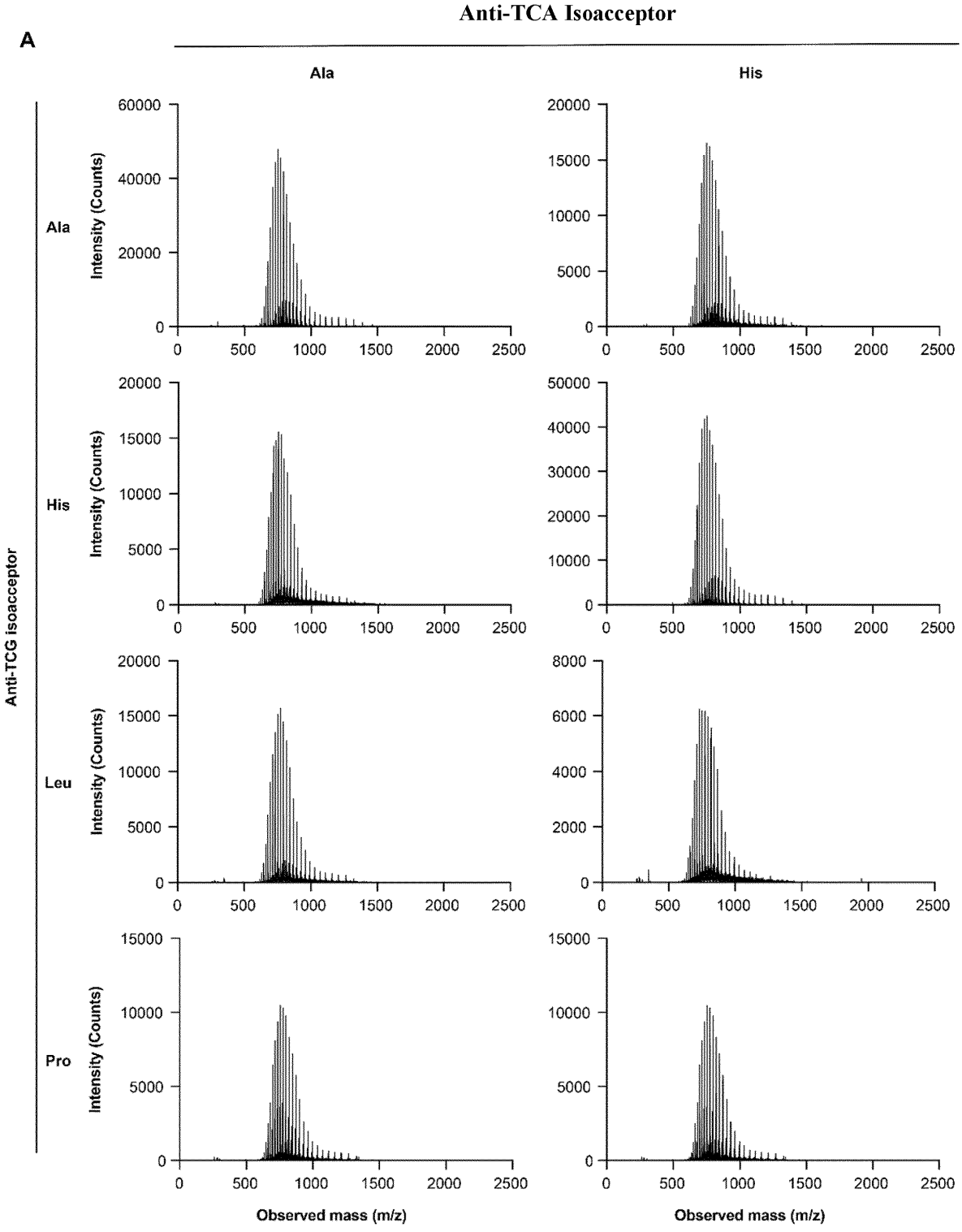

A
Anti-TCA Isoacceptor
Ala
His
Anti-TCG isoacceptor
Ala
His
Leu
Pro
Intensity (Counts)
Observed mass (m/z)

Figure 12 (cont)

A (cont)

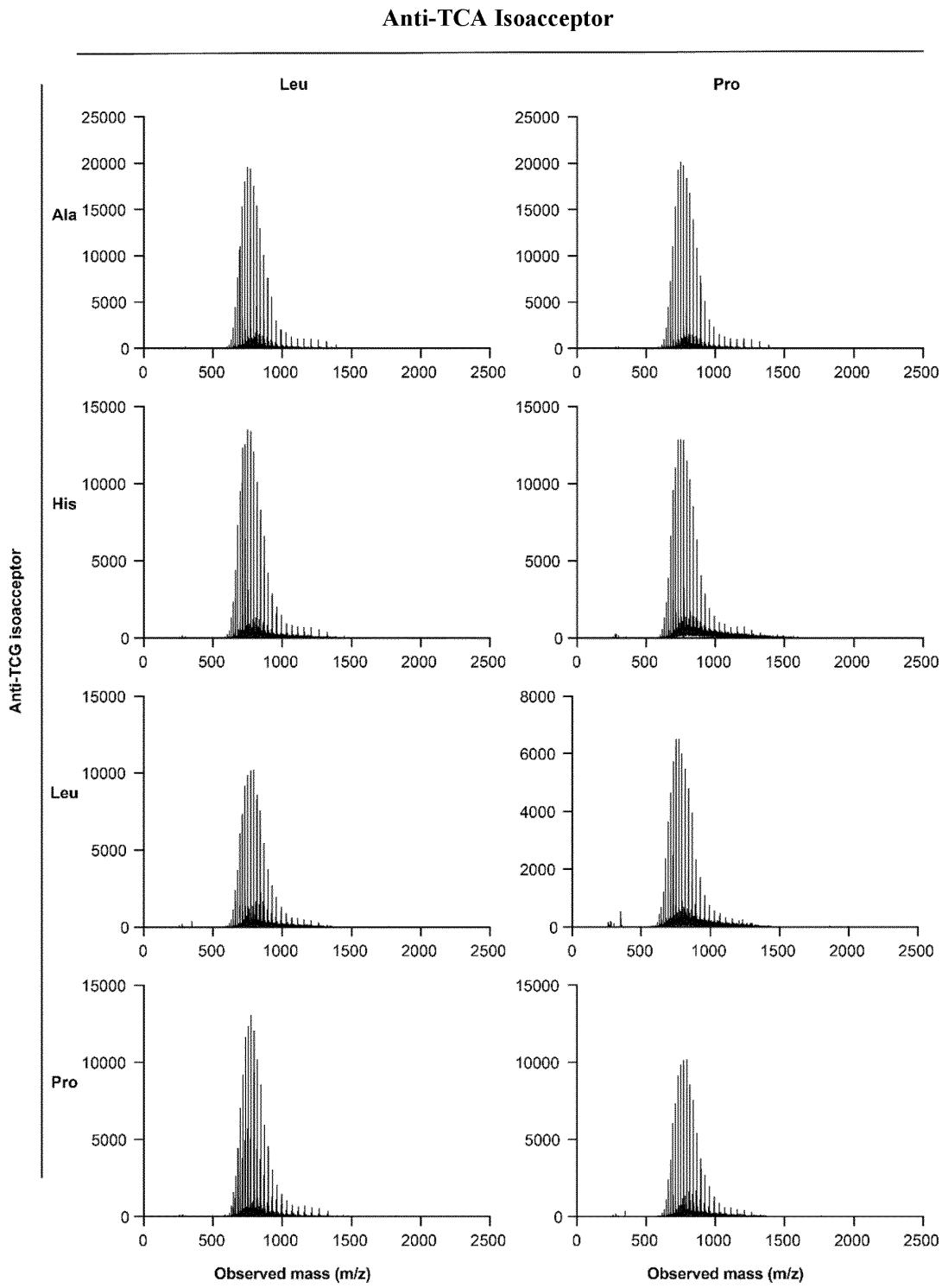


Figure 12 (cont)

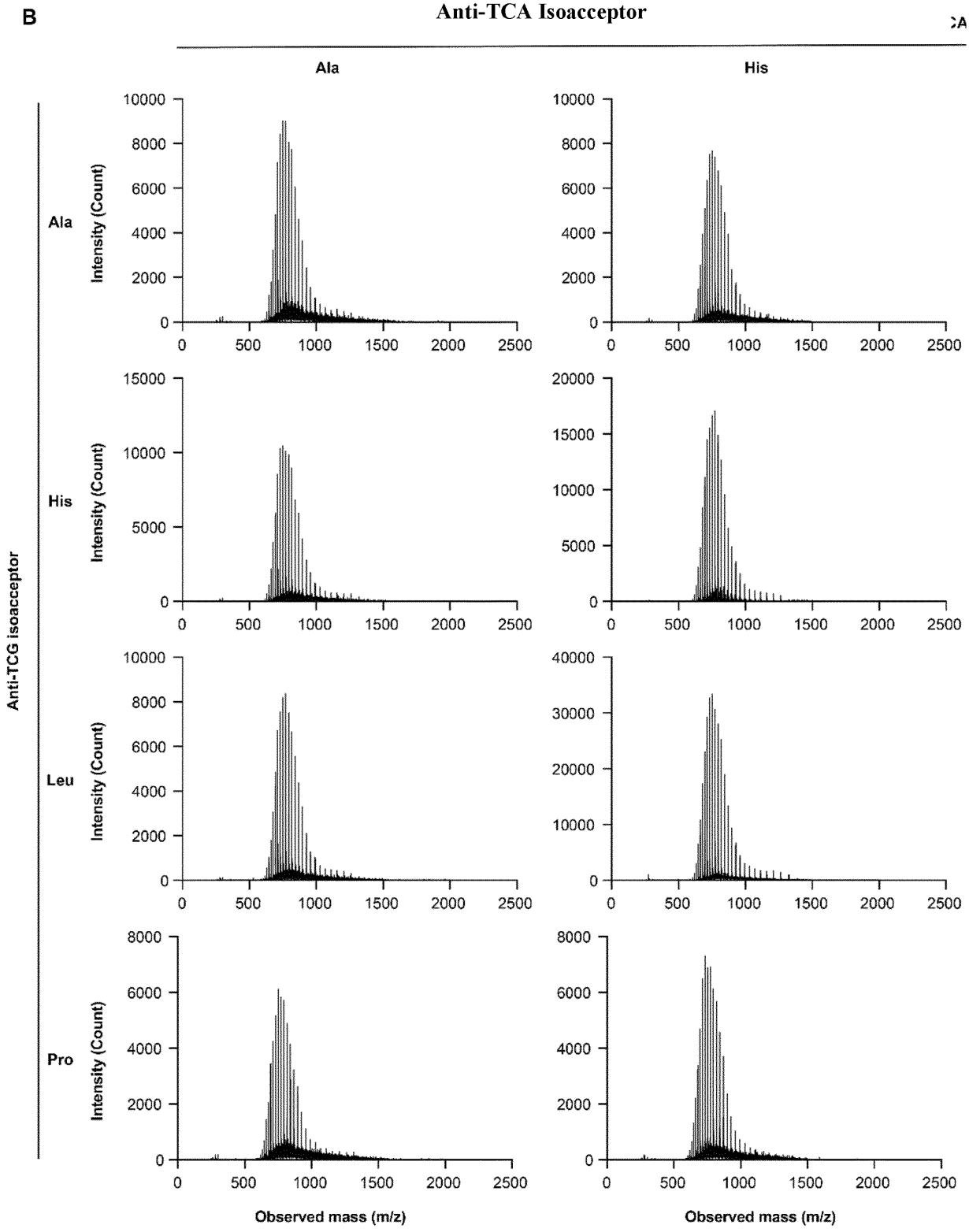


Figure 12 (cont)

B (cont)

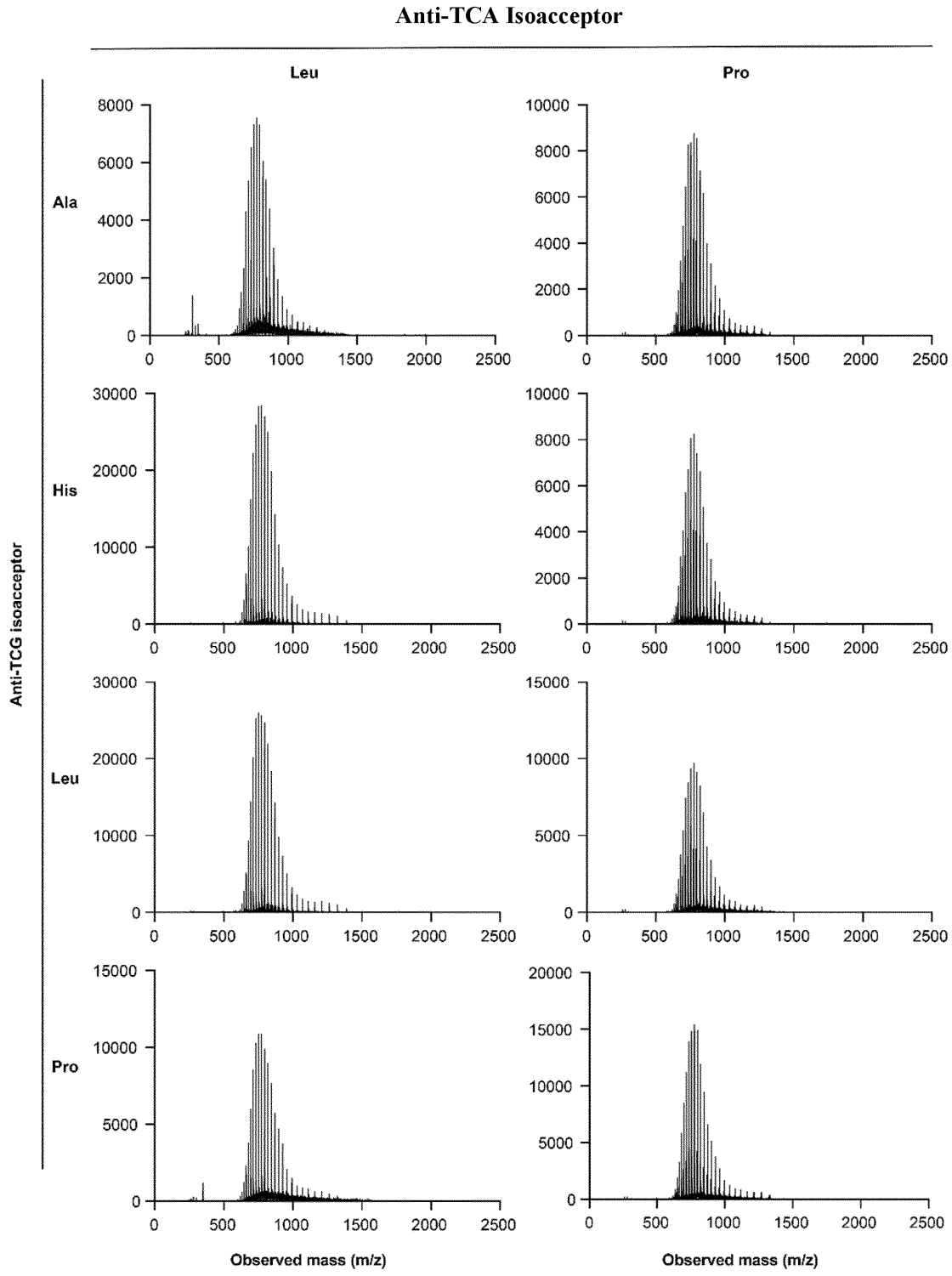


Figure 13

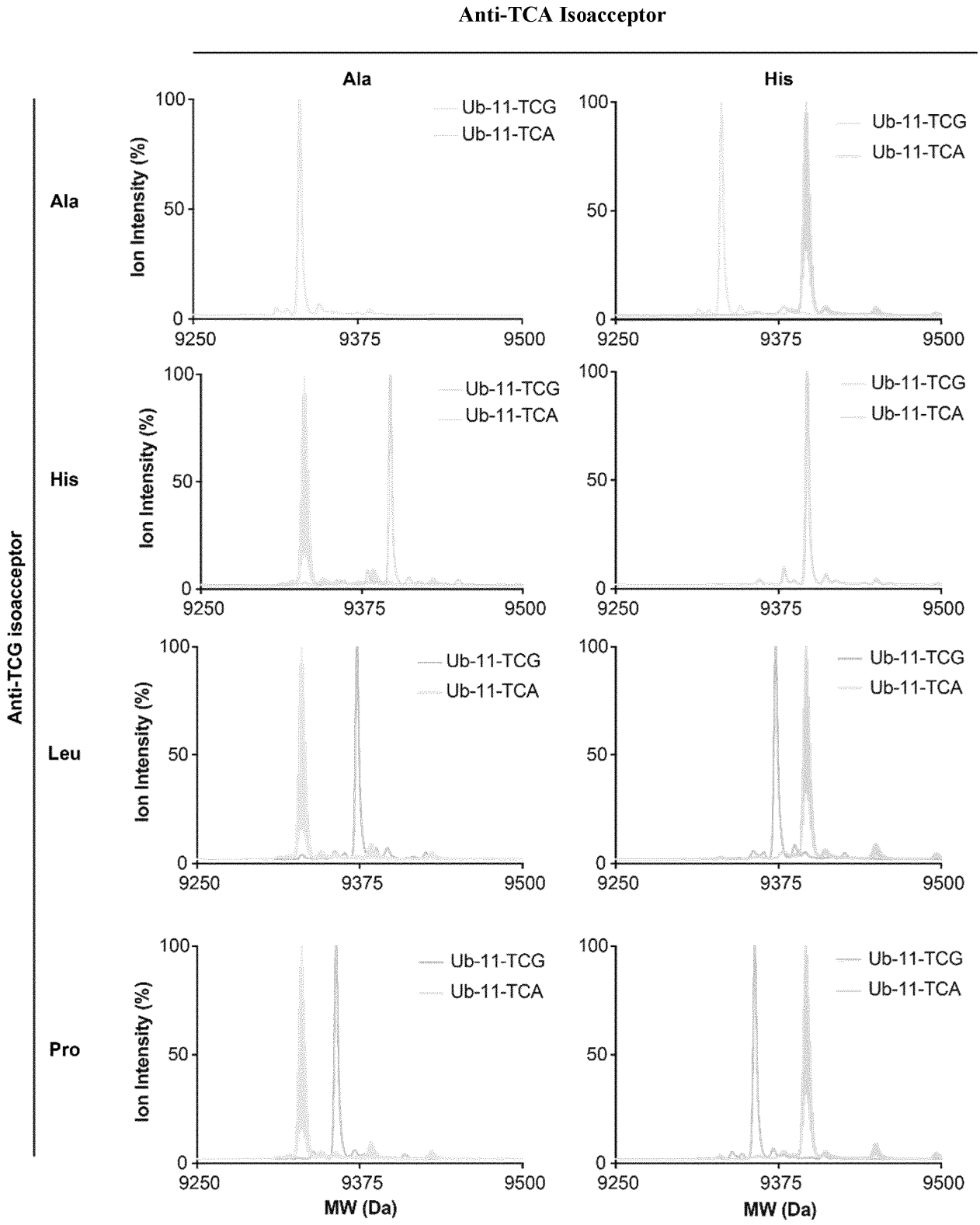

Anti-TCA Isoacceptor
Ala
His
Anti-TCG isoacceptor
Ala
His
Leu
Pro
Ion Intensity (%)
100
50
0
9250
9375
9500
MW (Da)
Ub-11-TCG
Ub-11-TCA

Figure 13 (cont)

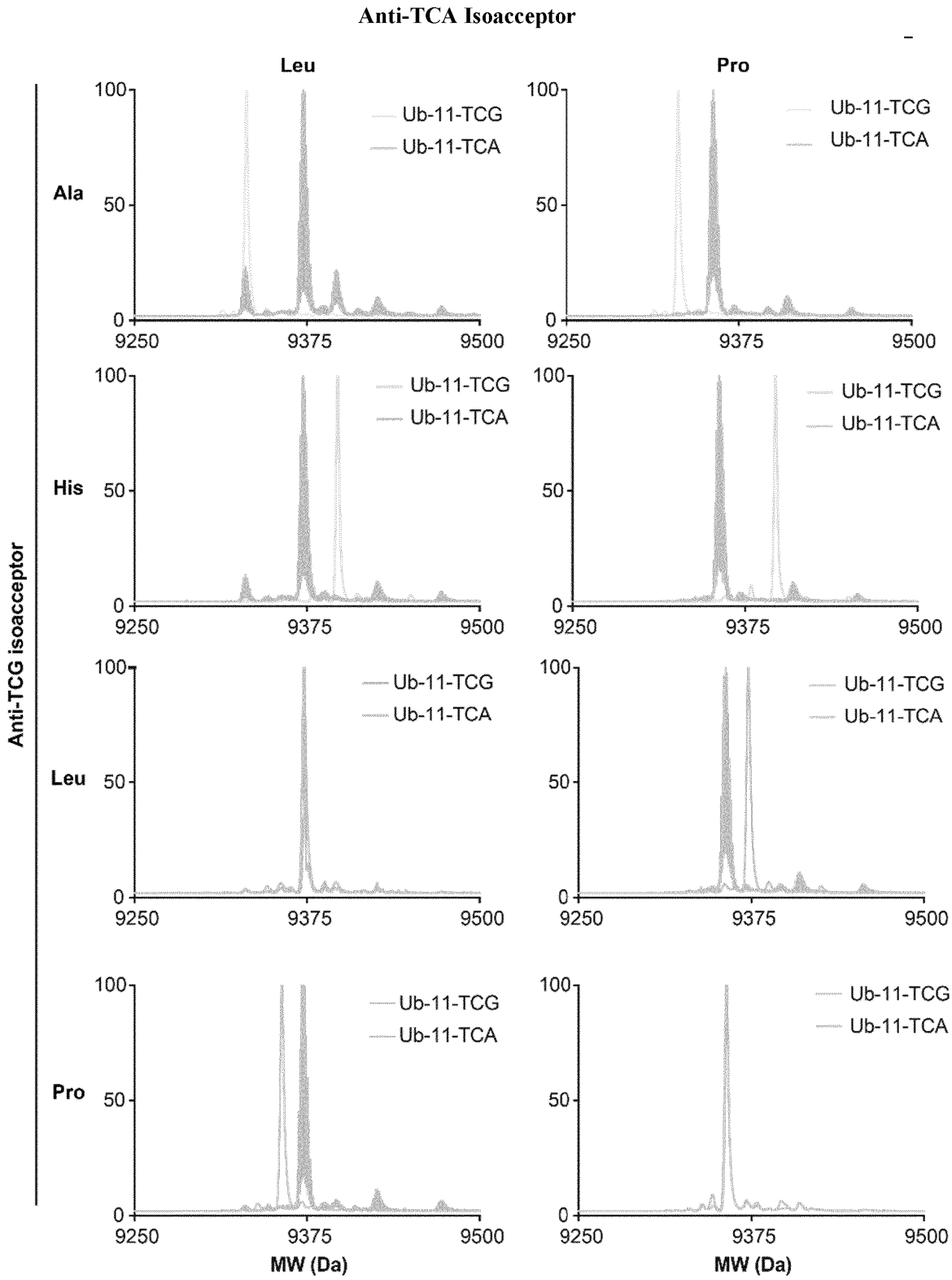


Figure 14

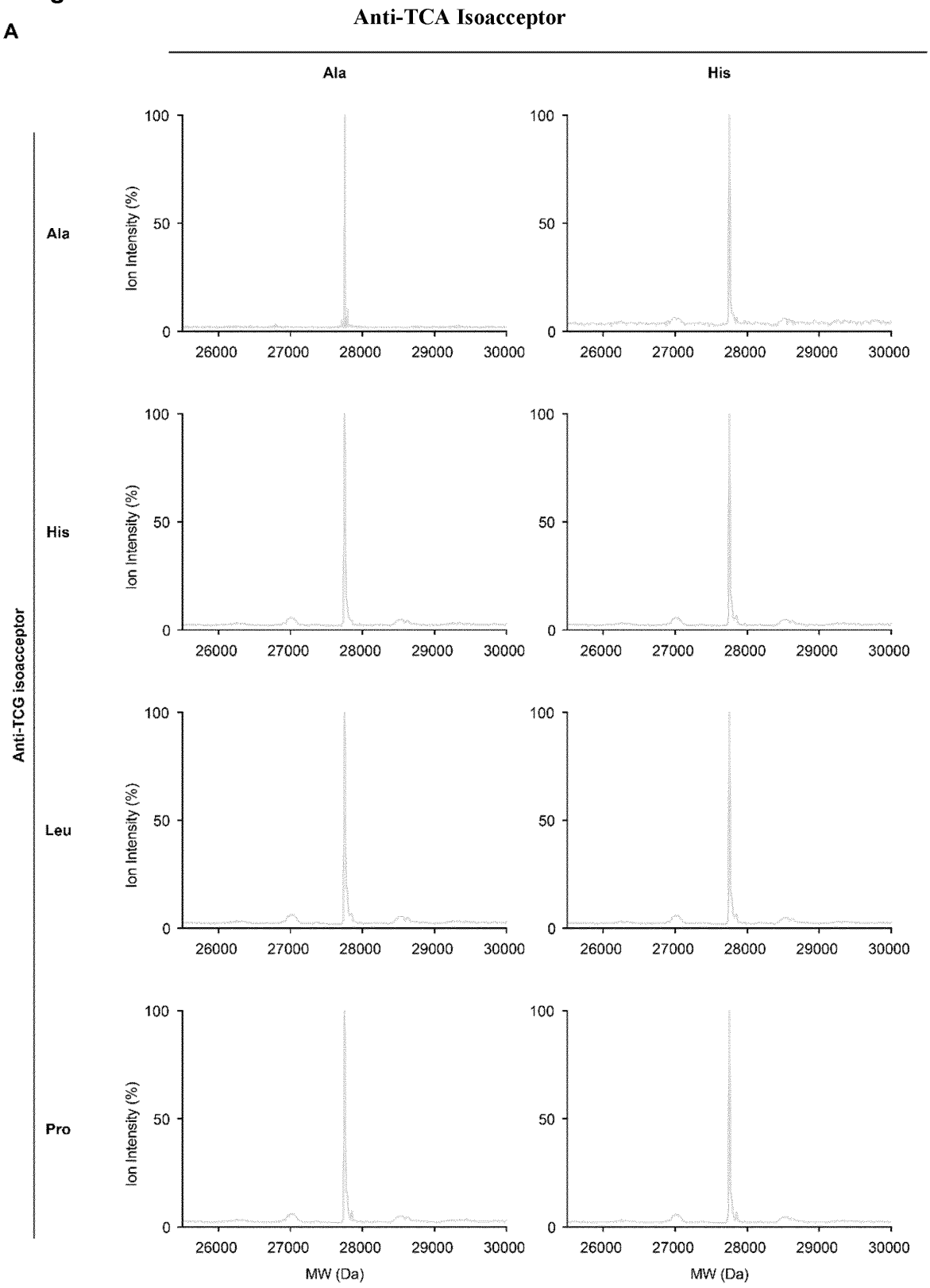

A
Anti-TCA Isoacceptor
Ala
His
Anti-TCG isoacceptor
Ala
His
Leu
Pro
Ion Intensity (%)
100
50
0
26000
27000
28000
29000
30000
MW (Da)

Figure 14 (cont) A (cont)

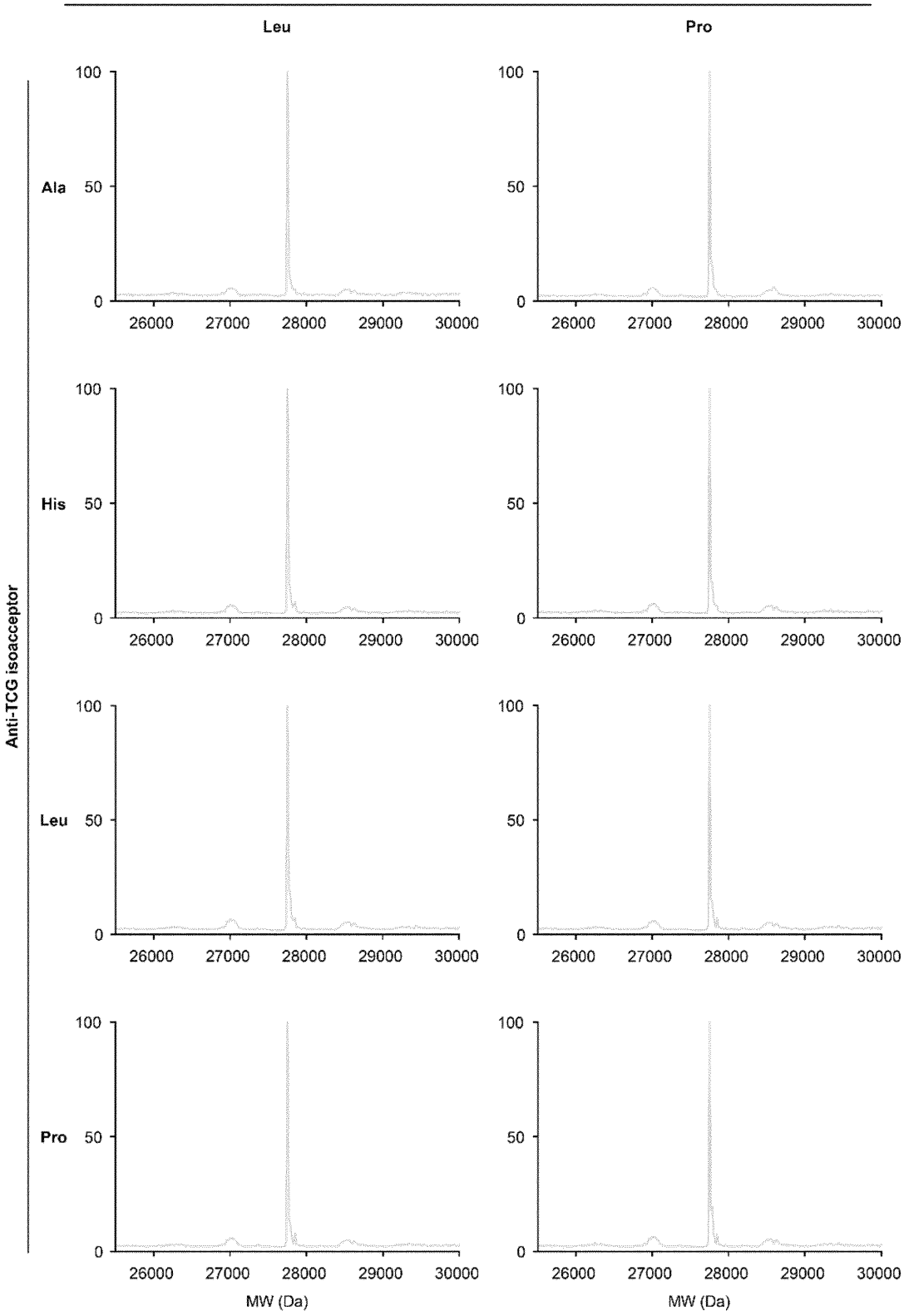


Figure 14 (cont)

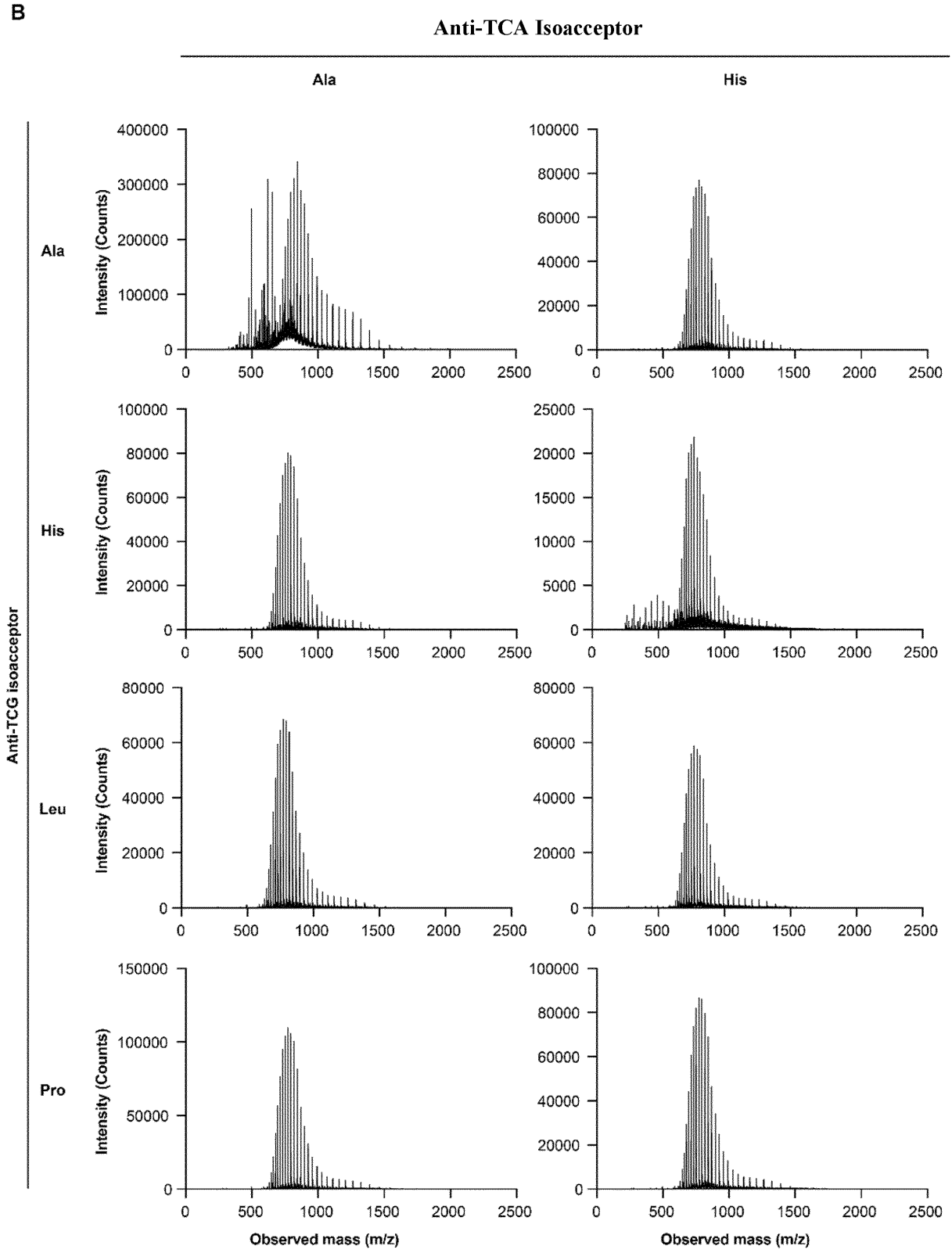


Figure 14 (cont) B (cont)

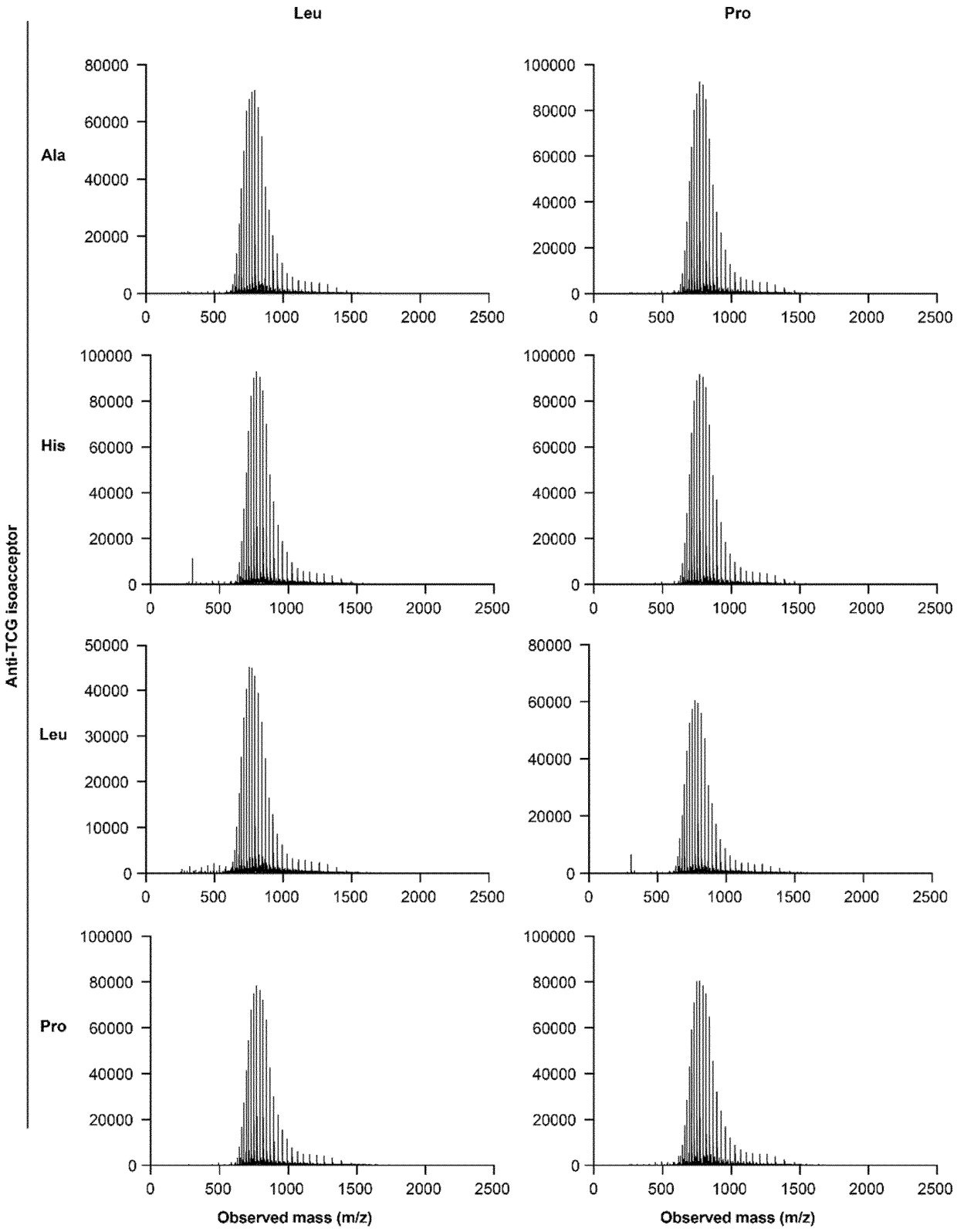


Figure 15

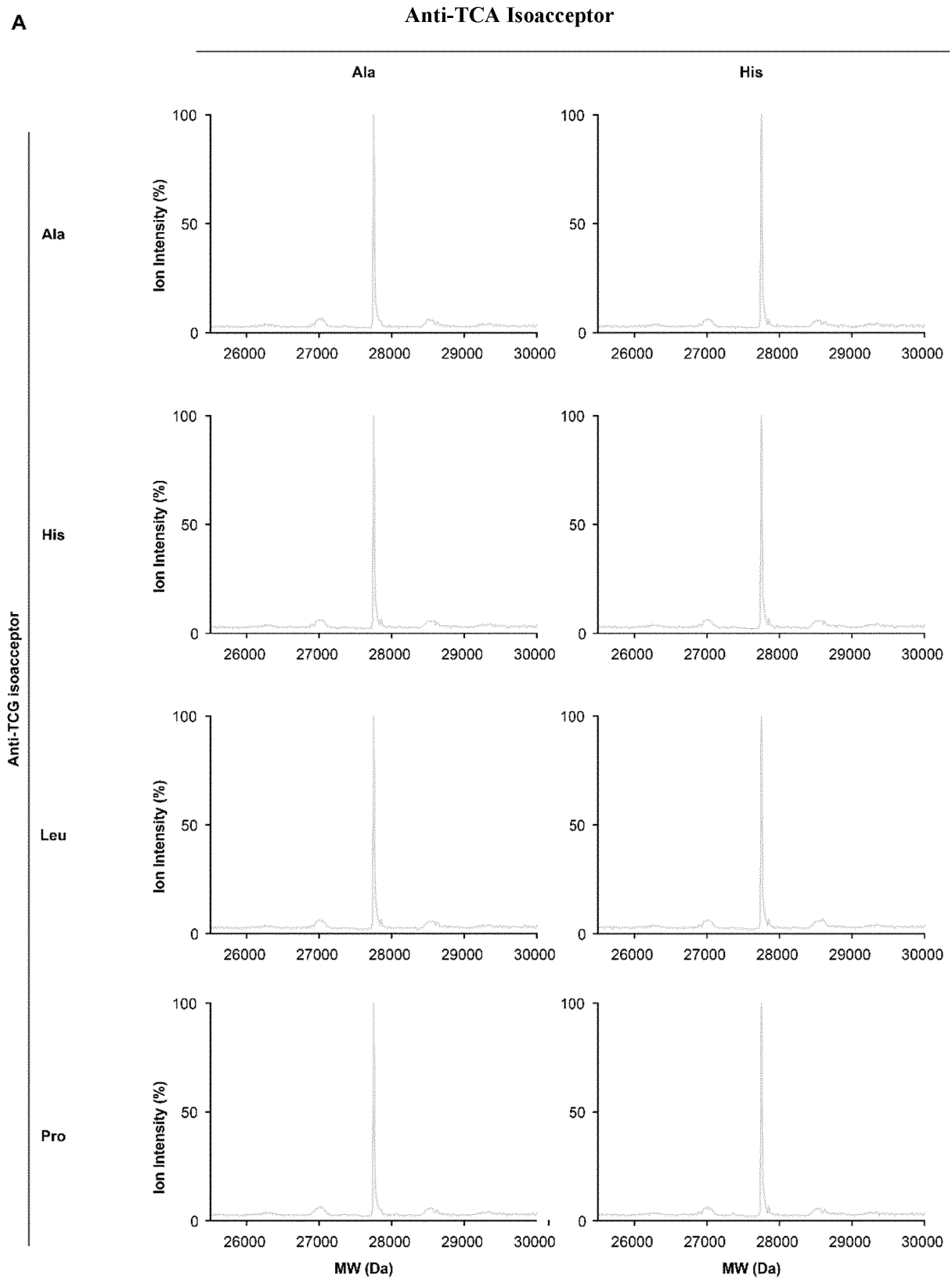

A
Anti-TCA Isoacceptor
Ala
His
Anti-TCG isoacceptor
Ala
His
Leu
Pro
Ion Intensity (%)
100
50
0
26000
27000
28000
29000
30000
MW (Da)

Figure 15 (cont) A (cont)

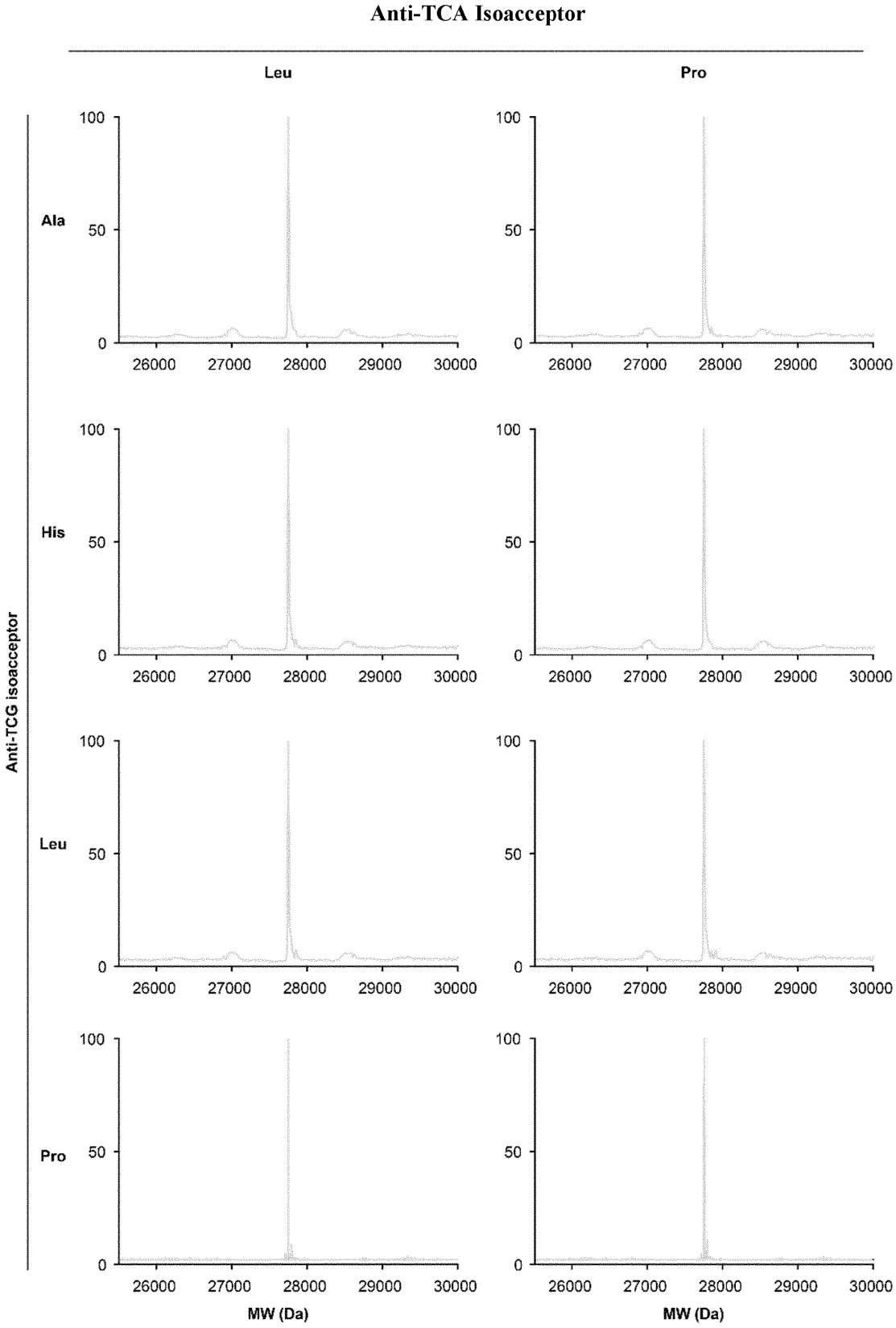


Figure 15 (cont)

B

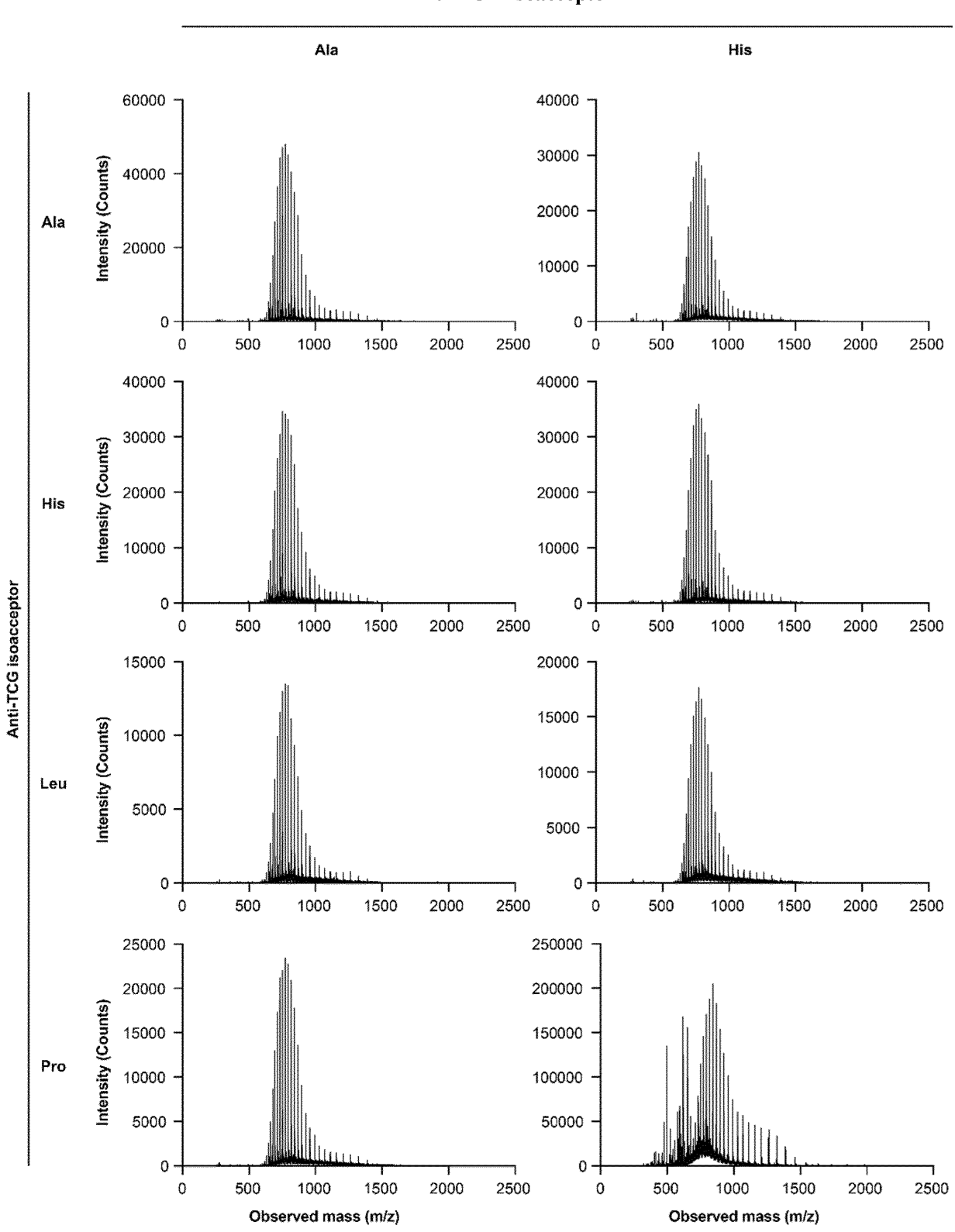


Figure 15 (cont) B (cont)

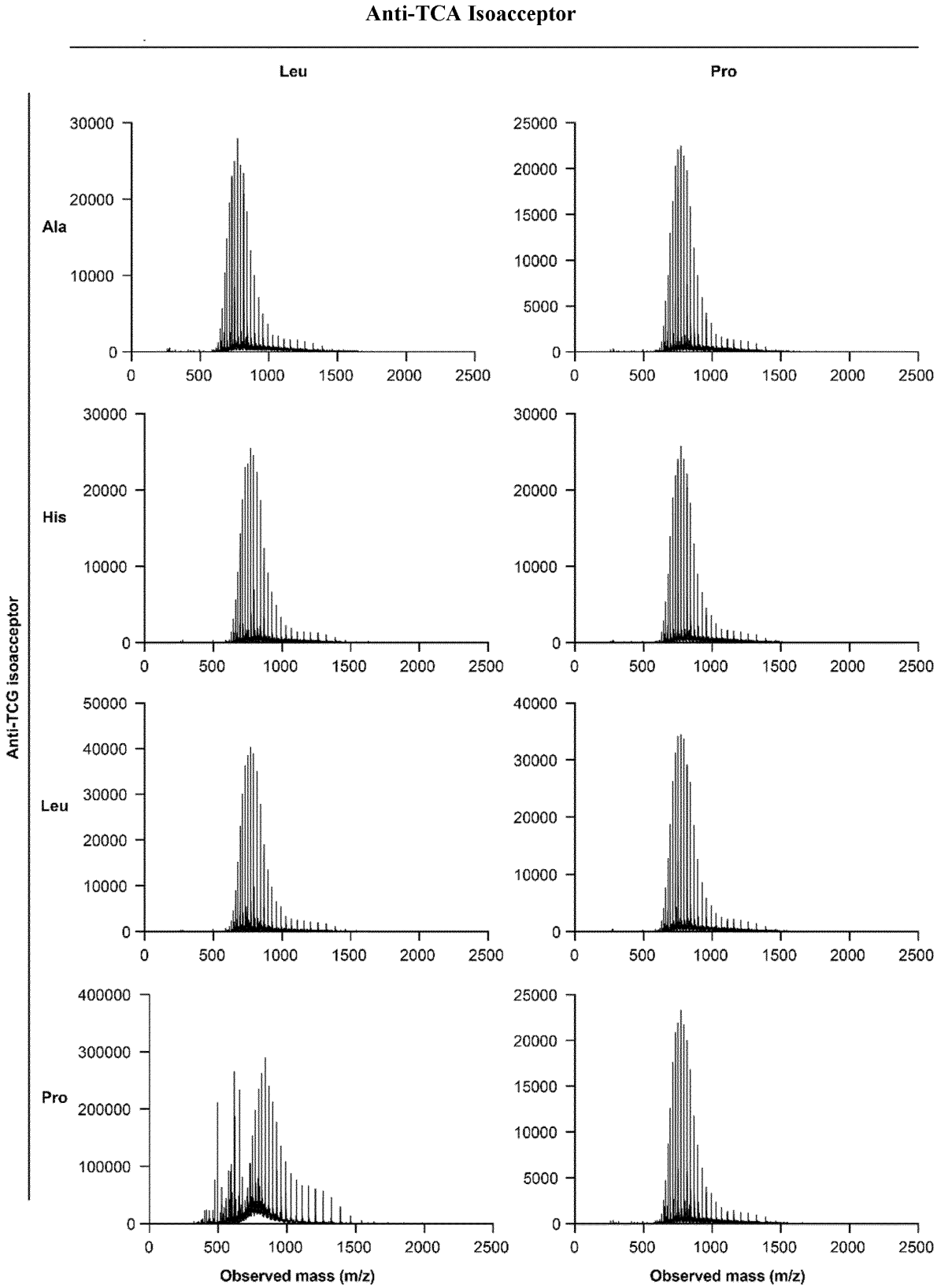


Figure 16

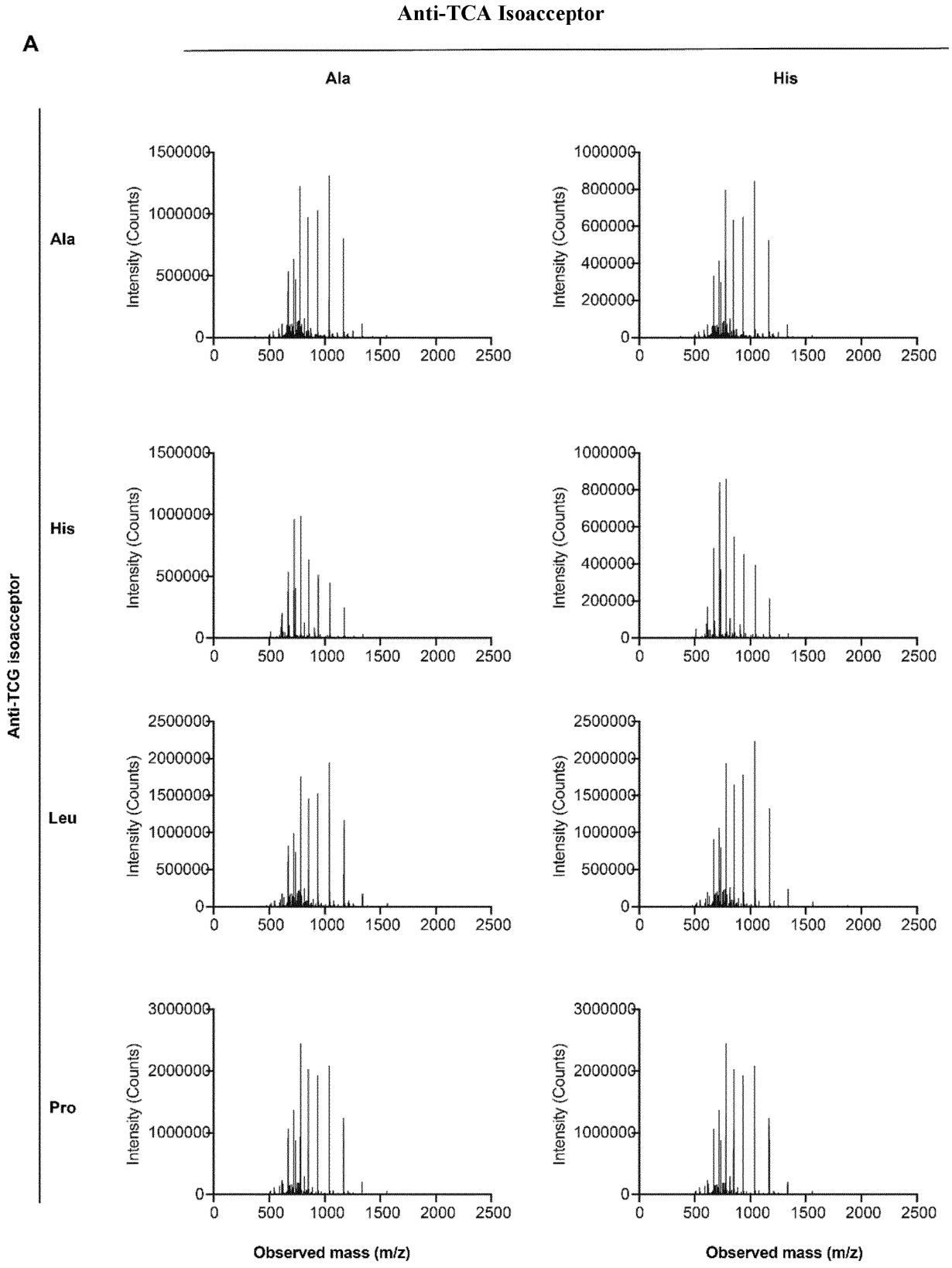

Anti-TCA Isoacceptor
A
Ala
His
Anti-TCG isoacceptor
Ala
His
Leu
Pro
Intensity (Counts)
Observed mass (m/z)
0
500
1000
1500
2000
2500

Figure 16 (cont) A (cont) Anti-TCA Isoacceptor

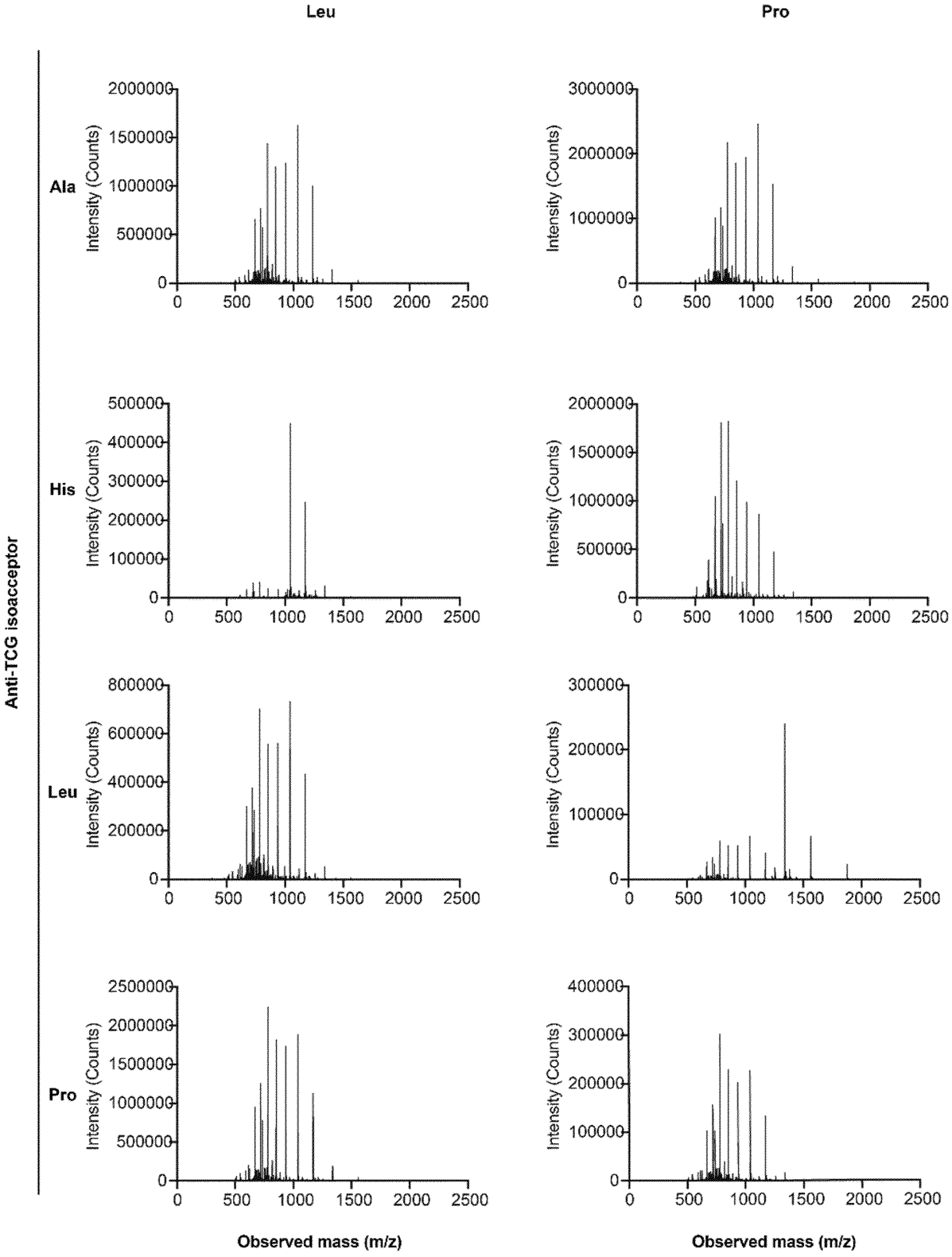


Figure 16 (cont)

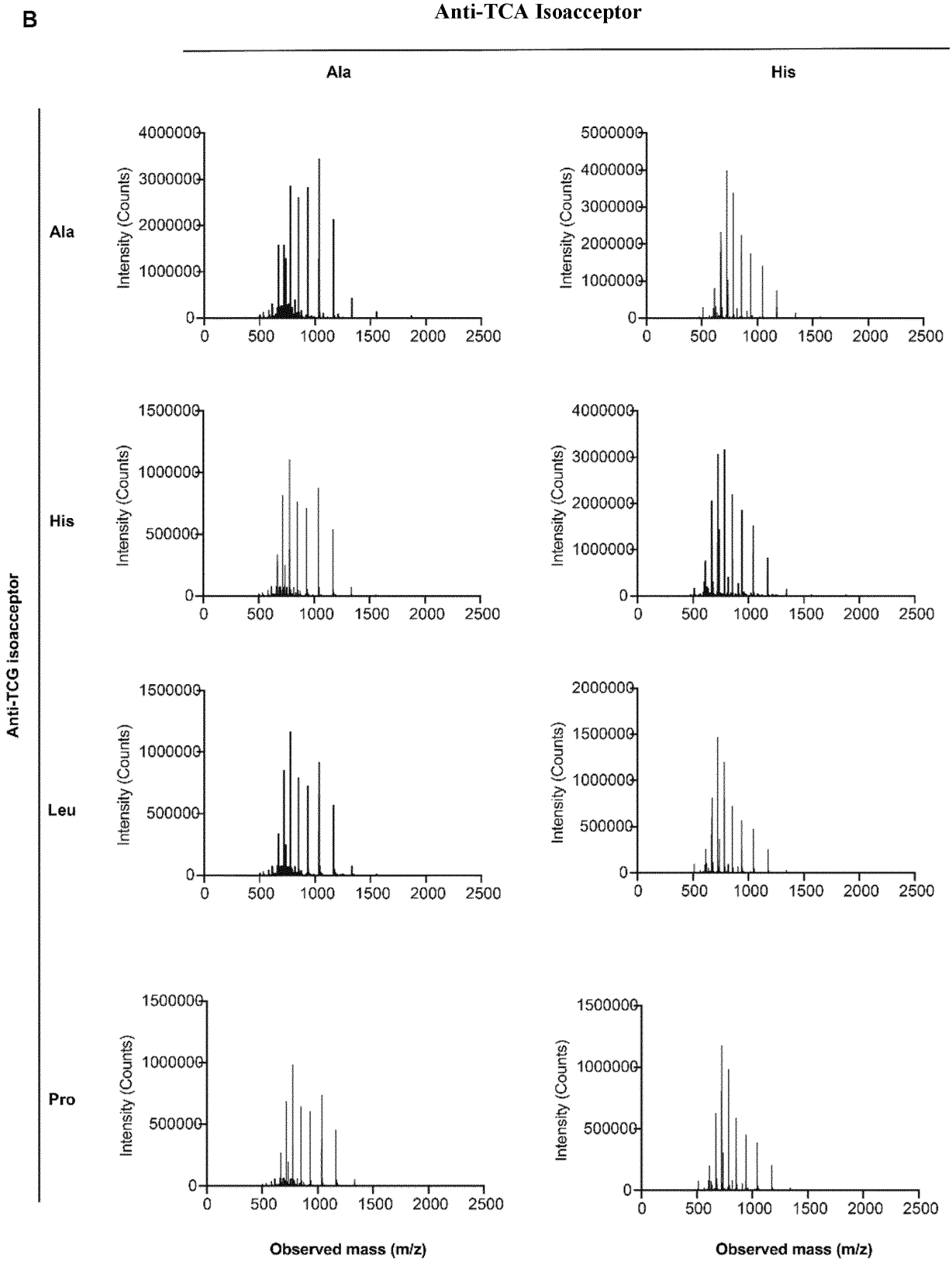


Figure 16 (cont) B (cont) Anti-TCA Isoacceptor

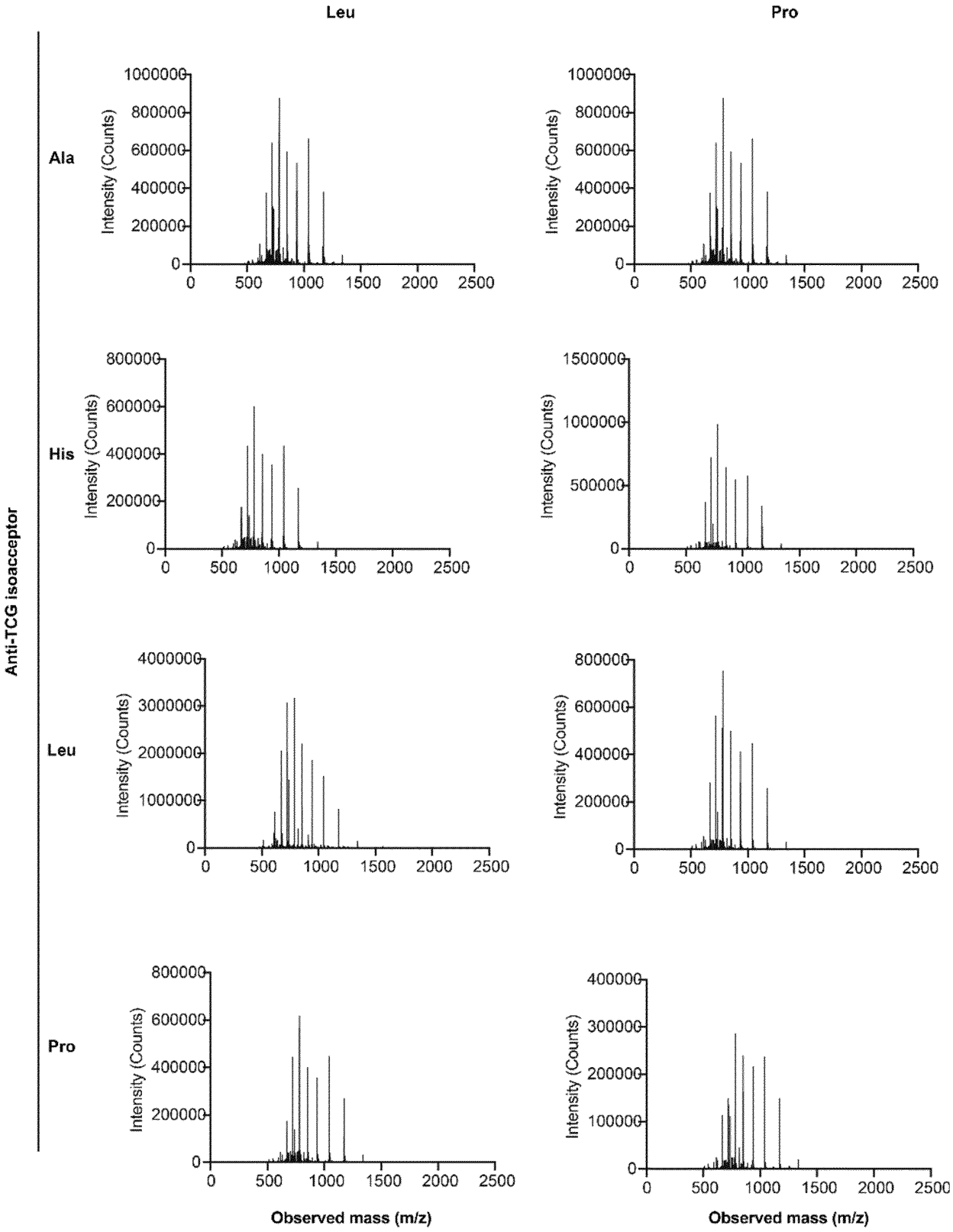
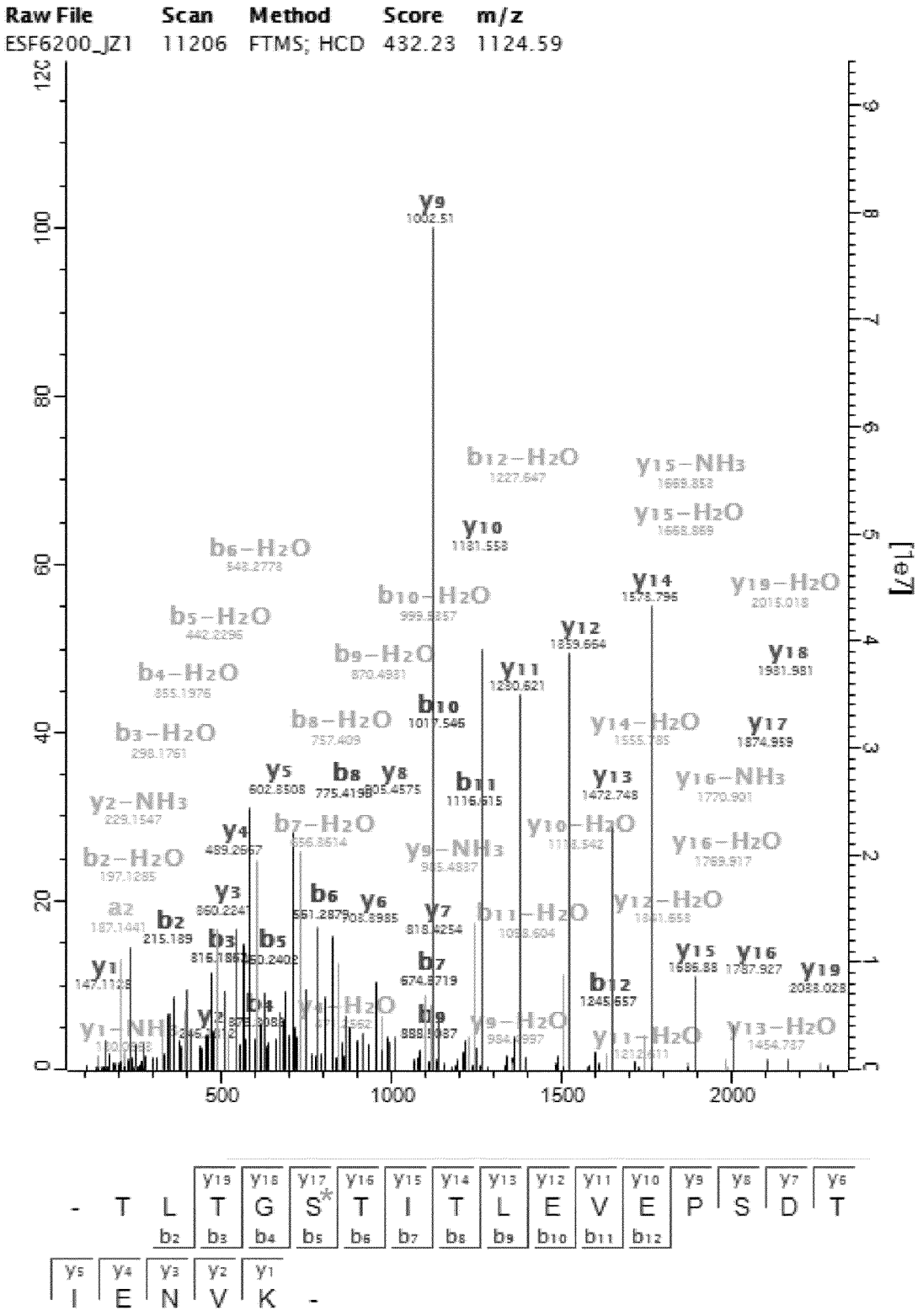


Figure 17

Ub-11-TCG

tRNA-UGA-Ser

A

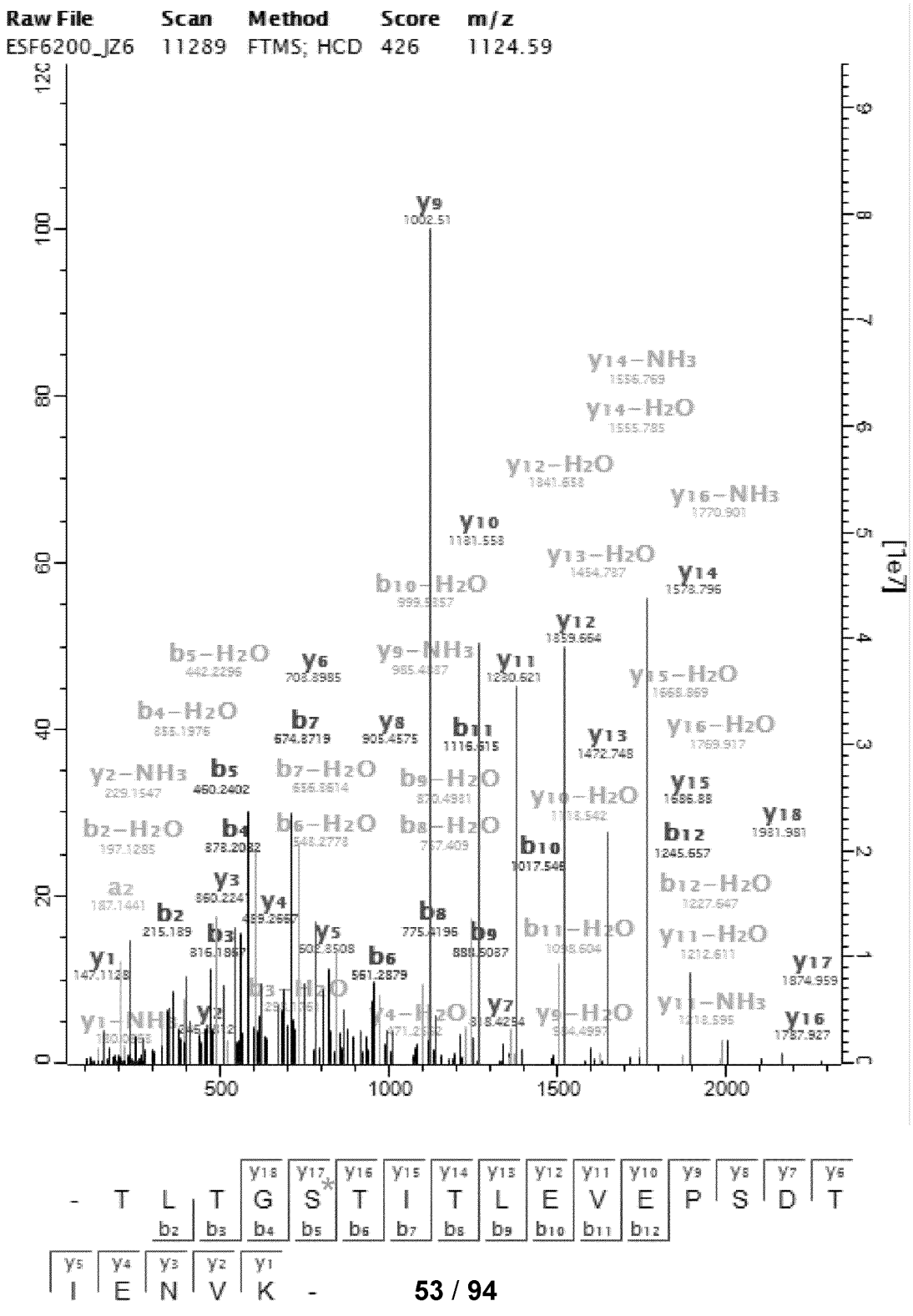


Ub-11-TCA

tRNA-UGA-Ser

Figure 17 (cont)

B



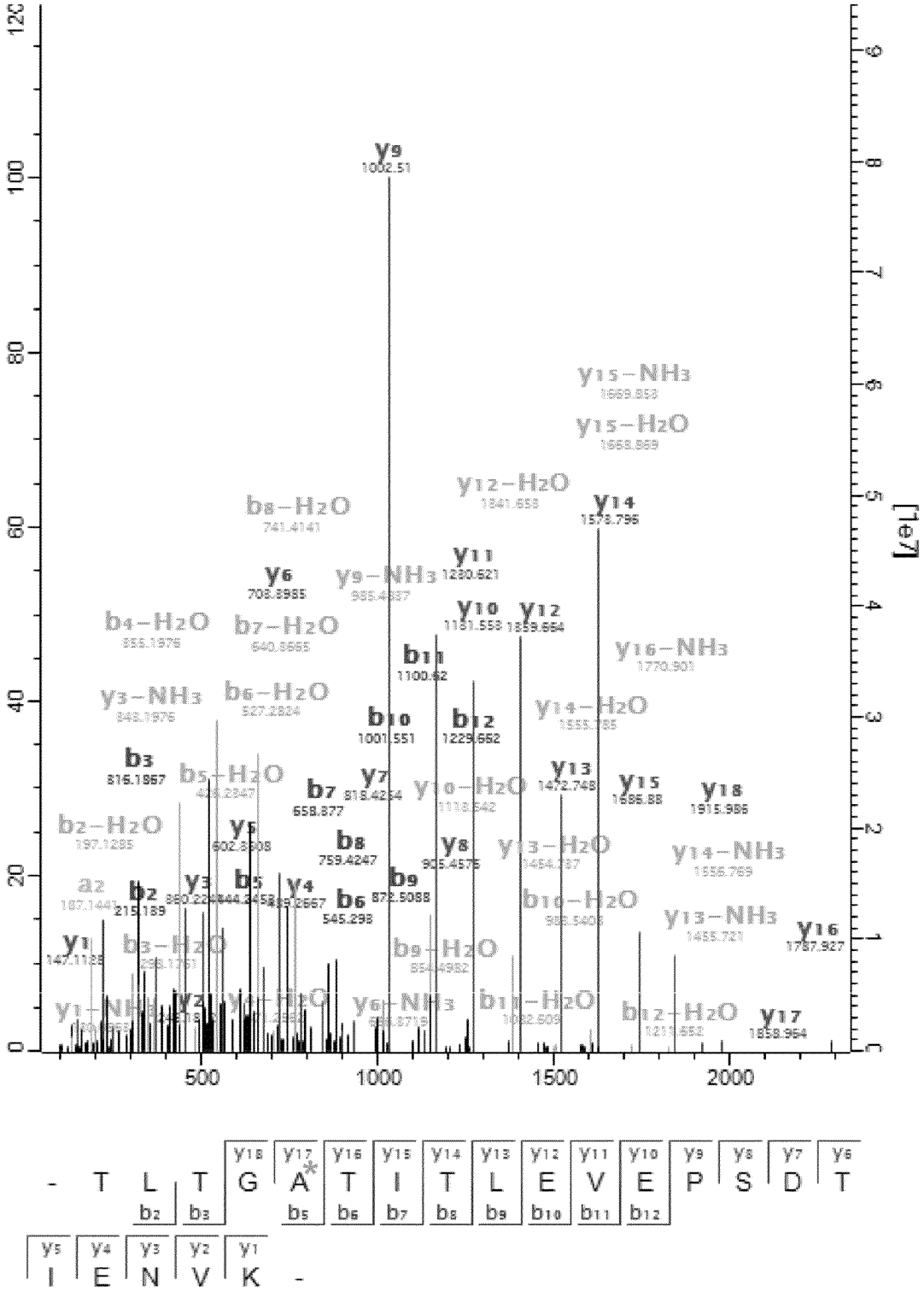
Ub-11-TCG

tRNA-CGA-Ala

Figure 18

A

Raw File	Scan	Method	Score	m/z
ESF6200_JZ2	12617	FTMS; HCD	401.72	1116.59



Ub-11-TCA

tRNA-UGA- Ala

Figure 18 (cont)

B

Raw File	Scan	Method	Score	m/z
ESF6200_JZ7	10888	FTMS; HCD	436.26	1116.59

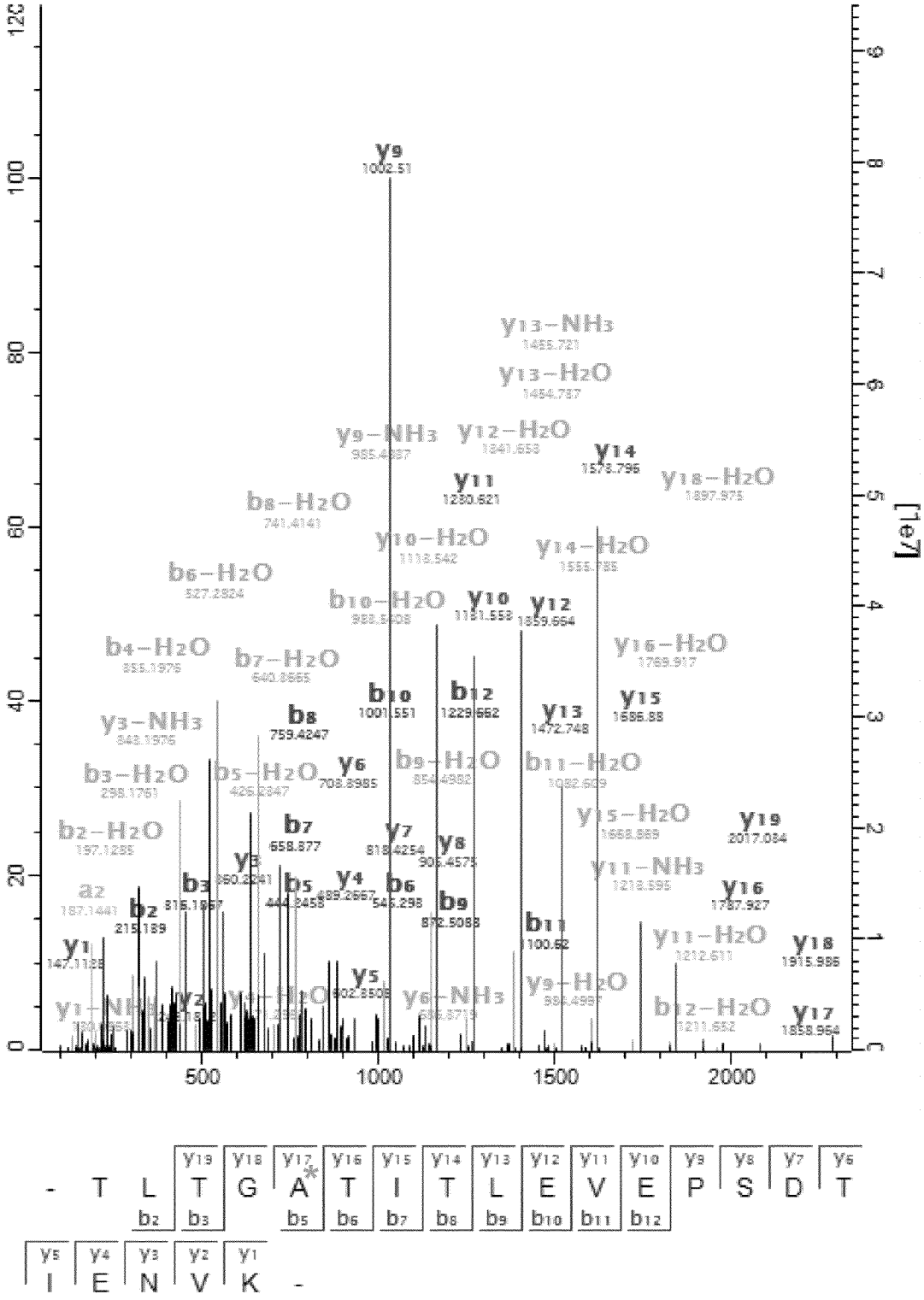


Figure 19

Ub-11-TCG

tRNA-CGA-His

A

Raw File	Scan	Method	Score	m/z
ESF6200_JZ3	9223	FTMS; HCD	576.6	1149.6

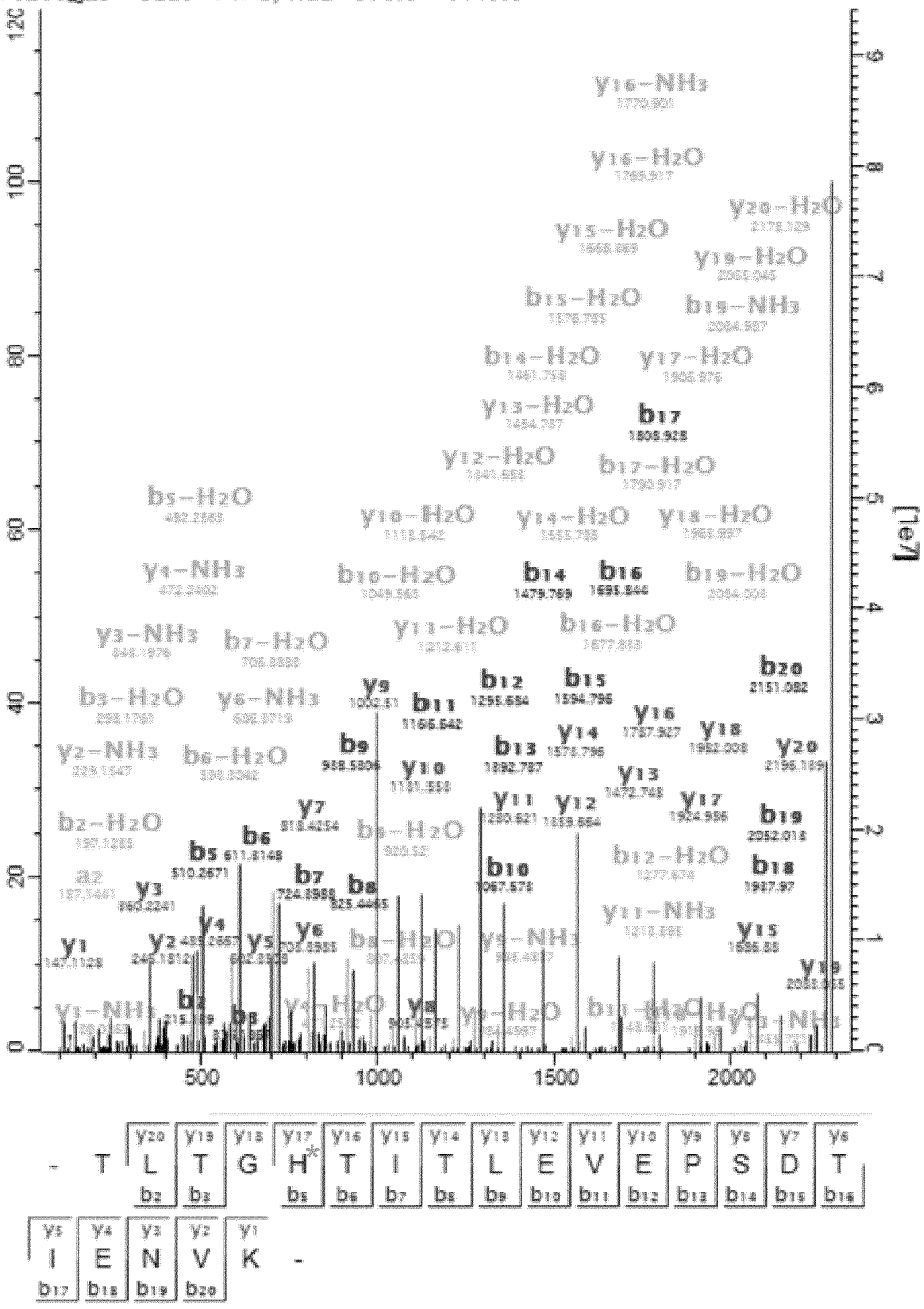


Figure 19 (cont)

Ub-11-TCA

tRNA-UGA-His

B

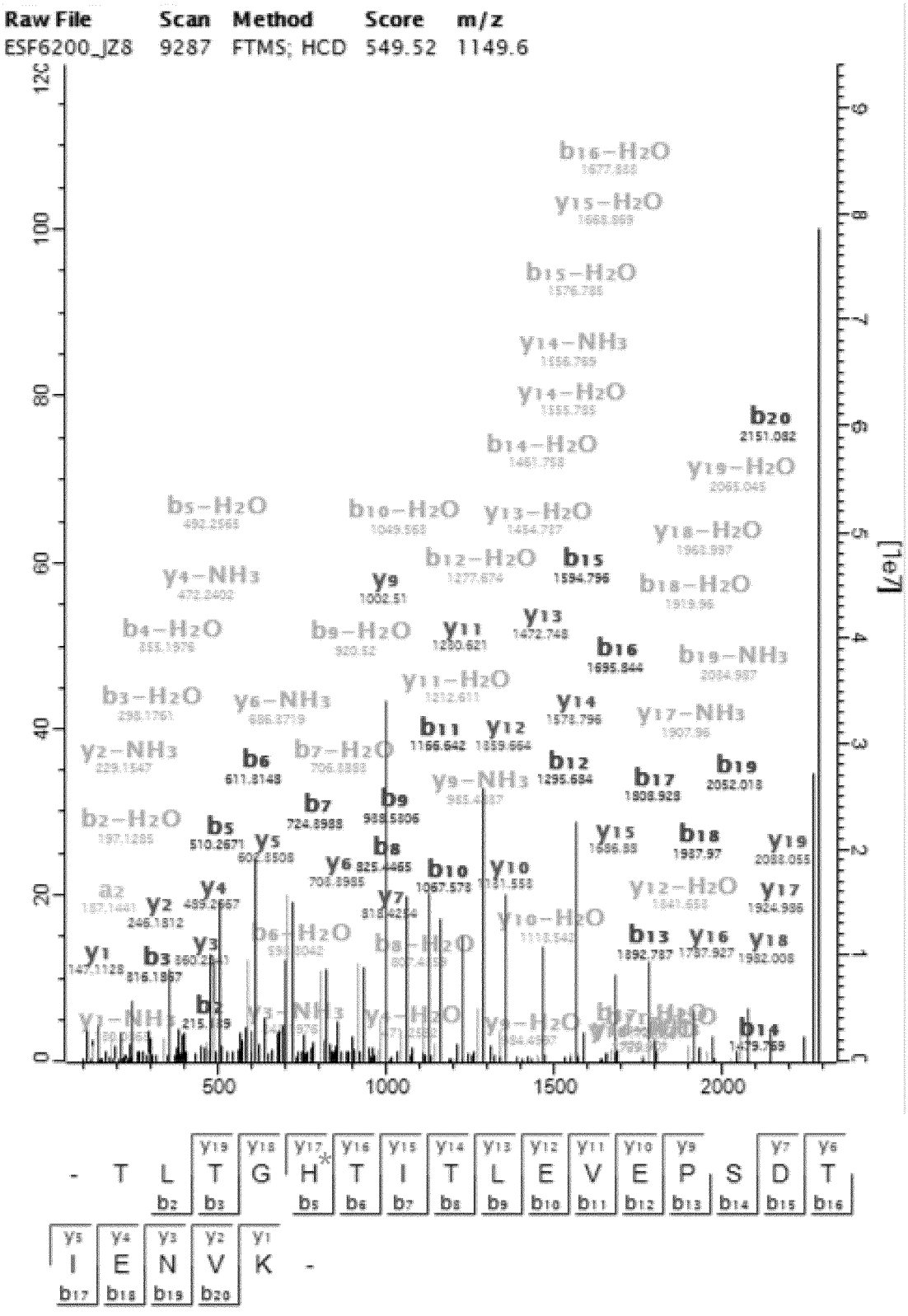


Figure 20

Ub-11-TCG

tRNA-CGA-Leu

A

Raw File	Scan	Method	Score	m/z
ESF6200_JZ4	14404	FTMS; HCD	451.59	1137.61

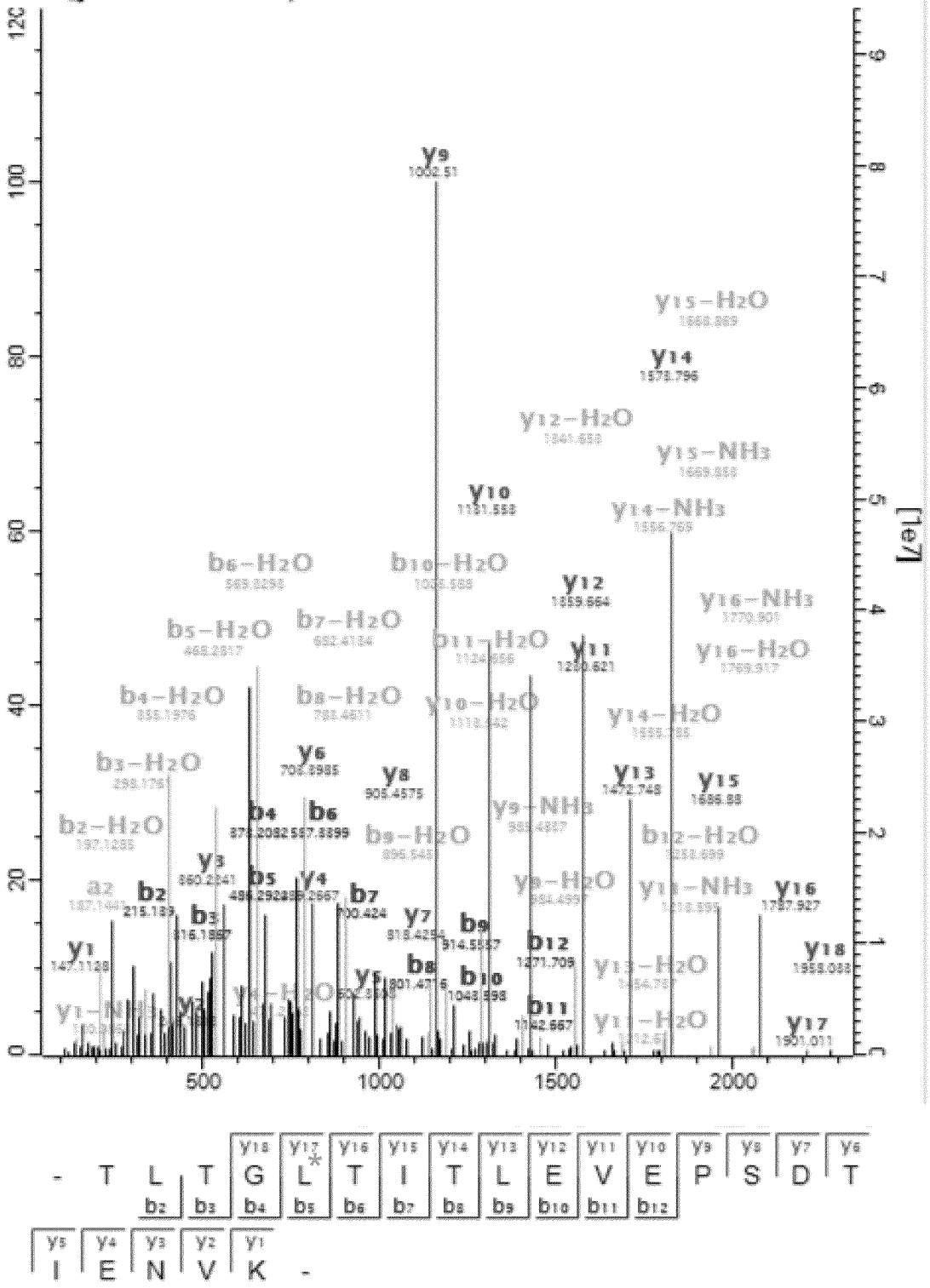


Figure 20 (cont)

Ub-11-TCA

tRNA-UGA-Leu

B

Raw File	Scan	Method	Score	m/z
ESF6200_JZ9	13111	FTMS; HCD	419.46	1137.61

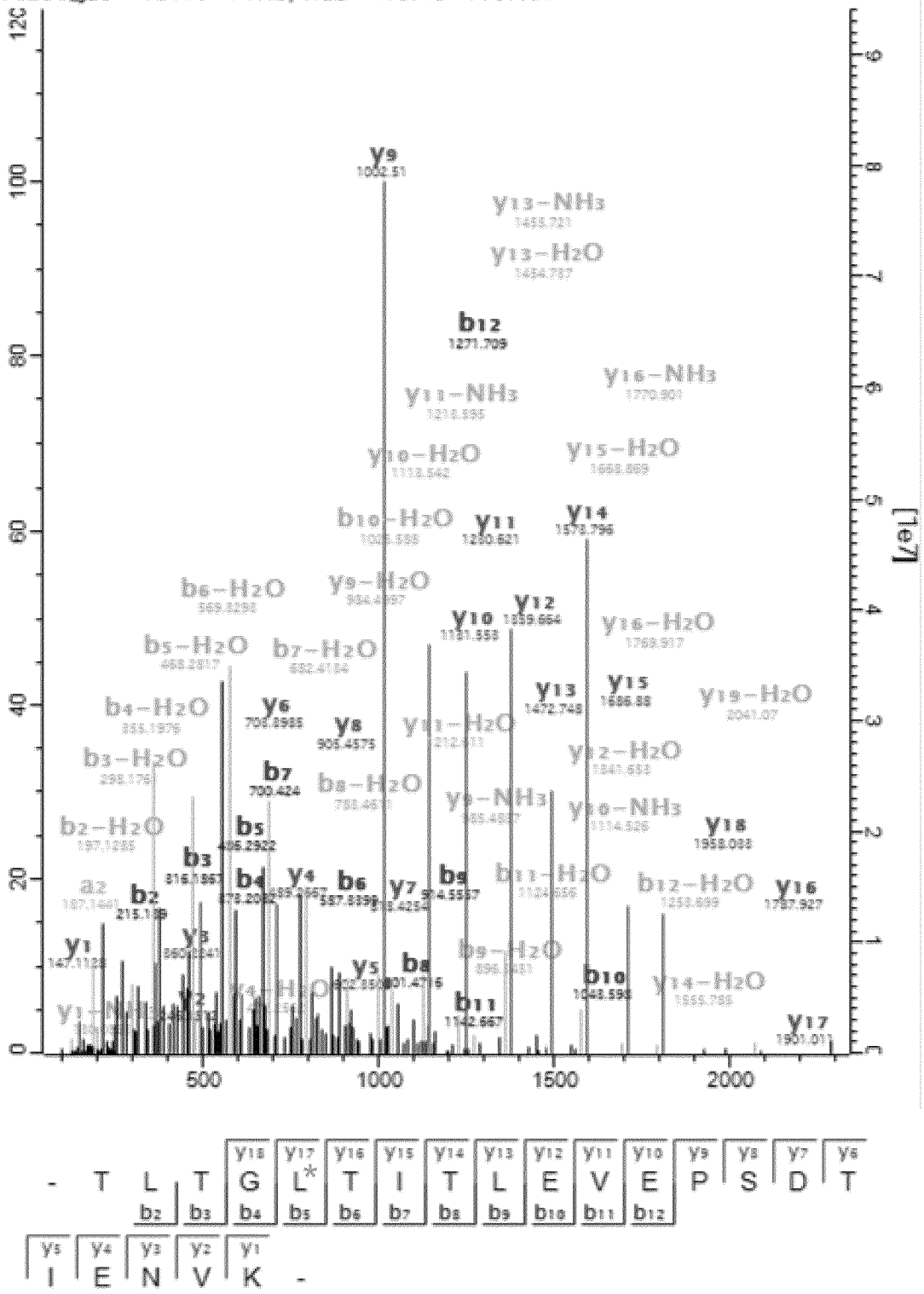


Figure 21

Ub-11-TCG

tRNA-CGA-Pro

A

Raw File	Scan	Method	Score	m/z
ESF6200_JZ5	10850	FTMS; HCD	416.46	1129.6

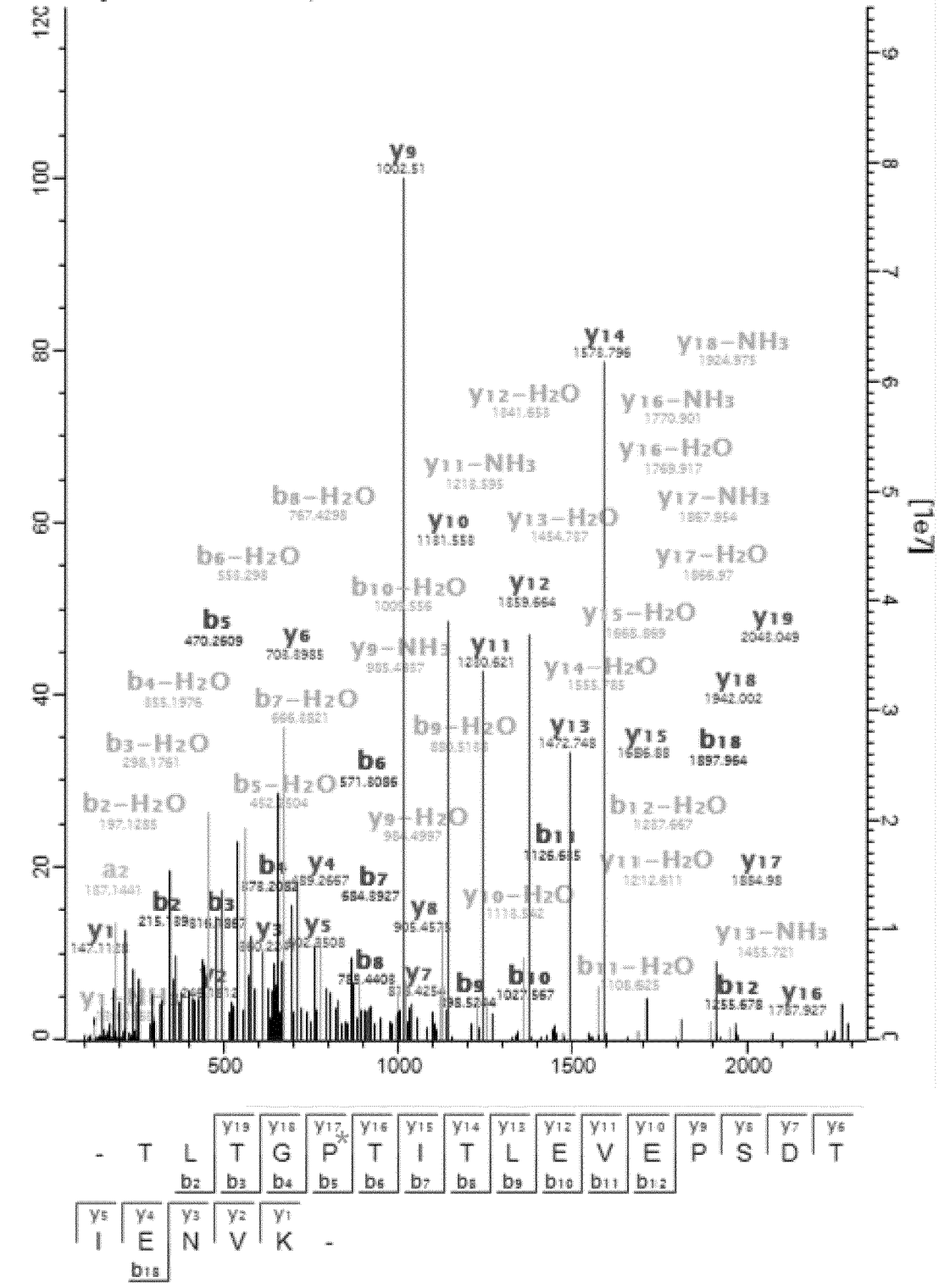


Figure 21 (cont)

Ub-11-TCA

tRNA-UGA-Pro

B

Raw File	Scan	Method	Score	m/z
ESF6200_JZ10	11246	FTMS; HCD	358.01	1129.1

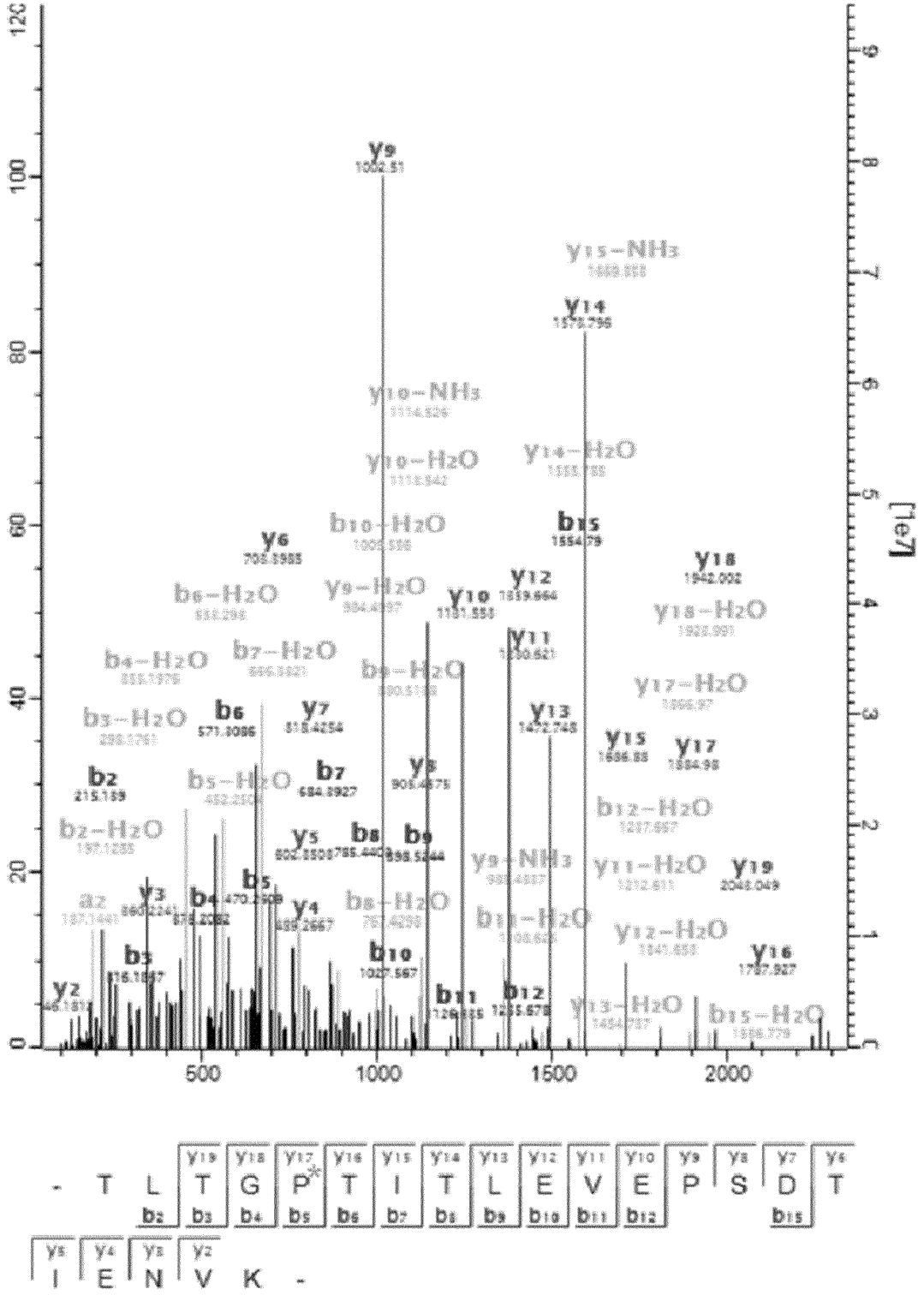


Figure 22

A

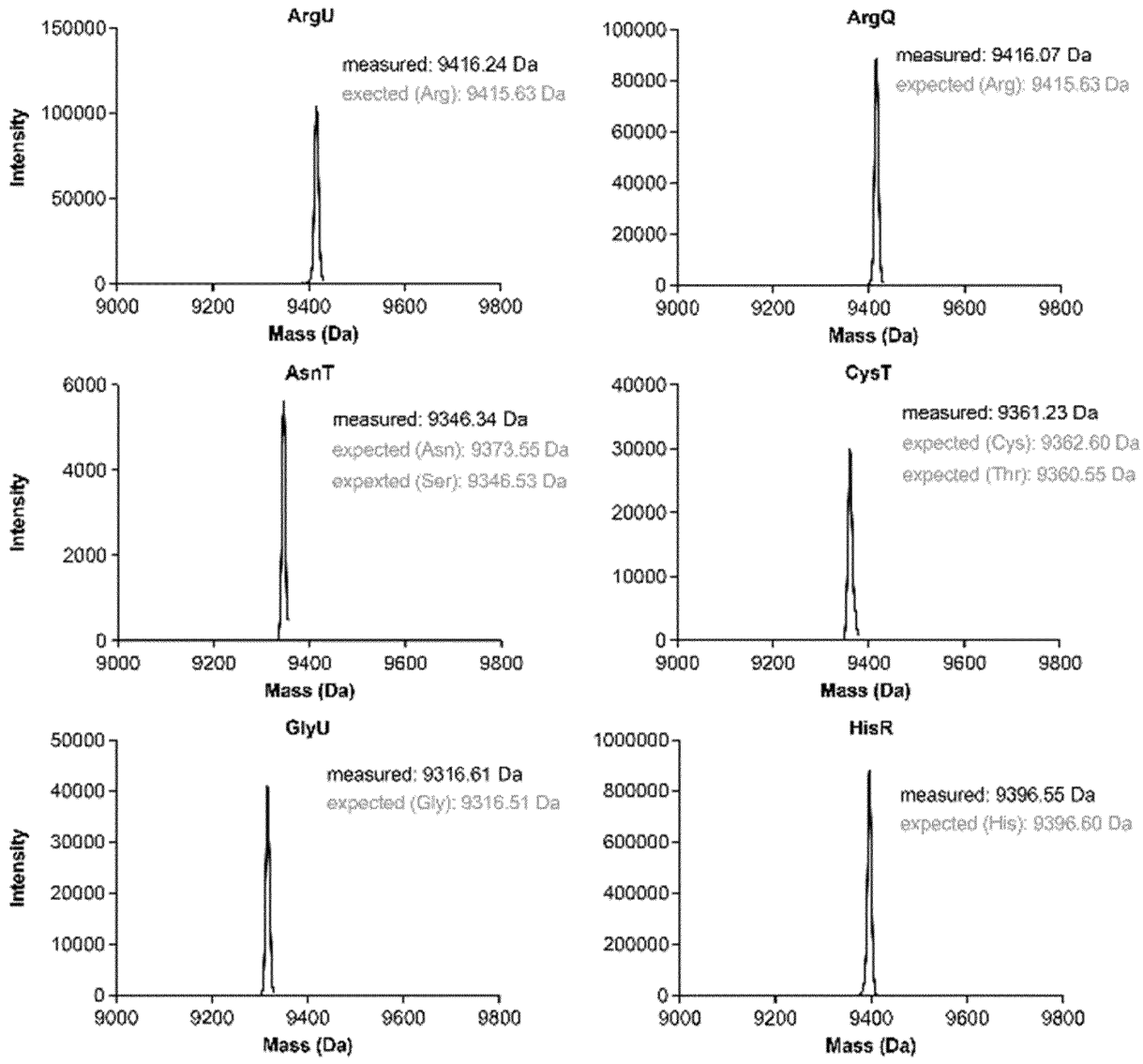

ArgU
measured: 9416.24 Da
exected (Arg): 9415.63 Da
ArgQ
measured: 9416.07 Da
expected (Arg): 9415.63 Da
AsnT
measured: 9346.34 Da
expected (Asn): 9373.55 Da
expexted (Ser): 9346.53 Da
CysT
measured: 9361.23 Da
expected (Cys): 9362.60 Da
expected (Thr): 9360.55 Da
GlyU
measured: 9316.61 Da
expected (Gly): 9316.51 Da
HisR
measured: 9396.55 Da
expected (His): 9396.60 Da
Intensity
Mass (Da)

Figure 22 (cont) A (cont)

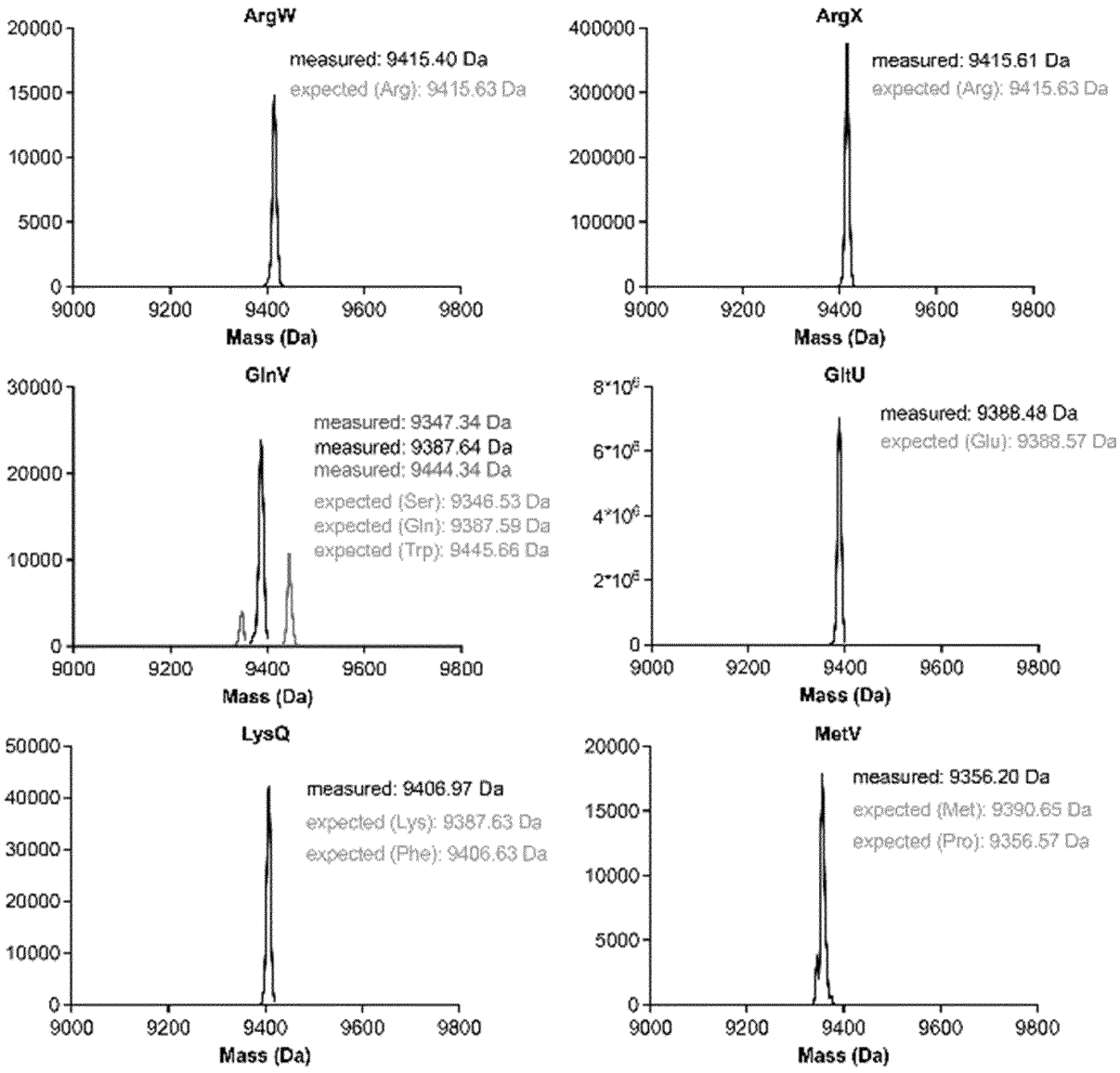


Figure 22 (cont) (A) (cont)

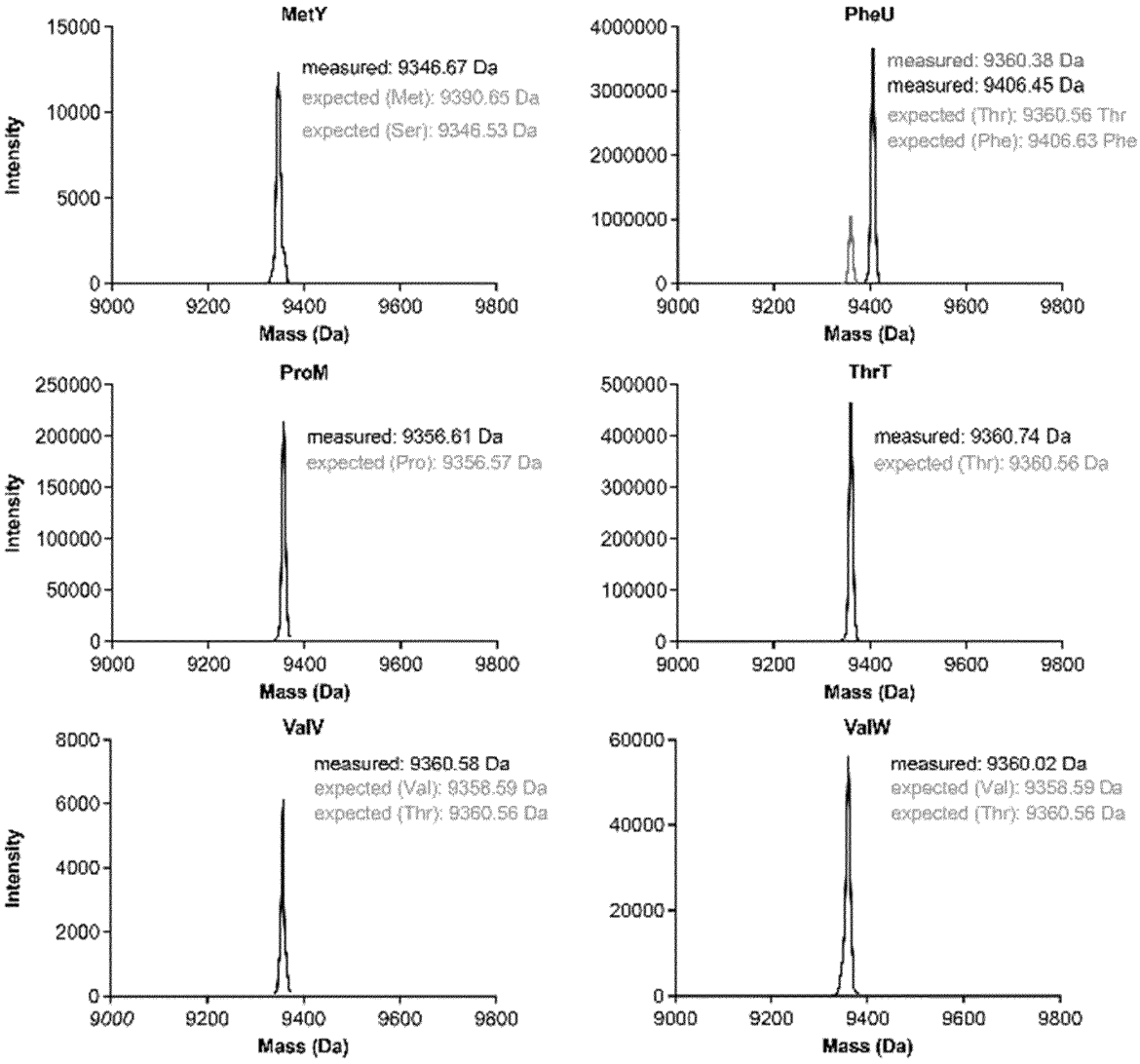


Figure 22 (cont) (A) (cont)

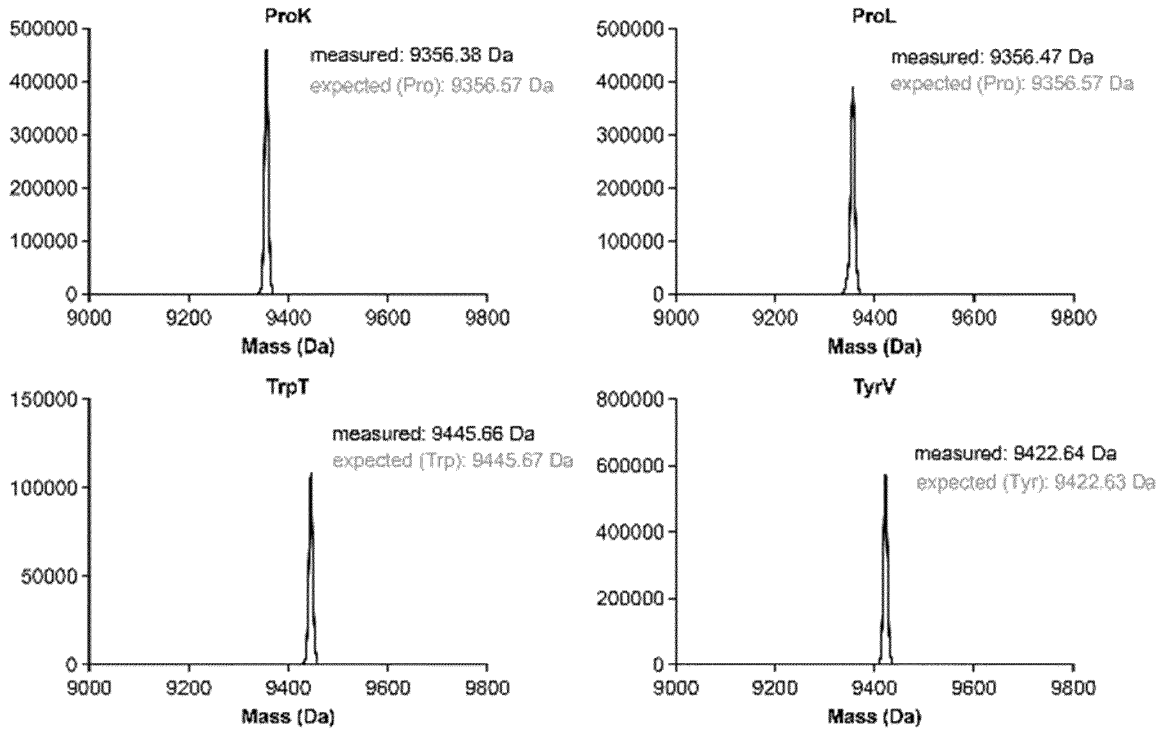
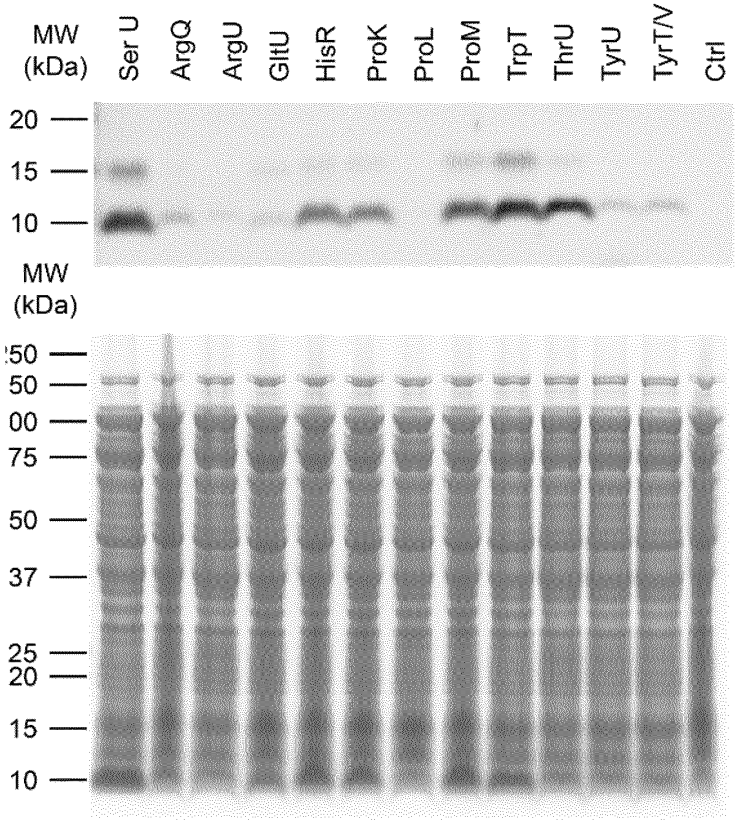


Figure 22 (cont)

B

Replicate 1



Replicate 2

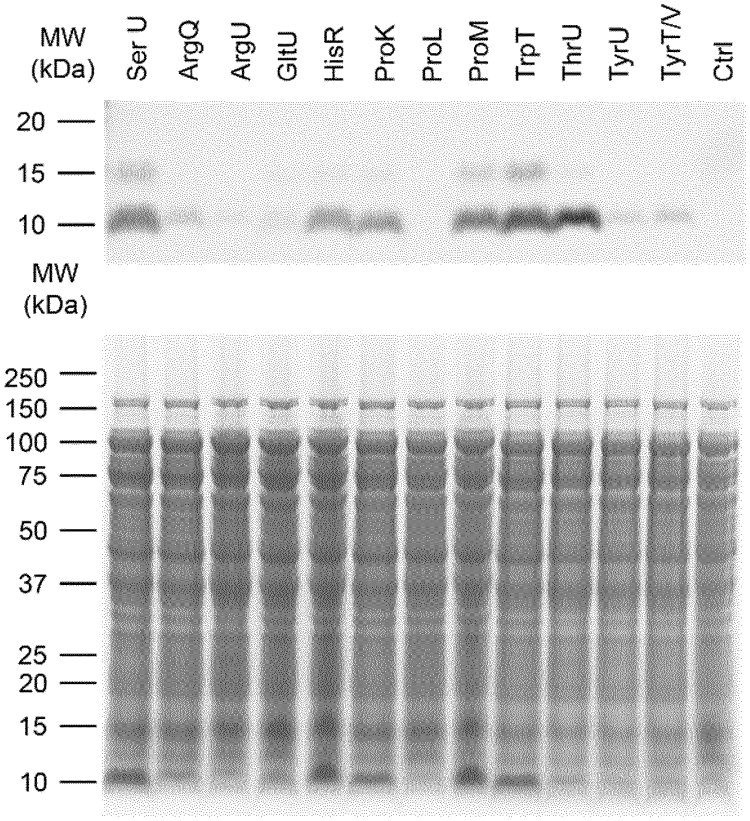


Figure 22 (cont)
B (cont)

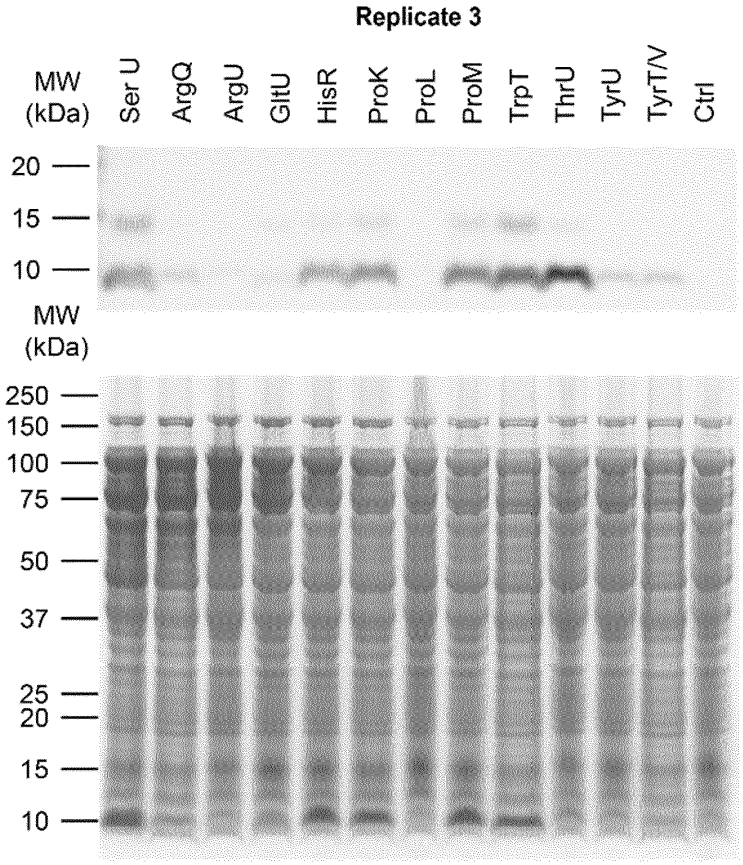


Figure 23

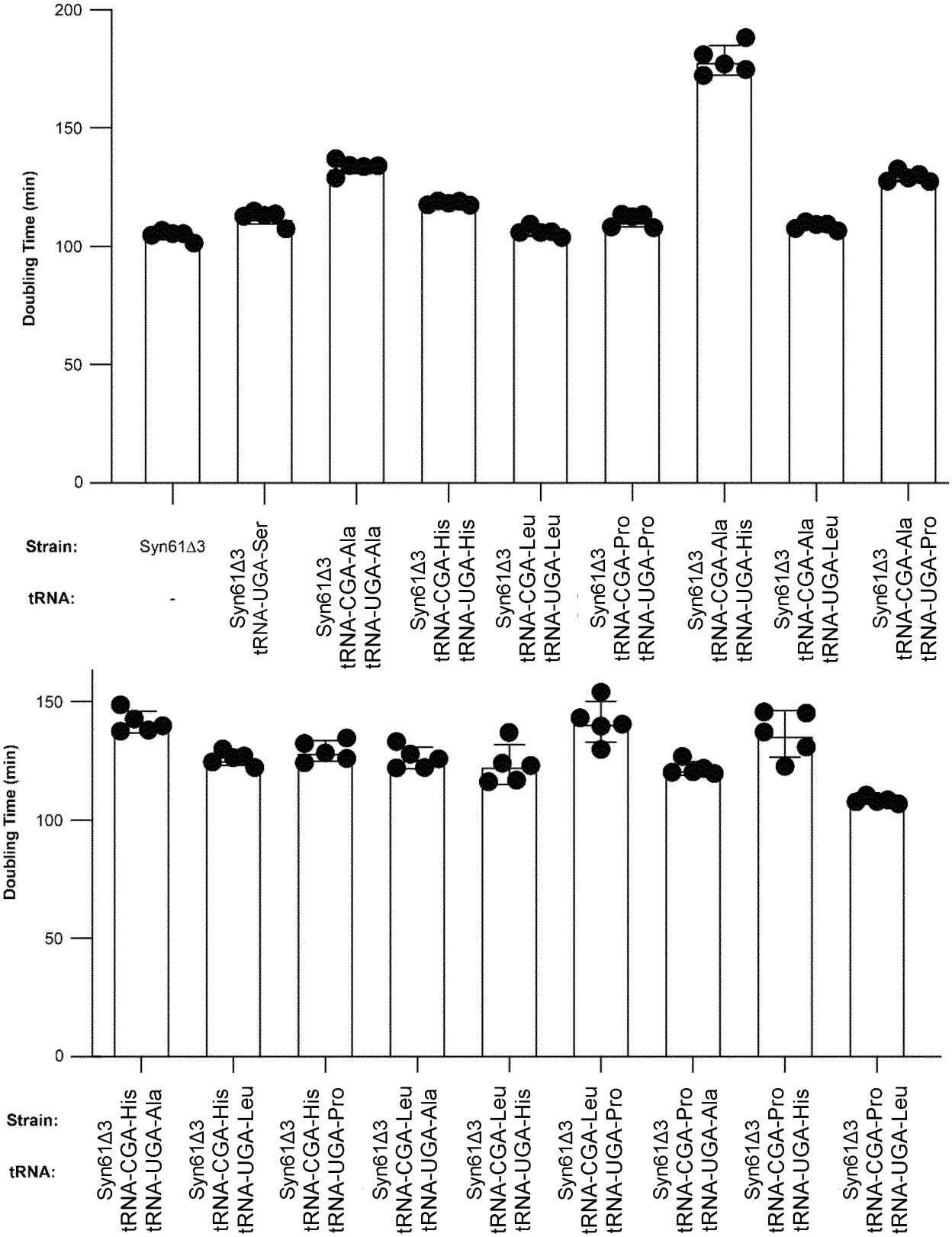

Doubling Time (min)
200
150
100
50
0
Strain:
tRNA:
Syn61Δ3
-
Syn61Δ3 tRNA-UGA-Ser
Syn61Δ3 tRNA-CGA-Ala tRNA-UGA-Ala
Syn61Δ3 tRNA-CGA-His tRNA-UGA-His
Syn61Δ3 tRNA-CGA-Leu tRNA-UGA-Leu
Syn61Δ3 tRNA-CGA-Pro tRNA-UGA-Pro
Syn61Δ3 tRNA-CGA-Ala tRNA-UGA-His
Syn61Δ3 tRNA-CGA-Ala tRNA-UGA-Leu
Syn61Δ3 tRNA-CGA-Ala tRNA-UGA-Pro
Doubling Time (min)
150
100
50
0
Strain:
tRNA:
Syn61Δ3 tRNA-CGA-His tRNA-UGA-Ala
Syn61Δ3 tRNA-CGA-His tRNA-UGA-Leu
Syn61Δ3 tRNA-CGA-His tRNA-UGA-Pro
Syn61Δ3 tRNA-CGA-Leu tRNA-UGA-Ala
Syn61Δ3 tRNA-CGA-Leu tRNA-UGA-His
Syn61Δ3 tRNA-CGA-Leu tRNA-UGA-Pro
Syn61Δ3 tRNA-CGA-Pro tRNA-UGA-Ala
Syn61Δ3 tRNA-CGA-Pro tRNA-UGA-His
Syn61Δ3 tRNA-CGA-Pro tRNA-UGA-Leu

Figure 24 A

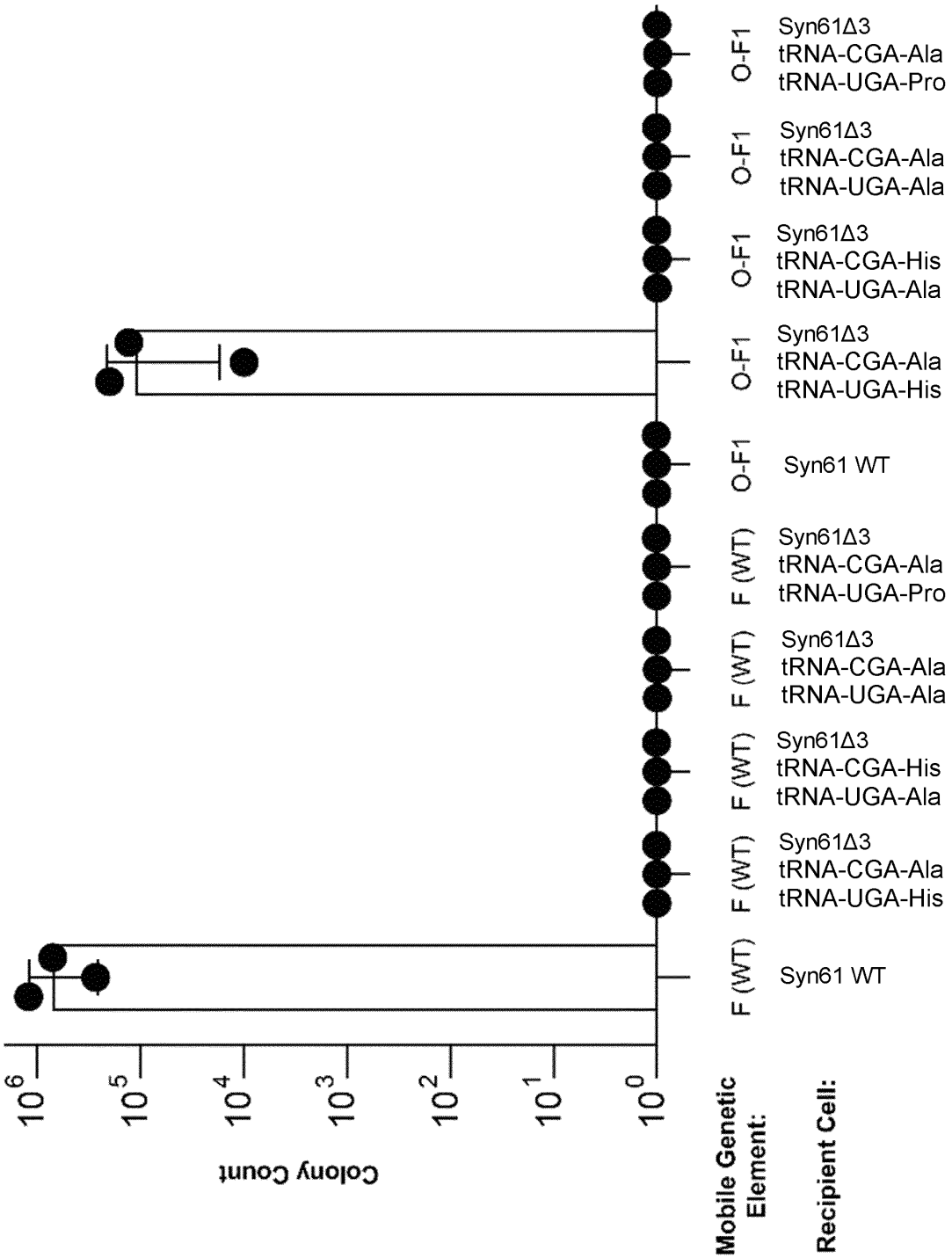

Colony Count
10^6
10^5
10^4
10^3
10^2
10^1
10^0
Mobile Genetic Element:
Recipient Cell:
O-F1
Syn61Δ3 tRNA-CGA-Ala tRNA-UGA-Pro
O-F1
Syn61Δ3 tRNA-CGA-Ala tRNA-UGA-Ala
O-F1
Syn61Δ3 tRNA-CGA-His tRNA-UGA-Ala
O-F1
Syn61Δ3 tRNA-CGA-Ala tRNA-UGA-His
O-F1
Syn61 WT
F (WT)
Syn61Δ3 tRNA-CGA-Ala tRNA-UGA-Pro
F (WT)
Syn61Δ3 tRNA-CGA-Ala tRNA-UGA-Ala
F (WT)
Syn61Δ3 tRNA-CGA-His tRNA-UGA-Ala
F (WT)
Syn61Δ3 tRNA-CGA-Ala tRNA-UGA-His
F (WT)
Syn61 WT

Figure 24 (cont) A (cont)

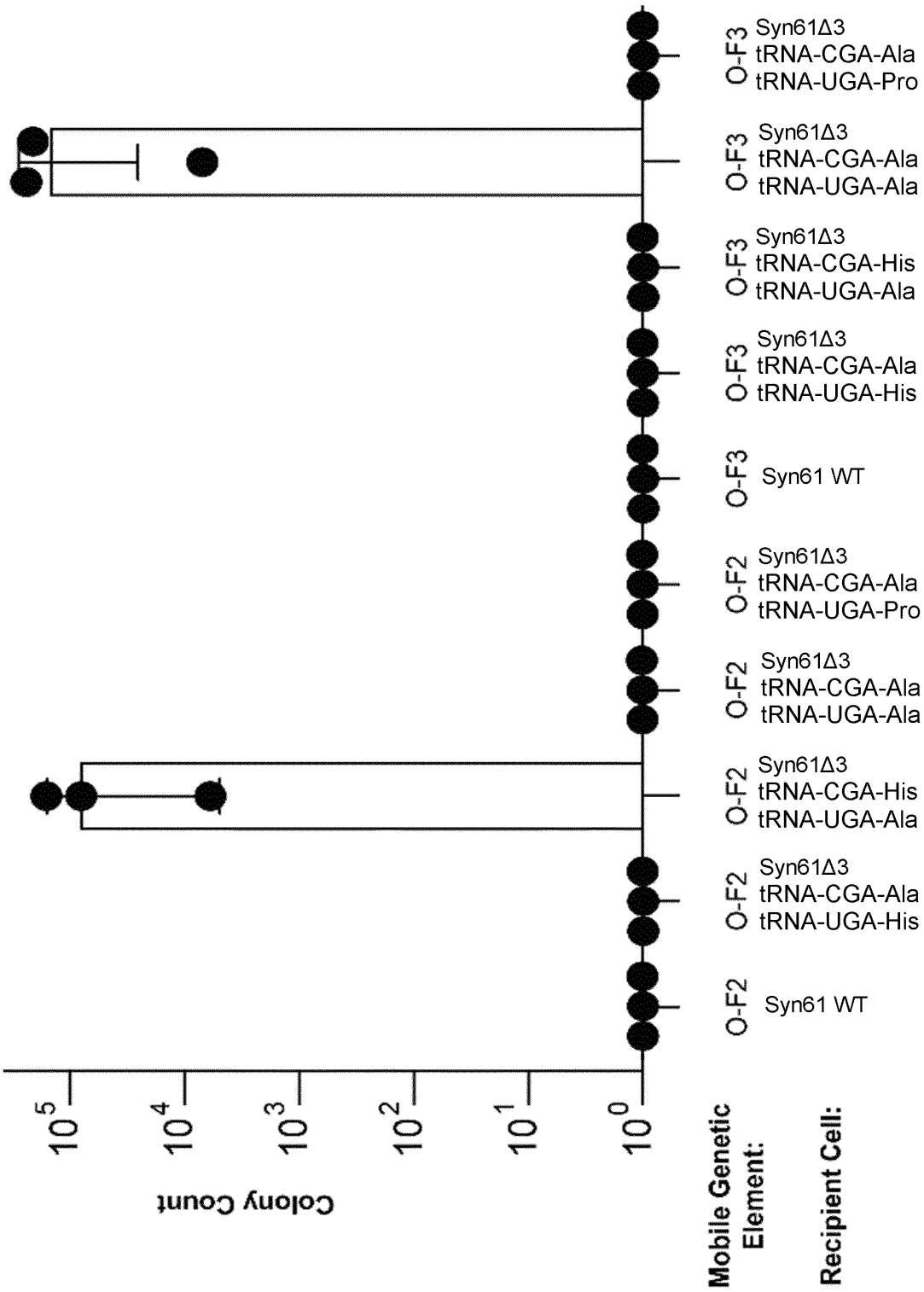


Figure 24 (cont) A (cont)

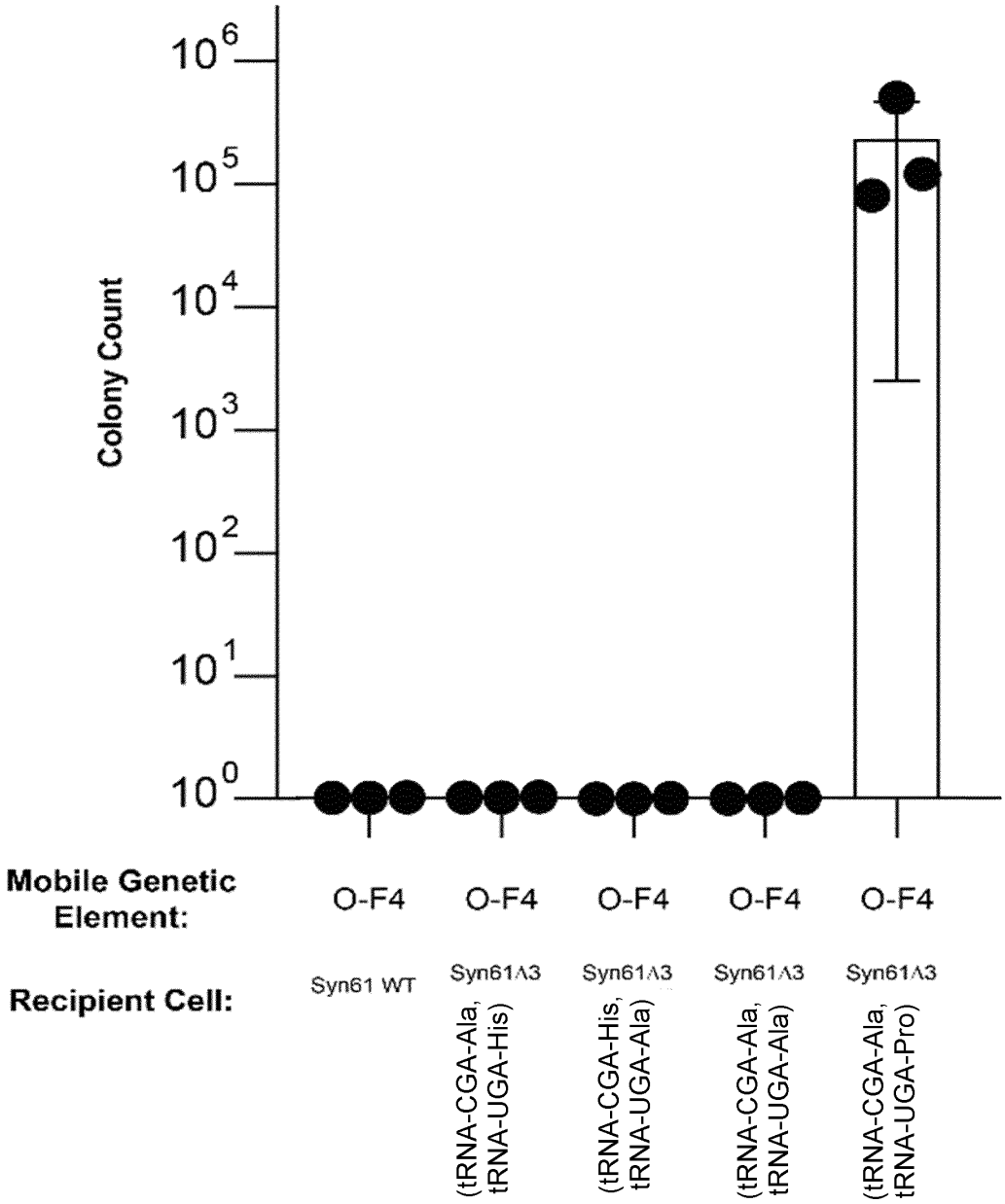


Figure 25

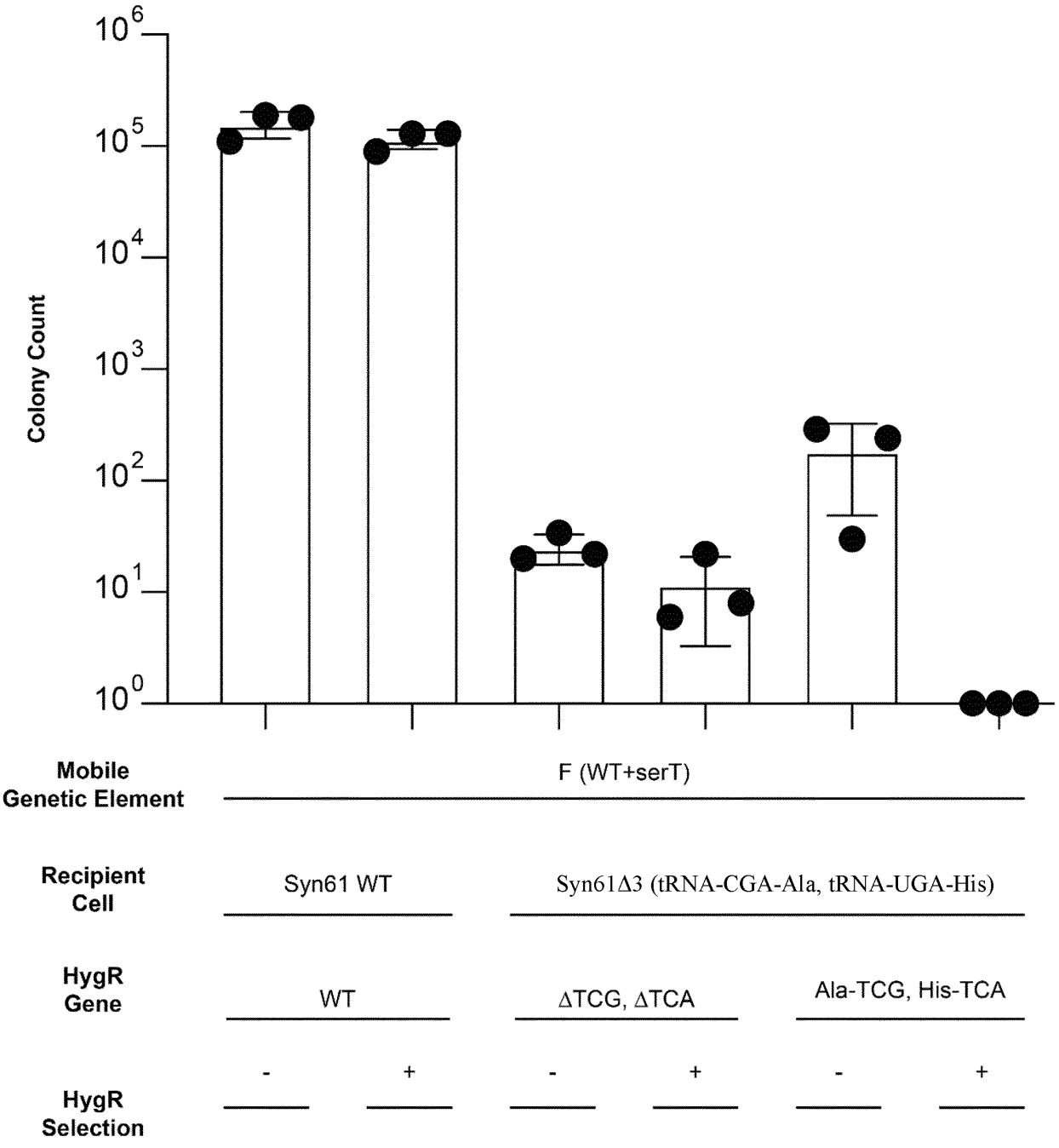

Colony Count
10^6
10^5
10^4
10^3
10^2
10^1
10^0
Mobile Genetic Element
F (WT+serT)
Recipient Cell
Syn61 WT
Syn61Δ3 (tRNA-CGA-Ala, tRNA-UGA-His)
HygR Gene
WT
ΔTCG, ΔTCA
Ala-TCG, His-TCA
HygR Selection
- + - + - +

Figure 26

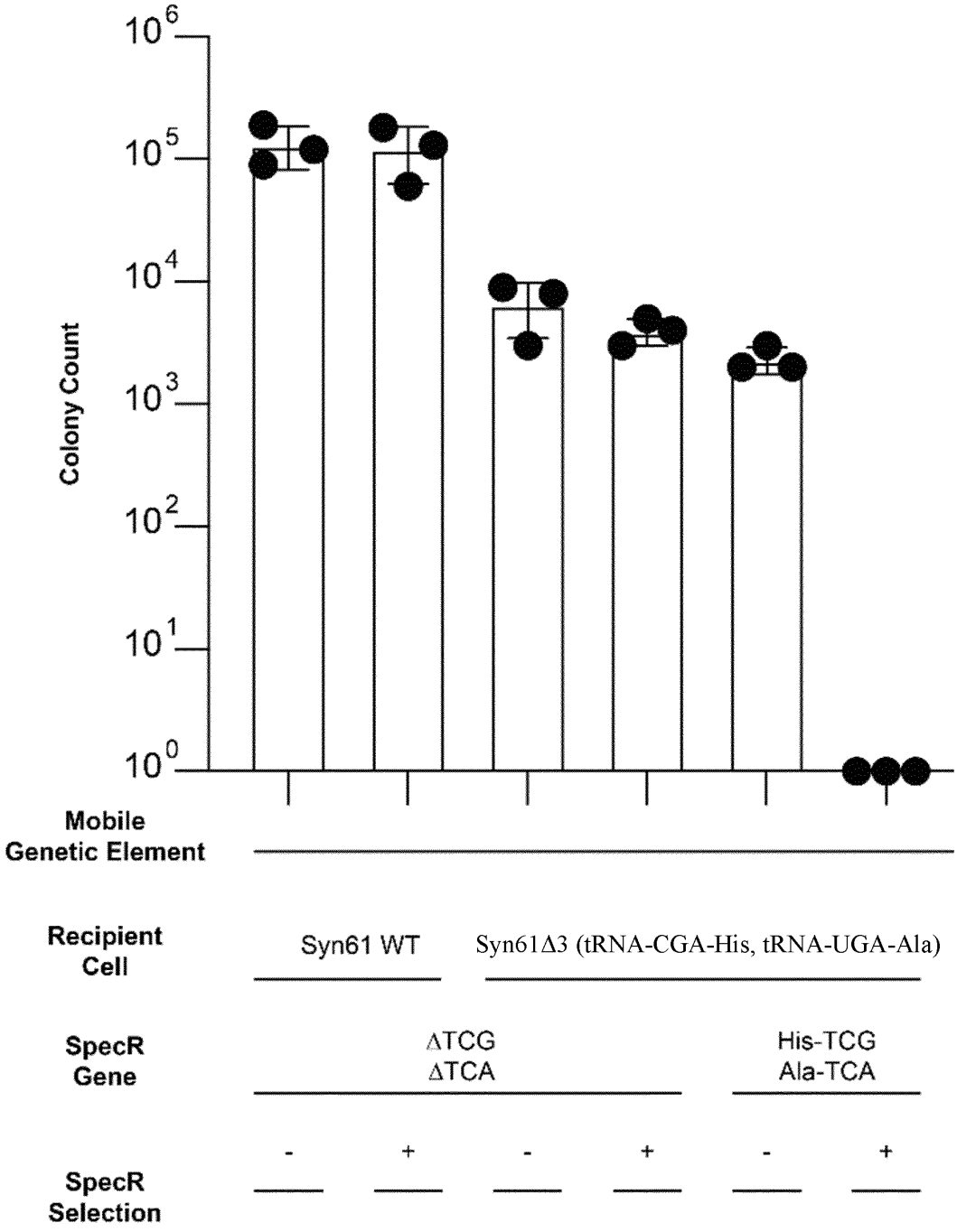

10^6
10^5
10^4
10^3
10^2
10^1
10^0
Colony Count
Mobile Genetic Element
Recipient Cell
Syn61 WT
Syn61Δ3 (tRNA-CGA-His, tRNA-UGA-Ala)
SpecR Gene
ΔTCG ΔTCA
His-TCG Ala-TCA
- + - + - +
SpecR Selection

Figure 26 (cont)

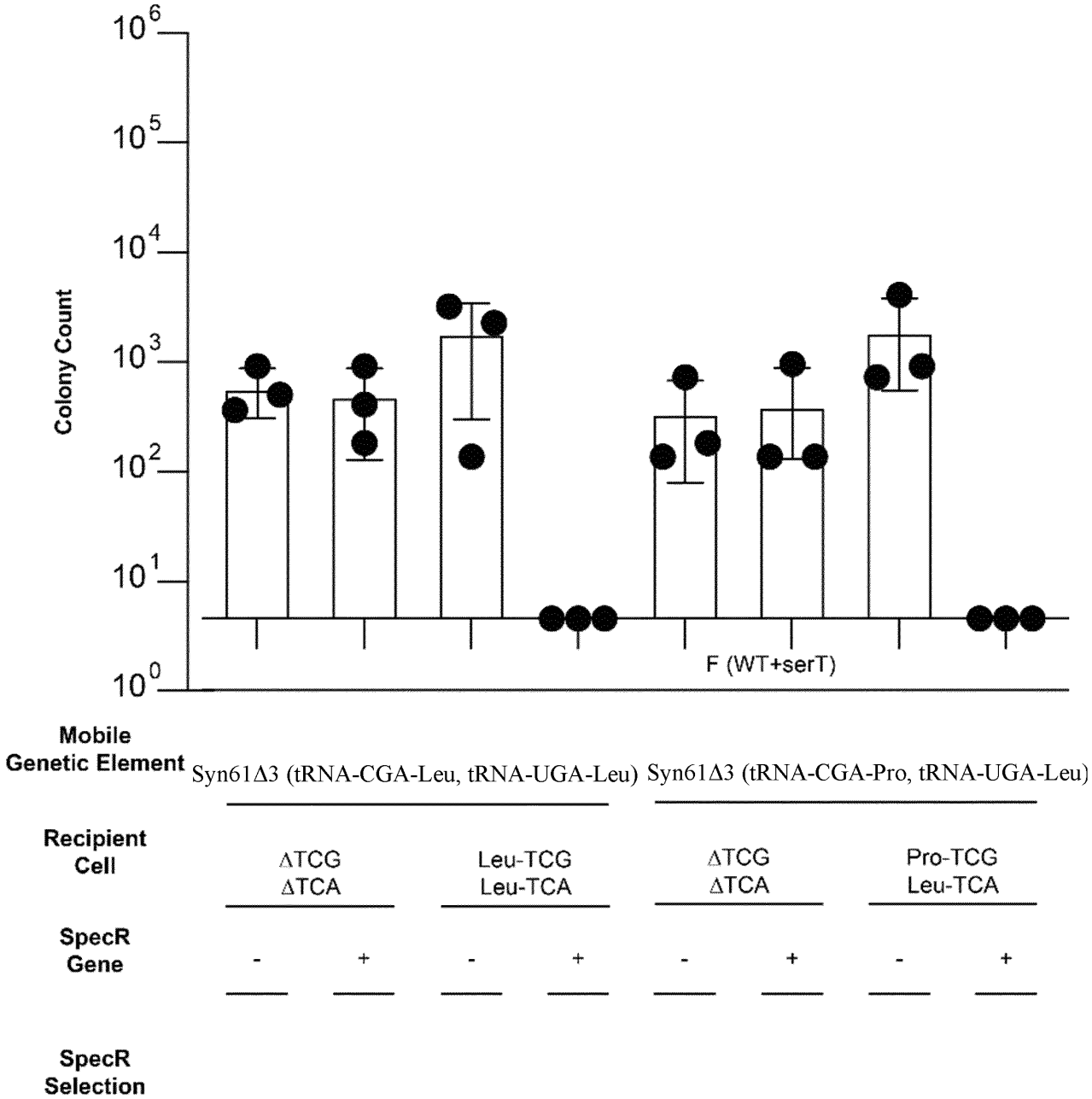


Figure 26 (cont)

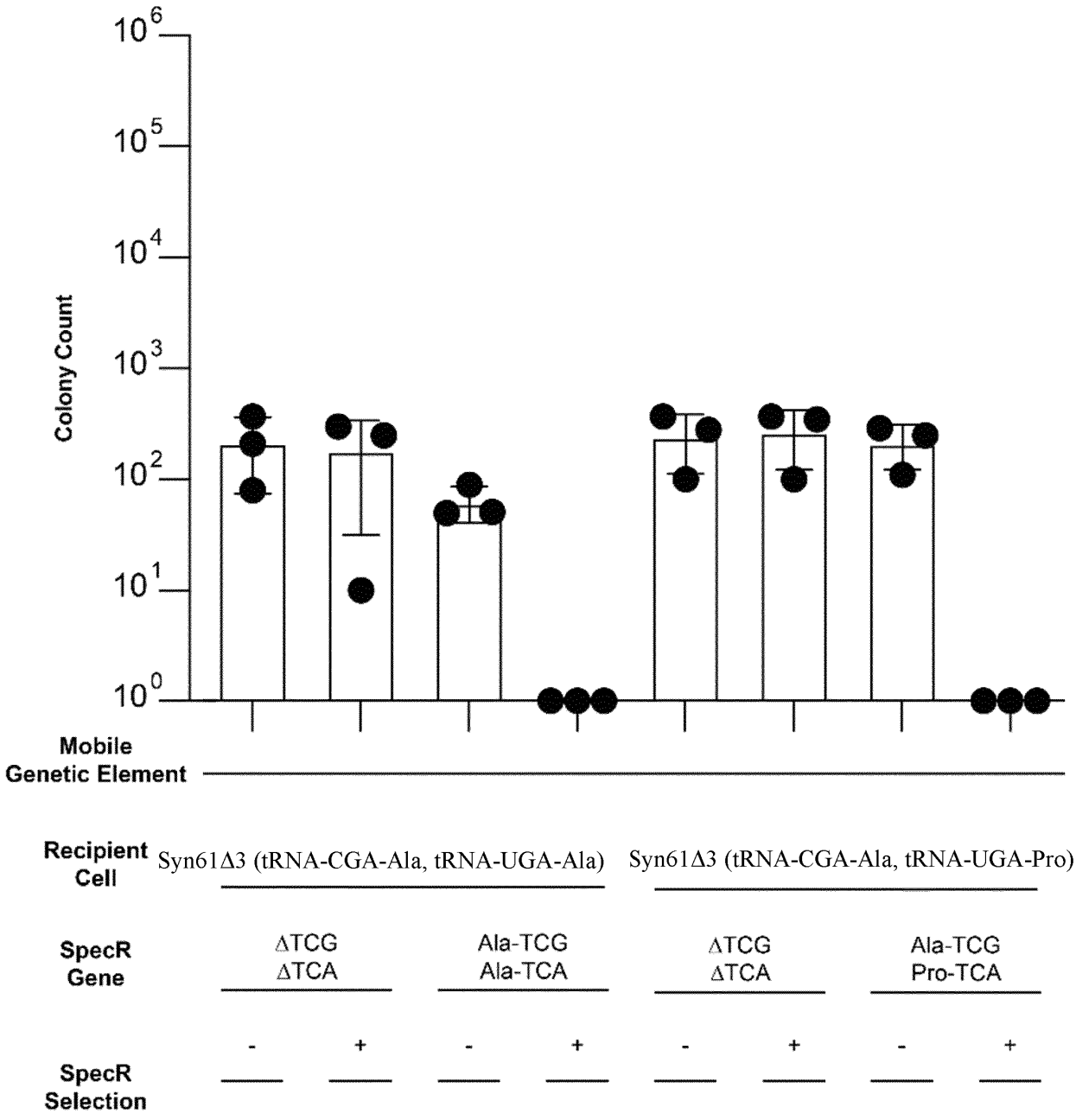


Figure 27

A

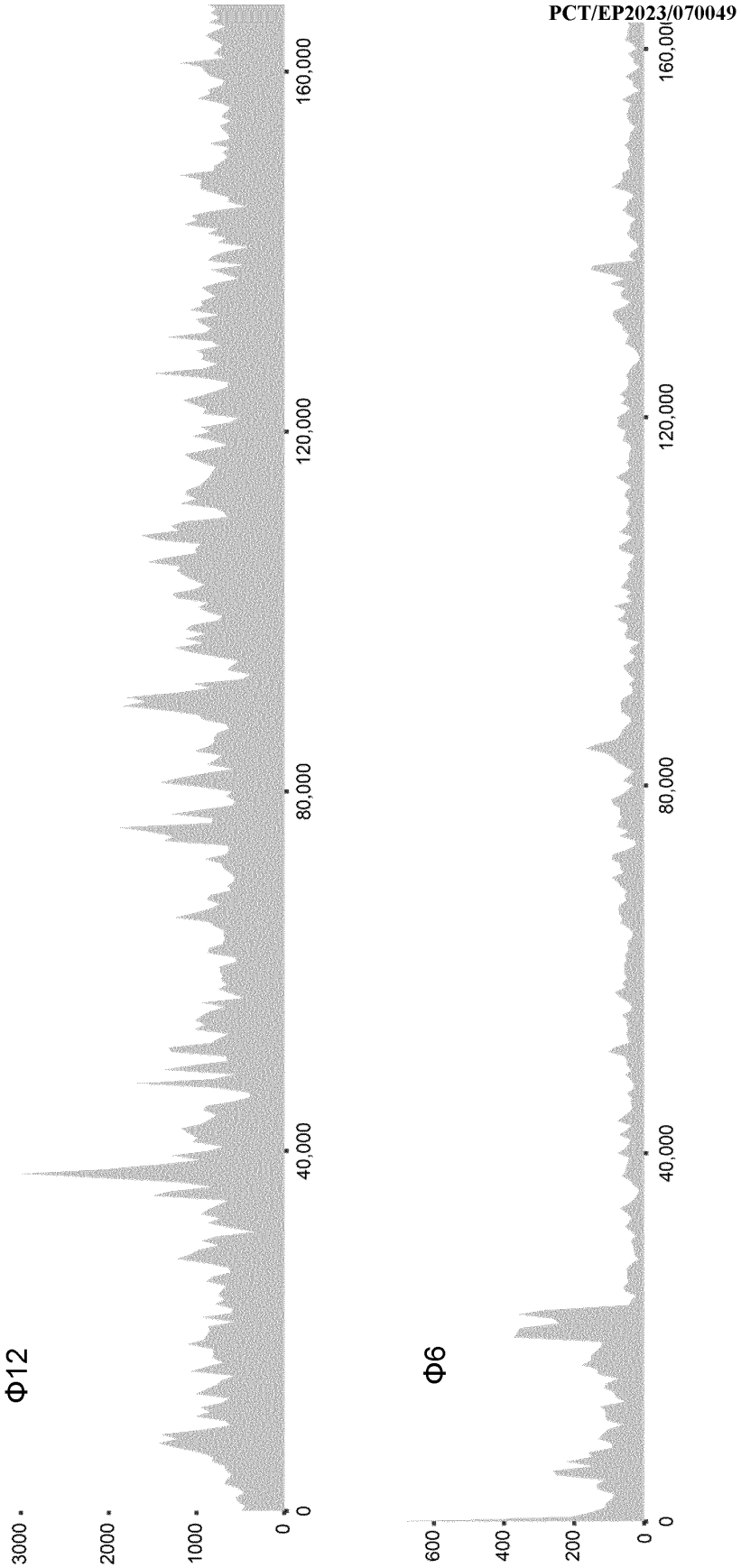
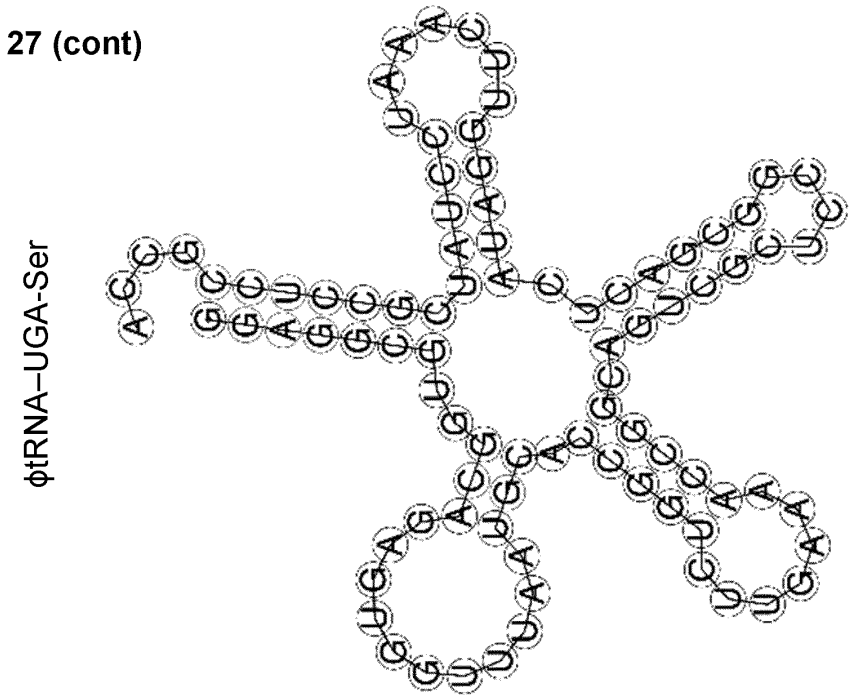

Φ12
3000
2000
1000
0
0
40,000
80,000
120,000
160,000
Φ6
600
400
200
0
0
40,000
80,000
120,000
160,000

Figure 27 (cont)
B



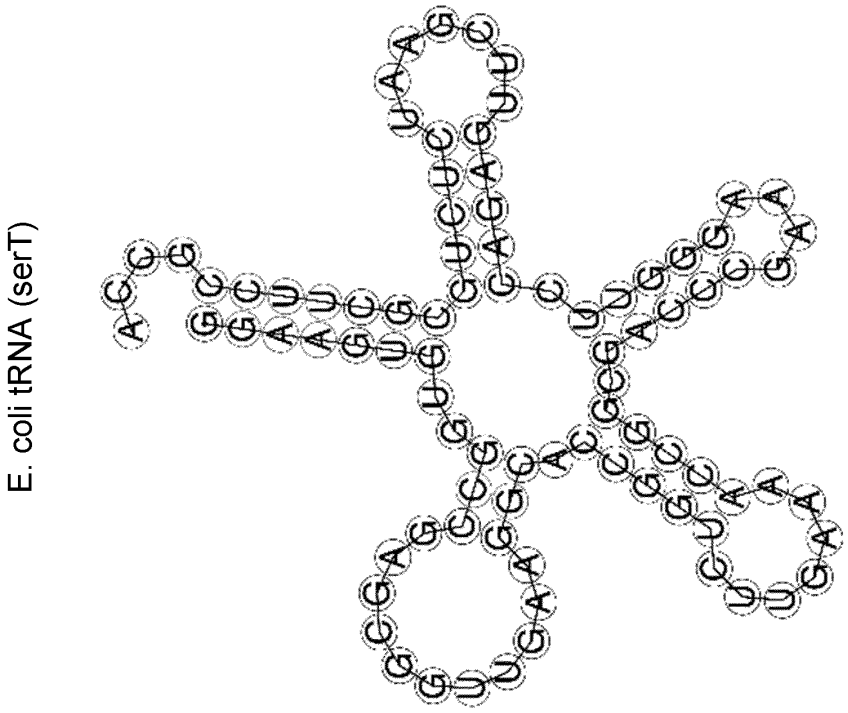
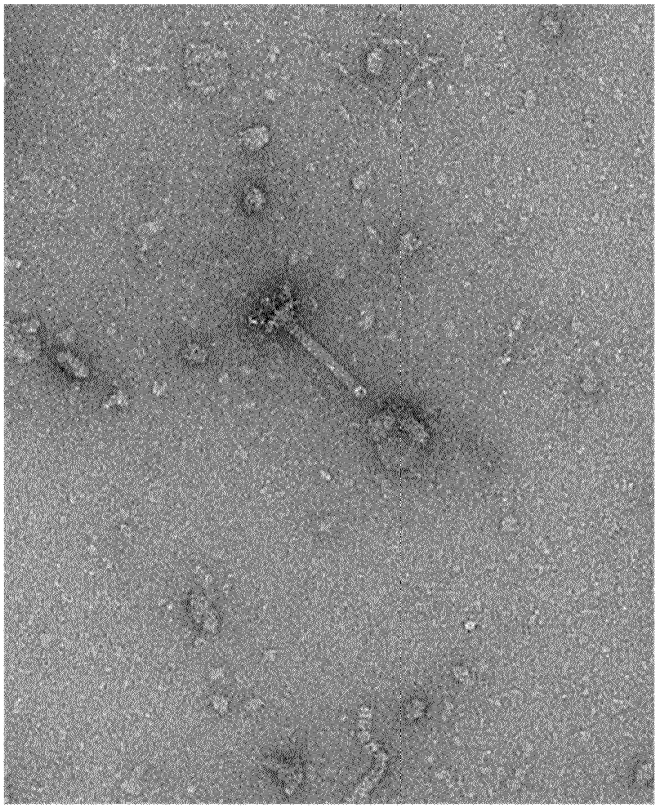


Figure 27 (cont)
C

Φ 06



Φ 12

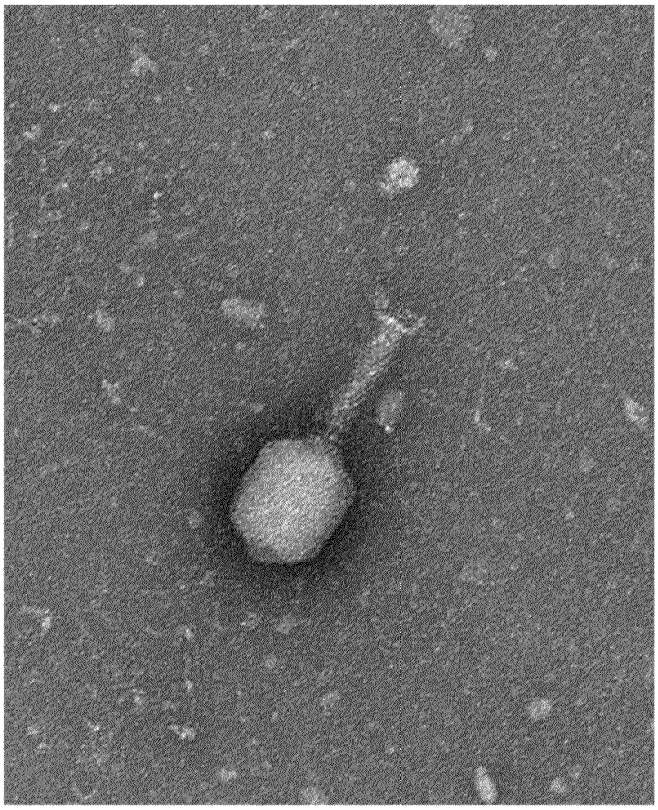


Figure 28

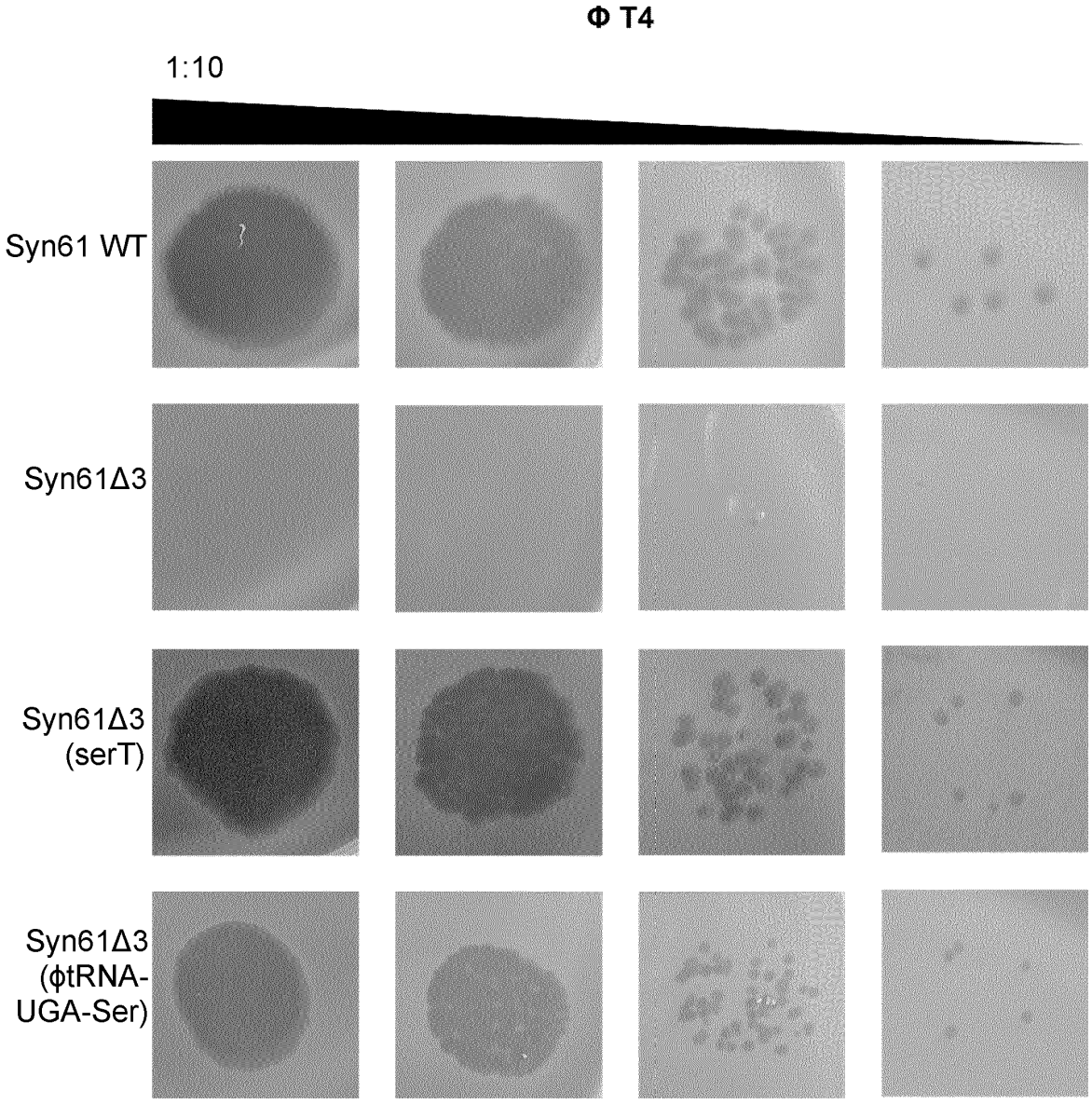


Figure 29

A

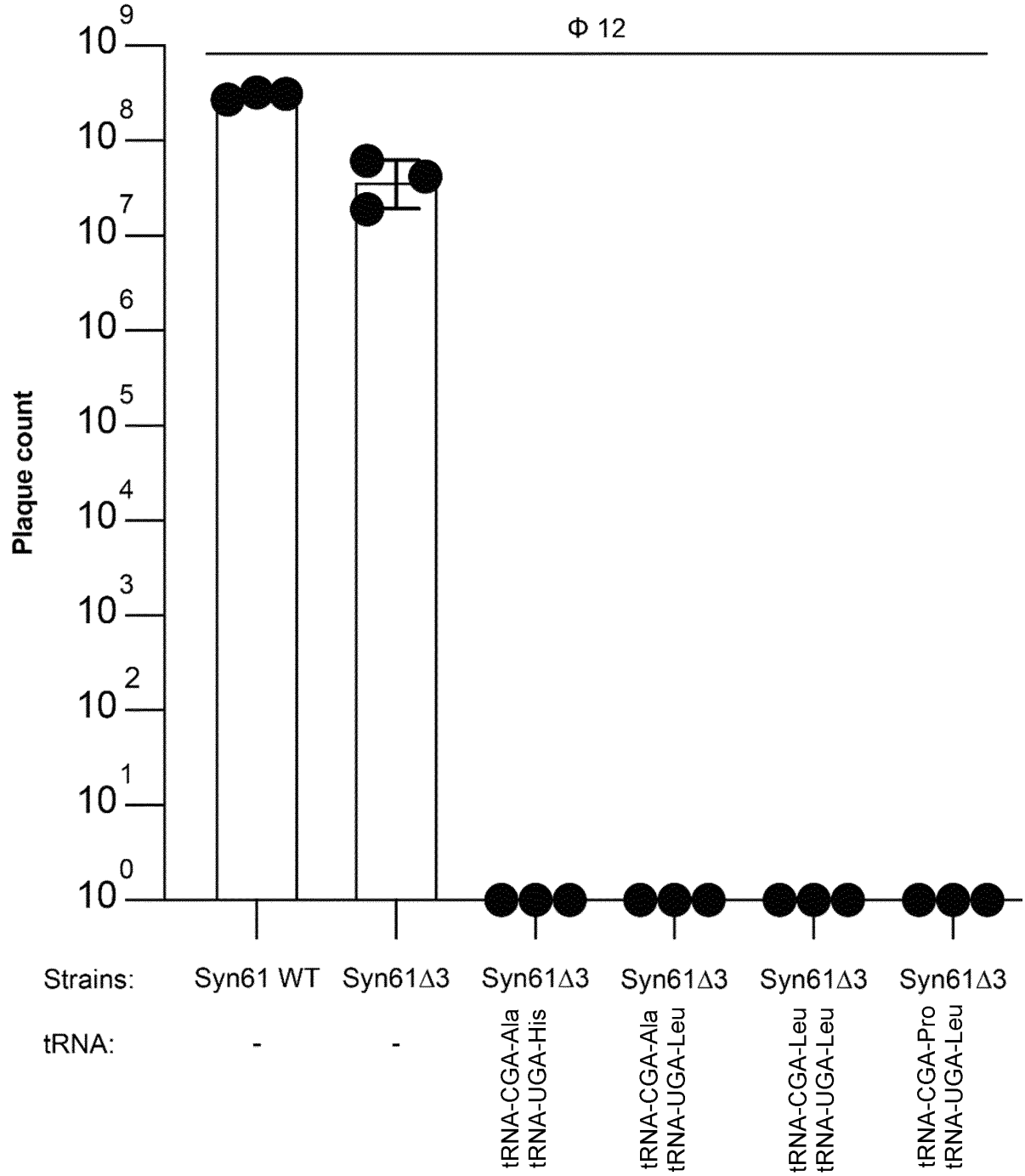

Φ 12
Plaque count
10^9
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0
Strains:
Syn61 WT
Syn61Δ3
Syn61Δ3
Syn61Δ3
Syn61Δ3
Syn61Δ3
tRNA:
-
-
tRNA-CGA-Ala tRNA-UGA-His
tRNA-CGA-Ala tRNA-UGA-Leu
tRNA-CGA-Leu tRNA-UGA-Leu
tRNA-CGA-Pro tRNA-UGA-Leu

Figure 29 (cont)

A (cont)

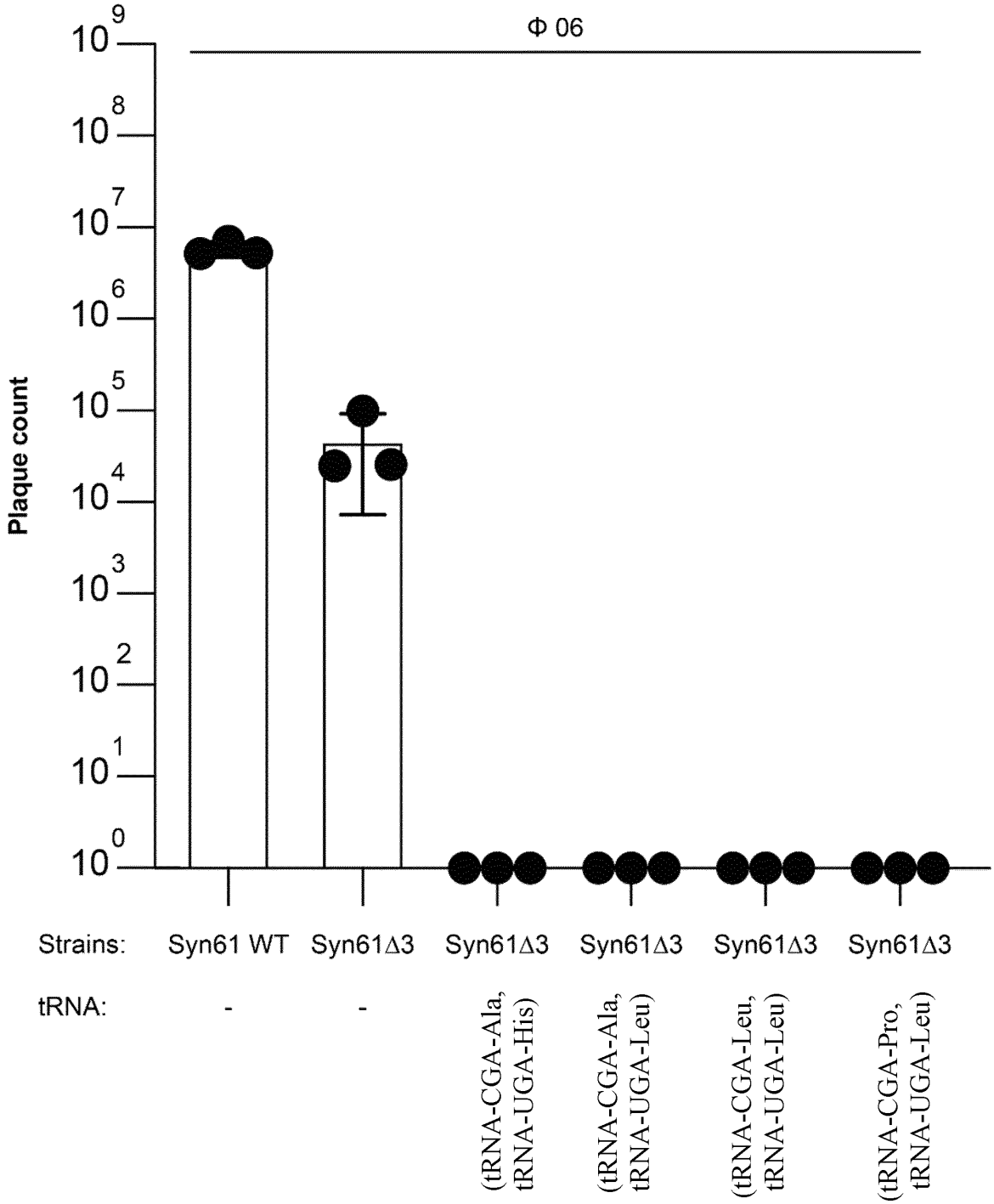


Figure 29 (cont)
B

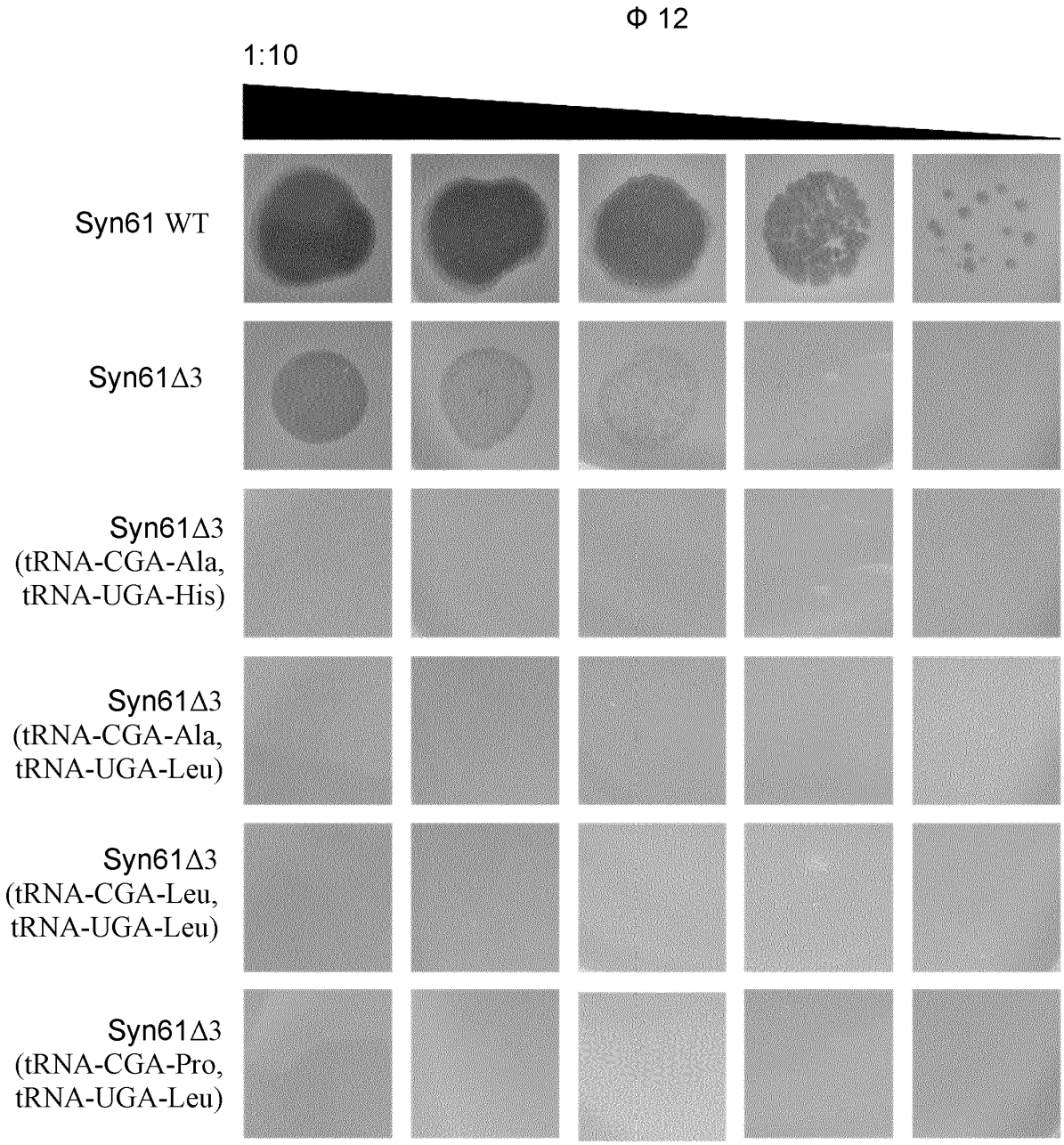


Figure 29 (cont)
B (cont)

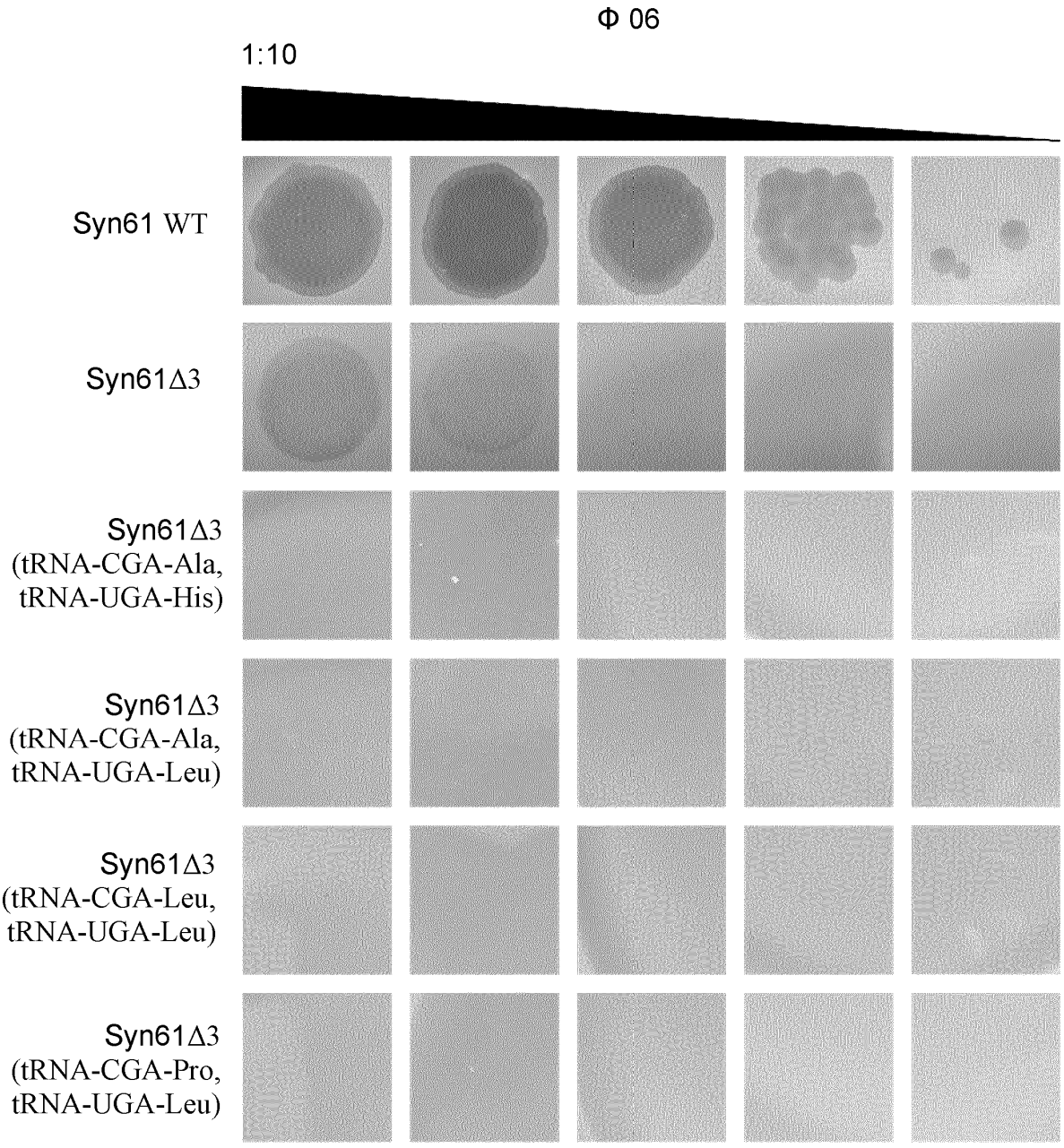


Figure 30

A

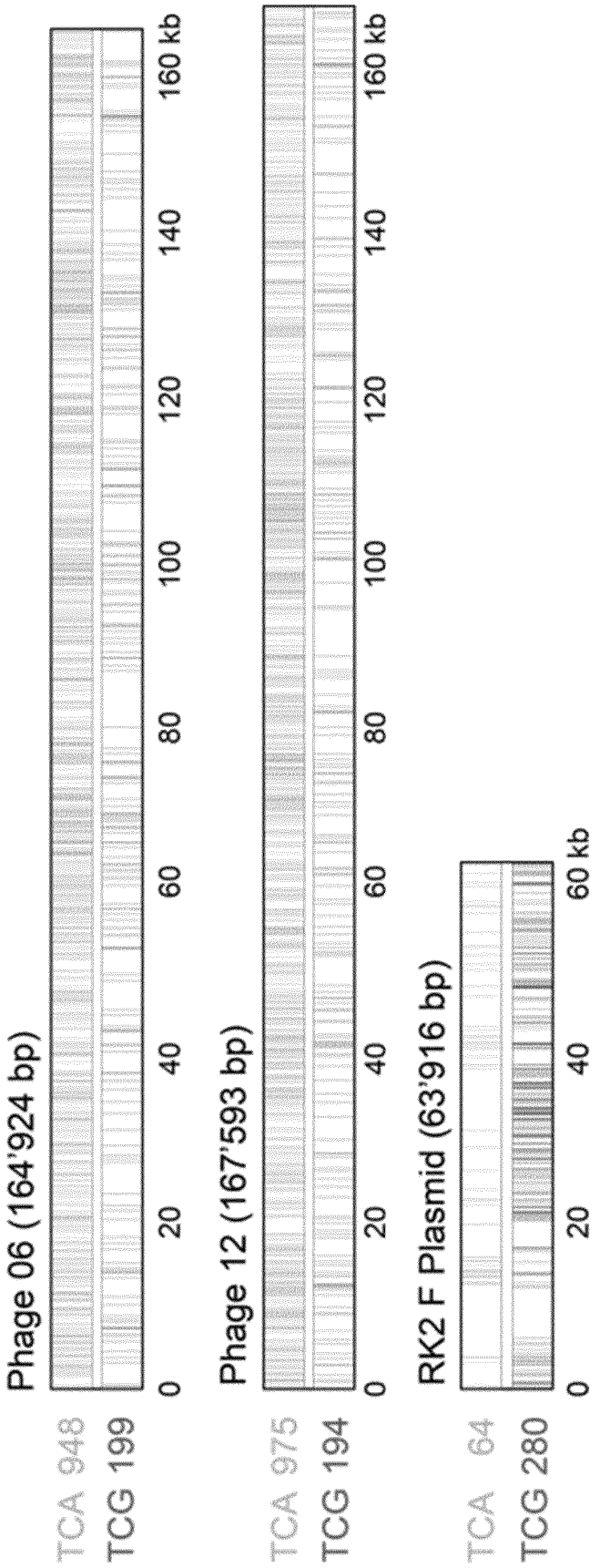

Phage 06 (164'924 bp)
0
20
40
60
80
100
120
140
160 kb
TCA 948
TCG 199
Phage 12 (167'593 bp)
0
20
40
60
80
100
120
140
160 kb
TCA 975
TCG 194
RK2 F Plasmid (63'916 bp)
0
20
40
60 kb
TCA 64
TCG 280

Figure 30 (cont)

B

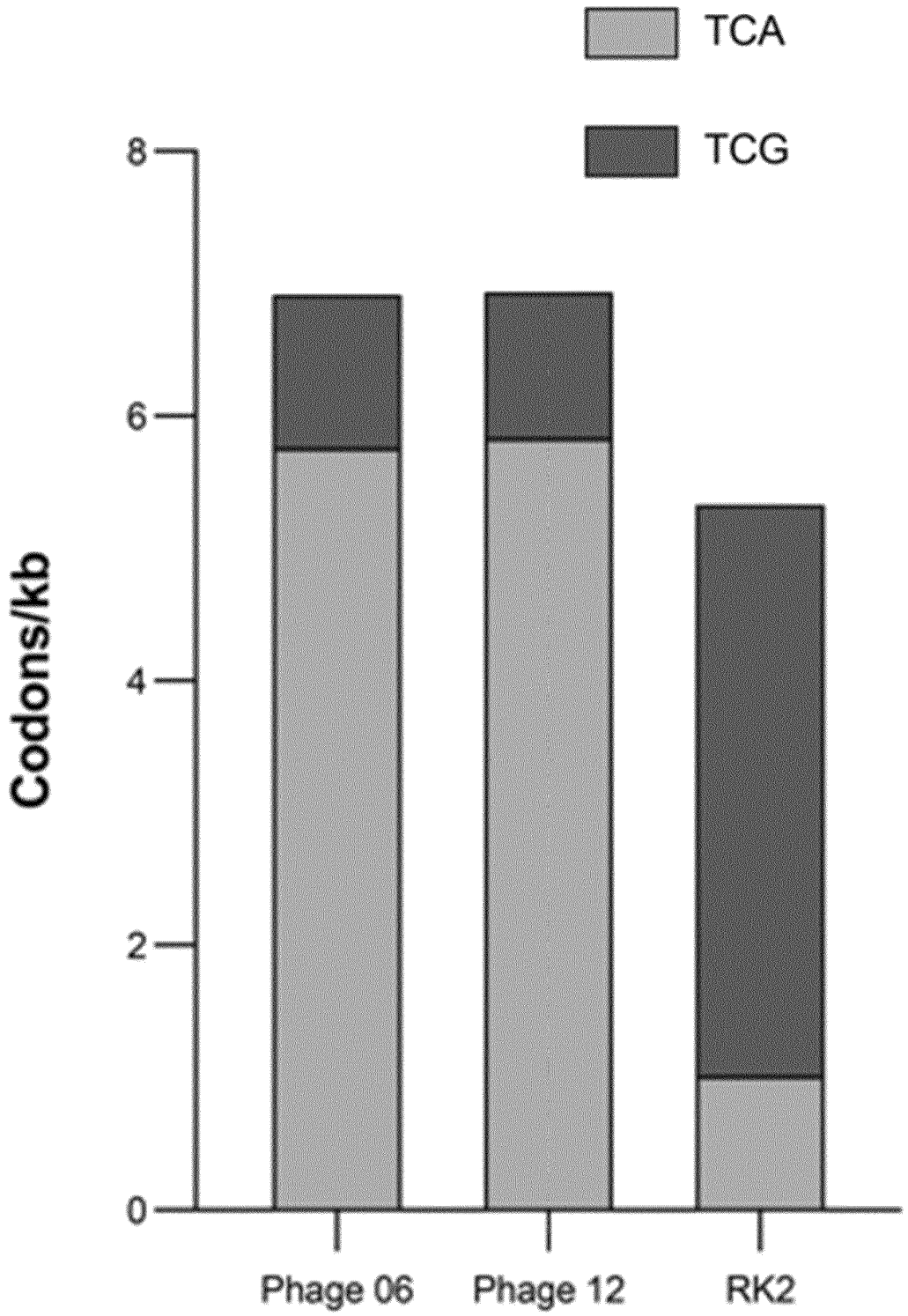


Figure 30 (cont)

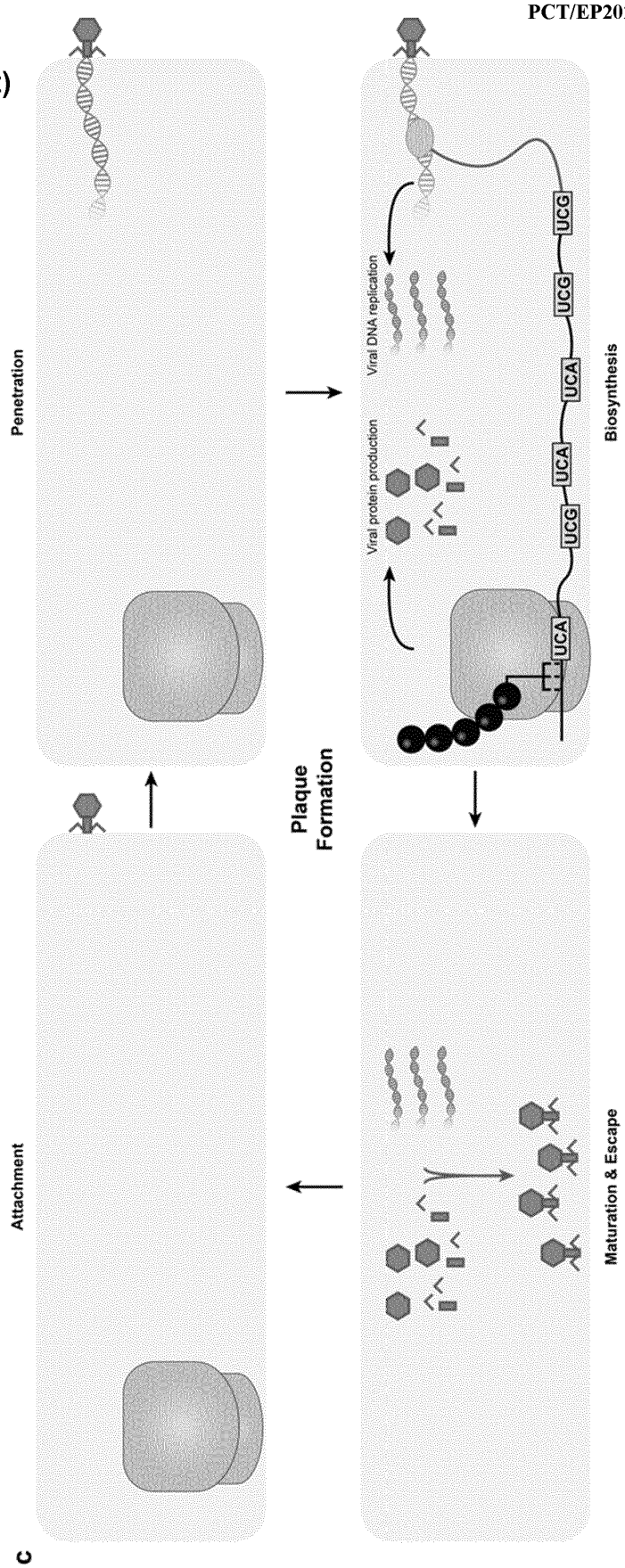


Figure 30 (cont)

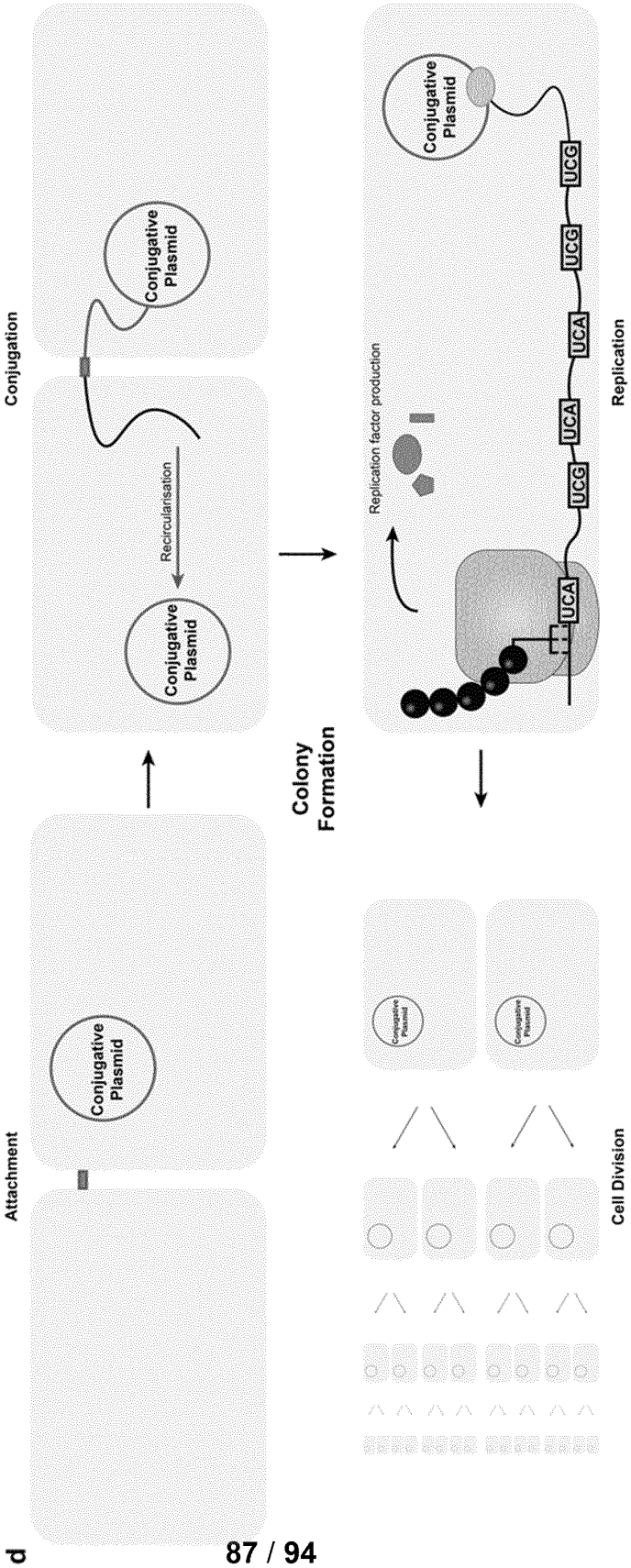


Figure 31

A

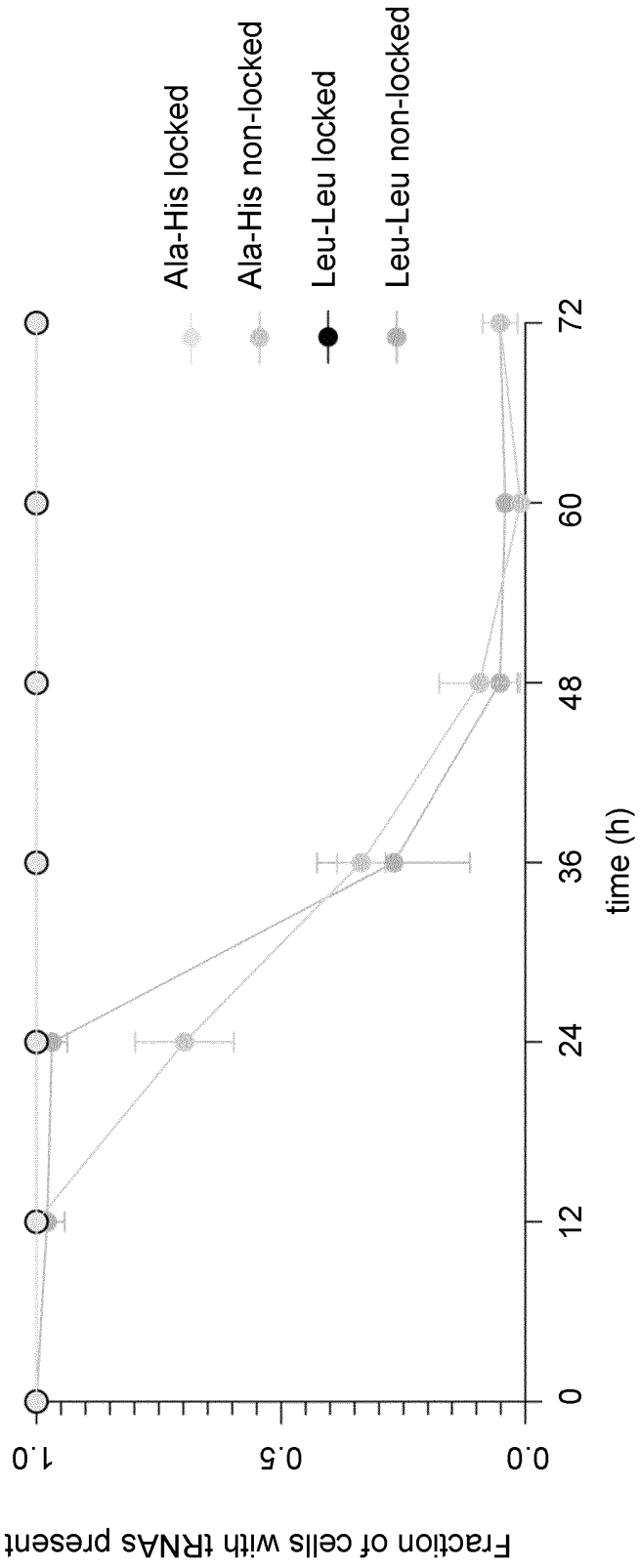

Ala-His locked
Ala-His non-locked
Leu-Leu locked
Leu-Leu non-locked
Fraction of cells with tRNAs present
0.0
0.5
1.0
time (h)
0
12
24
36
48
60
72

Figure 31 (cont)

B

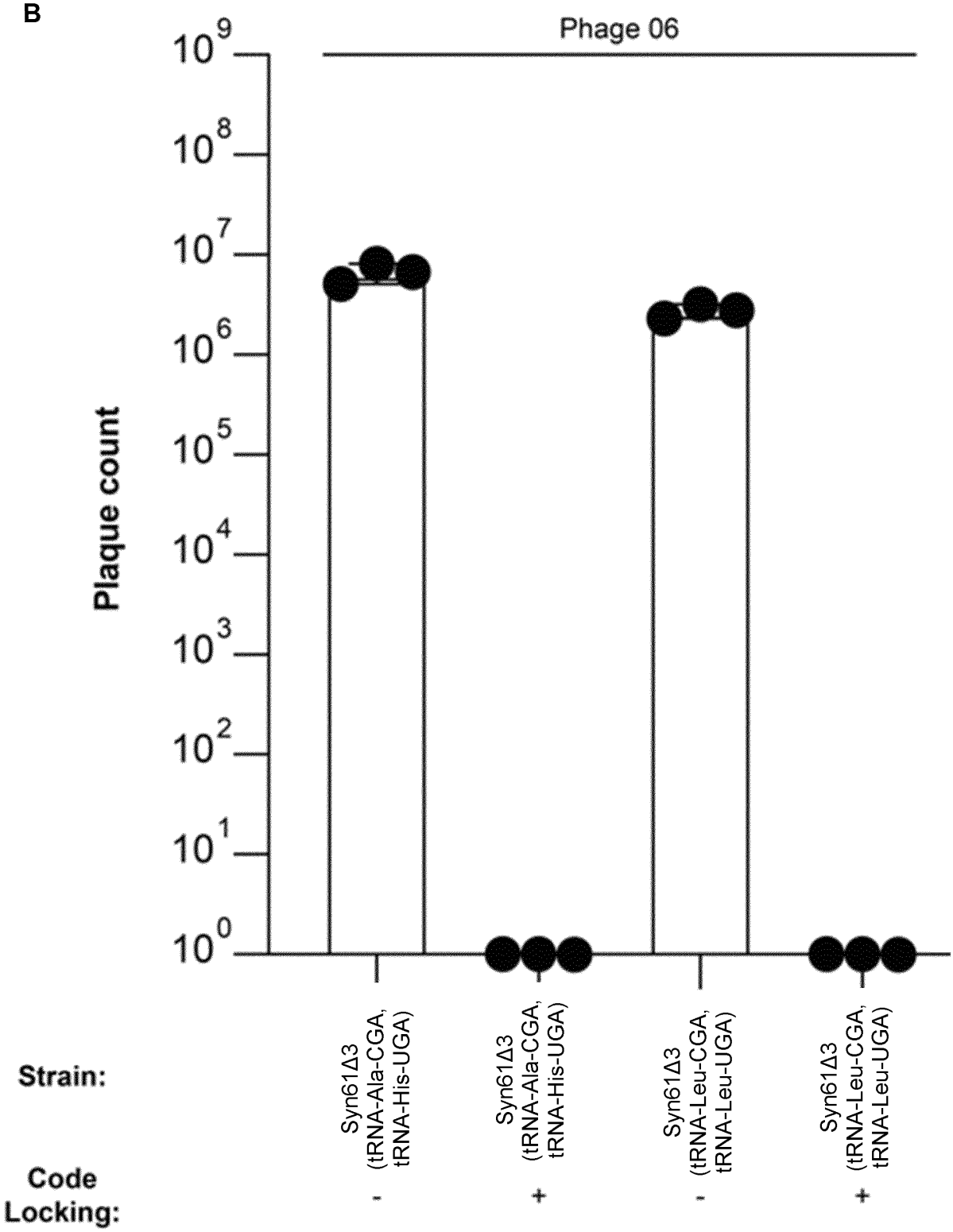


Figure 31 (cont)
B (cont)

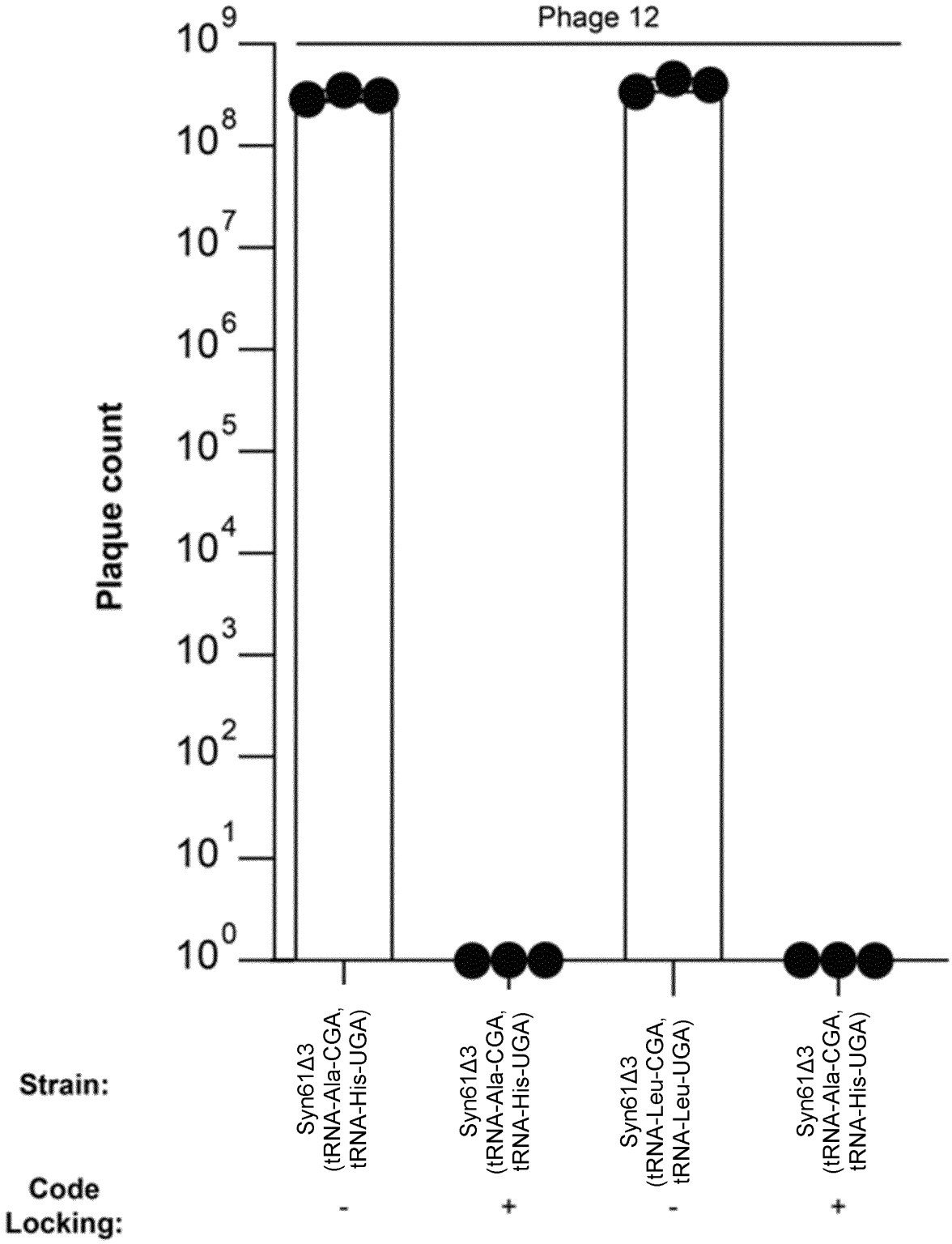


Figure 32

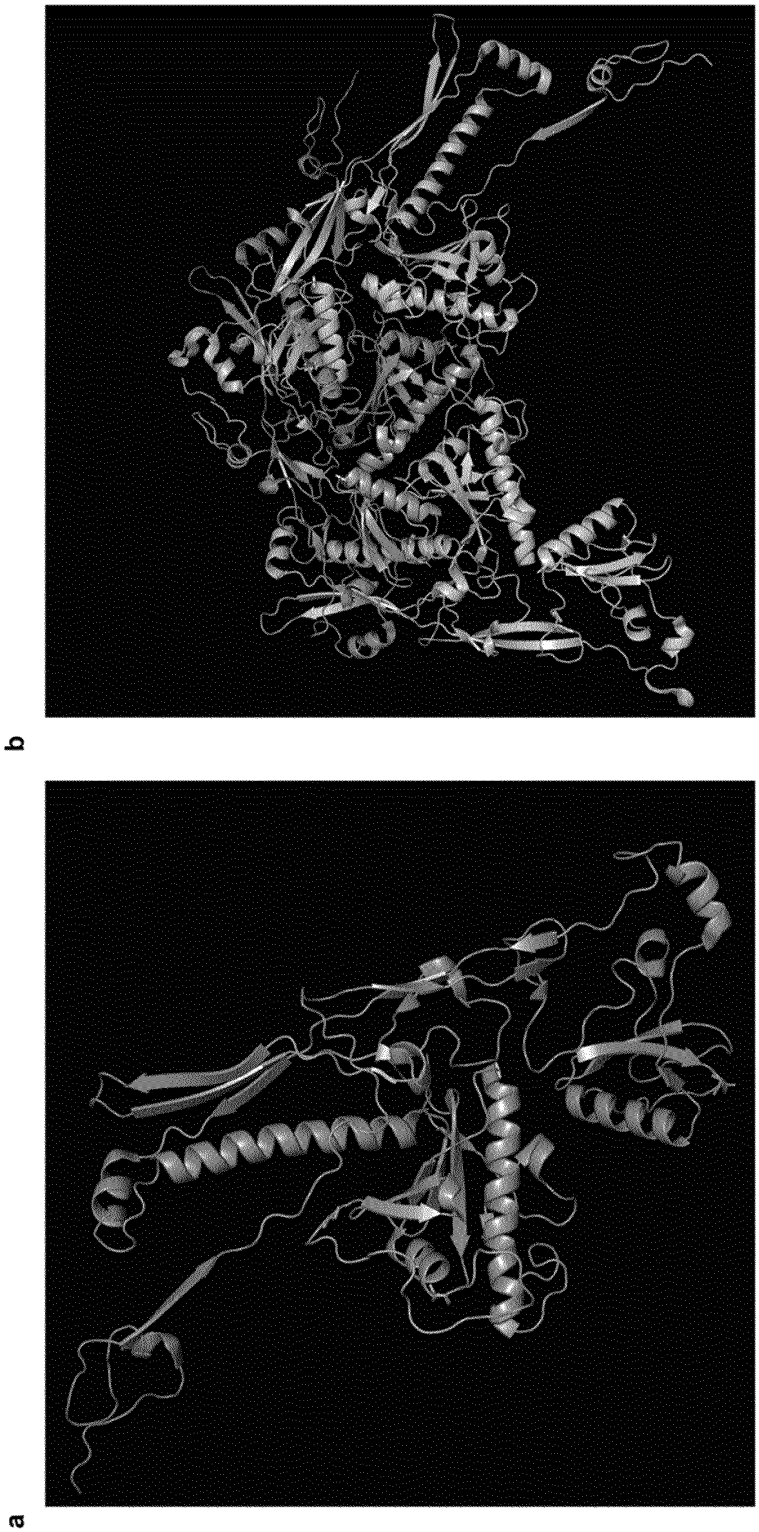


Figure 33

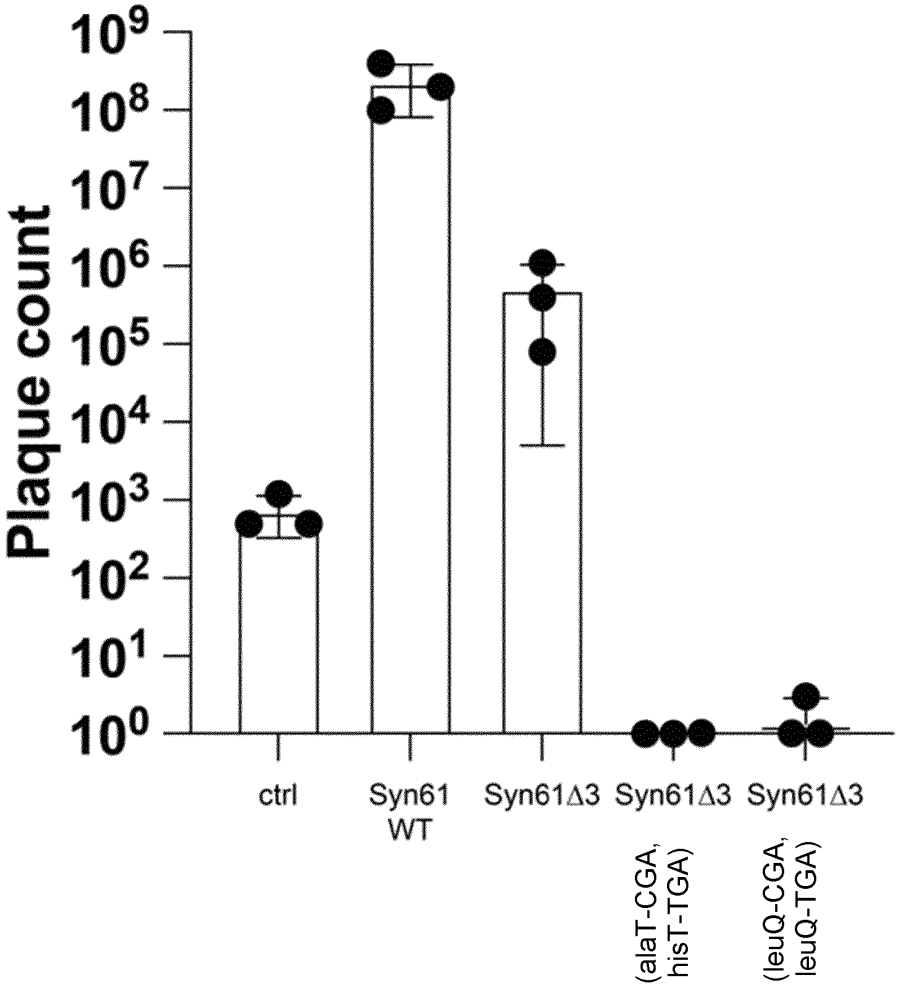

Plaque count
10^9
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0
ctrl
Syn61 WT
Syn61Δ3
Syn61Δ3 (alaT-CGA, hisT-TGA)
Syn61Δ3 (leuQ-CGA, leuQ-TGA)

Figure 34

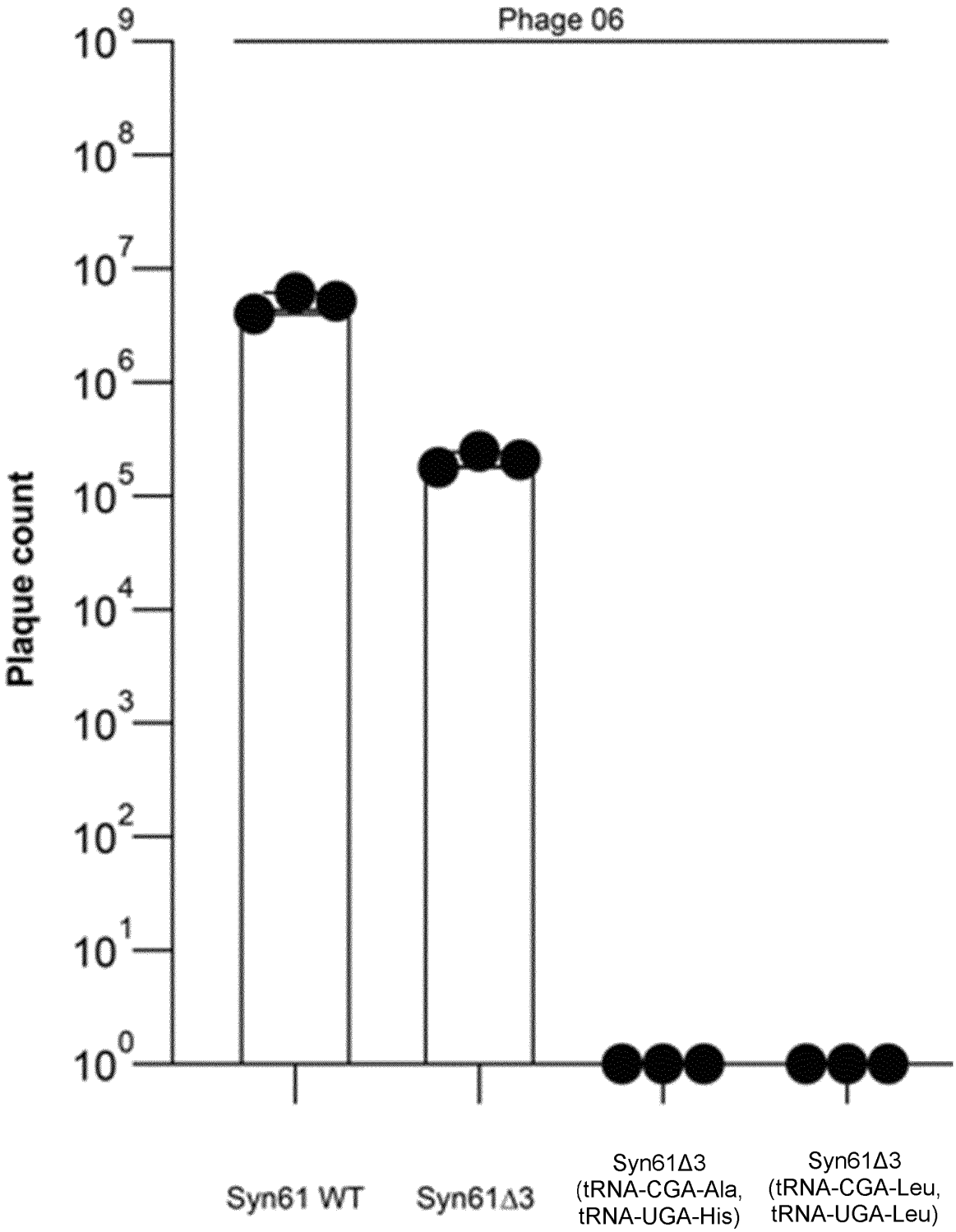

Phage 06
Plaque count
10^9
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0
Syn61 WT
Syn61Δ3
Syn61Δ3 (tRNA-CGA-Ala, tRNA-UGA-His)
Syn61Δ3 (tRNA-CGA-Leu, tRNA-UGA-Leu)

Figure 34 (cont)

