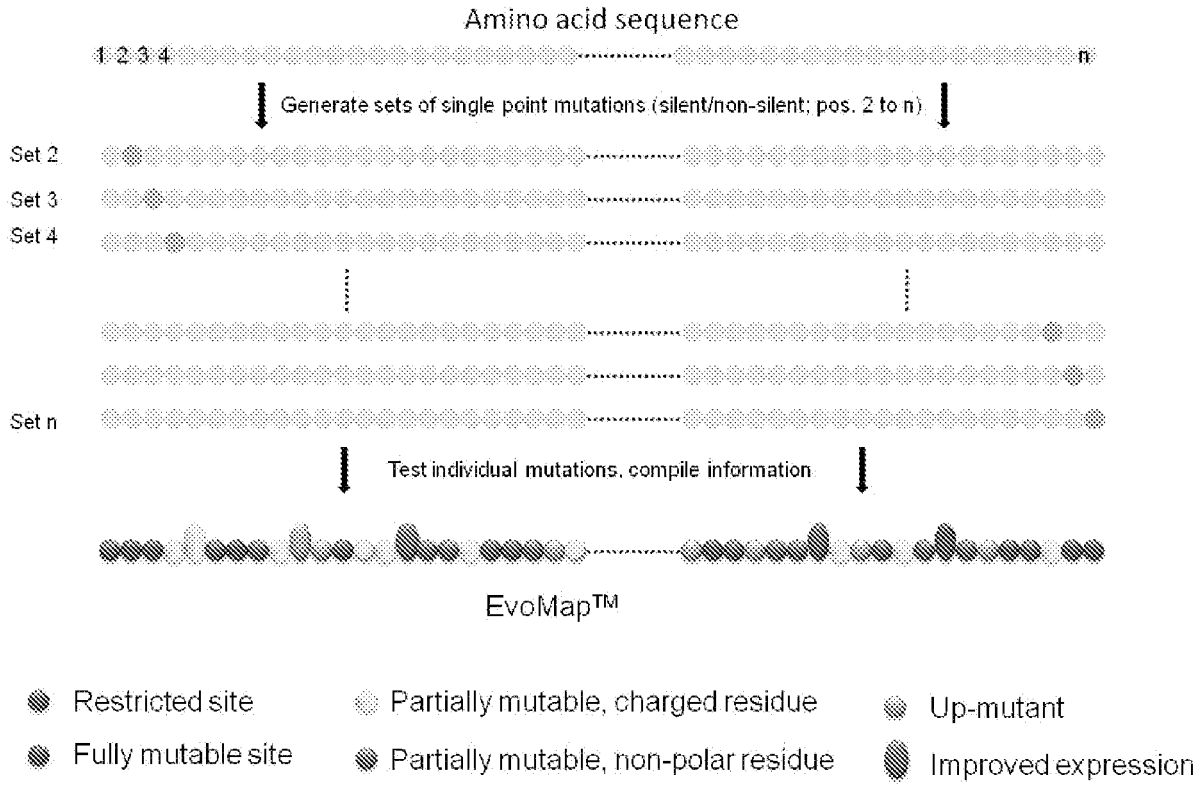

Amino acid sequence
1 2 3 4
n
Generate sets of single point mutations (silent/non-silent; pos. 2 to n)
Set 2
Set 3
Set 4
Set n
Test individual mutations, compile information
EvoMap™
Restricted site
Fully mutable site
Partially mutable, charged residue
Partially mutable, non-polar residue
Up-mutant
Improved expression

FIGURE 1

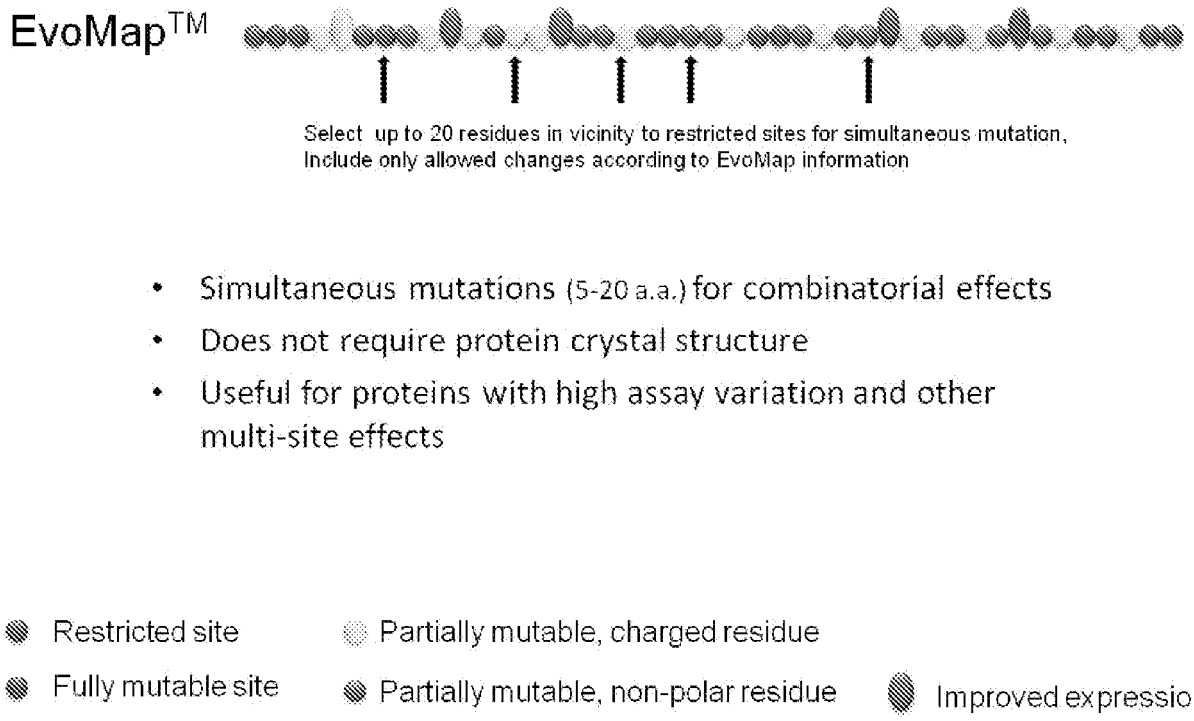

EvoMap™
Select up to 20 residues in vicinity to restricted sites for simultaneous mutation,
Include only allowed changes according to EvoMap information
• Simultaneous mutations (5-20 a.a.) for combinatorial effects
• Does not require protein crystal structure
• Useful for proteins with high assay variation and other multi-site effects
Restricted site
Fully mutable site
Partially mutable, charged residue
Partially mutable, non-polar residue
Improved expression

FIGURE 2

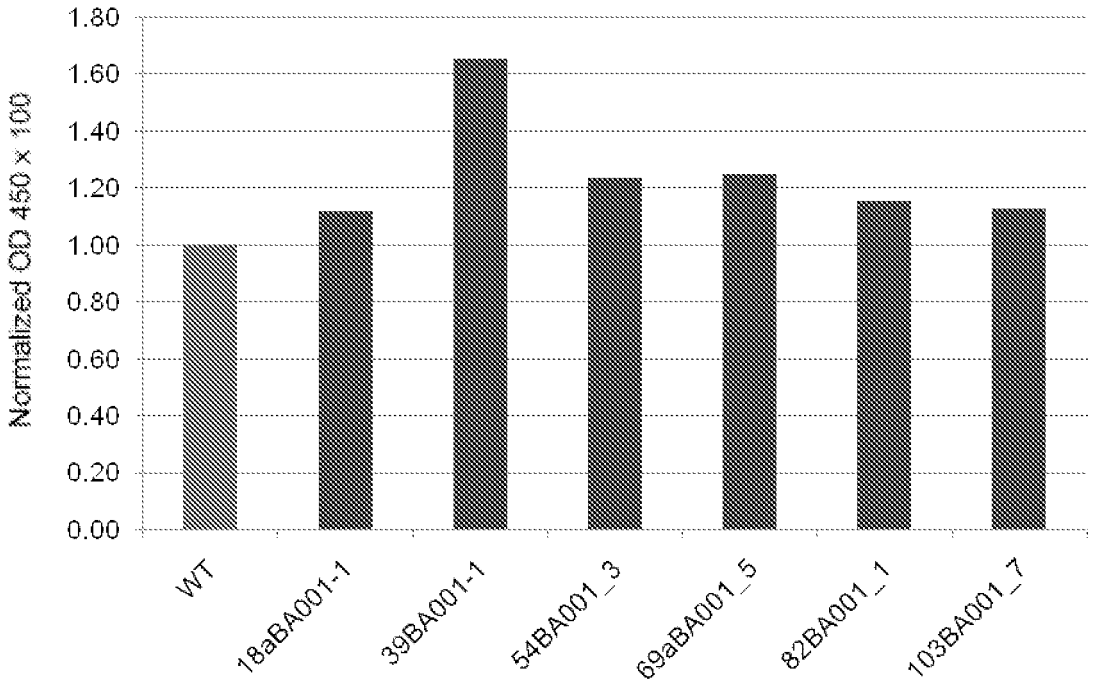

Normalized OD 450 x 100
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
WT
18aBA001-1
39BA001-1
54BA001_3
69aBA001_5
82BA001_1
103BA001_7

FIGURE 3

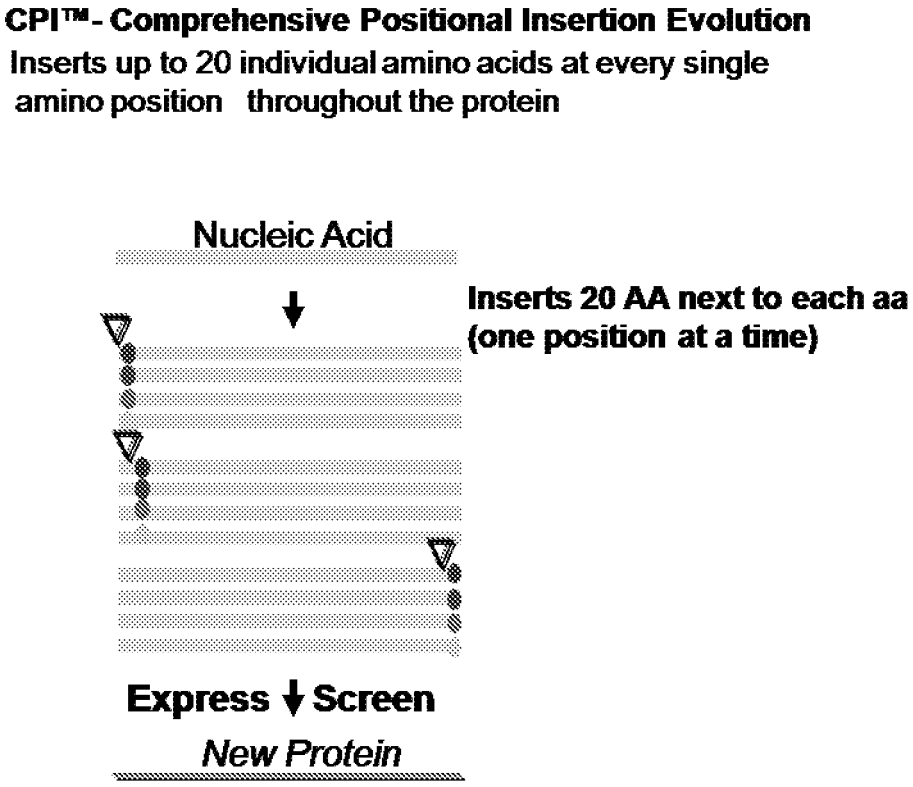

CPI™- Comprehensive Positional Insertion Evolution
Inserts up to 20 individual amino acids at every single amino position throughout the protein
Nucleic Acid
Inserts 20 AA next to each aa (one position at a time)
Express
Screen
New Protein

FIGURE 4

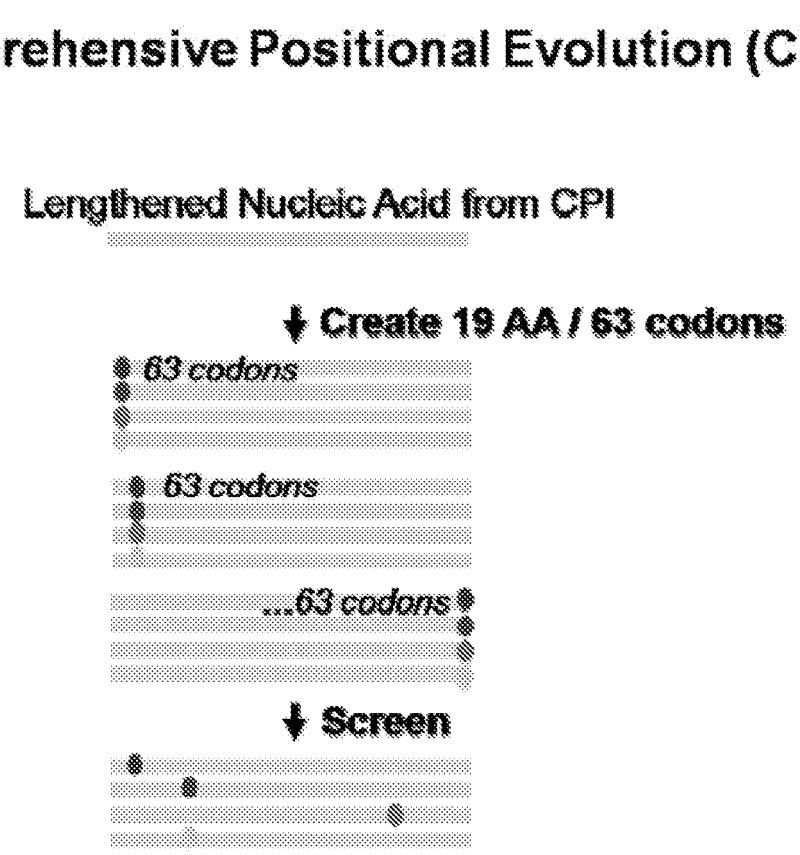

Comprehensive Positional Evolution (CPE™)
Lengthened Nucleic Acid from CPI
Create 19 AA / 63 codons
63 codons
63 codons
...63 codons
Screen

FIGURE 5

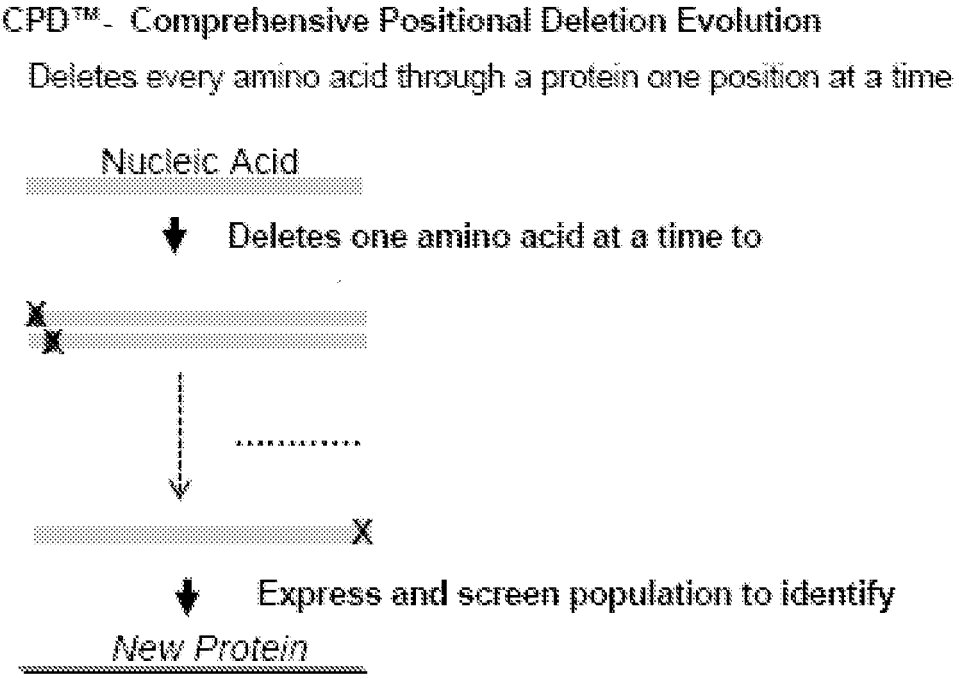

CPD™- Comprehensive Positional Deletion Evolution
Deletes every amino acid through a protein one position at a time
Nucleic Acid
Deletes one amino acid at a time to
Express and screen population to identify
New Protein

FIGURE 6

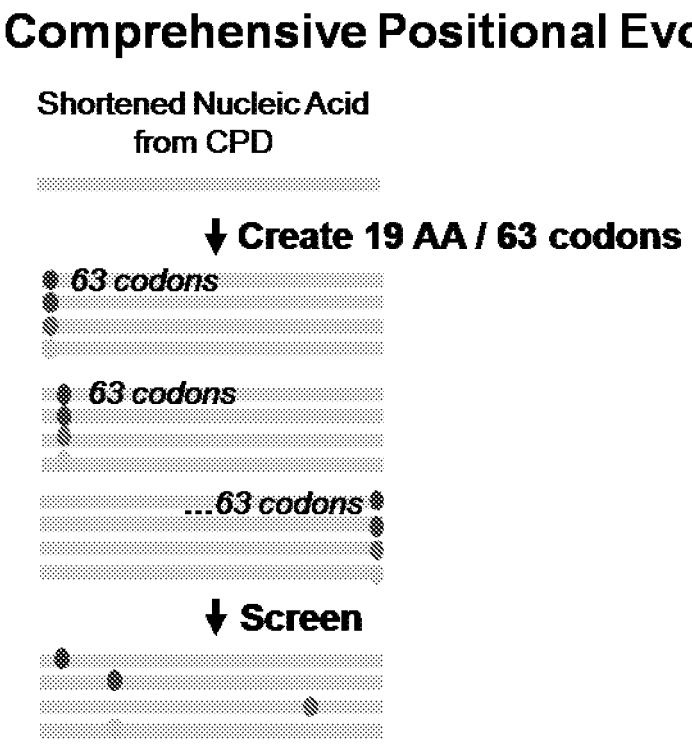

Comprehensive Positional Evolution (CPE™)
Shortened Nucleic Acid from CPD
Create 19 AA / 63 codons
63 codons
63 codons
...63 codons
Screen
Optimized Gene/Protein

FIGURE 7

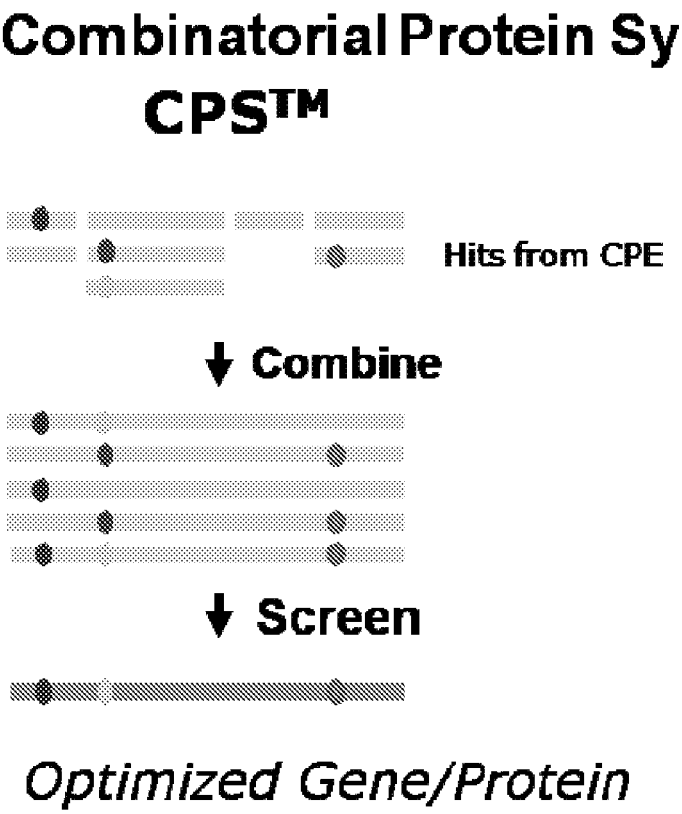

Combinatorial Protein Synthesis
CPS™
Hits from CPE
Combine
Screen
Optimized Gene/Protein

FIGURE 8

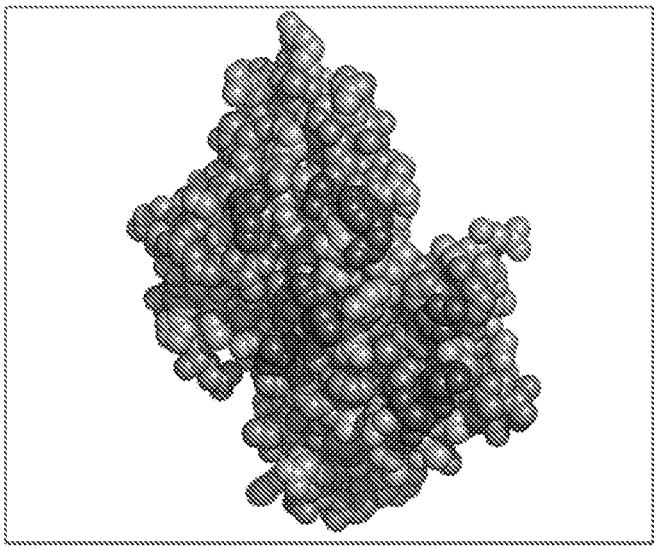

Comprehensive Positional Evolution Map
(Antibody LC/HC variable domains)
Up-mutants
Non-mutable sites
Partially mutable sites
Fully mutable sites

FIGURE 9

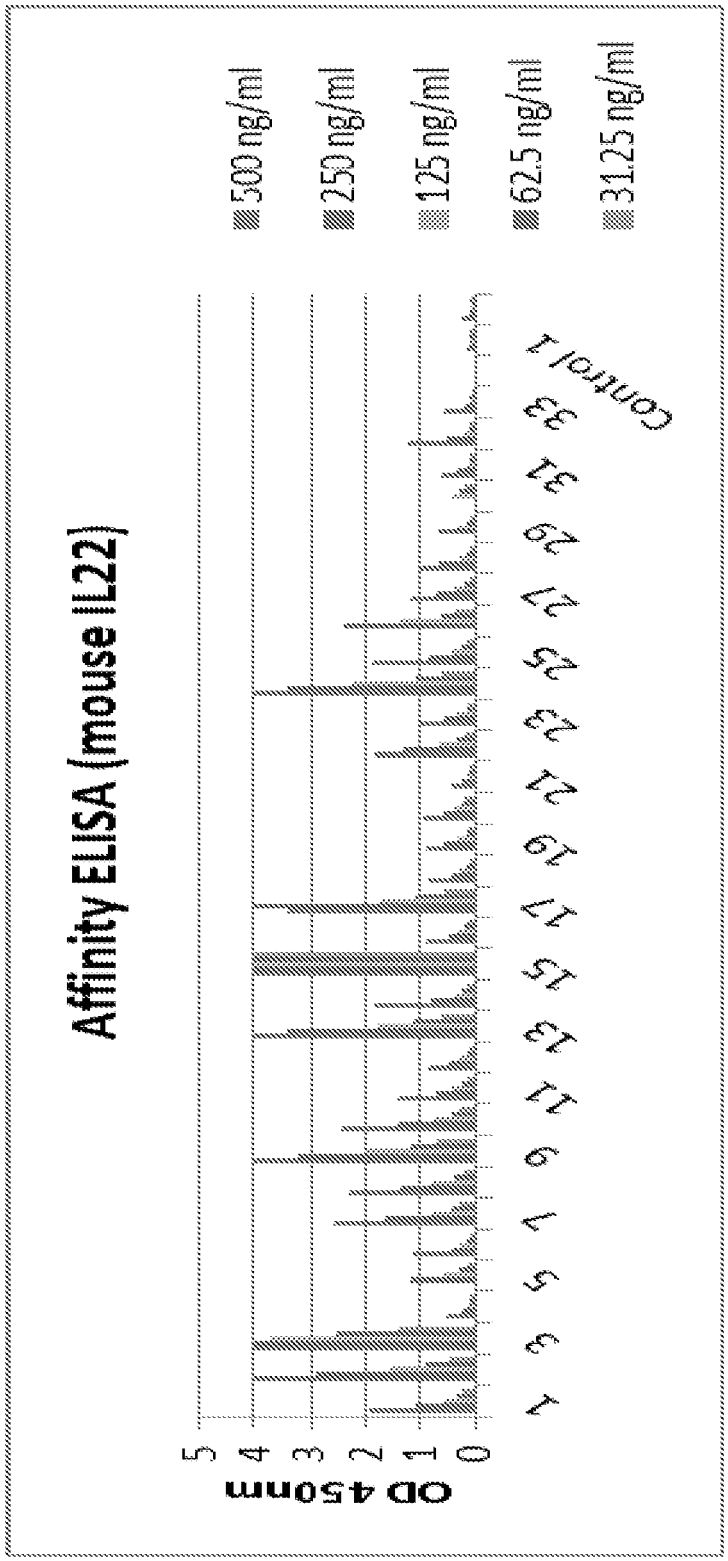

Affinity ELISA (mouse IL22)
OD 450nm
0
1
2
3
4
5
500 ng/ml
250 ng/ml
125 ng/ml
62.5 ng/ml
31.25 ng/ml
Control 1
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33

Figure 10

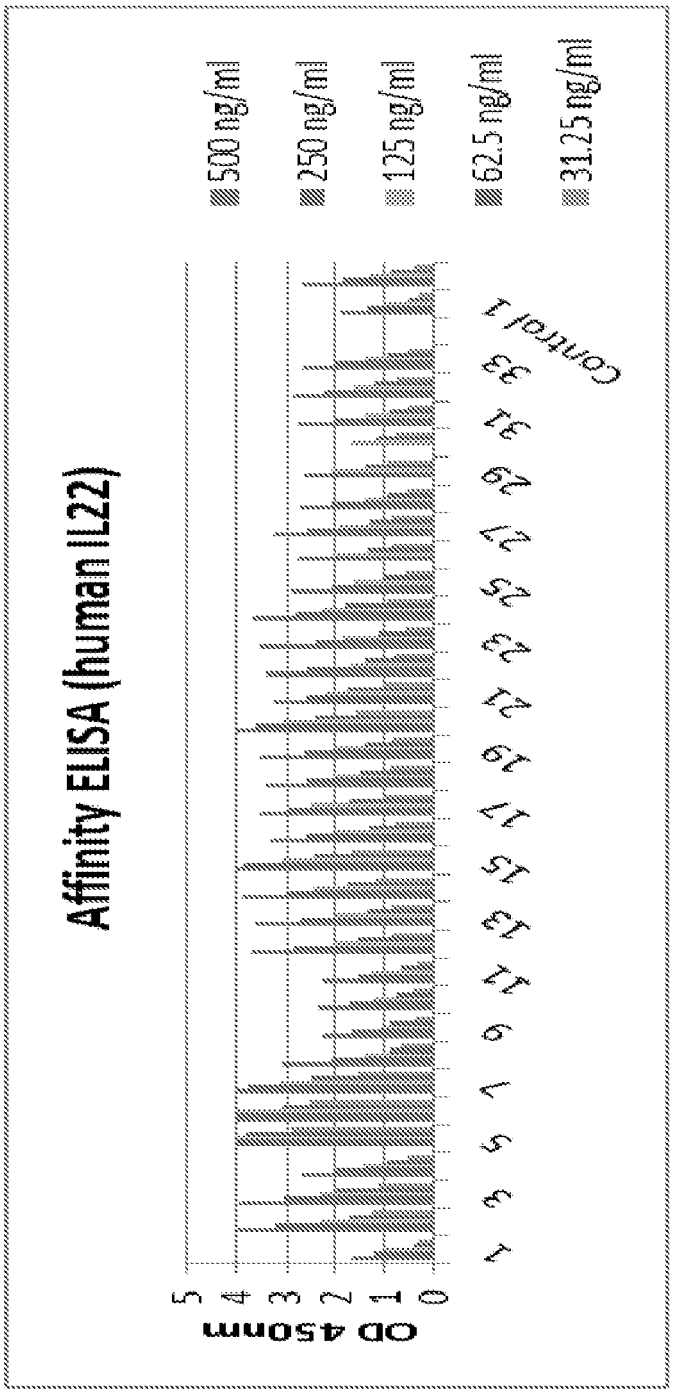

Affinity ELISA (human IL22)
OD 450nm
0
1
2
3
4
5
500 ng/ml
250 ng/ml
125 ng/ml
62.5 ng/ml
31.25 ng/ml
Control 1

Figure 11

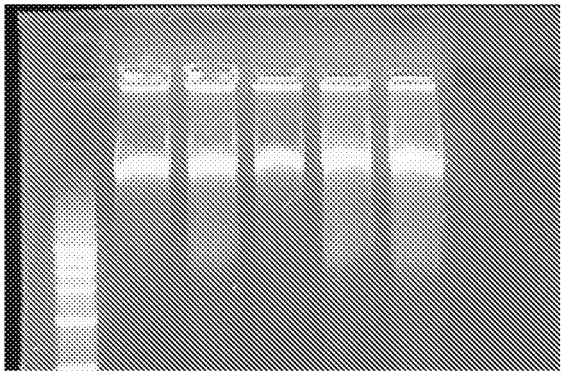


Figure 12