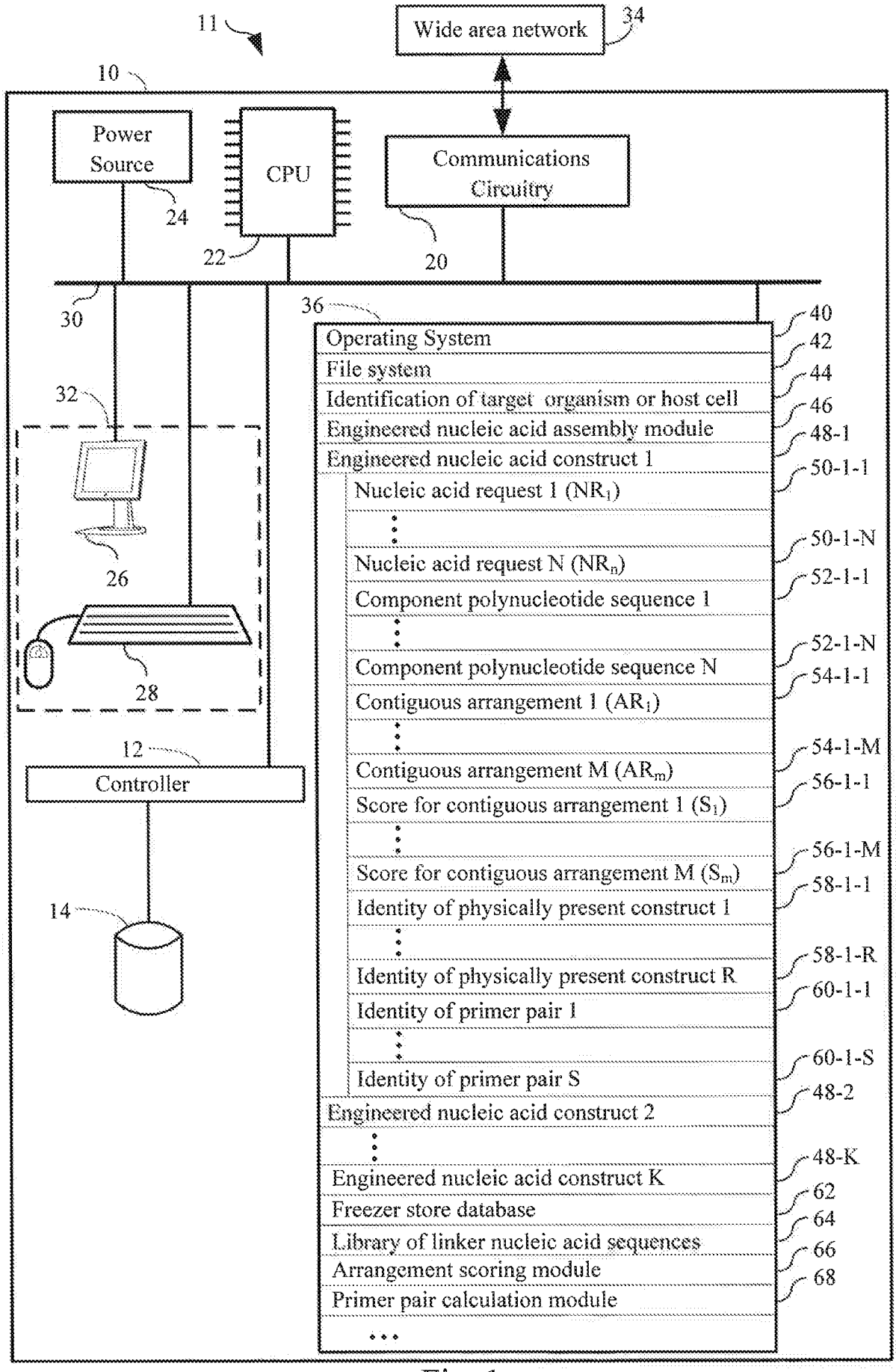

11
Wide area network
34
10
Power Source
24
CPU
22
Communications Circuitry
20
30
36
32
26
28
12
Controller
14
Operating System
File system
Identification of target organism or host cell
Engineered nucleic acid assembly module
Engineered nucleic acid construct 1
Nucleic acid request 1 (NR1)
Nucleic acid request N (NRn)
Component polynucleotide sequence 1
Component polynucleotide sequence N
Contiguous arrangement 1 (AR1)
Contiguous arrangement M (ARm)
Score for contiguous arrangement 1 (S1)
Score for contiguous arrangement M (Sm)
Identity of physically present construct 1
Identity of physically present construct R
Identity of primer pair 1
Identity of primer pair S
Engineered nucleic acid construct 2
Engineered nucleic acid construct K
Freezer store database
Library of linker nucleic acid sequences
Arrangement scoring module
Primer pair calculation module
40
42
44
46
48-1
50-1-1
50-1-N
52-1-1
52-1-N
54-1-1
54-1-M
56-1-1
56-1-M
58-1-1
58-1-R
60-1-1
60-1-S
48-2
48-K
62
64
66
68

Fig. 1

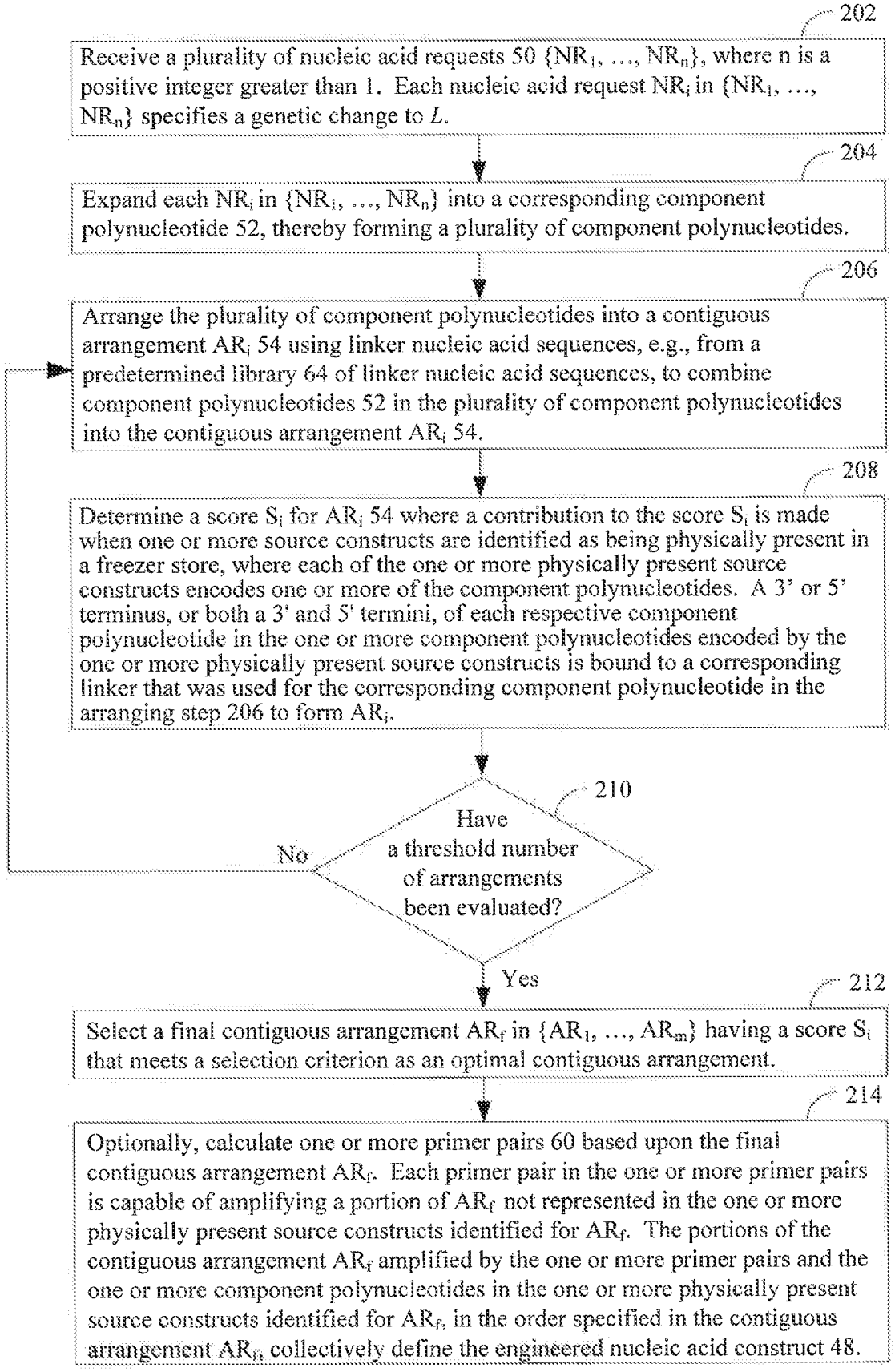

202
Receive a plurality of nucleic acid requests 50 {NR1, ..., NRn}, where n is a positive integer greater than 1. Each nucleic acid request NRi in {NR1, ..., NRn} specifies a genetic change to L.
204
Expand each NRi in {NR1, ..., NRn} into a corresponding component polynucleotide 52, thereby forming a plurality of component polynucleotides.
206
Arrange the plurality of component polynucleotides into a contiguous arrangement ARj 54 using linker nucleic acid sequences, e.g., from a predetermined library 64 of linker nucleic acid sequences, to combine component polynucleotides 52 in the plurality of component polynucleotides into the contiguous arrangement ARj 54.
208
Determine a score Sj for ARj 54 where a contribution to the score Sj is made when one or more source constructs are identified as being physically present in a freezer store, where each of the one or more physically present source constructs encodes one or more of the component polynucleotides. A 3' or 5' terminus, or both a 3' and 5' termini, of each respective component polynucleotide in the one or more component polynucleotides encoded by the one or more physically present source constructs is bound to a corresponding linker that was used for the corresponding component polynucleotide in the arranging step 206 to form ARj.
210
Have a threshold number of arrangements been evaluated?
No
Yes
212
Select a final contiguous arrangement ARf in {AR1, ..., ARm} having a score Si that meets a selection criterion as an optimal contiguous arrangement.
214
Optionally, calculate one or more primer pairs 60 based upon the final contiguous arrangement ARf. Each primer pair in the one or more primer pairs is capable of amplifying a portion of ARf not represented in the one or more physically present source constructs identified for ARf. The portions of the contiguous arrangement ARf amplified by the one or more primer pairs and the one or more component polynucleotides in the one or more physically present source constructs identified for ARf, in the order specified in the contiguous arrangement ARf, collectively define the engineered nucleic acid construct 48.

Fig. 2

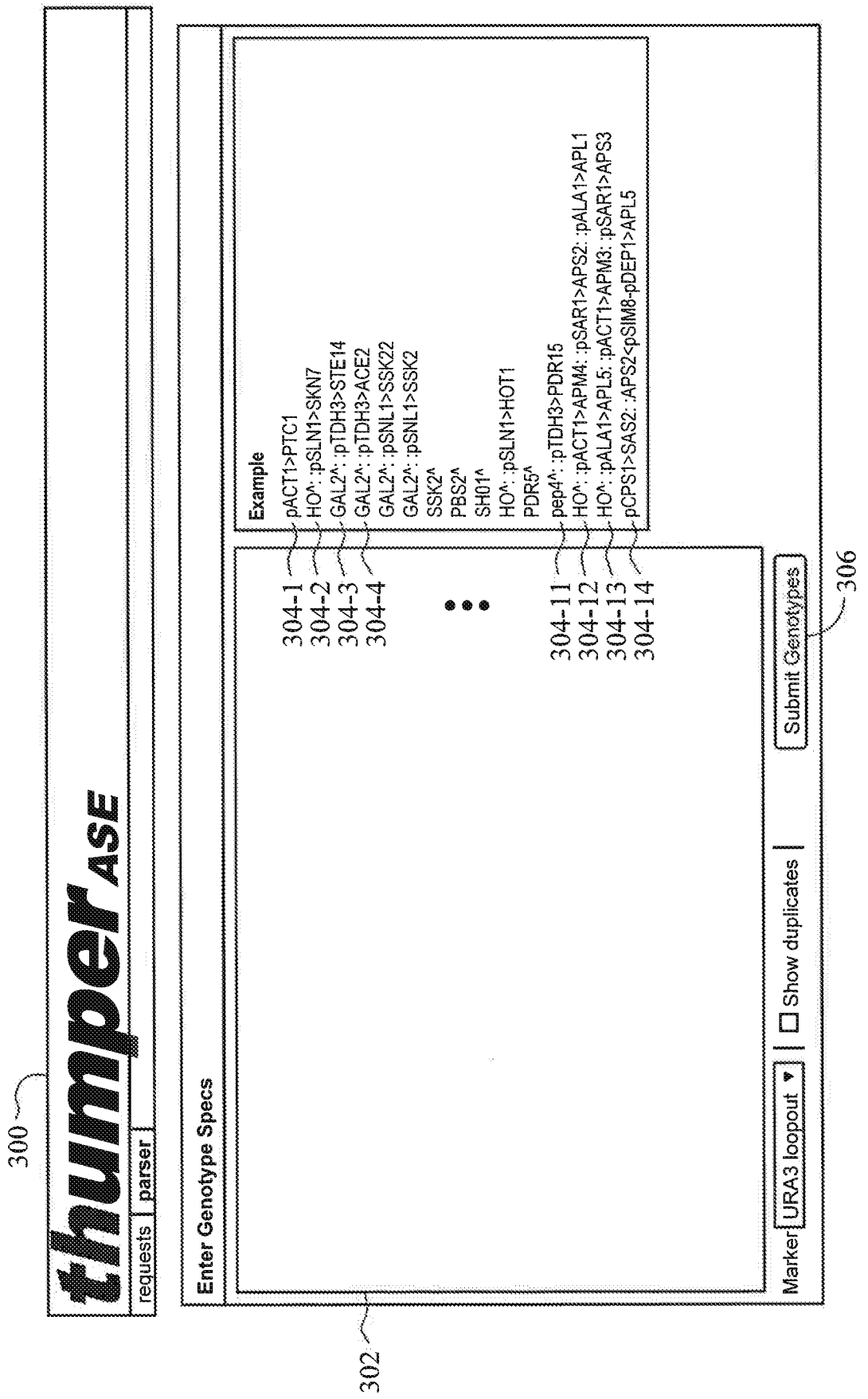

300
thumperASE
requests
parser
Enter Genotype Specs
Example
pACT1>PTC1
HO^: :pSLN1>SKN7
GAL2^: :pTDH3>STE14
GAL2^: :pTDH3>ACE2
GAL2^: :pSNL1>SSK22
GAL2^: :pSNL1>SSK2
SSK2^
PBS2^
SH01^
HO^: :pSLN1>HOT1
PDR5^
pep4^: :pTDH3>PDR15
HO^: :pACT1>APM4: :pSAR1>APS2: :pALA1>APL1
HO^: :pALA1>APL5: :pACT1>APM3: :pSAR1>APS3
pCPS1>SAS2: :APS2<pSIM8-pDEP1>APL5
304-1
304-2
304-3
304-4
304-11
304-12
304-13
304-14
Marker
URA3 loopout
Show duplicates
Submit Genotypes
306
302

Fig. 3

Component polynucleotides for the exemplary plurality of nucleic acid requests: HO^::pFBA>ADH2::pSLN1>ADH1

5' – LA_1 – pFBA1 – LB_1 – 3'

5' – LA_2 – ADH2– LB_2 – 3'

5' – LA_3 – pSLN1– LB_3 – 3'

5' – LA_4 – ADH1– LB_4 – 3'

Fig. 4A

5' – LA_1 – pFBA1 – LB_1 – ADH2– LB_2 – pSLN1– LB_3 – ADH1– LB_4 – 3'

5' – LA_1 – pFBA1 – LB_1 – ADH2– LB_2 – pSLN1– LB_3 – 3'

5' – LA_2 – ADH2– LB_2 – pSLN1– LB_3 – ADH1– LB_4 – 3'

5' – LA_1 – pFBA1 – LB_1 – ADH2– LB_2 – 3'

5' – LA_3 – pSLN1– LB_3 – ADH1– LB_4 – 3'

5'– LA_2 – ADH2– LB_2 – pSLN1– LB_3 – 3'

Fig. 4B

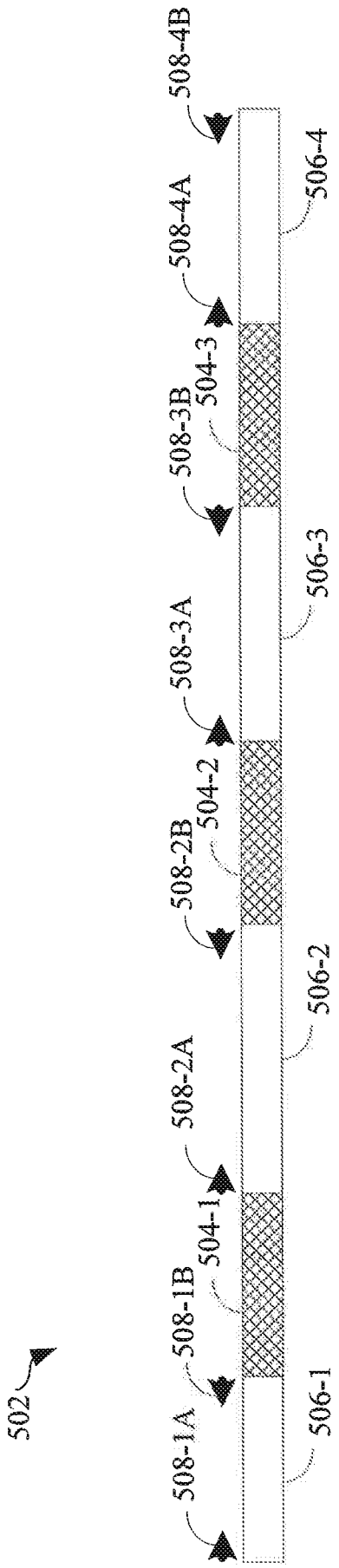

502
508-1A
508-1B
504-1
508-2A
508-2B
504-2
508-3A
508-3B
504-3
508-4A
508-4B
506-1
506-2
506-3
506-4

Fig. 5

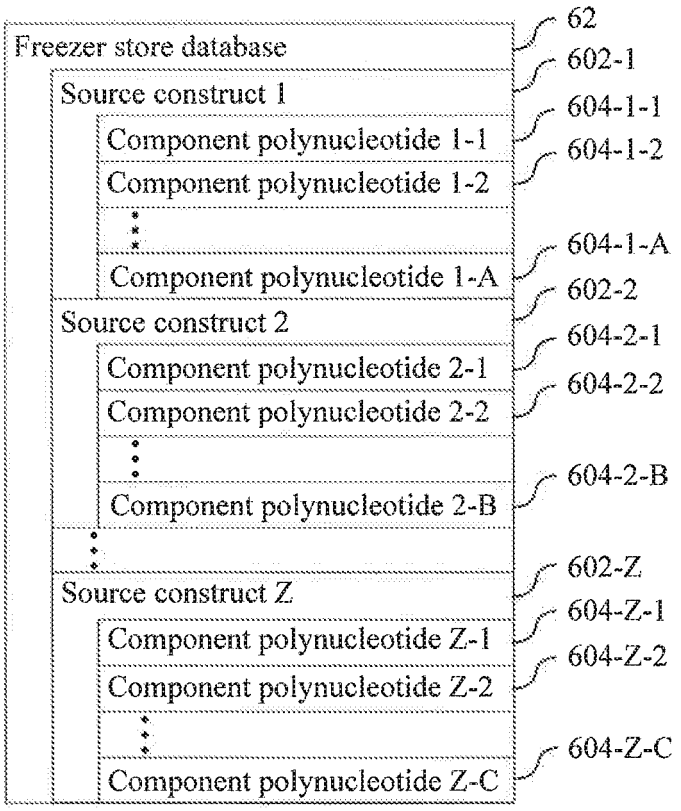

Freezer store database
62
602-1
Source construct 1
604-1-1
Component polynucleotide 1-1
604-1-2
Component polynucleotide 1-2
604-1-A
Component polynucleotide 1-A
602-2
Source construct 2
604-2-1
Component polynucleotide 2-1
604-2-2
Component polynucleotide 2-2
604-2-B
Component polynucleotide 2-B
602-Z
Source construct Z
604-Z-1
Component polynucleotide Z-1
604-Z-2
Component polynucleotide Z-2
604-Z-C
Component polynucleotide Z-C

Fig. 6A

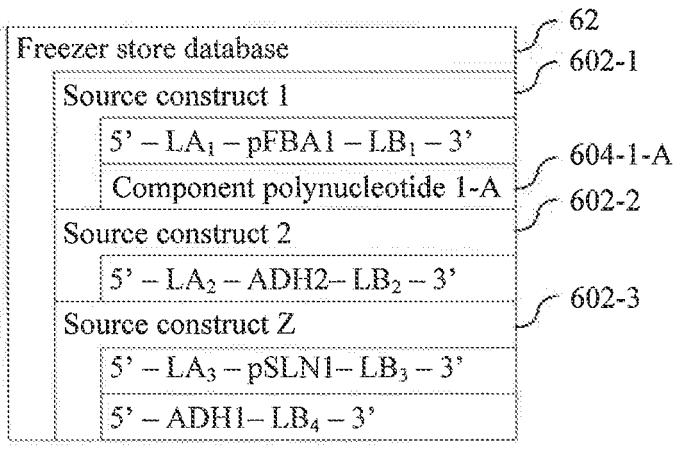

Freezer store database
62
602-1
Source construct 1
5' – LA1 – pFBA1 – LB1 – 3'
604-1-A
Component polynucleotide 1-A
602-2
Source construct 2
5' – LA2 – ADH2– LB2 – 3'
602-3
Source construct Z
5' – LA3 – pSLN1– LB3 – 3'
5' – ADH1– LB4 – 3'

Fig. 6B

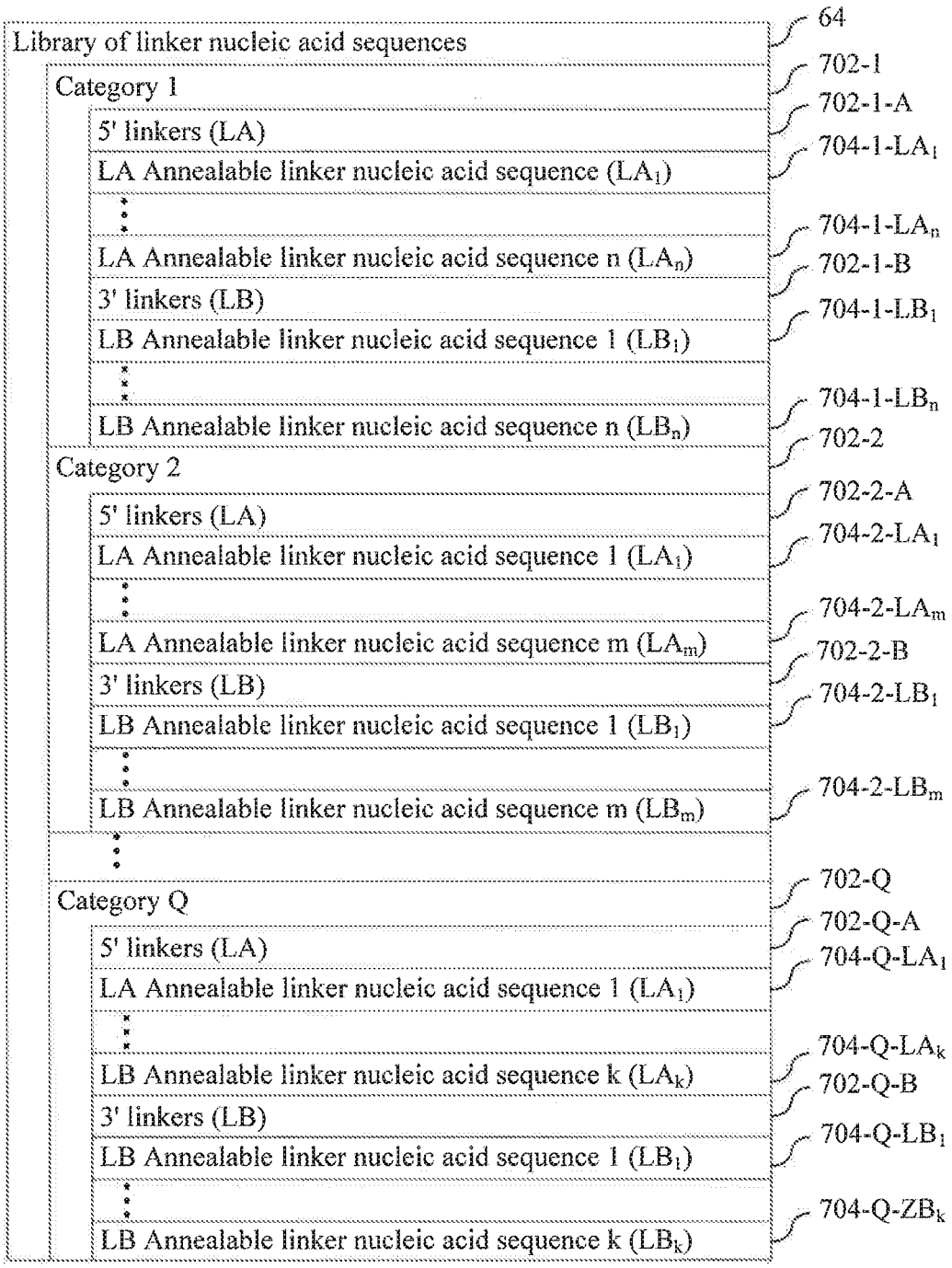

Library of linker nucleic acid sequences
64
Category 1
702-1
5' linkers (LA)
702-1-A
LA Annealable linker nucleic acid sequence (LA1)
704-1-LA1
LA Annealable linker nucleic acid sequence n (LAn)
704-1-LAn
3' linkers (LB)
702-1-B
LB Annealable linker nucleic acid sequence 1 (LB1)
704-1-LB1
LB Annealable linker nucleic acid sequence n (LBn)
704-1-LBn
Category 2
702-2
5' linkers (LA)
702-2-A
LA Annealable linker nucleic acid sequence 1 (LA1)
704-2-LA1
LA Annealable linker nucleic acid sequence m (LAm)
704-2-LAm
3' linkers (LB)
702-2-B
LB Annealable linker nucleic acid sequence 1 (LB1)
704-2-LB1
LB Annealable linker nucleic acid sequence m (LBm)
704-2-LBm
Category Q
702-Q
5' linkers (LA)
702-Q-A
LA Annealable linker nucleic acid sequence 1 (LA1)
704-Q-LA1
LA Annealable linker nucleic acid sequence k (LAk)
704-Q-LAk
3' linkers (LB)
702-Q-B
LB Annealable linker nucleic acid sequence 1 (LB1)
704-Q-LB1
LB Annealable linker nucleic acid sequence k (LBk)
704-Q-ZBk

Fig. 7

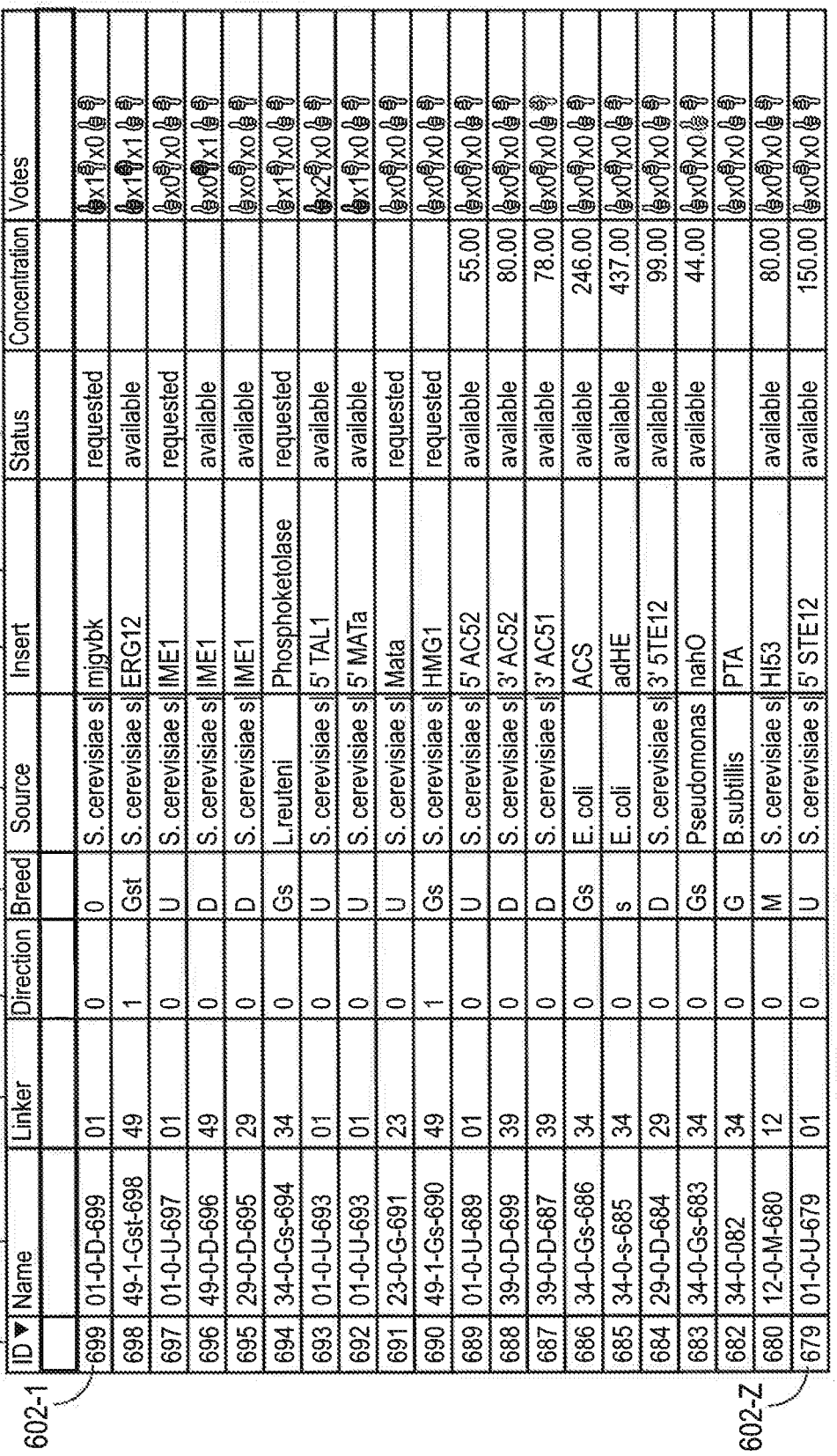

802
804
806
808
810
812
814
816
818
820
822
602-1
602-Z
ID
Name
Linker
Direction
Breed
Source
Insert
Status
Concentration
Votes
699 01-0-D-699 01 0 0 S. cerevisiae s migvbk requested 6x1 1x0 6 6
698 49-1-Gst-698 49 1 Gst S. cerevisiae s ERG12 available 6x1 1x1 6 6
697 01-0-U-697 01 0 U S. cerevisiae s IME1 requested 6x0 1x0 6 6
696 49-0-D-696 49 0 D S. cerevisiae s IME1 available 6x0 1x1 6 6
695 29-0-D-695 29 0 D S. cerevisiae s IME1 available 6x0 1x0 6 6
694 34-0-Gs-694 34 0 Gs L.reuteni Phosphoketolase requested 6x1 1x0 6 6
693 01-0-U-693 01 0 U S. cerevisiae s 5' TAL1 available 6x2 1x0 6 6
692 01-0-U-693 01 0 U S. cerevisiae s 5' MATa available 6x1 1x0 6 6
691 23-0-G-691 23 0 U S. cerevisiae s Mata requested 6x0 1x0 6 6
690 49-1-Gs-690 49 1 Gs S. cerevisiae s HMG1 requested 6x0 1x0 6 6
689 01-0-U-689 01 0 U S. cerevisiae s 5' AC52 available 55.00 6x0 1x0 6 6
688 39-0-D-699 39 0 D S. cerevisiae s 3' AC52 available 80.00 6x0 1x0 6 6
687 39-0-D-687 39 0 D S. cerevisiae s 3' AC51 available 78.00 6x0 1x0 6 6
686 34-0-Gs-686 34 0 Gs E. coli ACS available 246.00 6x0 1x0 6 6
685 34-0-s-685 34 0 s E. coli adHE available 437.00 6x0 1x0 6 6
684 29-0-D-684 29 0 D S. cerevisiae s 3' STE12 available 99.00 6x0 1x0 6 6
683 34-0-Gs-683 34 0 Gs Pseudomonas nahO available 44.00 6x0 1x0 6 6
682 34-0-082 34 0 G B.subtillis PTA 6x0 1x0 6 6
680 12-0-M-680 12 0 M S. cerevisiae s HI53 available 80.00 6x0 1x0 6 6
679 01-0-U-679 01 0 U S. cerevisiae s 5' STE12 available 150.00 6x0 1x0 6 6

FIG. 8

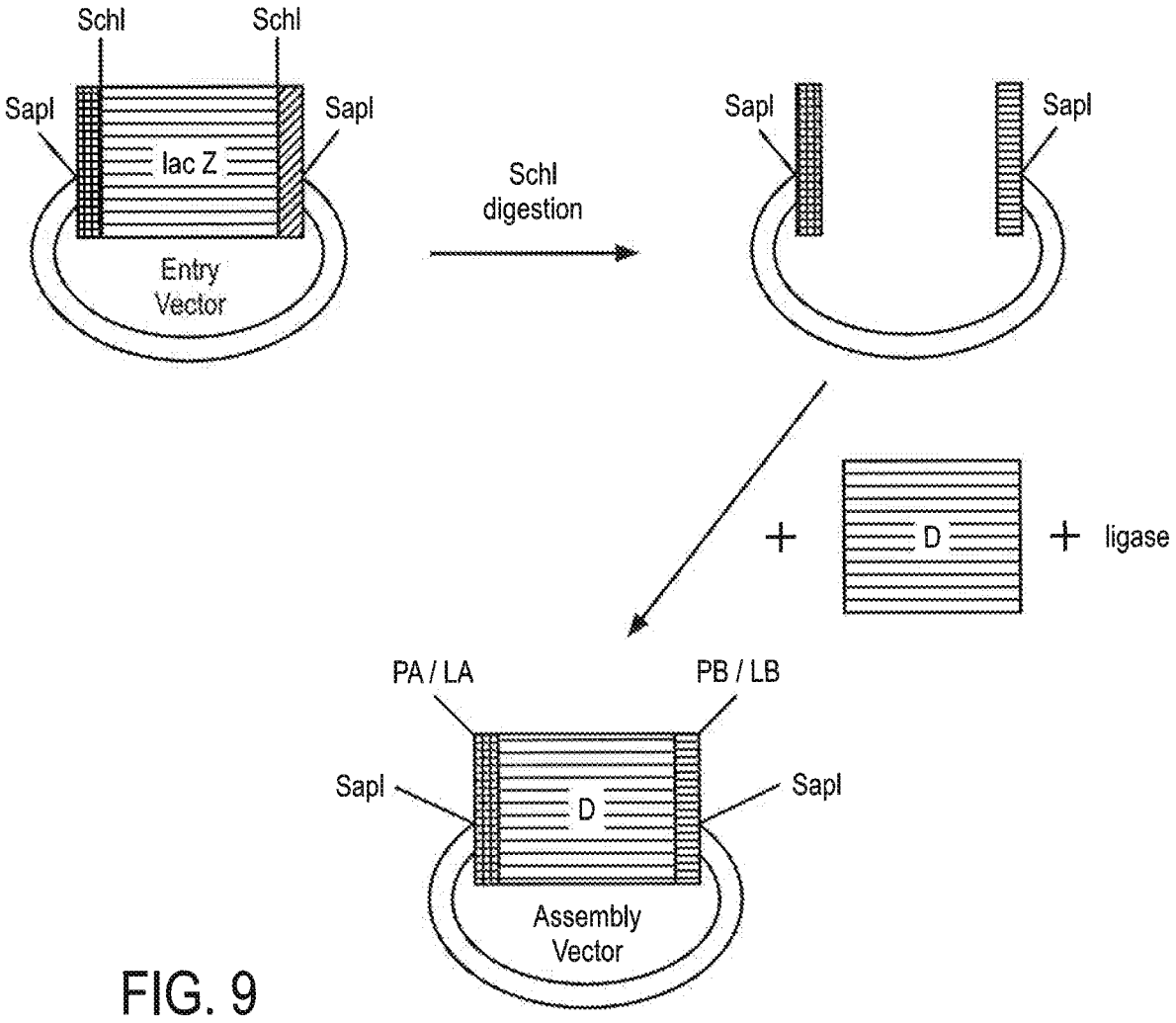

SchI
SchI
SapI
SapI
lac Z
Entry
Vector
SchI
digestion
SapI
SapI
+
D
+
ligase
PA / LA
PB / LB
SapI
SapI
D
Assembly
Vector

FIG. 9

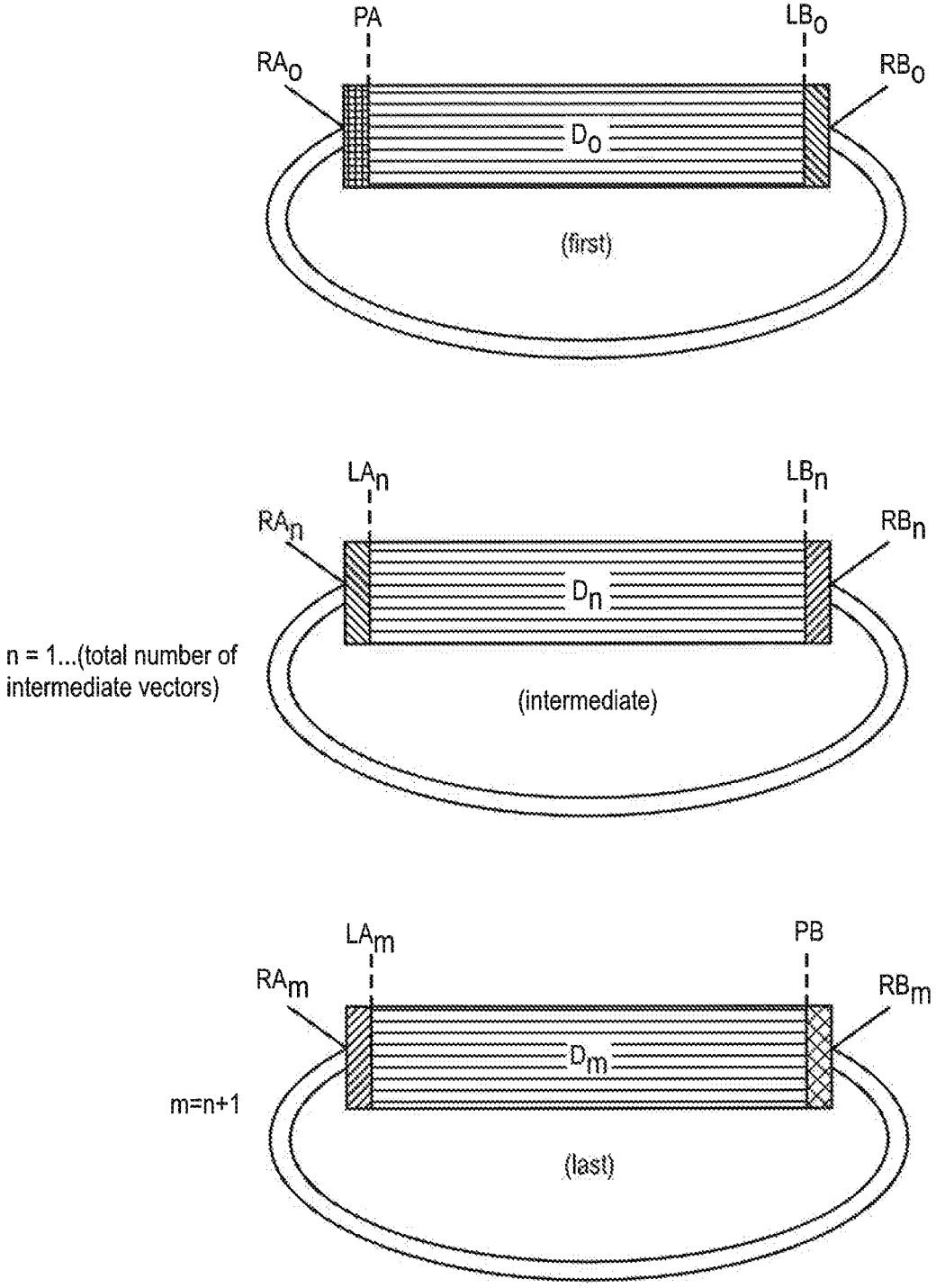

PA
LB0
RA0
RB0
D0
(first)
LAn
LBn
RAn
RBn
Dn
n = 1...(total number of intermediate vectors)
(intermediate)
LAm
PB
RAm
RBm
Dm
m=n+1
(last)

FIG. 10

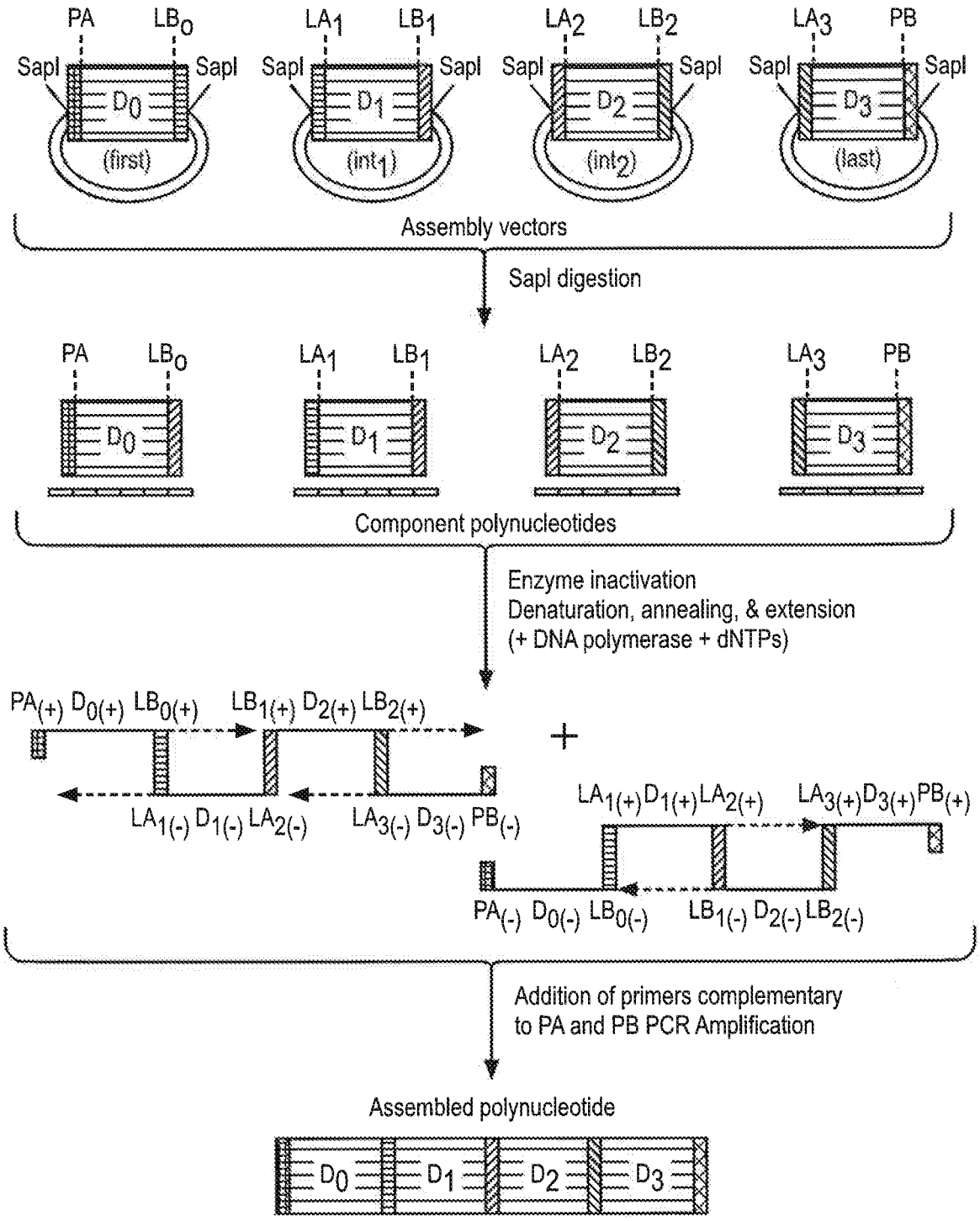

PA
LB0
LA1
LB1
LA2
LB2
LA3
PB
SapI
D0
D1
D2
D3
(first)
(int1)
(int2)
(last)
Assembly vectors
SapI digestion
Component polynucleotides
Enzyme inactivation
Denaturation, annealing, & extension
(+ DNA polymerase + dNTPs)
PA(+) D0(+) LB0(+)
LB1(+) D2(+) LB2(+)
LA1(-) D1(-) LA2(-)
LA3(-) D3(-) PB(-)
LA1(+)D1(+)LA2(+)
LA3(+)D3(+)PB(+)
PA(-) D0(-) LB0(-)
LB1(-) D2(-) LB2(-)
Addition of primers complementary
to PA and PB PCR Amplification
Assembled polynucleotide

FIG. 11

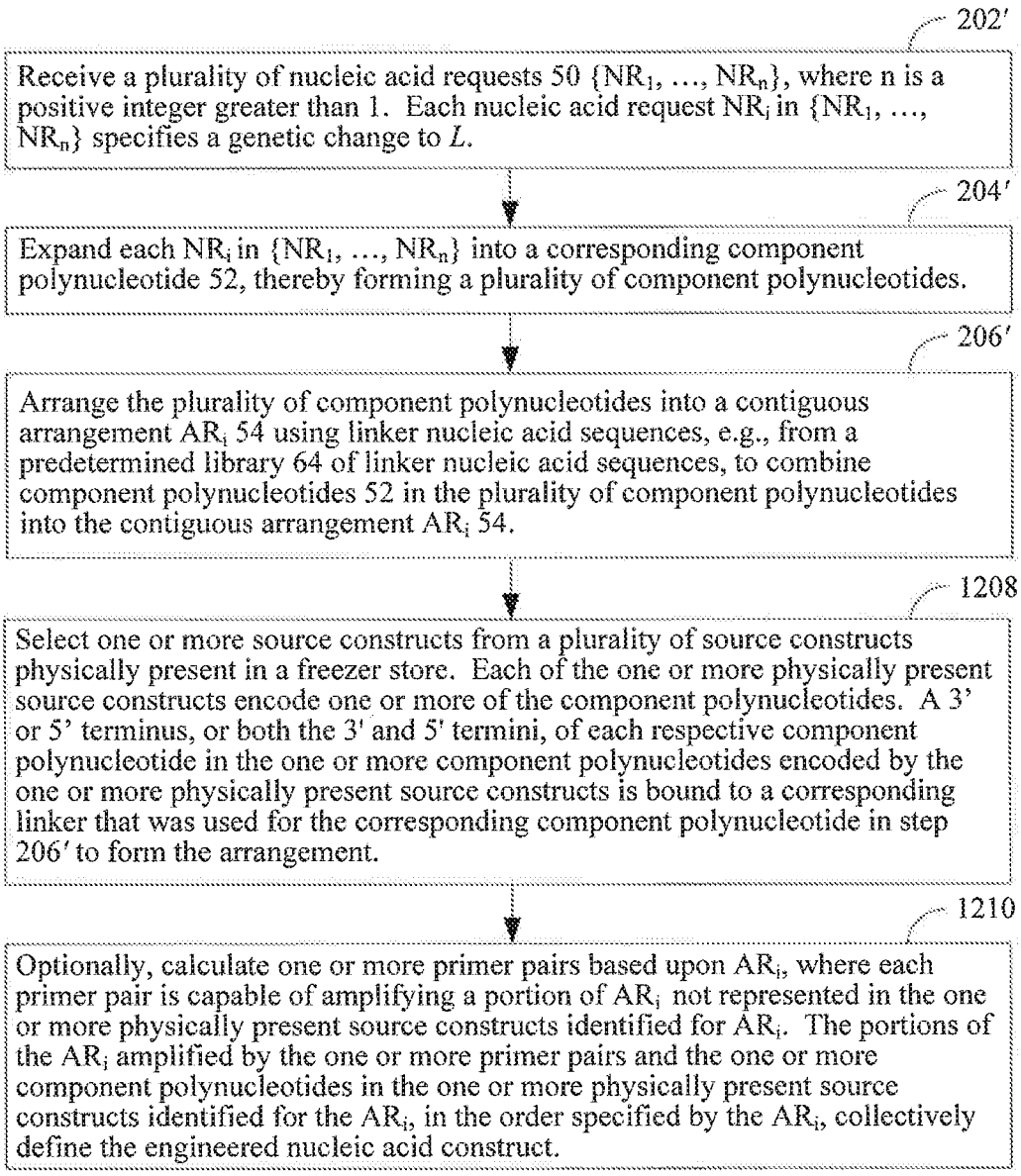

202′
Receive a plurality of nucleic acid requests 50 {NR1, …, NRn}, where n is a positive integer greater than 1. Each nucleic acid request NRi in {NR1, …, NRn} specifies a genetic change to L.
204′
Expand each NRi in {NR1, …, NRn} into a corresponding component polynucleotide 52, thereby forming a plurality of component polynucleotides.
206′
Arrange the plurality of component polynucleotides into a contiguous arrangement ARi 54 using linker nucleic acid sequences, e.g., from a predetermined library 64 of linker nucleic acid sequences, to combine component polynucleotides 52 in the plurality of component polynucleotides into the contiguous arrangement ARi 54.
1208
Select one or more source constructs from a plurality of source constructs physically present in a freezer store. Each of the one or more physically present source constructs encode one or more of the component polynucleotides. A 3' or 5' terminus, or both the 3' and 5' termini, of each respective component polynucleotide in the one or more component polynucleotides encoded by the one or more physically present source constructs is bound to a corresponding linker that was used for the corresponding component polynucleotide in step 206′ to form the arrangement.
1210
Optionally, calculate one or more primer pairs based upon ARi, where each primer pair is capable of amplifying a portion of ARi not represented in the one or more physically present source constructs identified for ARi. The portions of the ARi amplified by the one or more primer pairs and the one or more component polynucleotides in the one or more physically present source constructs identified for the ARi, in the order specified by the ARi, collectively define the engineered nucleic acid construct.

Fig. 12

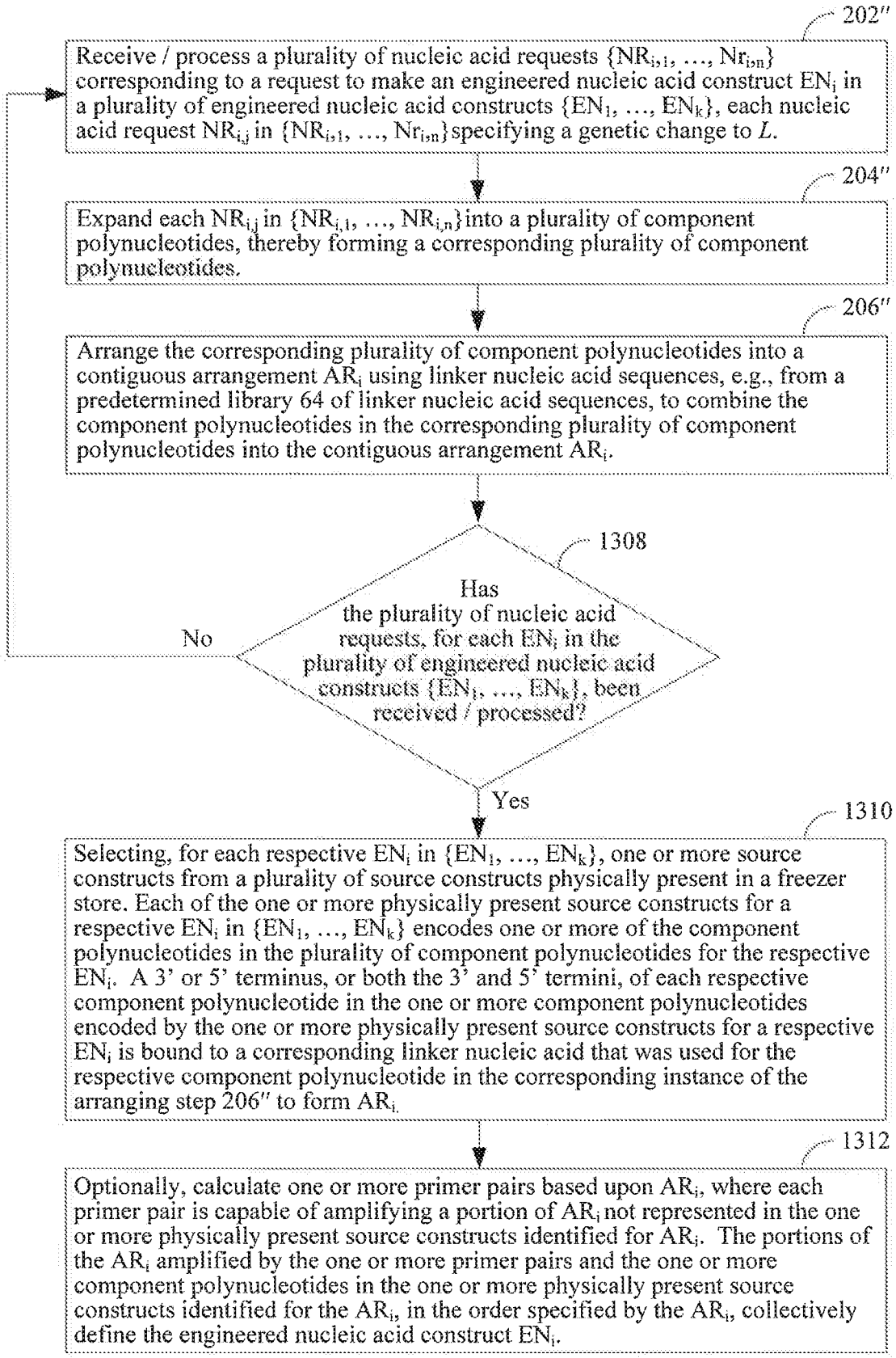

202″
Receive / process a plurality of nucleic acid requests {NR$_{i,1}$, …, Nr$_{i,n}$} corresponding to a request to make an engineered nucleic acid construct EN$_i$ in a plurality of engineered nucleic acid constructs {EN$_1$, …, EN$_k$}, each nucleic acid request NR$_{i,j}$ in {NR$_{i,1}$, …, Nr$_{i,n}$} specifying a genetic change to *L*.
204″
Expand each NR$_{i,j}$ in {NR$_{i,1}$, …, NR$_{i,n}$} into a plurality of component polynucleotides, thereby forming a corresponding plurality of component polynucleotides.
206″
Arrange the corresponding plurality of component polynucleotides into a contiguous arrangement AR$_i$ using linker nucleic acid sequences, e.g., from a predetermined library 64 of linker nucleic acid sequences, to combine the component polynucleotides in the corresponding plurality of component polynucleotides into the contiguous arrangement AR$_i$.
1308
Has the plurality of nucleic acid requests, for each EN$_i$ in the plurality of engineered nucleic acid constructs {EN$_1$, …, EN$_k$}, been received / processed?
No
Yes
1310
Selecting, for each respective EN$_i$ in {EN$_1$, …, EN$_k$}, one or more source constructs from a plurality of source constructs physically present in a freezer store. Each of the one or more physically present source constructs for a respective EN$_i$ in {EN$_1$, …, EN$_k$} encodes one or more of the component polynucleotides in the plurality of component polynucleotides for the respective EN$_i$. A 3' or 5' terminus, or both the 3' and 5' termini, of each respective component polynucleotide in the one or more component polynucleotides encoded by the one or more physically present source constructs for a respective EN$_i$ is bound to a corresponding linker nucleic acid that was used for the respective component polynucleotide in the corresponding instance of the arranging step 206″ to form AR$_i$.
1312
Optionally, calculate one or more primer pairs based upon AR$_i$, where each primer pair is capable of amplifying a portion of AR$_i$ not represented in the one or more physically present source constructs identified for AR$_i$. The portions of the AR$_i$ amplified by the one or more primer pairs and the one or more component polynucleotides in the one or more physically present source constructs identified for the AR$_i$, in the order specified by the AR$_i$, collectively define the engineered nucleic acid construct EN$_i$.

Fig. 13

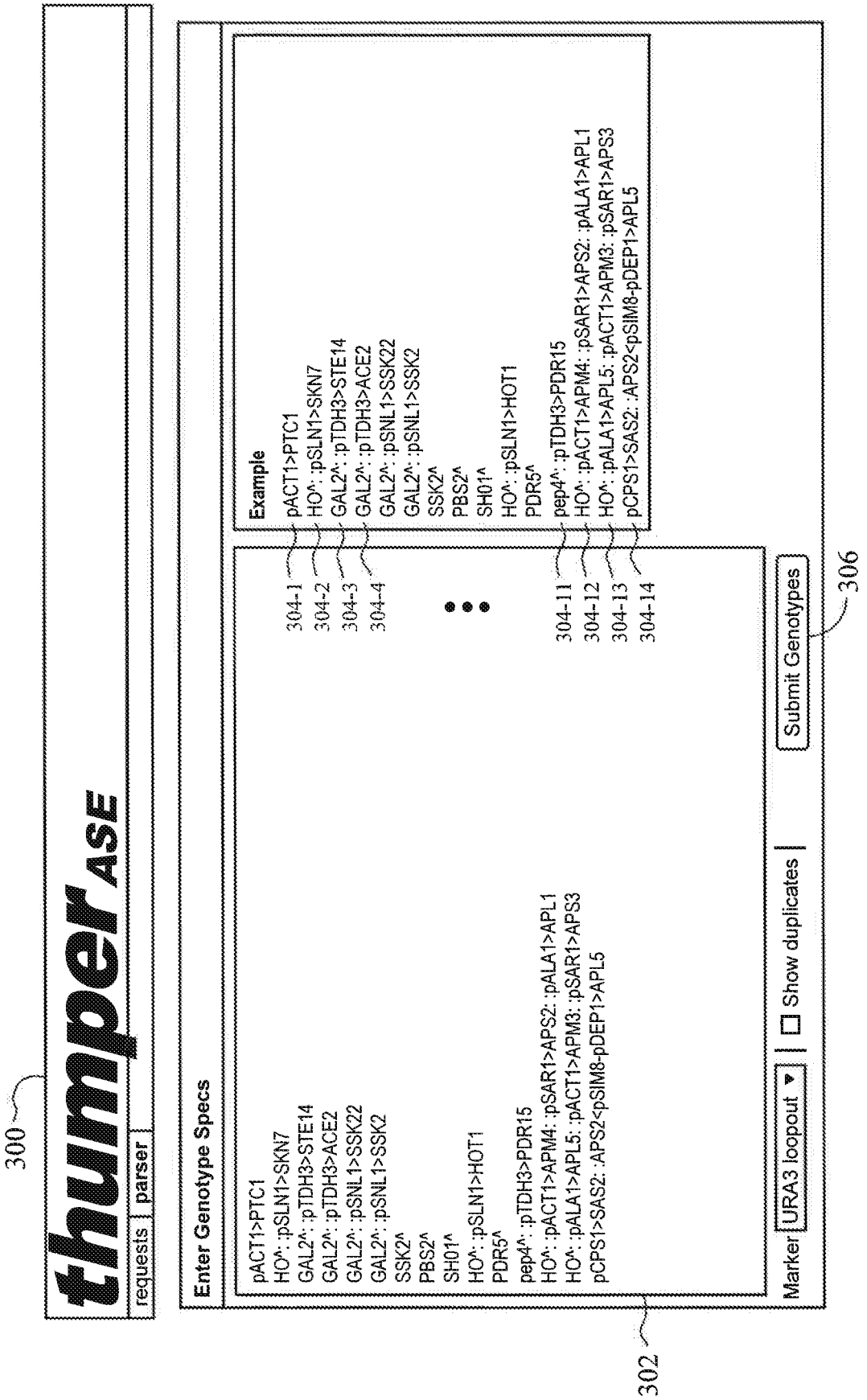

300
thumperASE
requests
parser
Enter Genotype Specs
pACT1>PTC1
HO^: :pSLN1>SKN7
GAL2^: :pTDH3>STE14
GAL2^: :pTDH3>ACE2
GAL2^: :pSNL1>SSK22
GAL2^: :pSNL1>SSK2
SSK2^
PBS2^
SH01^
HO^: :pSLN1>HOT1
PDR5^
pep4^: :pTDH3>PDR15
HO^: :pACT1>APM4: :pSAR1>APS2: :pALA1>APL1
HO^: :pALA1>APL5: :pACT1>APM3: :pSAR1>APS3
pCPS1>SAS2: :APS2<pSIM8-pDEP1>APL5
302
Example
304-1
304-2
304-3
304-4
304-11
304-12
304-13
304-14
pACT1>PTC1
HO^: :pSLN1>SKN7
GAL2^: :pTDH3>STE14
GAL2^: :pTDH3>ACE2
GAL2^: :pSNL1>SSK22
GAL2^: :pSNL1>SSK2
SSK2^
PBS2^
SH01^
HO^: :pSLN1>HOT1
PDR5^
pep4^: :pTDH3>PDR15
HO^: :pACT1>APM4: :pSAR1>APS2: :pALA1>APL1
HO^: :pALA1>APL5: :pACT1>APM3: :pSAR1>APS3
pCPS1>SAS2: :APS2<pSIM8-pDEP1>APL5
Marker
URA3 loopout
Show duplicates
Submit Genotypes
306

Fig. 14

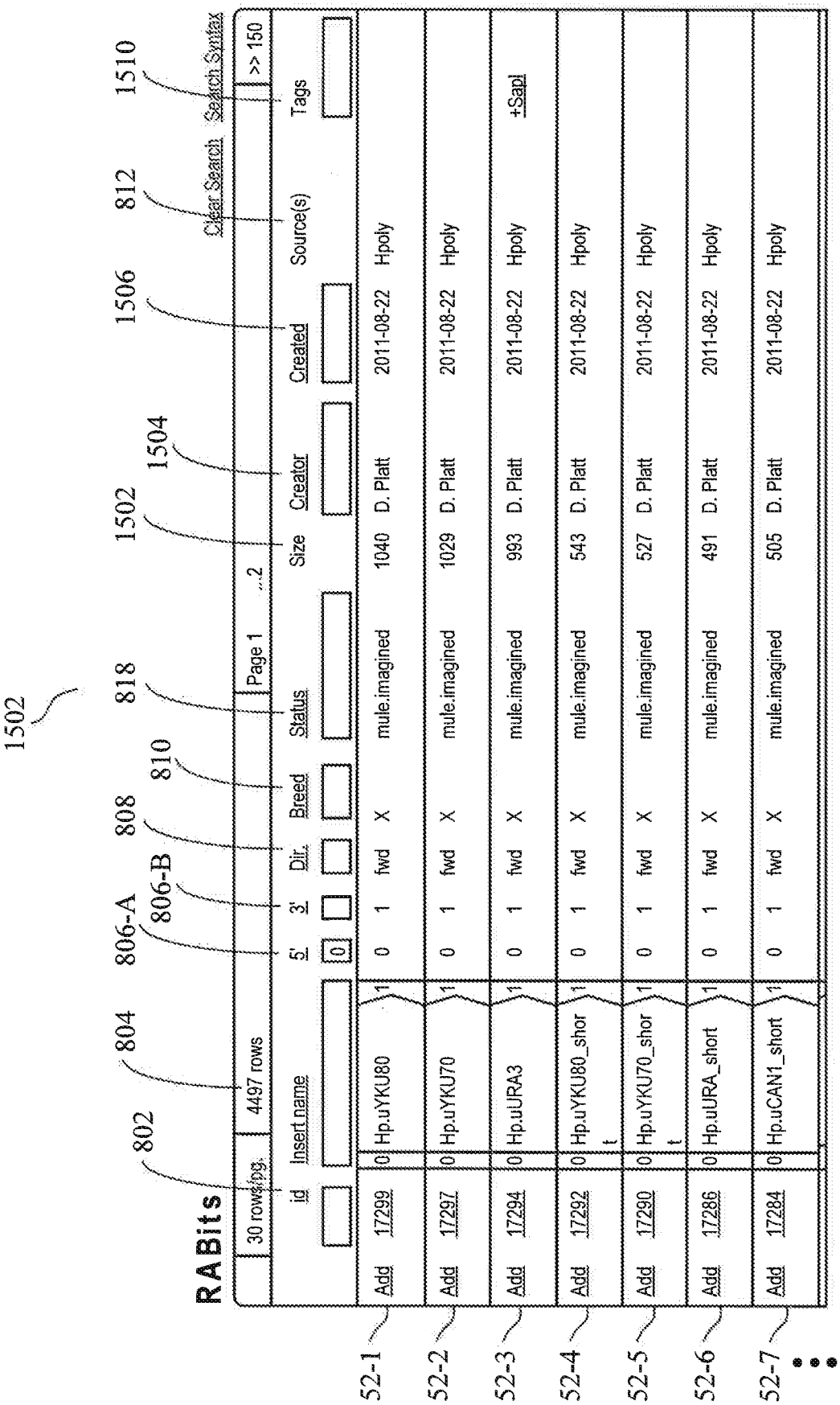

1502
802
804
806-A
806-B
808
810
818
1502
1504
1506
812
1510
RABits
30 rows/pg
4497 rows
Page 1 ...2
Clear Search
Search Syntax
>> 150
id
Insert name
5'
3'
Dir.
Breed
Status
Size
Creator
Created
Source(s)
Tags
Add
17299
Hp.uYKU80
0 1 fwd X
mule.imagined
1040
D. Platt
2011-08-22
Hpoly
Add
17297
Hp.uYKU70
0 1 fwd X
mule.imagined
1029
D. Platt
2011-08-22
Hpoly
Add
17294
Hp.uURA3
0 1 fwd X
mule.imagined
993
D. Platt
2011-08-22
Hpoly
+Sapl
Add
17292
Hp.uYKU80_shor t
0 1 fwd X
mule.imagined
543
D. Platt
2011-08-22
Hpoly
Add
17290
Hp.uYKU70_shor t
0 1 fwd X
mule.imagined
527
D. Platt
2011-08-22
Hpoly
Add
17286
Hp.uURA_short
0 1 fwd X
mule.imagined
491
D. Platt
2011-08-22
Hpoly
Add
17284
Hp.uCAN1_short
0 1 fwd X
mule.imagined
505
D. Platt
2011-08-22
Hpoly
52-1
52-2
52-3
52-4
52-5
52-6
52-7
52-N

Fig. 15

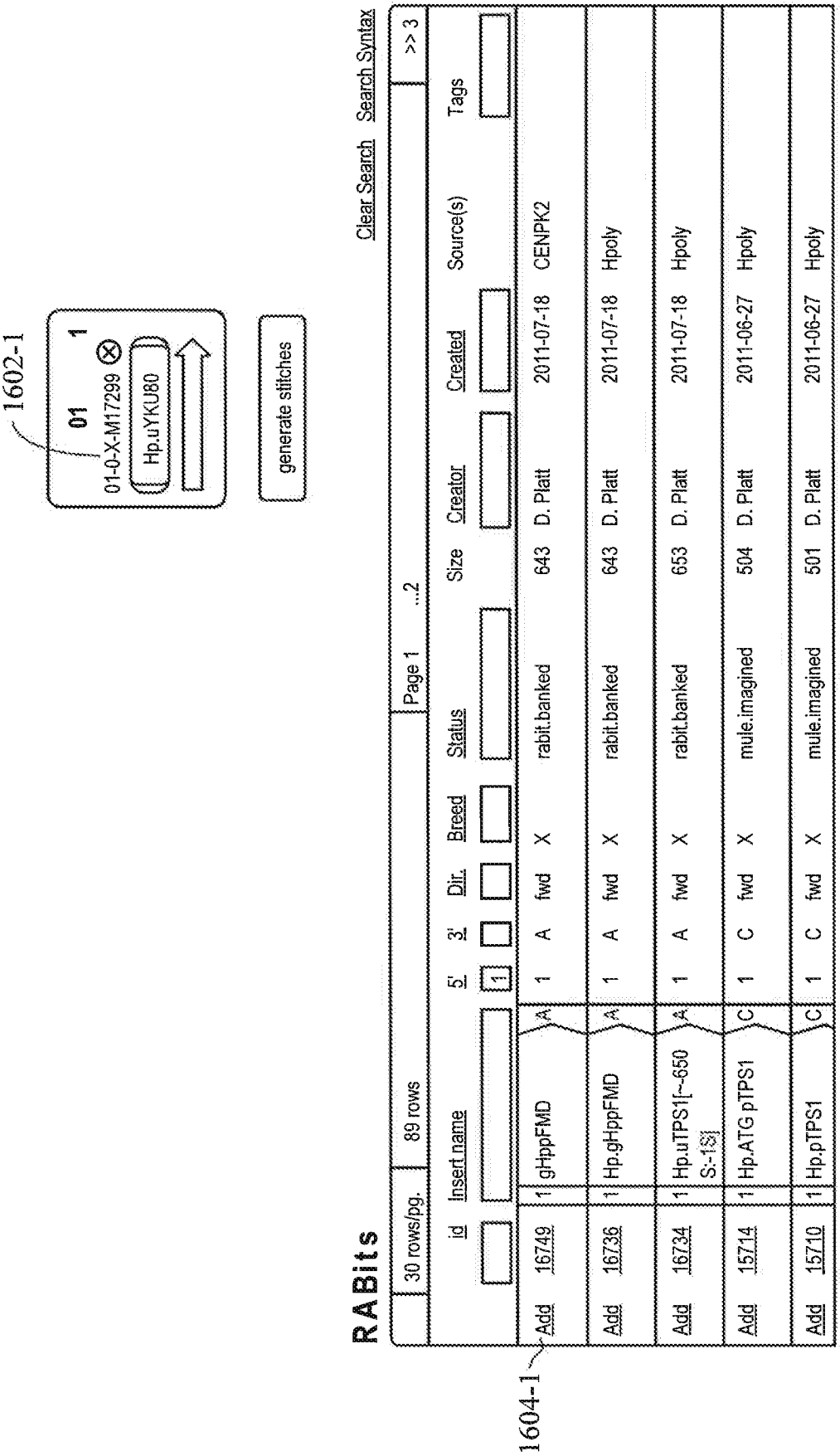

1602-1
01 1
01-0-X-M17299
Hp.uYKU80
generate stitches
RABits
Clear Search
Search Syntax
30 rows/pg.
89 rows
Page 1
...2
>> 3
id
Insert name
5'
3'
Dir.
Breed
Status
Size
Creator
Created
Source(s)
Tags
Add 16749 11 gHppFMD A 1 A fwd X rabit.banked 643 D. Platt 2011-07-18 CENPK2
Add 16736 11 Hp.gHppFMD A 1 A fwd X rabit.banked 643 D. Platt 2011-07-18 Hpoly
Add 16734 11 Hp.uTPS1[~650 S:-1$] A 1 A fwd X rabit.banked 653 D. Platt 2011-07-18 Hpoly
Add 15714 11 Hp.ATG pTPS1 C 1 C fwd X mule.imagined 504 D. Platt 2011-06-27 Hpoly
Add 15710 11 Hp.pTPS1 C 1 C fwd X mule.imagined 501 D. Platt 2011-06-27 Hpoly
1604-1

Fig. 16

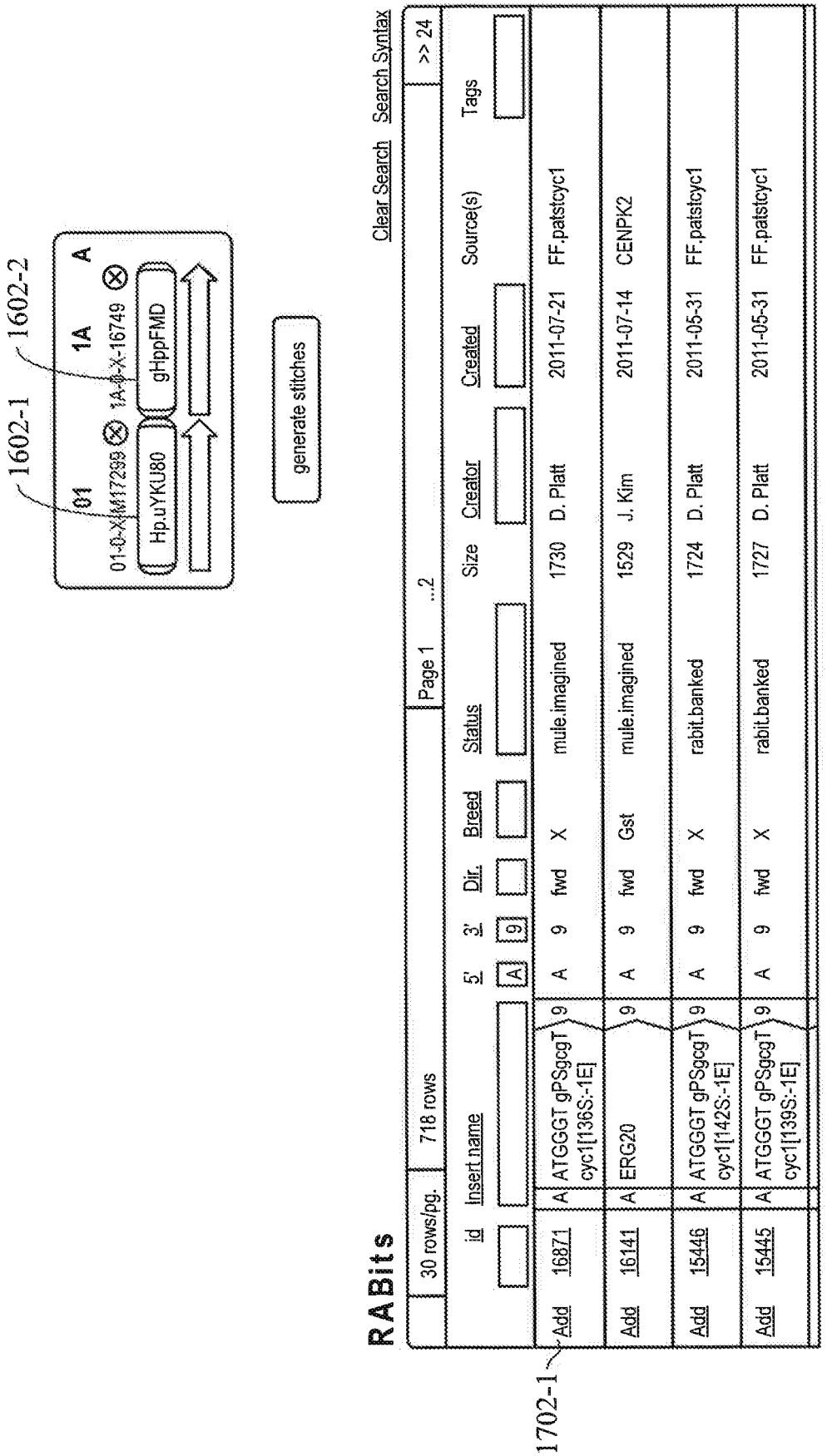

1602-1
1602-2
01
1A
A
01-0-X-M17239
1A-0-X-16749
Hp.uYKU80
gHppFMD
generate stitches
RABits
Clear Search
Search Syntax
30 rows/pg.
718 rows
Page 1
...2
>> 24
id
Insert name
5'
3'
Dir.
Breed
Status
Size
Creator
Created
Source(s)
Tags
Add
16871
A
ATGGGT gPSgcgT cyc1[136S:-1E]
9
A
9
fwd
X
mule.imagined
1730
D. Platt
2011-07-21
FF.patstcyc1
Add
16141
A
ERG20
9
A
9
fwd
Gst
mule.imagined
1529
J. Kim
2011-07-14
CENPK2
Add
15446
A
ATGGGT gPSgcgT cyc1[142S:-1E]
9
A
9
fwd
X
rabit.banked
1724
D. Platt
2011-05-31
FF.patstcyc1
Add
15445
A
ATGGGT gPSgcgT cyc1[139S:-1E]
9
A
9
fwd
X
rabit.banked
1727
D. Platt
2011-05-31
FF.patstcyc1
1702-1

Fig. 17

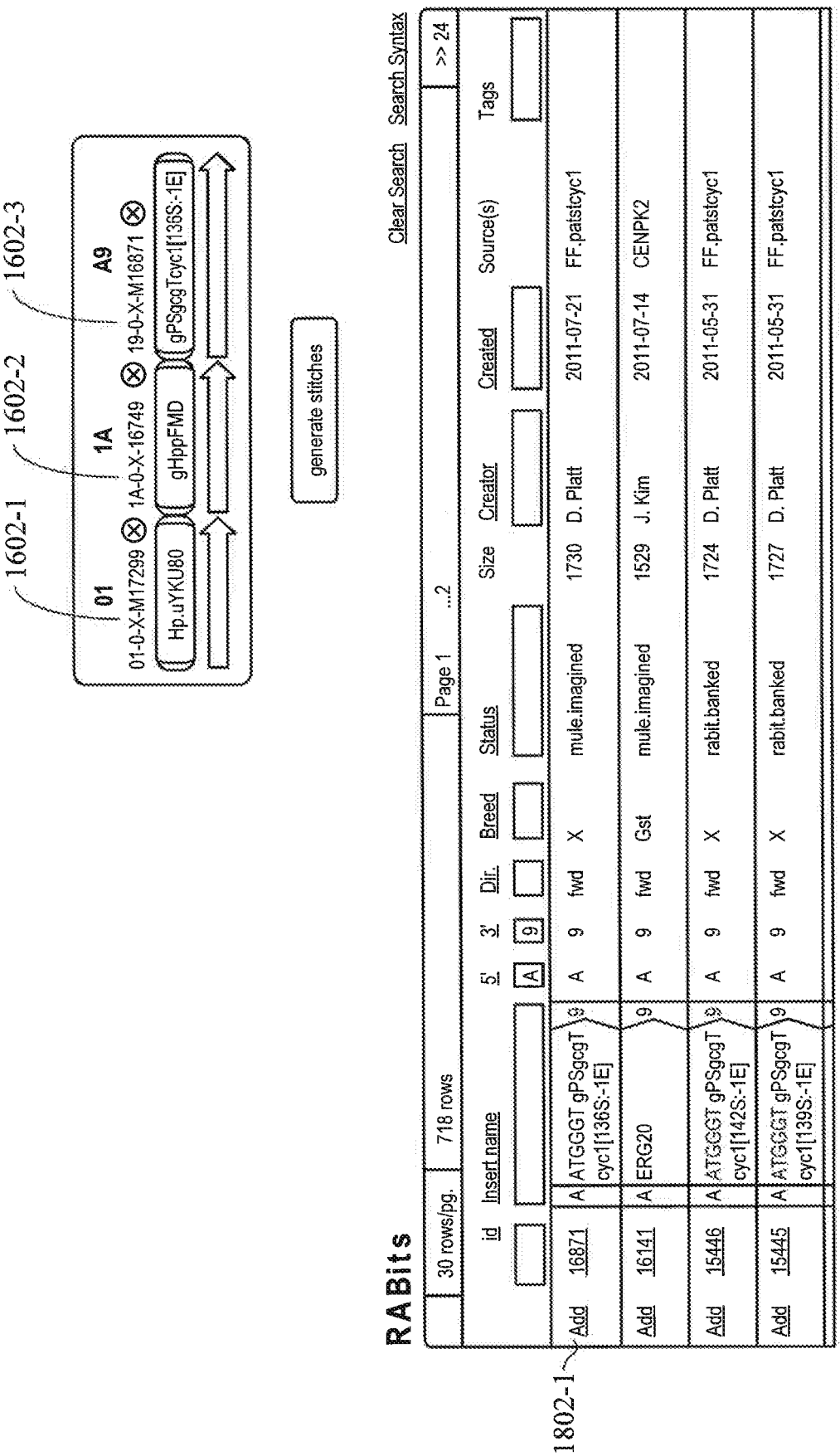

RABits
Clear Search
Search Syntax
30 rows/pg.
718 rows
Page 1 ...2
>> 24
id
Insert name
5'
3'
Dir.
Breed
Status
Size
Creator
Created
Source(s)
Tags
Add
16871
ATGGGT gPSgcgT cyc1[136S:-1E]
A
9
fwd
X
mule.imagined
1730
D. Platt
2011-07-21
FF.patstcyc1
Add
16141
ERG20
A
9
fwd
Gst
mule.imagined
1529
J. Kim
2011-07-14
CENPK2
Add
15446
ATGGGT gPSgcgT cyc1[142S:-1E]
A
9
fwd
X
rabit.banked
1724
D. Platt
2011-05-31
FF.patstcyc1
Add
15445
ATGGGT gPSgcgT cyc1[139S:-1E]
A
9
fwd
X
rabit.banked
1727
D. Platt
2011-05-31
FF.patstcyc1
1802-1
generate stitches
1602-1
1602-2
1602-3
01
01-0-X-M17299
1A
1A-0-X-16749
A9
19-0-X-M16871
Hp.uYKU80
gHppFMD
gPSgcgTcyc1[136S:-1E]

Fig. 18

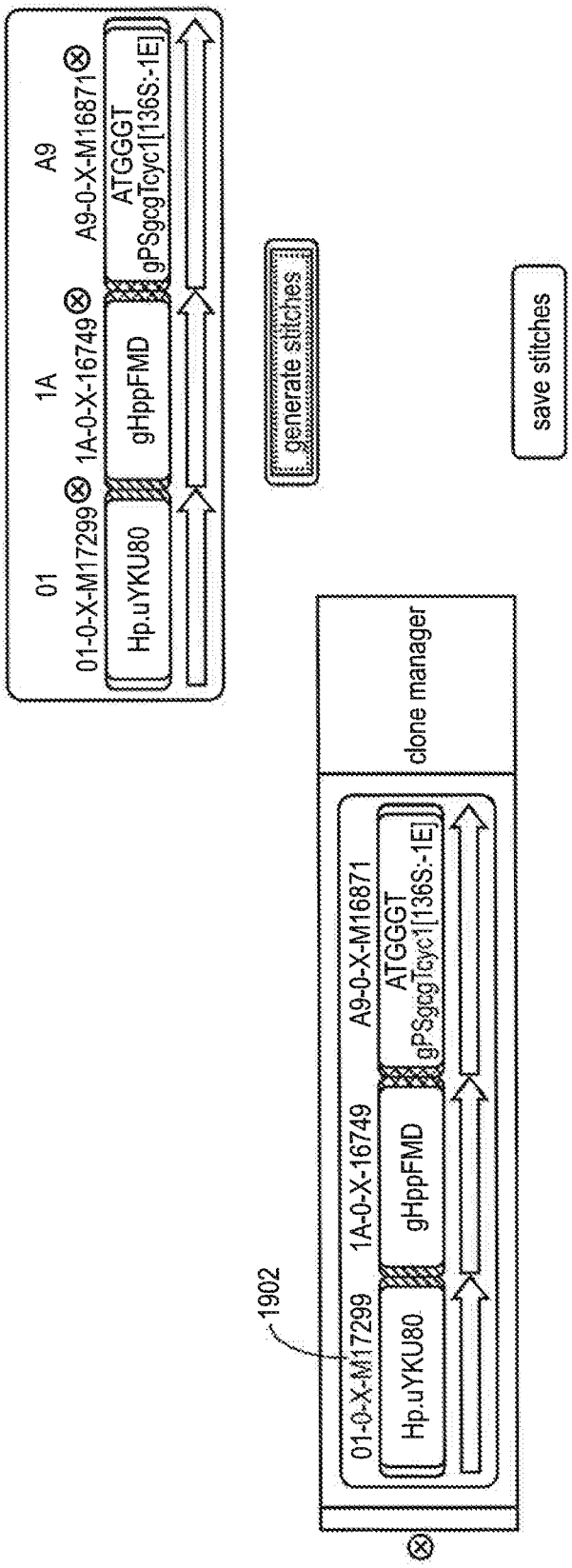
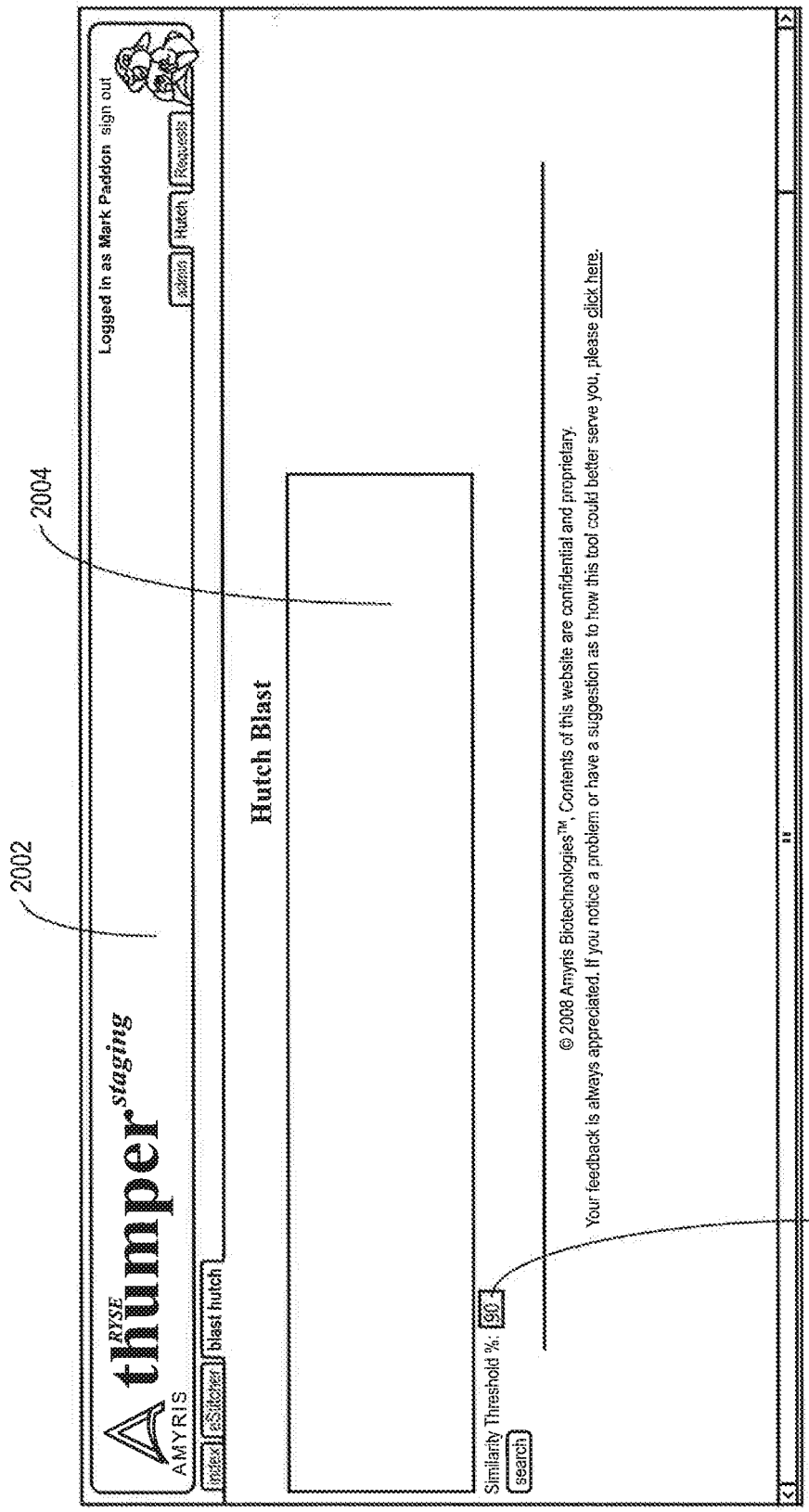

01
01-0-X-M17299
Hp.uYKU80
1A
1A-0-X-16749
gHppFMD
A9
A9-0-X-M16871
ATGGGT
gPSgcgTcyc1[136S:-1E]
generate stitches
save stitches
clone manager
1902
01-0-X-M17299
Hp.uYKU80
1A-0-X-16749
gHppFMD
A9-0-X-M16871
ATGGGT
gPSgcgTcyc1[136S:-1E]

FIG. 19


2002
2004
2006
AMYRIS
thumper staging
Logged in as Mark Paddon sign out
admin
Hutch
Requests
index
eSkitcher
blast hutch
Hutch Blast
Similarity Threshold %:
search
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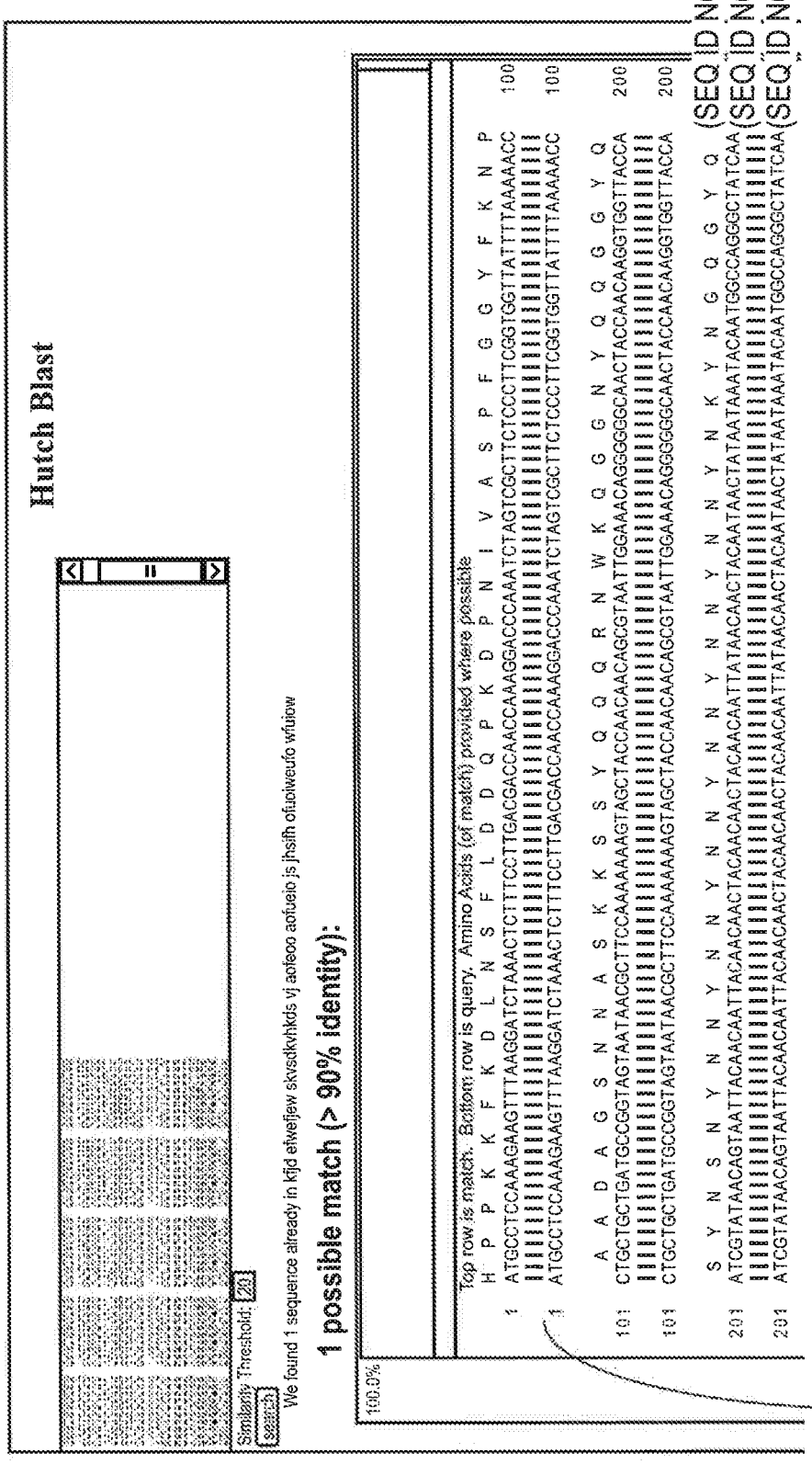
Your feedback is always appreciated. If you notice a problem or have a suggestion as to how this tool could better serve you, please click here.

FIG. 20



(SEQ ID NO. 1)
(SEQ ID NO. 2)
(SEQ ID NO. 3)

FIG. 21