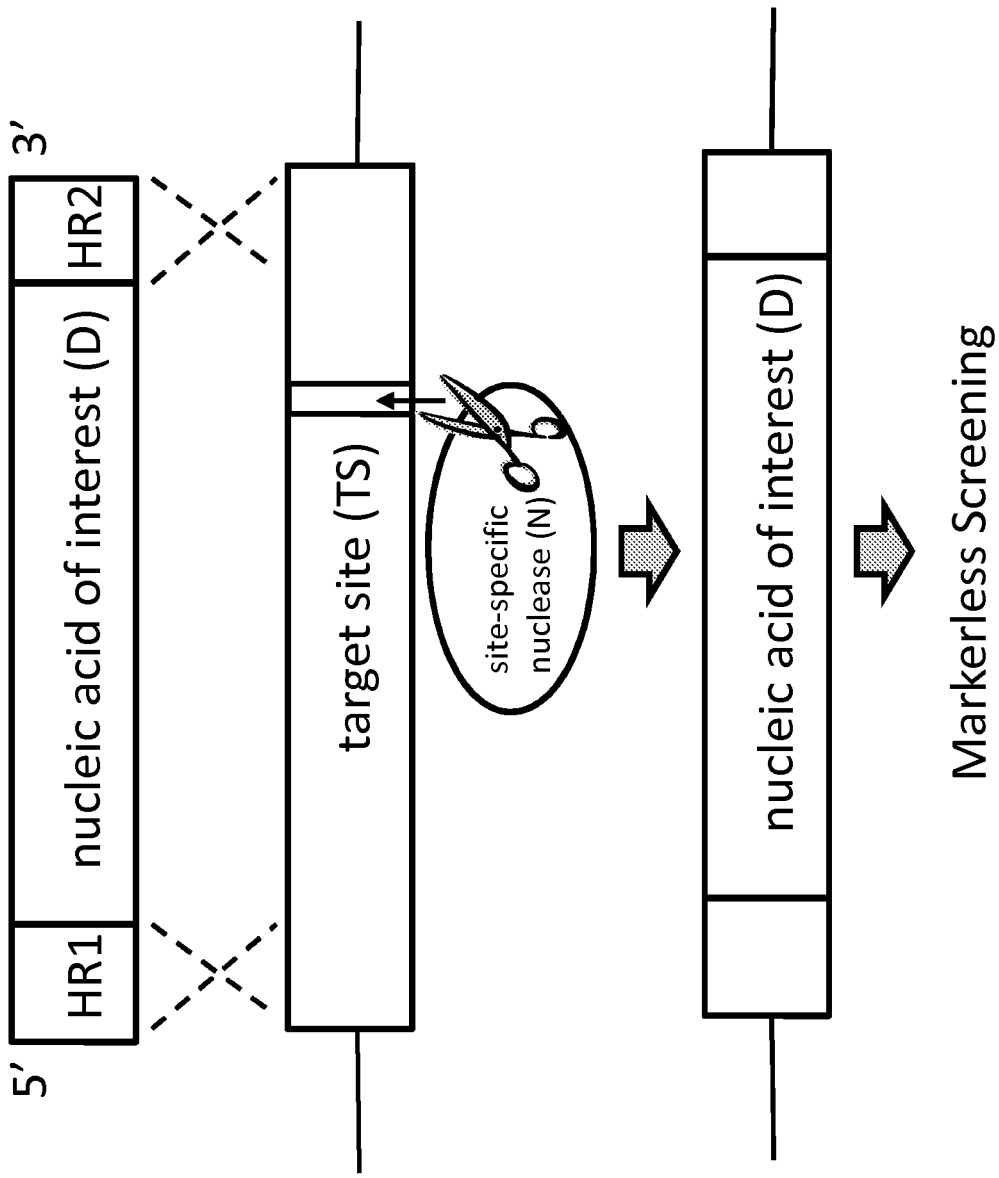

3'
HR2
nucleic acid of interest (D)
HR1
5'
target site (TS)
site-specific
nuclease (N)
nucleic acid of interest (D)
Markerless Screening

FIGURE 1

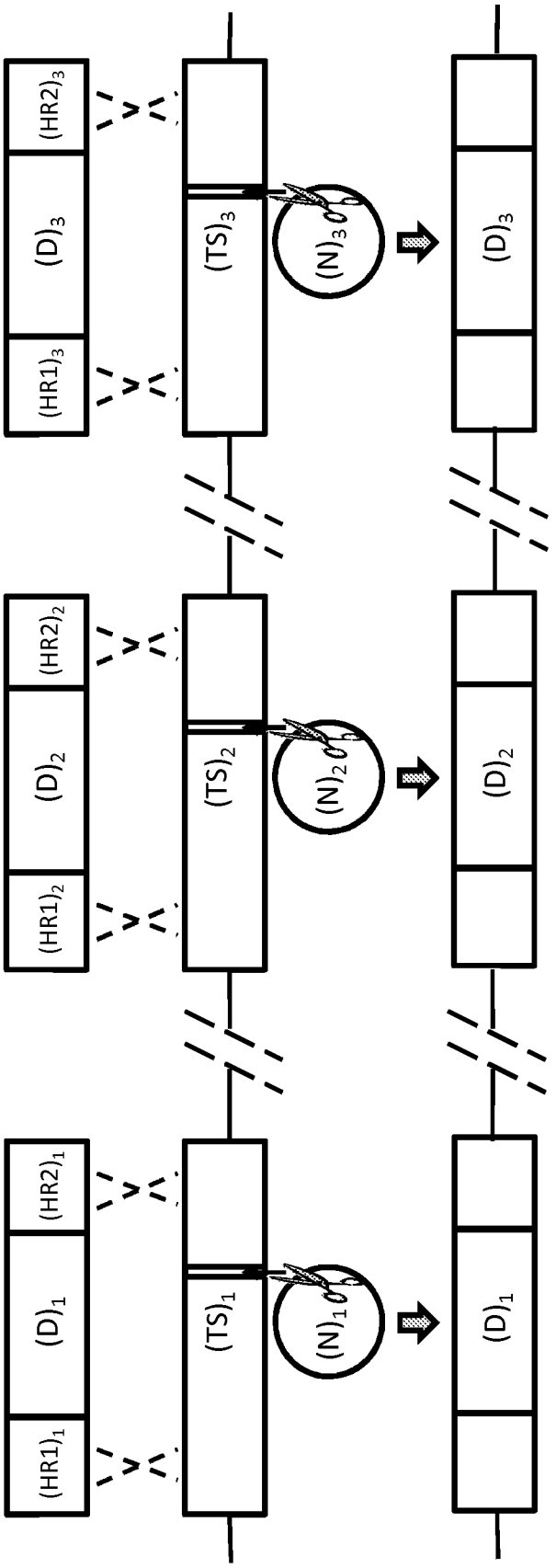

(HR2)3
(D)3
(HR1)3
(TS)3
(N)3
(D)3
(HR2)2
(D)2
(HR1)2
(TS)2
(N)2
(D)2
(HR2)1
(D)1
(HR1)1
(TS)1
(N)1
(D)1

FIGURE 2

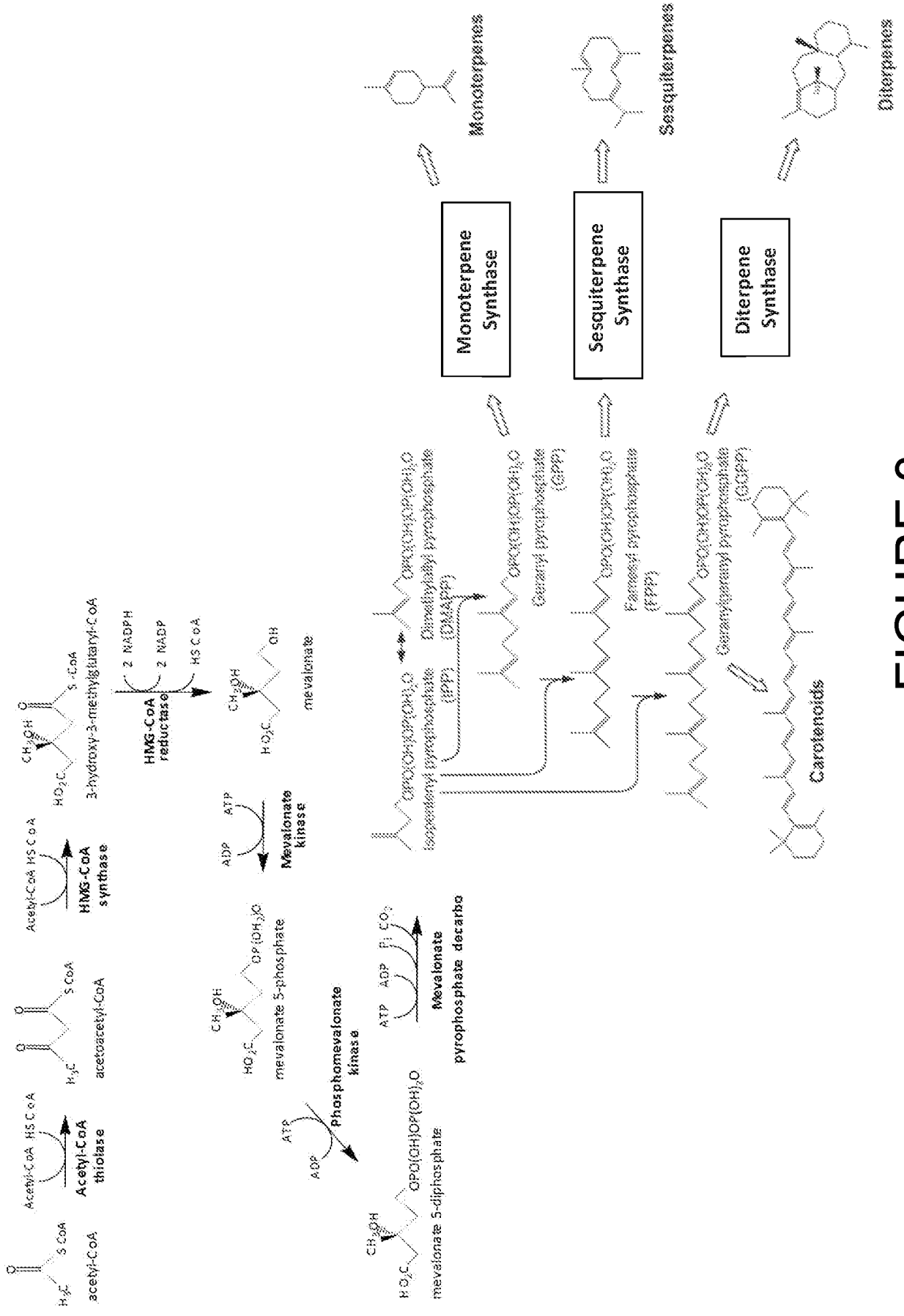

acetyl-CoA
Acetyl-CoA thiolase
acetoacetyl-CoA
HMG-CoA synthase
3-hydroxy-3-methylglutaryl-CoA
HMG-CoA reductase
2 NADPH
2 NADP
HS CoA
mevalonate
Mevalonate kinase
ATP
ADP
mevalonate 5-phosphate
Phosphomevalonate kinase
mevalonate 5-diphosphate
Mevalonate pyrophosphate decarbo
Isopentenyl pyrophosphate (IPP)
Dimethylallyl pyrophosphate (DMAPP)
Geranyl pyrophosphate (GPP)
Farnesyl pyrophosphate (FPP)
Geranylgeranyl pyrophosphate (GGPP)
Monoterpene Synthase
Sesquiterpene Synthase
Diterpene Synthase
Monoterpenes
Sesquiterpenes
Diterpenes
Carotenoids

FIGURE 3

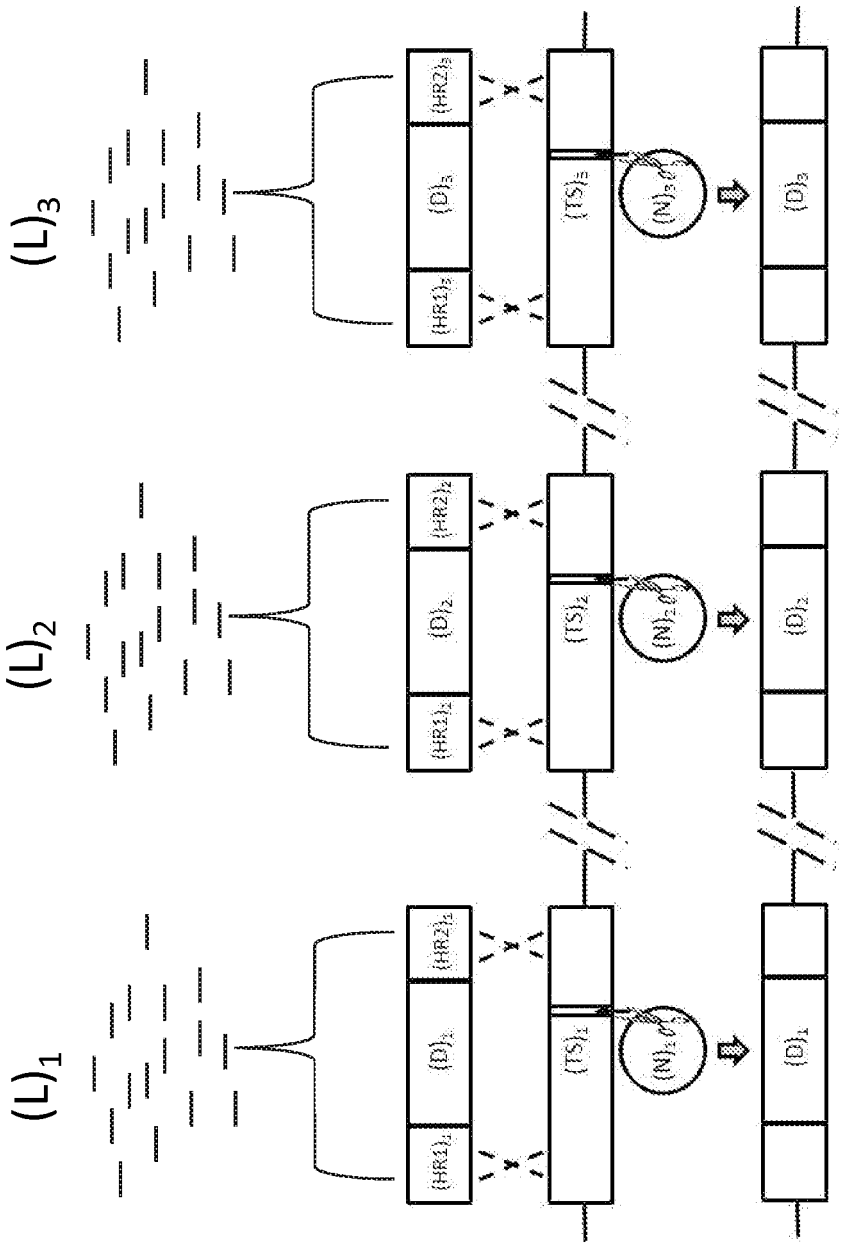

(L)1
(L)2
(L)3
{HR1}1
{D}1
{HR2}1
{TS}1
{N}1
{D}1
{HR1}2
{D}2
{HR2}2
{TS}2
{N}2
{D}2
{HR1}3
{D}3
{HR2}3
{TS}3
{N}3
{D}3

FIGURE 4

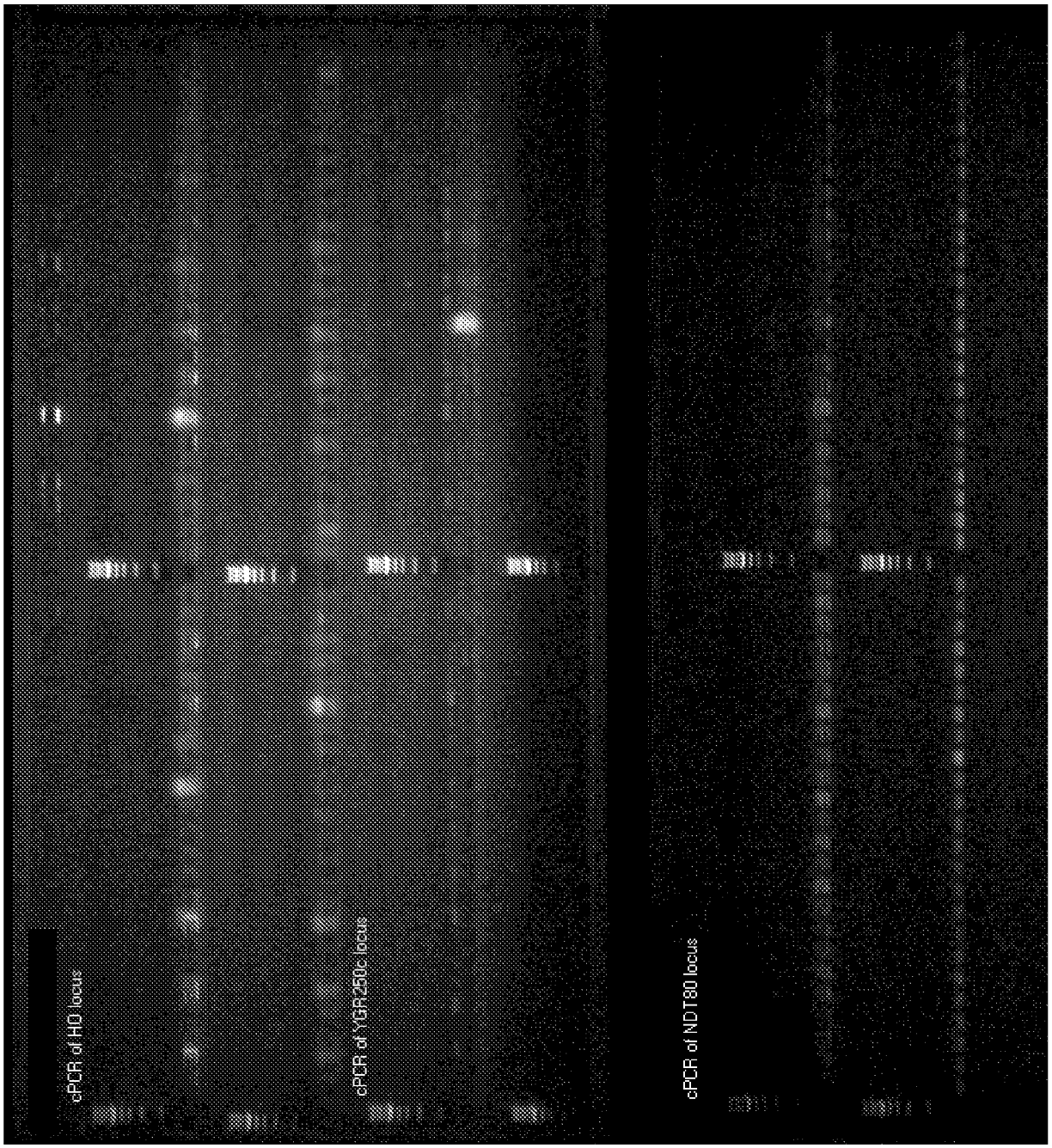


FIGURE 5

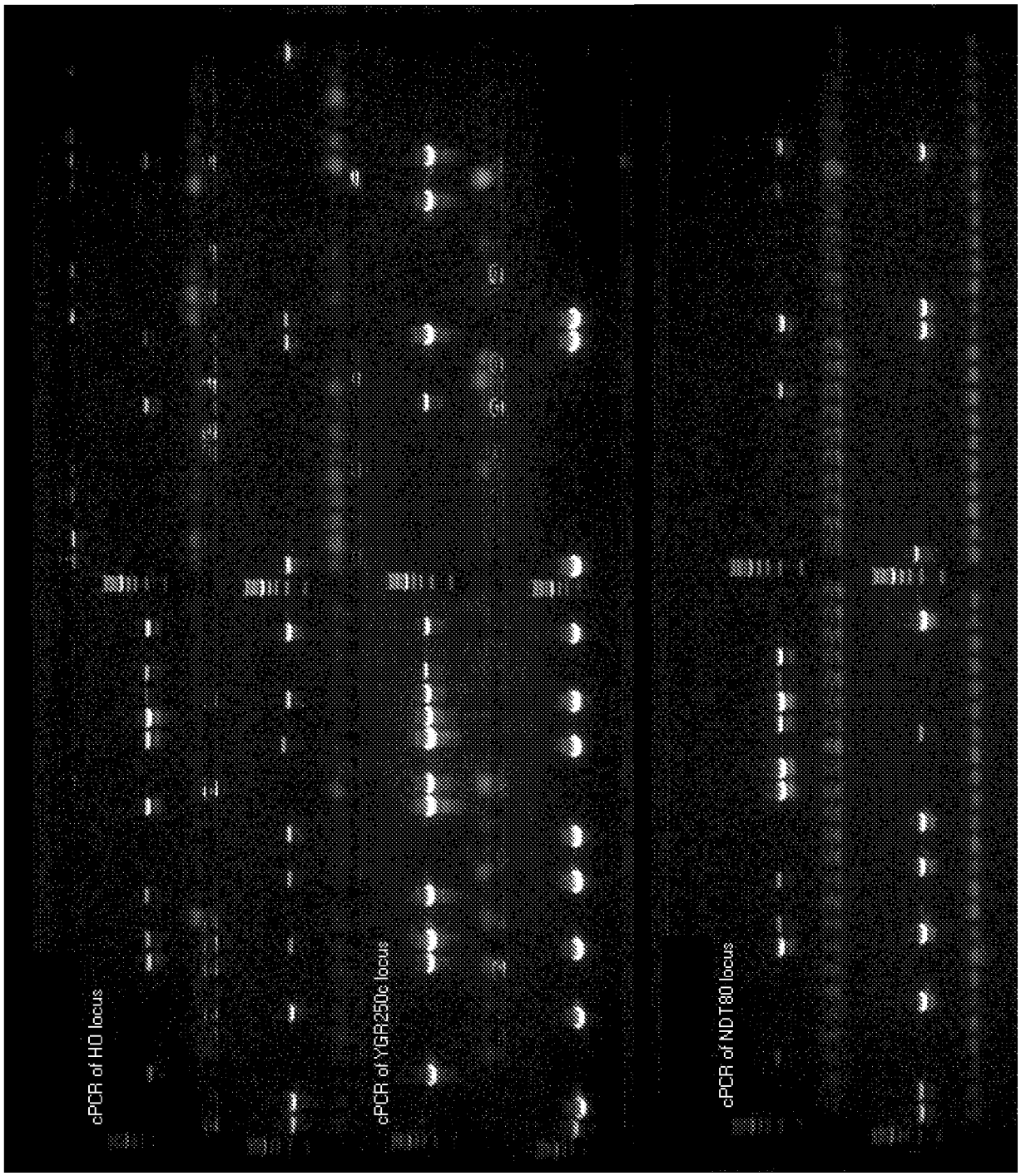


FIGURE 6

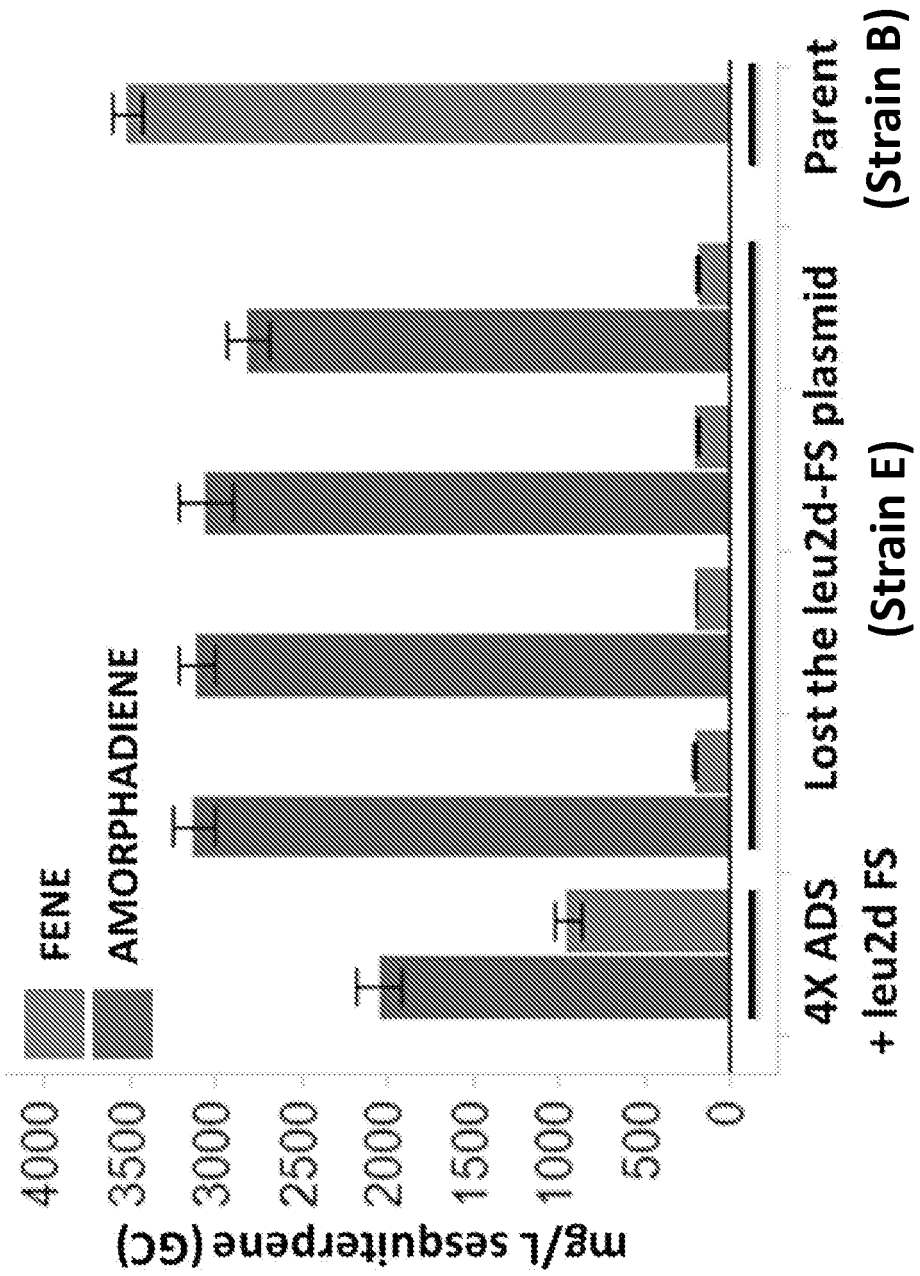

FENE
AMORPHADIENE
mg/L sesquiterpene (GC)
4000
3500
3000
2500
2000
1500
1000
500
0
4X ADS + leu2d FS (Strain D)
Lost the leu2d-FS plasmid (Strain E)
Parent (Strain B)

FIGURE 7

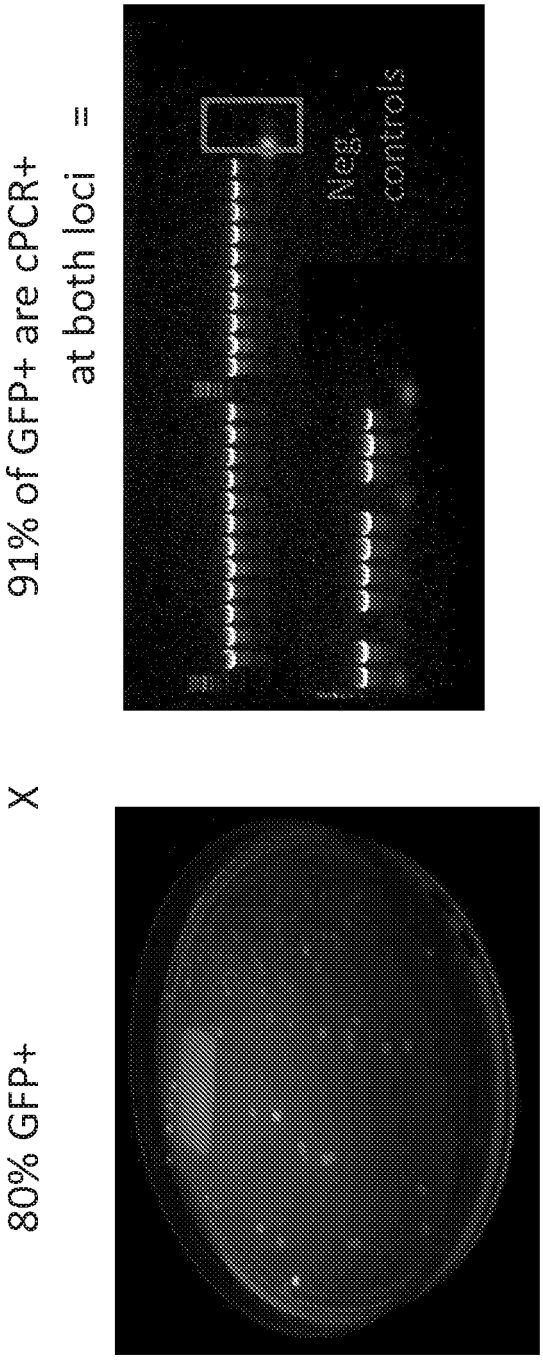

80% GFP+
X
91% of GFP+ are cPCR+
at both loci
=
72.8%
Double swap
at YJR, SFC1
Neg.
controls

FIGURE 8