Evolving a robust polypeptide backbone

Identify target conditions, based on analysis of solvent, pH, and temperature conditions in which starting polypeptide is operable

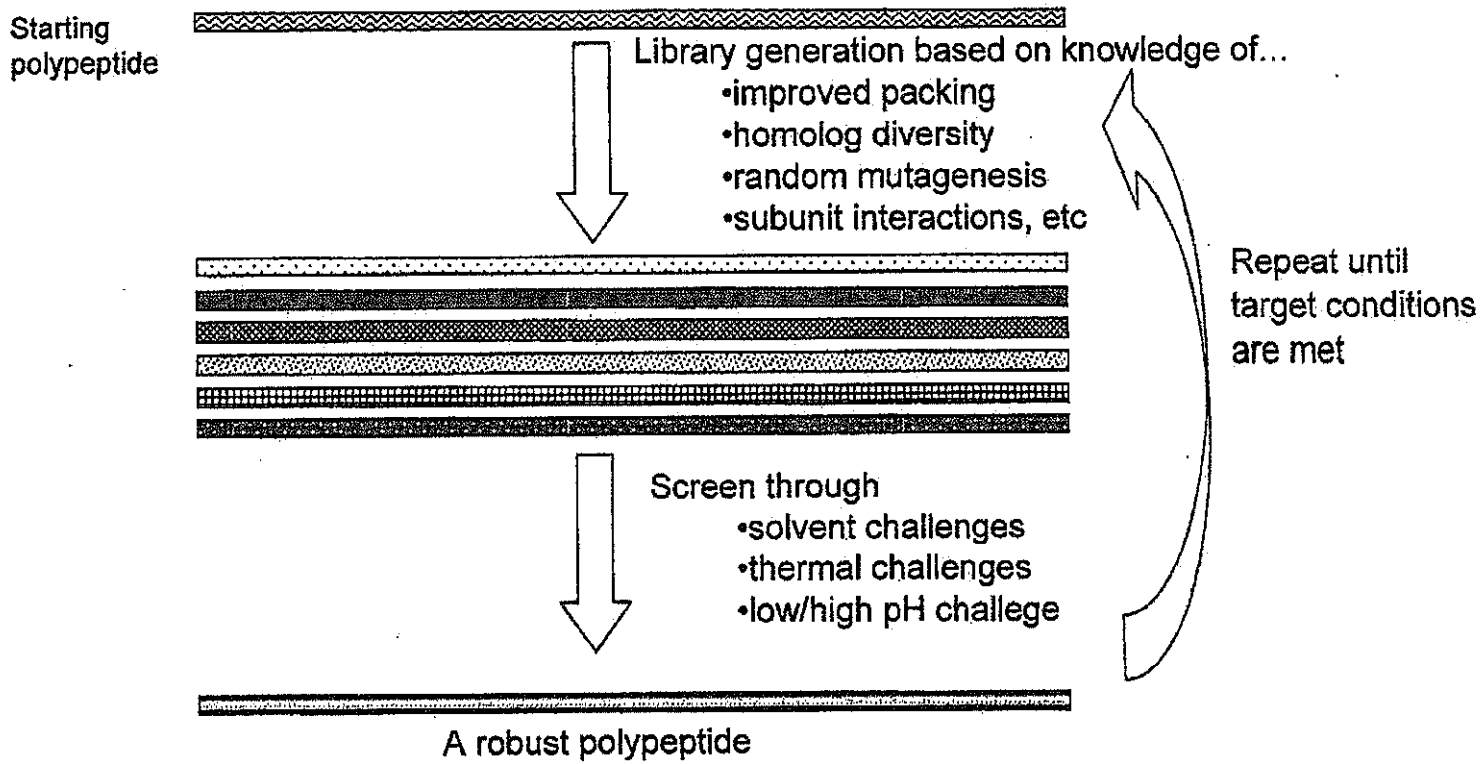
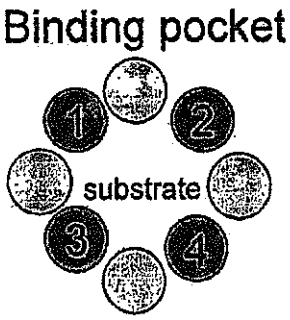


FIG. 1

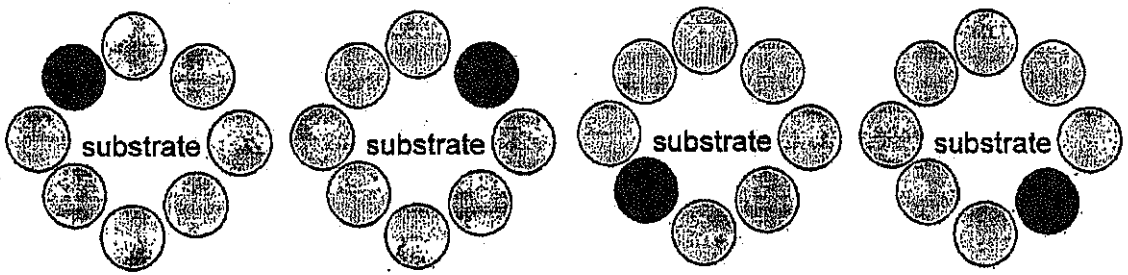
Generate Binding Pocket Diversity

- Identify positions with predicted influence on substrate specificity
  - Mutations reported in the literature
  - Structural modeling
  - Docking studies



Position of influence

- Individually randomize each position to test all 20 amino acids



- Design combinatorial libraries with mutations at multiple influential positions

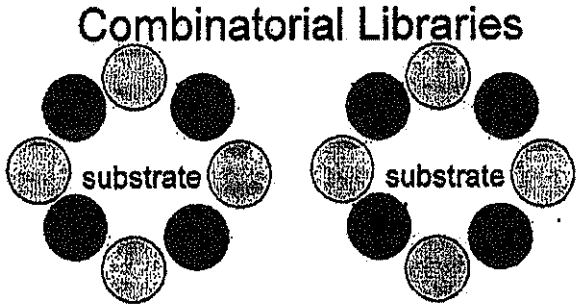


FIG. 2

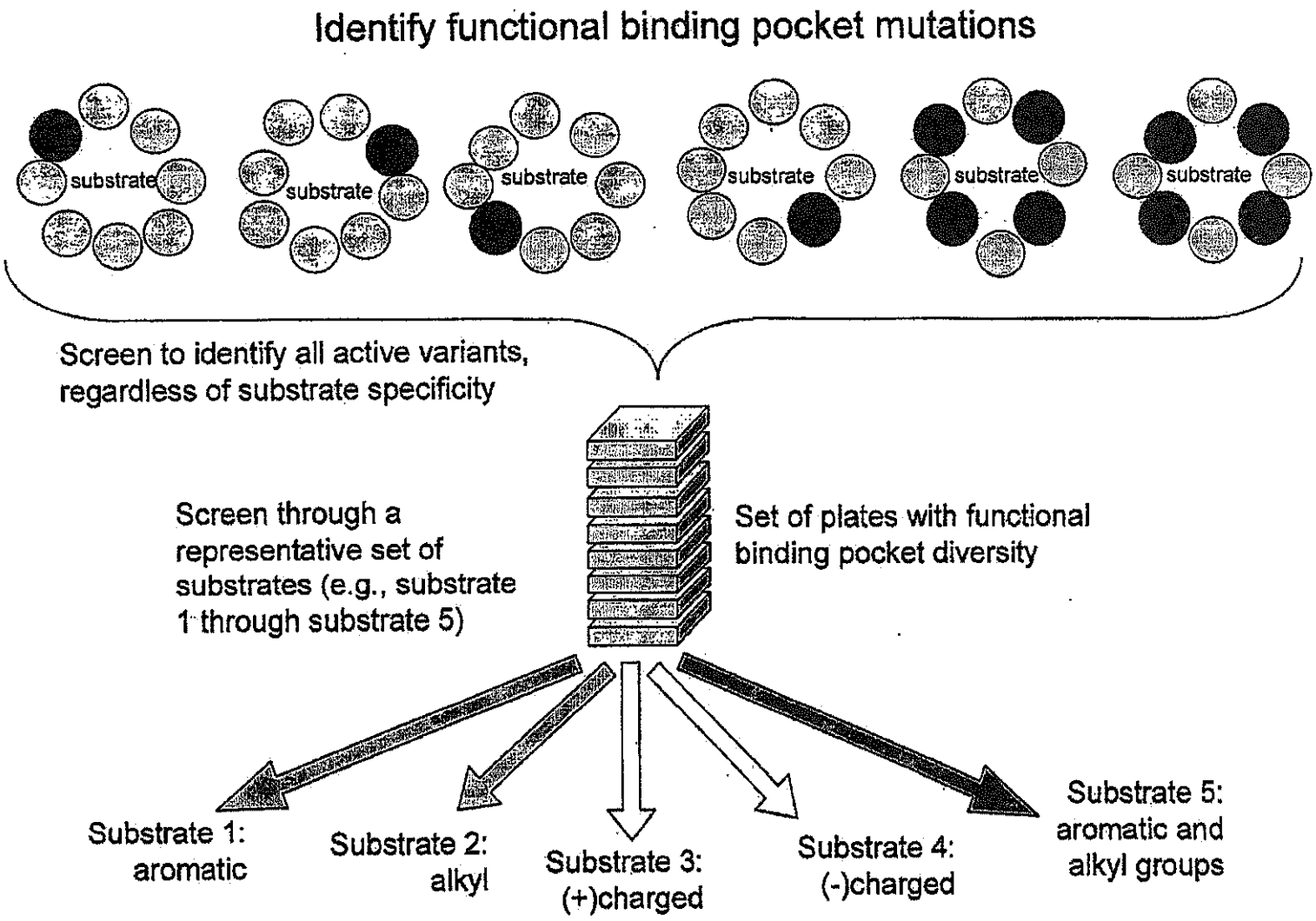

Identify functional binding pocket mutations
substrate
substrate
substrate
substrate
substrate
substrate
Screen to identify all active variants, regardless of substrate specificity
Screen through a representative set of substrates (e.g., substrate 1 through substrate 5)
Set of plates with functional binding pocket diversity
Substrate 1: aromatic
Substrate 2: alkyl
Substrate 3: (+)charged
Substrate 4: (-)charged
Substrate 5: aromatic and alkyl groups

FIG. 3

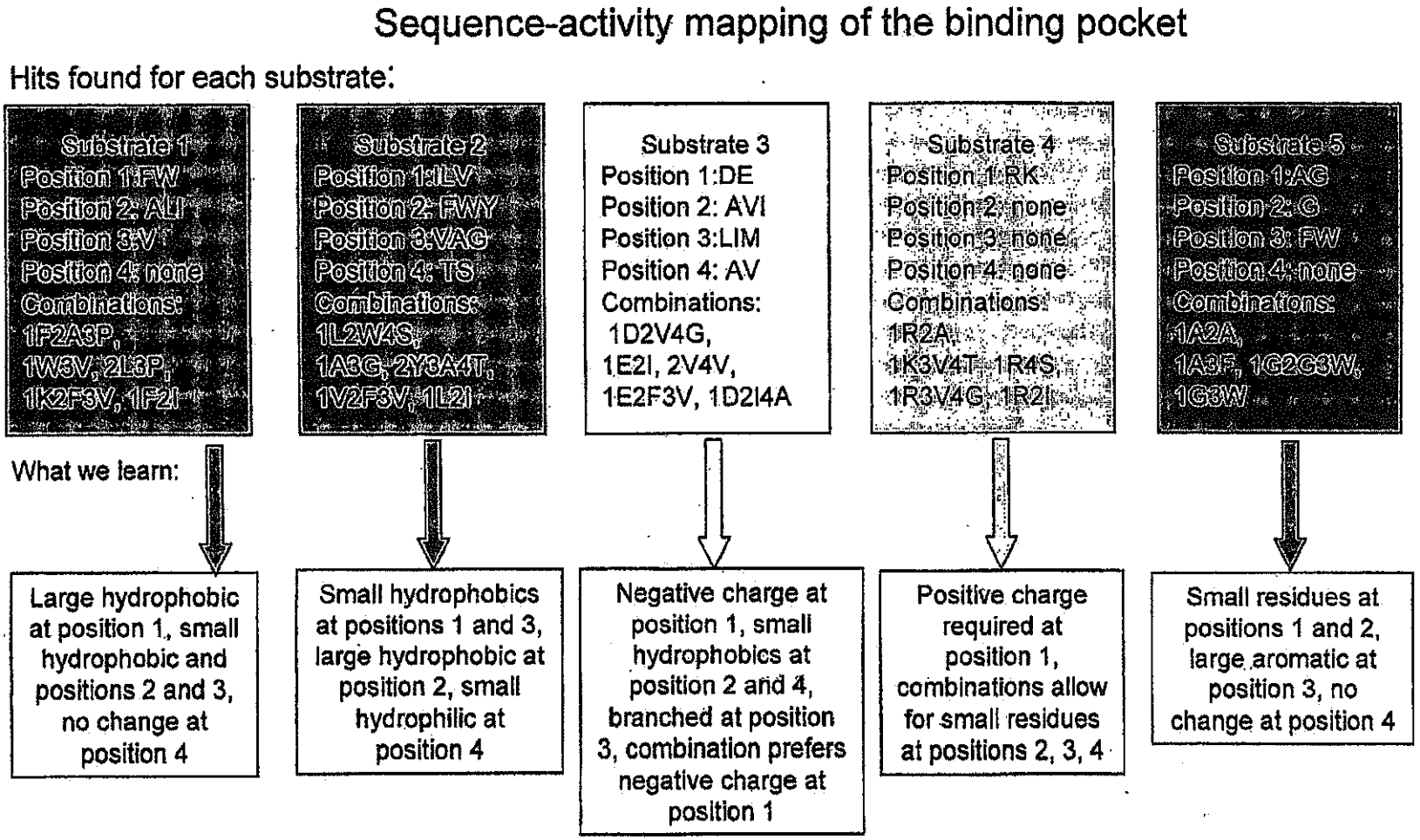

Sequence-activity mapping of the binding pocket
Hits found for each substrate:
Substrate 1
Position 1:FW
Position 2: ALI
Position 3:V
Position 4: none
Combinations:
1F2A3P,
1W3V, 2L3P,
1K2F3V, 1F2I
Substrate 2
Position 1:ILV
Position 2: FWY
Position 3:VAG
Position 4: TS
Combinations:
1L2W4S,
1A3G, 2Y3A4T,
1V2F3V, 1L2I
Substrate 3
Position 1:DE
Position 2: AVI
Position 3:LIM
Position 4: AV
Combinations:
1D2V4G,
1E2I, 2V4V,
1E2F3V, 1D2I4A
Substrate 4
Position 1:RK
Position 2: none
Position 3: none
Position 4: none
Combinations:
1R2A,
1K3V4T, 1R4S,
1R3V4G, 1R2I
Substrate 5
Position 1:AG
Position 2: G
Position 3: FW
Position 4: none
Combinations:
1A2A,
1A3F, 1G2G3W,
1G3W
What we learn:
Large hydrophobic at position 1, small hydrophobic and positions 2 and 3, no change at position 4
Small hydrophobics at positions 1 and 3, large hydrophobic at position 2, small hydrophilic at position 4
Negative charge at position 1, small hydrophobics at position 2 and 4, branched at position 3, combination prefers negative charge at position 1
Positive charge required at position 1, combinations allow for small residues at positions 2, 3, 4
Small residues at positions 1 and 2, large aromatic at position 3, no change at position 4

FIG. 4

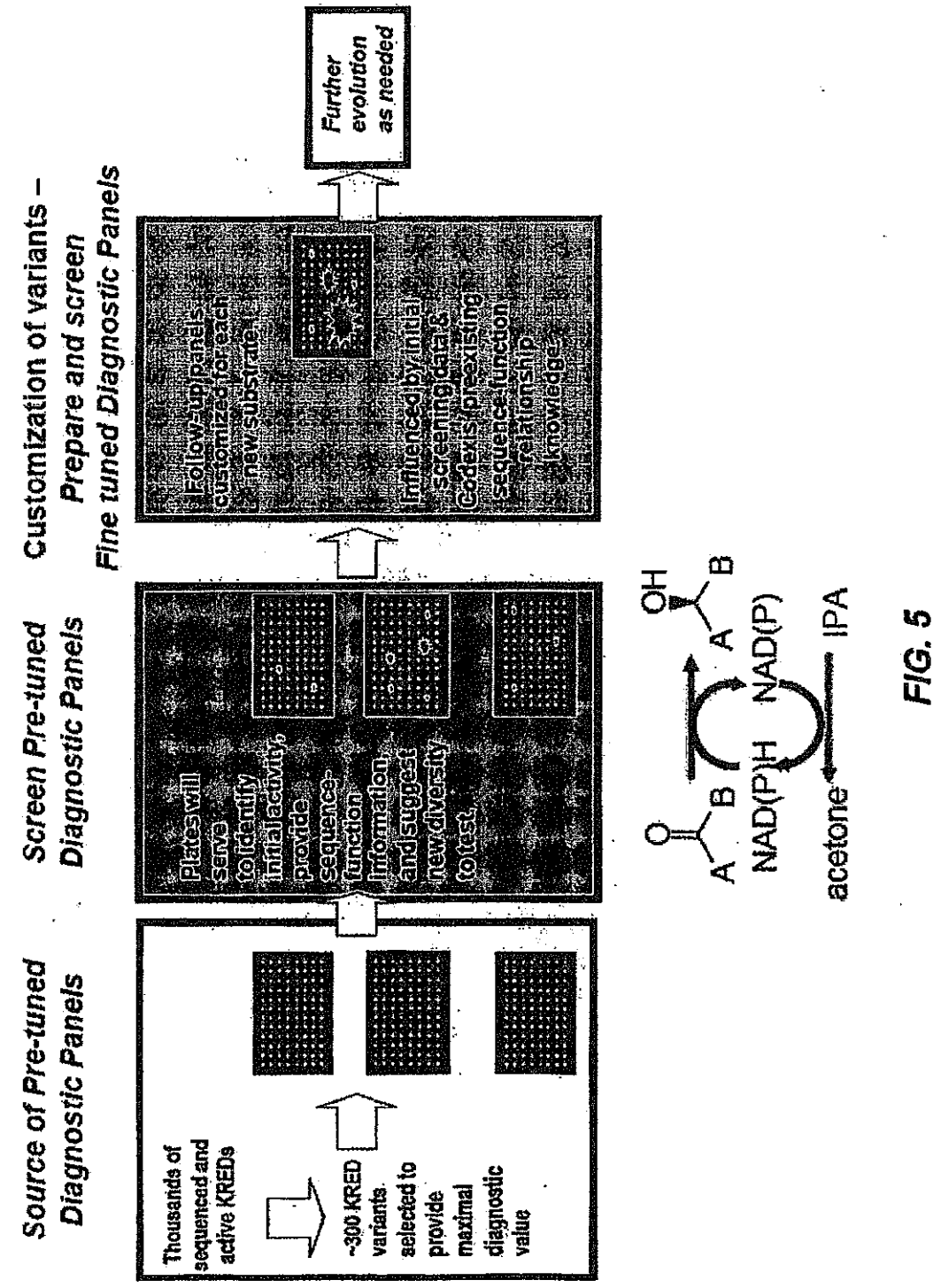

Source of Pre-tuned Diagnostic Panels
Screen Pre-tuned Diagnostic Panels
Customization of variants – Prepare and screen Fine tuned Diagnostic Panels
Thousands of sequenced and active KREDs
~300 KRED variants selected to provide maximal diagnostic value
Plates will serve to identify initial activity, provide sequence function information, and suggest new diversity to test
Follow-up panels customized for each new substrate
Influenced by initial screening data & Codexis pre-existing sequence-function relationship knowledge
Further evolution as needed
O
A
B
OH
A
B
NAD(P)H
NAD(P)
acetone
IPA

FIG. 5

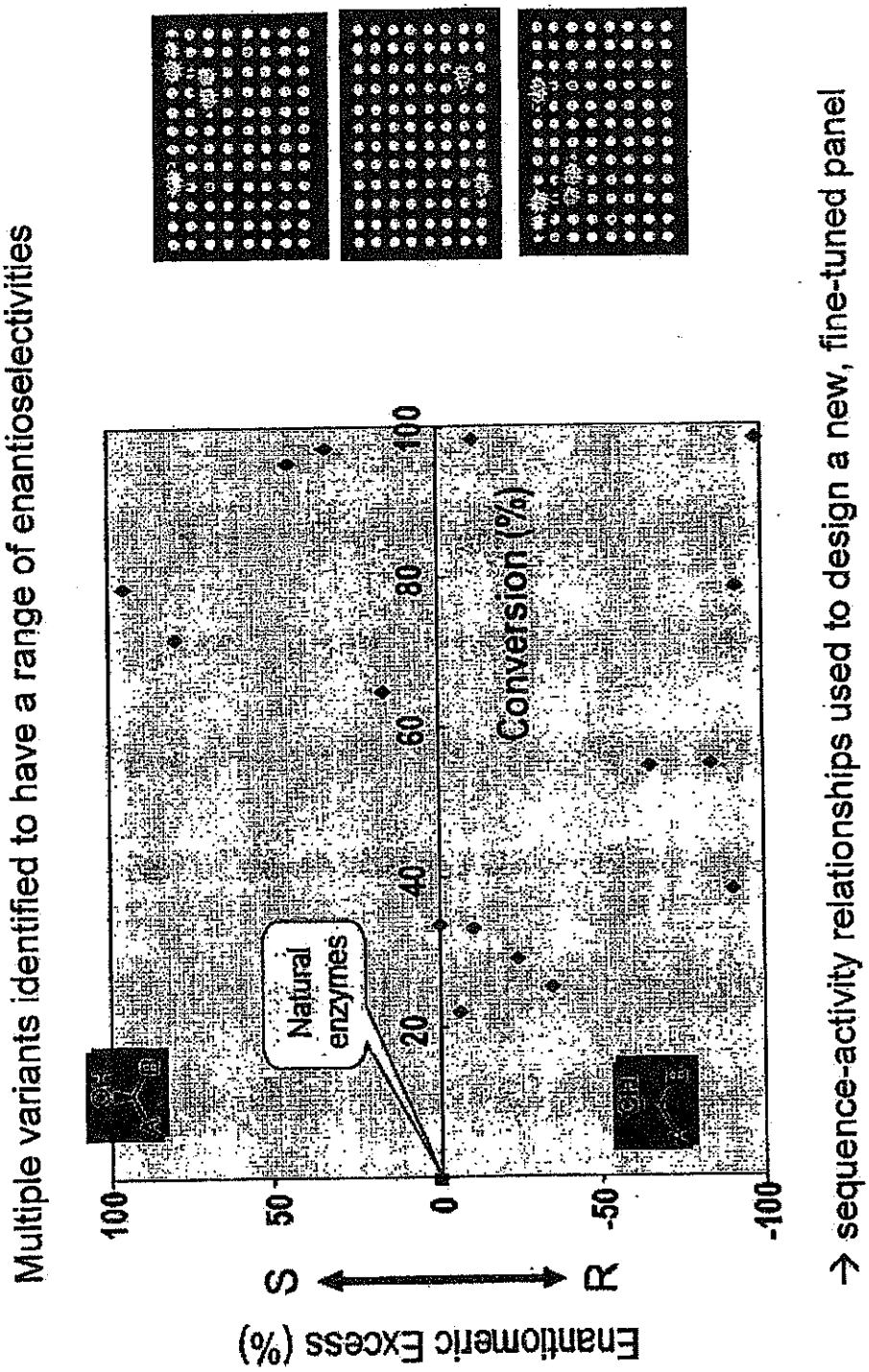

Multiple variants identified to have a range of enantioselectivities
Enantiomeric Excess (%)
S
R
100
50
0
-50
-100
Natural enzymes
Conversion (%)
20
40
60
80
100
→ sequence-activity relationships used to design a new, fine-tuned panel

FIG. 6

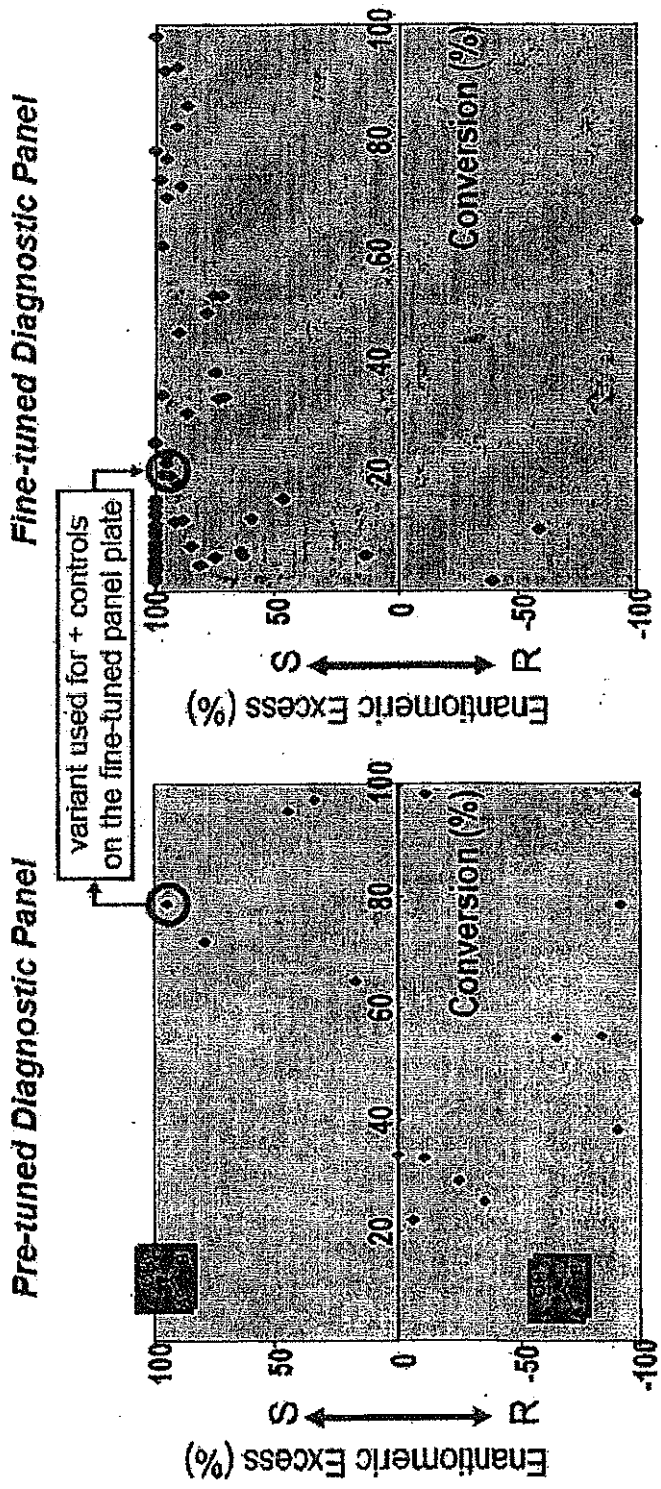

Pre-tuned Diagnostic Panel
Fine-tuned Diagnostic Panel
variant used for + controls on the fine-tuned panel plate
Enantiomeric Excess (%)
S
R
100
50
0
-50
-100
Conversion (%)
20
40
60
80
100
fine-tuned panel is then screened under same or different (e.g., more demanding) conditions

FIG. 7

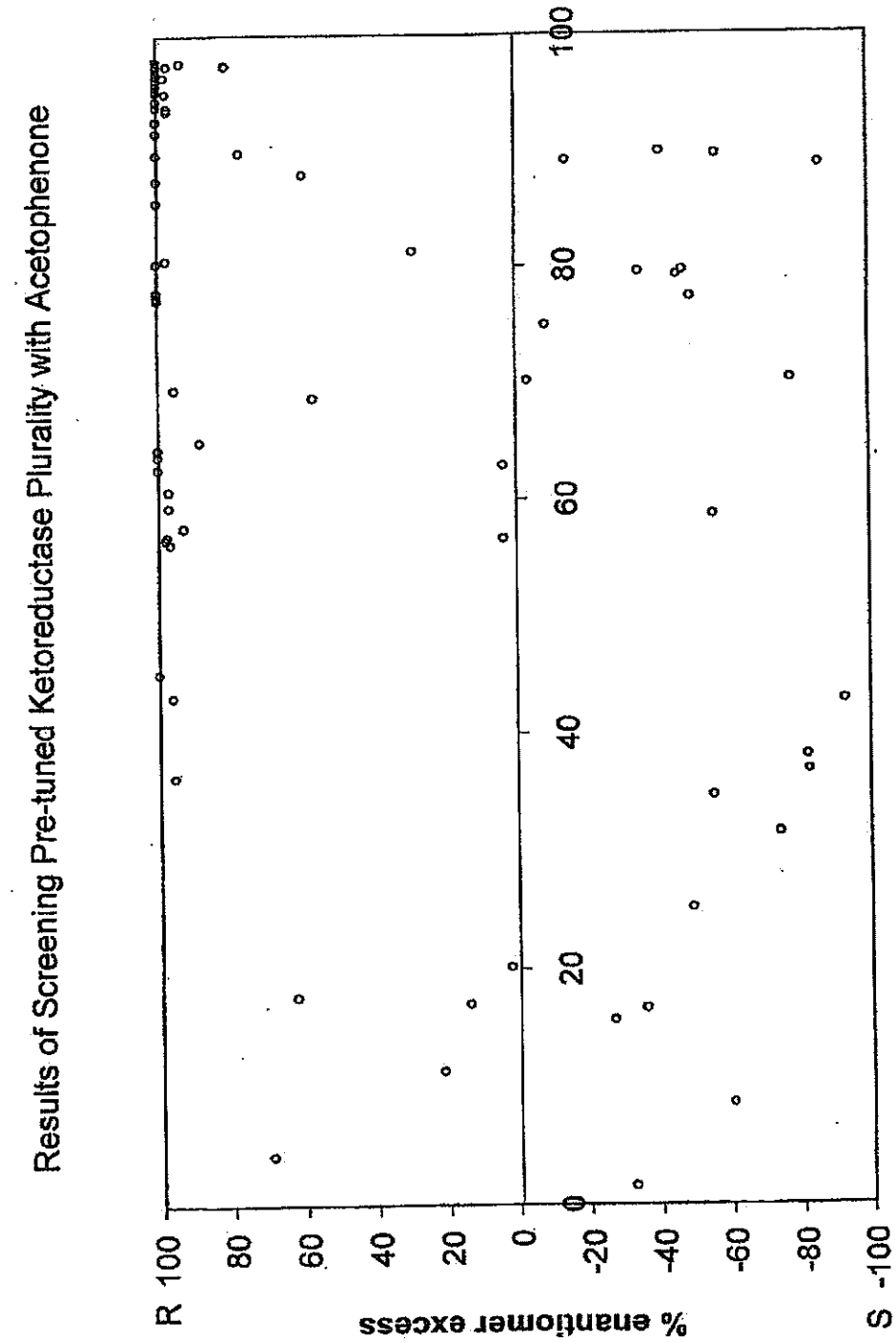

Results of Screening Pre-tuned Ketoreductase Plurality with Acetophenone
R 100
80
60
40
20
0
-20
-40
-60
-80
S -100
% enantiomer excess
0
20
40
60
80
100
% conversion

FIG. 8

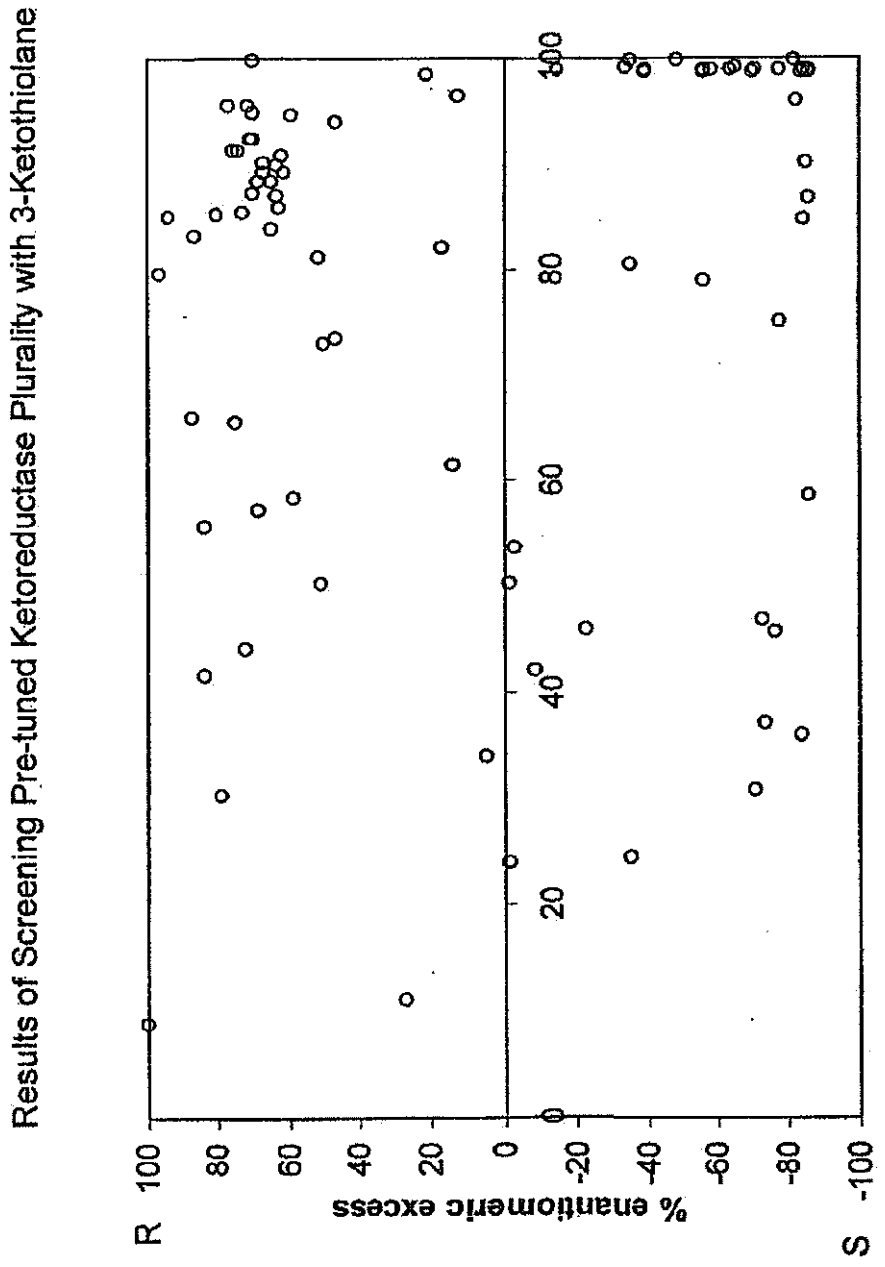

Results of Screening Pre-tuned Ketoreductase Plurality with 3-Ketothiolane
R
S
% enantiomeric excess
% conversion
100
80
60
40
20
0
-20
-40
-60
-80
-100
20
40
60
80
100

FIG. 9

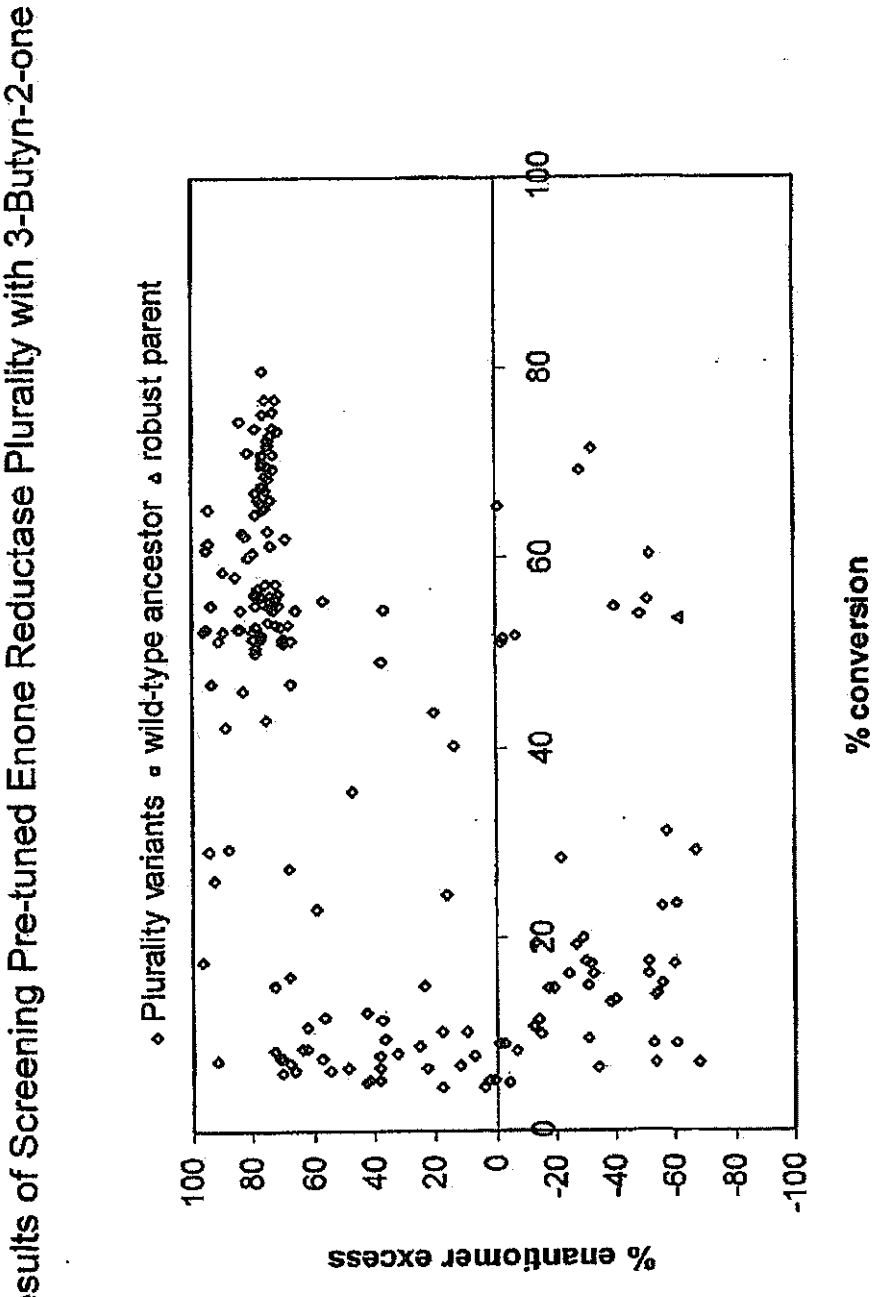

Results of Screening Pre-tuned Enone Reductase Plurality with 3-Butyn-2-one
◇ Plurality variants ▫ wild-type ancestor ▵ robust parent
% enantiomer excess
100
80
60
40
20
0
-20
-40
-60
-80
-100
0
20
40
60
80
100
% conversion

FIG. 10

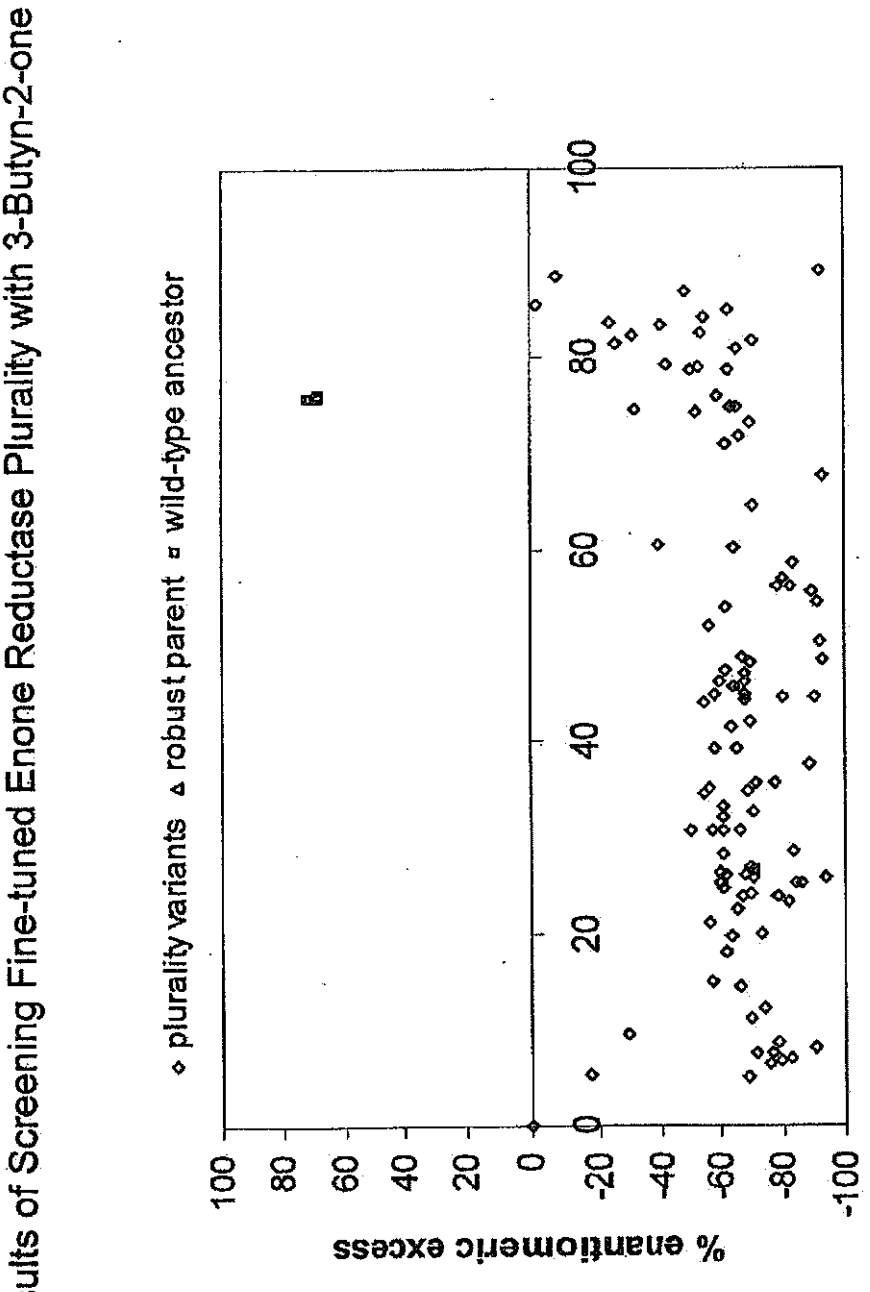

Results of Screening Fine-tuned Enone Reductase Plurality with 3-Butyn-2-one
plurality variants
robust parent
wild-type ancestor
% enantiomeric excess
100
80
60
40
20
0
-20
-40
-60
-80
-100
%Conversion

FIG. 11

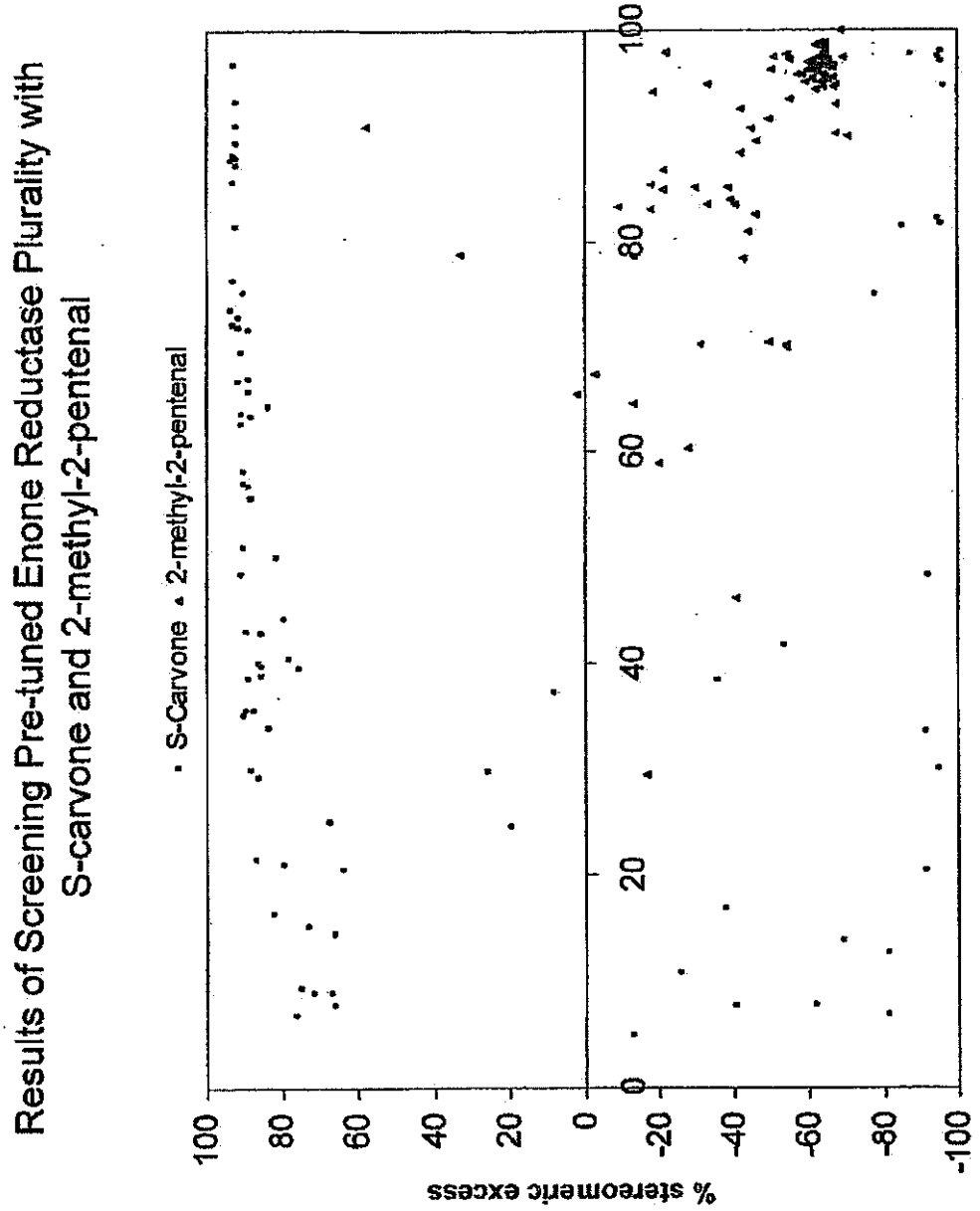

Results of Screening Pre-tuned Enone Reductase Plurality with S-carvone and 2-methyl-2-pentenal
S-Carvone
2-methyl-2-pentenal
% stereomeric excess
% conversion
100
80
60
40
20
0
-20
-40
-60
-80
-100
0
20
40
60
80
100

FIG. 12

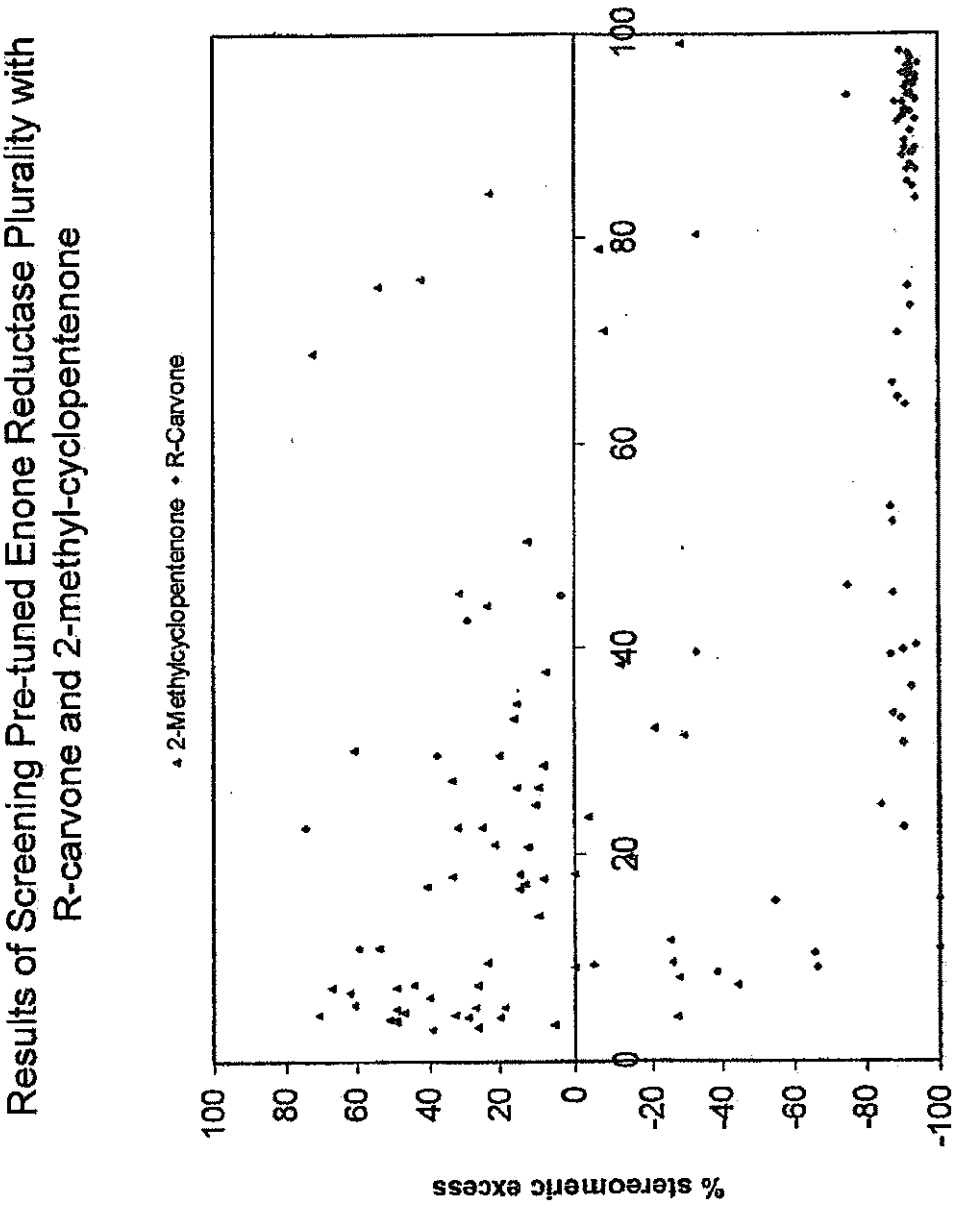

Results of Screening Pre-tuned Enone Reductase Plurality with R-carvone and 2-methyl-cyclopentenone
▲ 2-Methylcyclopentenone ♦ R-Carvone
% stereomeric excess
% conversion
100
80
60
40
20
0
-20
-40
-60
-80
-100
0
20
40
60
80
100

FIG. 13

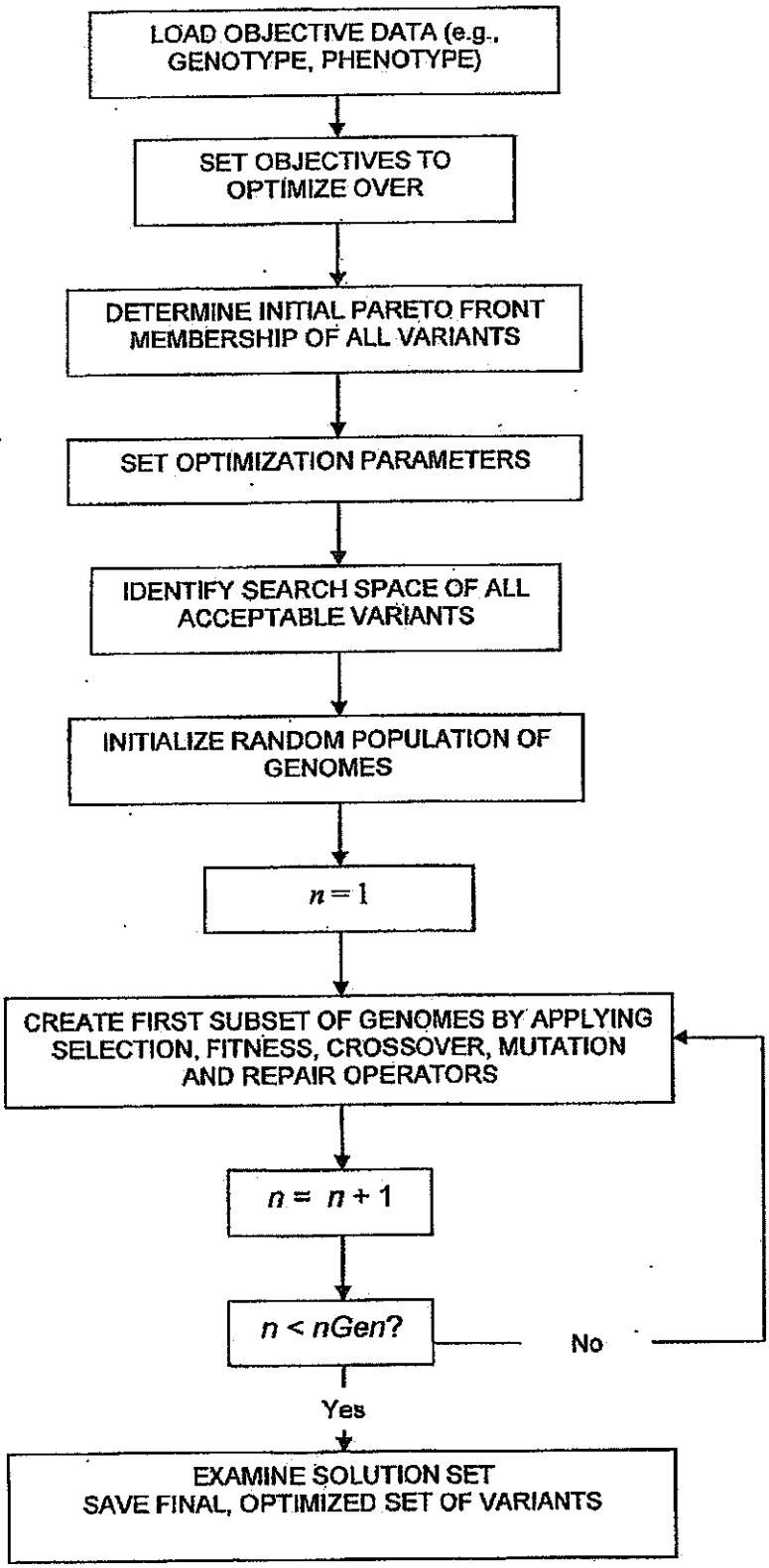

LOAD OBJECTIVE DATA (e.g., GENOTYPE, PHENOTYPE)
SET OBJECTIVES TO OPTIMIZE OVER
DETERMINE INITIAL PARETO FRONT MEMBERSHIP OF ALL VARIANTS
SET OPTIMIZATION PARAMETERS
IDENTIFY SEARCH SPACE OF ALL ACCEPTABLE VARIANTS
INITIALIZE RANDOM POPULATION OF GENOMES
n = 1
CREATE FIRST SUBSET OF GENOMES BY APPLYING SELECTION, FITNESS, CROSSOVER, MUTATION AND REPAIR OPERATORS
n = n + 1
n < nGen?
No
Yes
EXAMINE SOLUTION SET
SAVE FINAL, OPTIMIZED SET OF VARIANTS

FIG 14

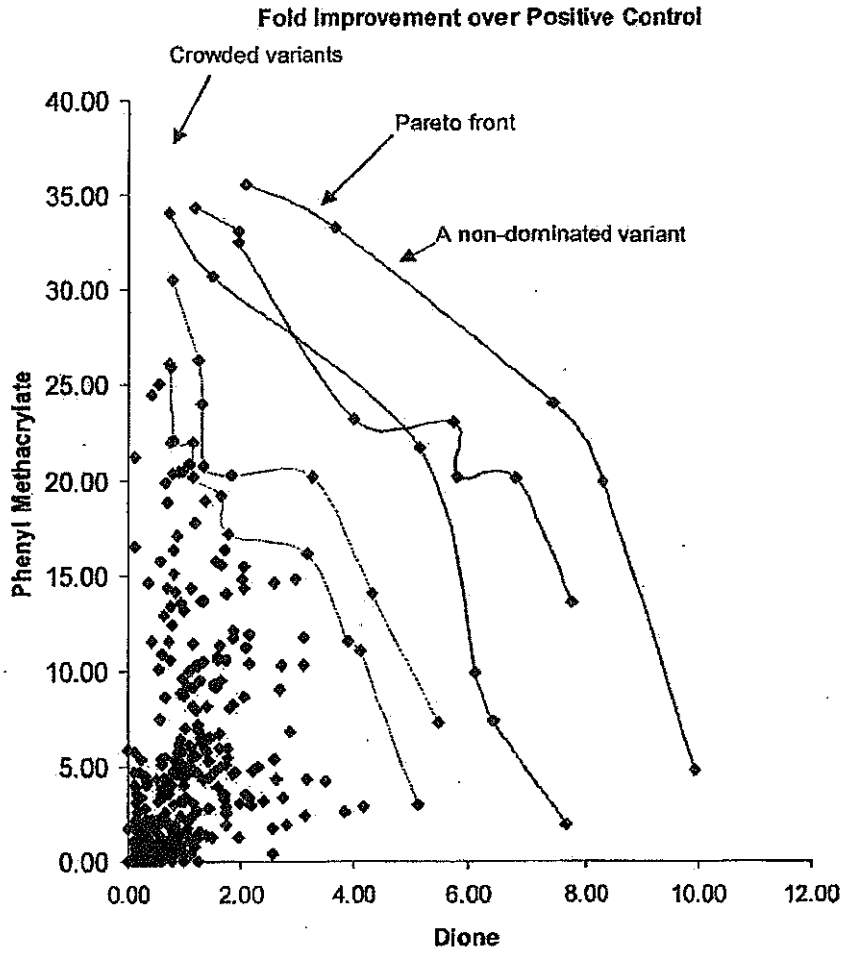

Fold Improvement over Positive Control
Crowded variants
Pareto front
A non-dominated variant
Phenyl Methacrylate
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
0.00
2.00
4.00
6.00
8.00
10.00
12.00
Dione

FIG 15

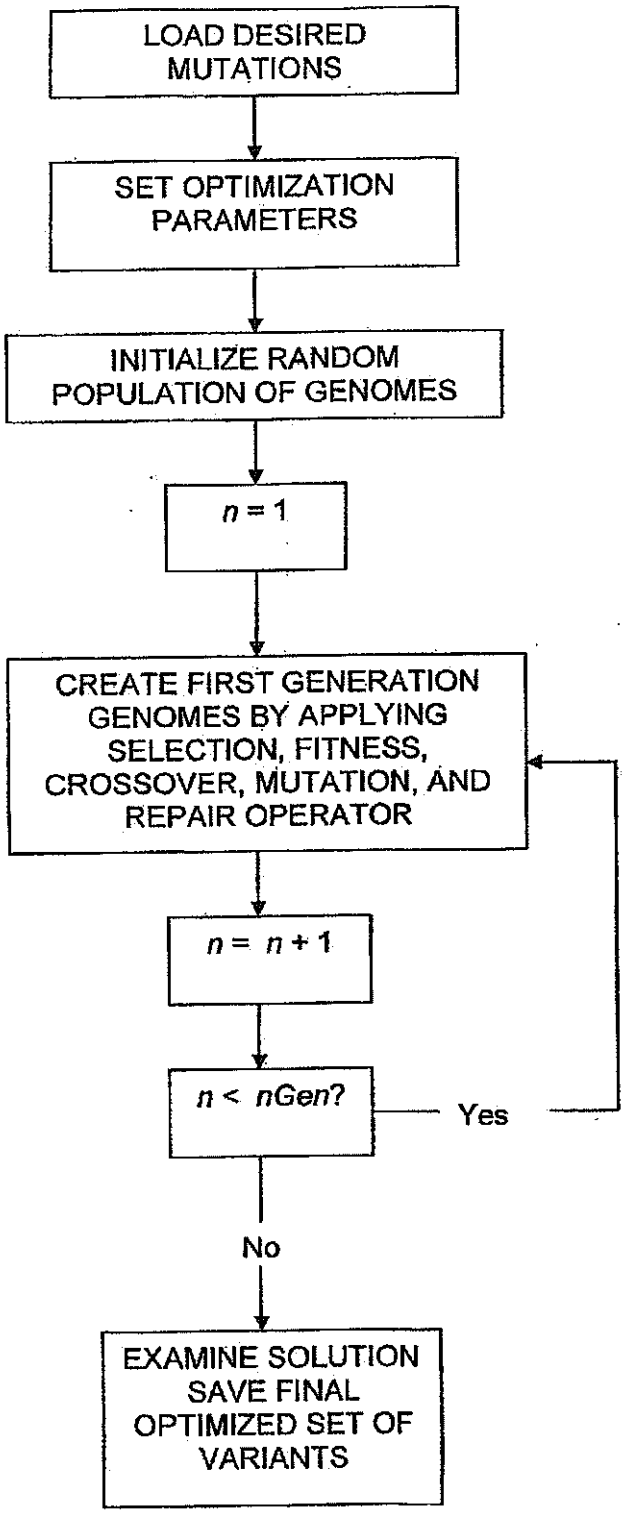

LOAD DESIRED MUTATIONS
SET OPTIMIZATION PARAMETERS
INITIALIZE RANDOM POPULATION OF GENOMES
n = 1
CREATE FIRST GENERATION GENOMES BY APPLYING SELECTION, FITNESS, CROSSOVER, MUTATION, AND REPAIR OPERATOR
n = n + 1
n < nGen?
Yes
No
EXAMINE SOLUTION SAVE FINAL OPTIMIZED SET OF VARIANTS

FIG 16