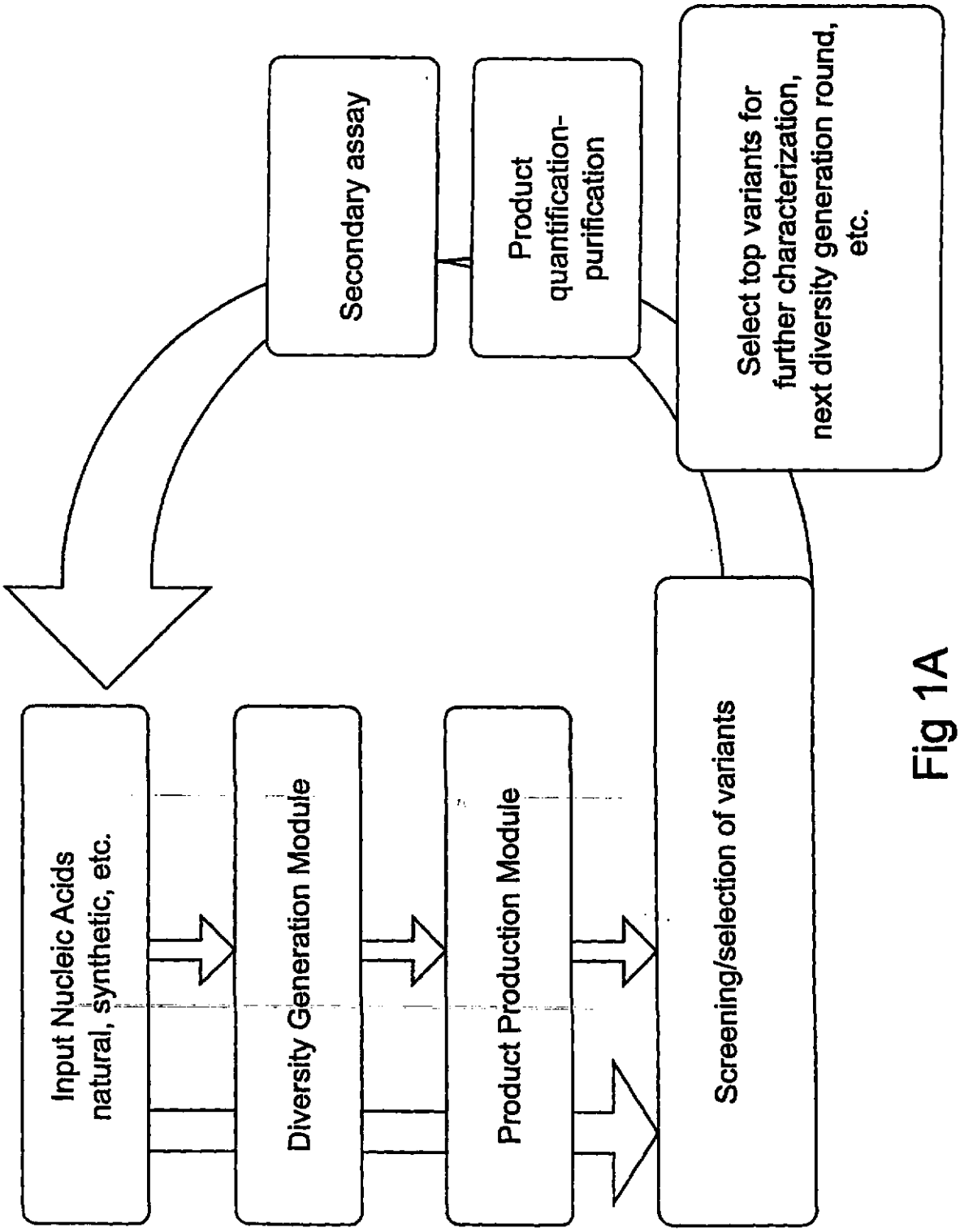

Input Nucleic Acids
natural, synthetic, etc.
Diversity Generation Module
Product Production Module
Screening/selection of variants
Secondary assay
Product quantification-purification
Select top variants for further characterization, next diversity generation round, etc.

Fig 1A

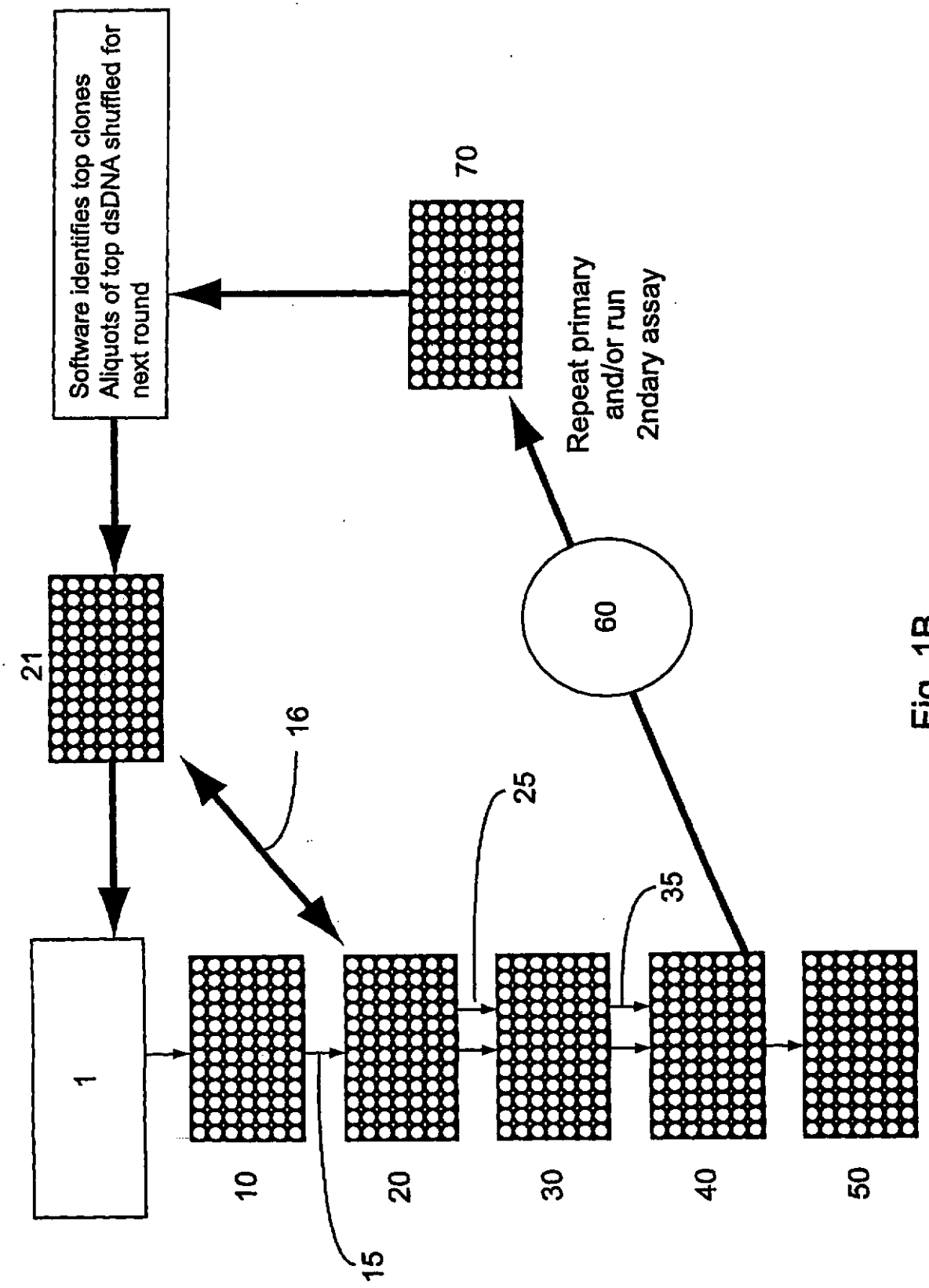

Software identifies top clones
Aliquots of top dsDNA shuffled for next round
70
Repeat primary and/or run 2ndary assay
60
21
16
25
35
1
10
15
20
30
40
50

Fig. 1B

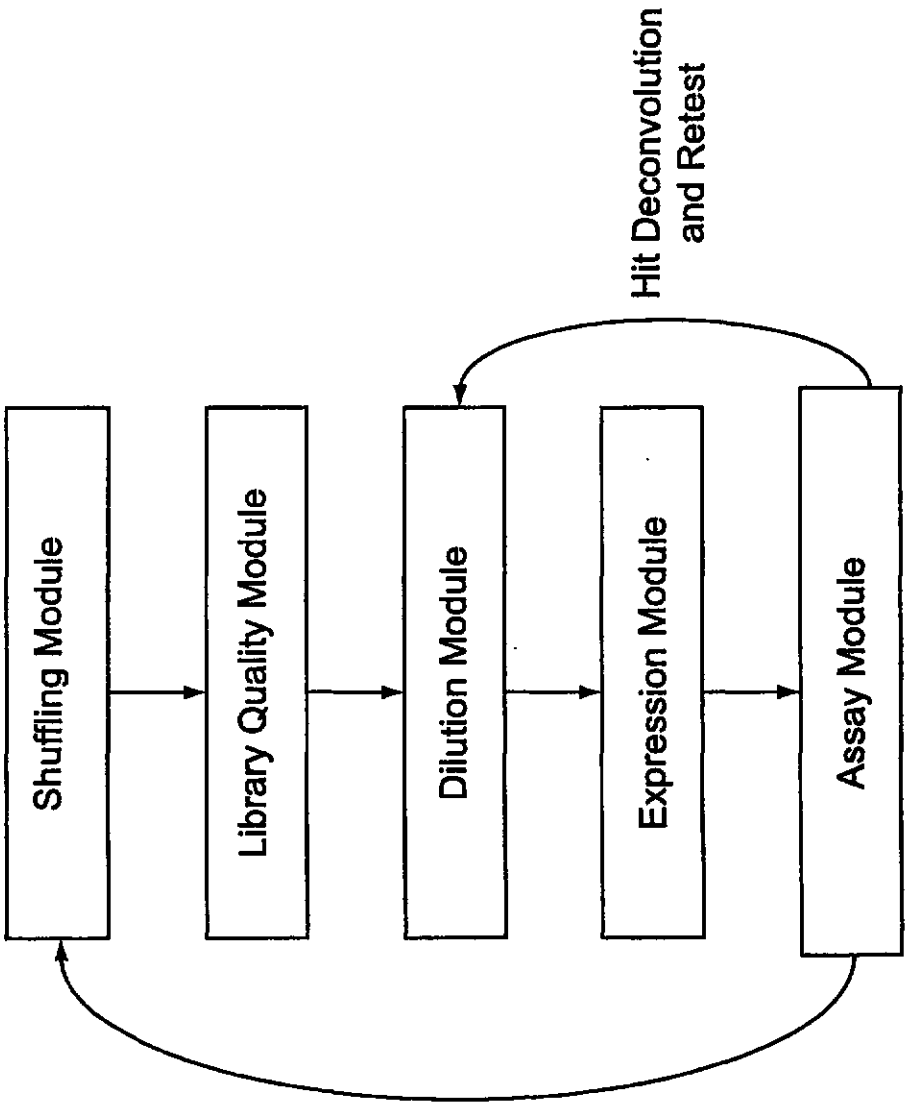

Hit Deconvolution and Retest
Shuffling Module
Library Quality Module
Dilution Module
Expression Module
Assay Module

Fig. 2

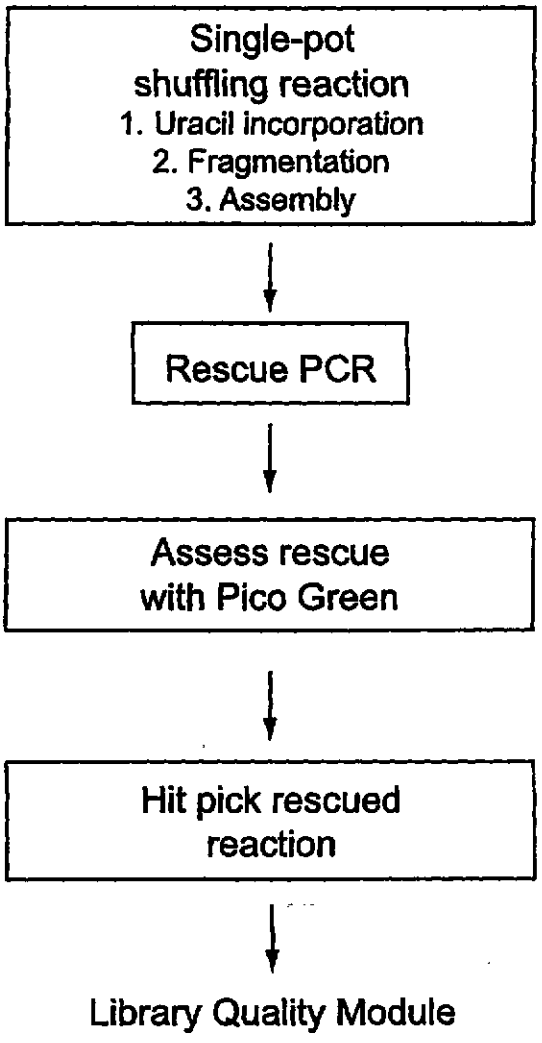

Single-pot
shuffling reaction
1. Uracil incorporation
2. Fragmentation
3. Assembly
Rescue PCR
Assess rescue
with Pico Green
Hit pick rescued
reaction
Library Quality Module

Fig. 3

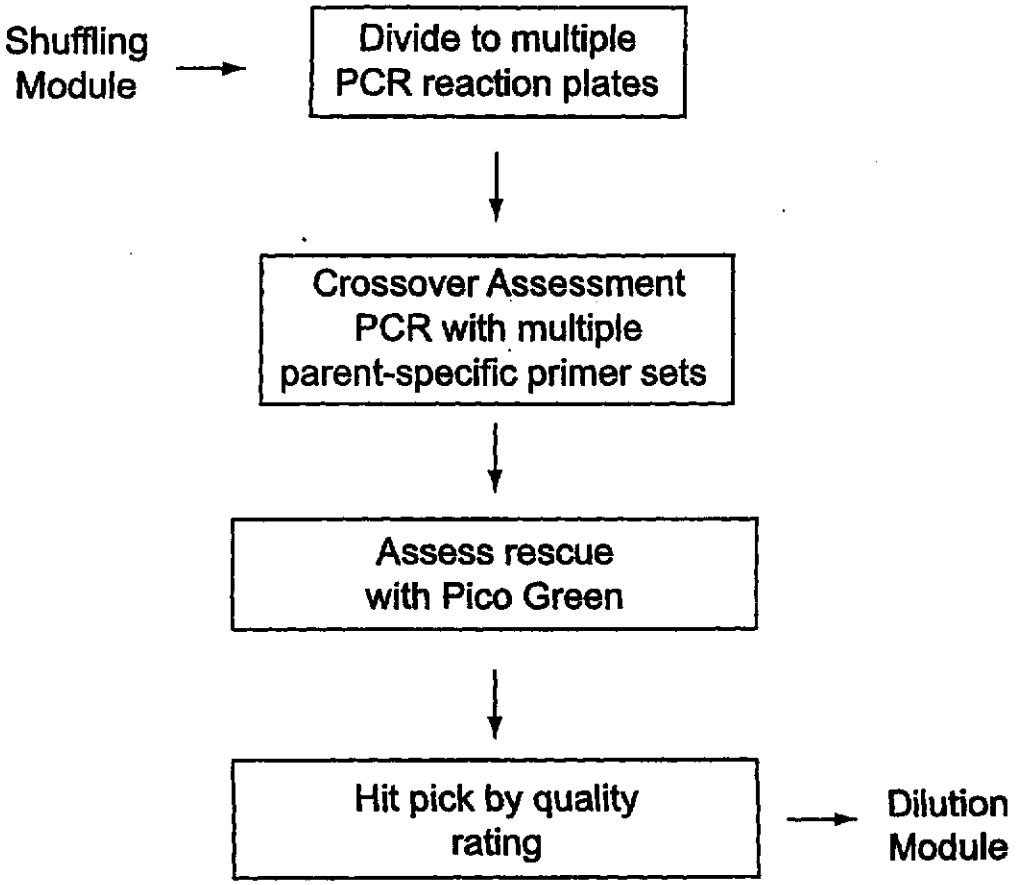

Shuffling Module
Divide to multiple PCR reaction plates
Crossover Assessment PCR with multiple parent-specific primer sets
Assess rescue with Pico Green
Hit pick by quality rating
Dilution Module

Fig. 4

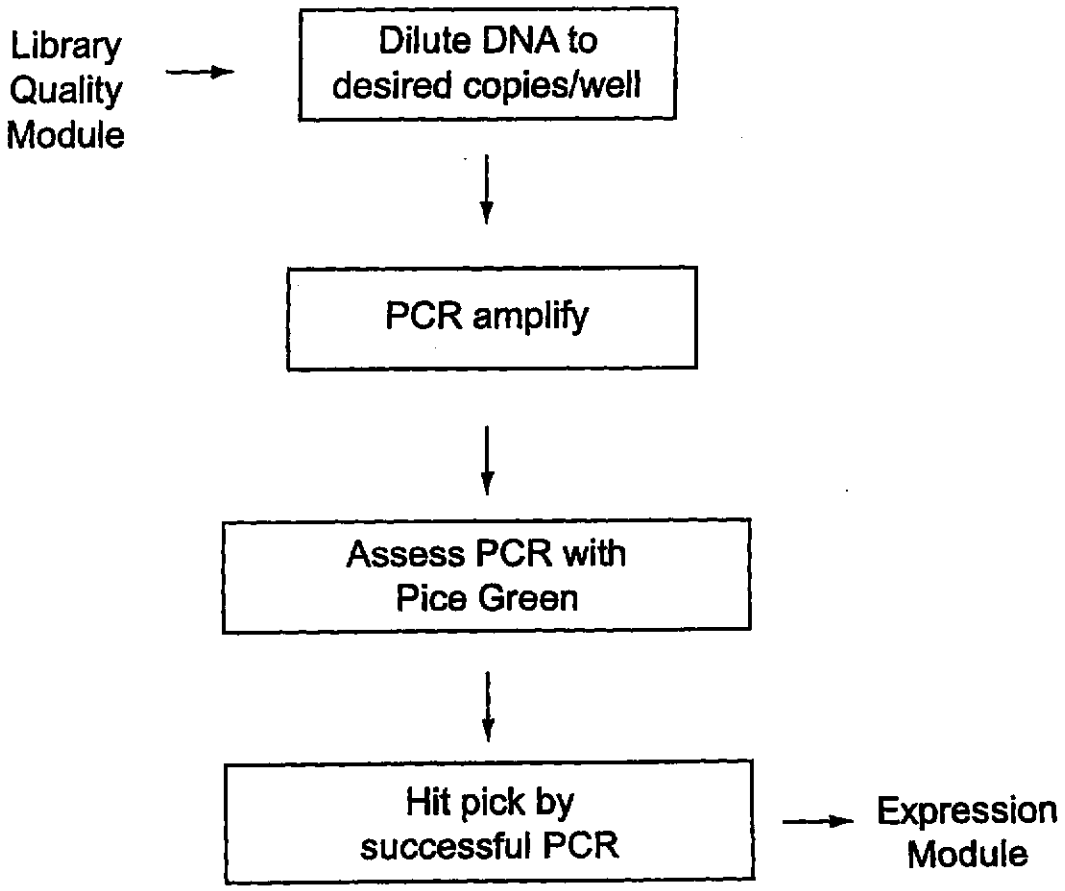

Library Quality Module
Dilute DNA to desired copies/well
PCR amplify
Assess PCR with Pice Green
Hit pick by successful PCR
Expression Module

Fig. 5

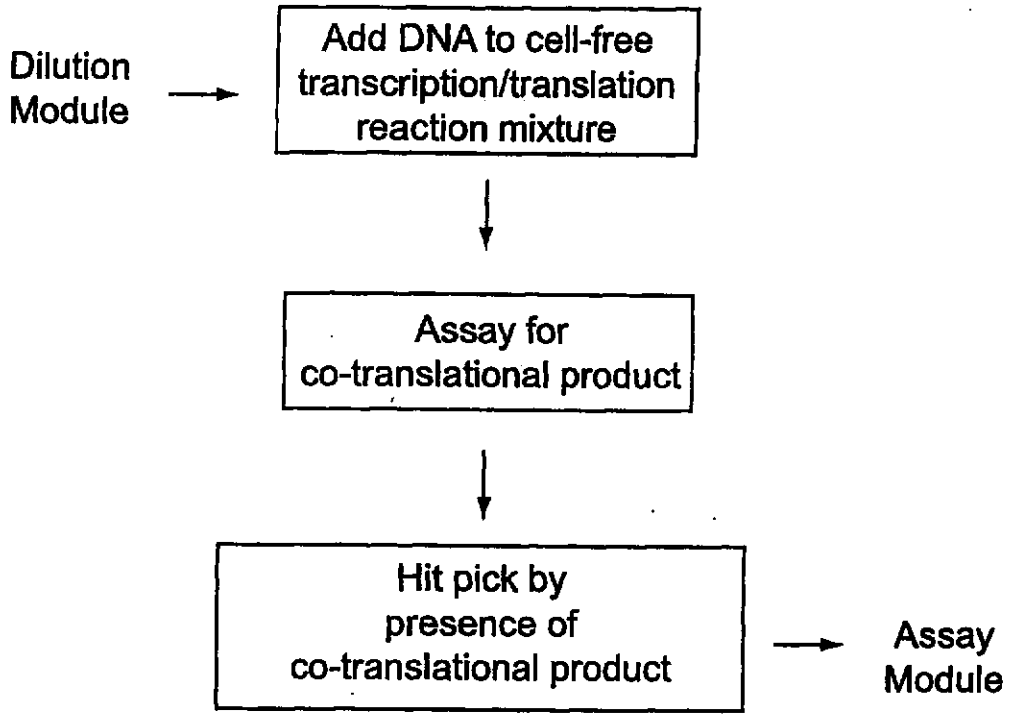

Dilution Module
Add DNA to cell-free transcription/translation reaction mixture
Assay for co-translational product
Hit pick by presence of co-translational product
Assay Module

Fig. 6

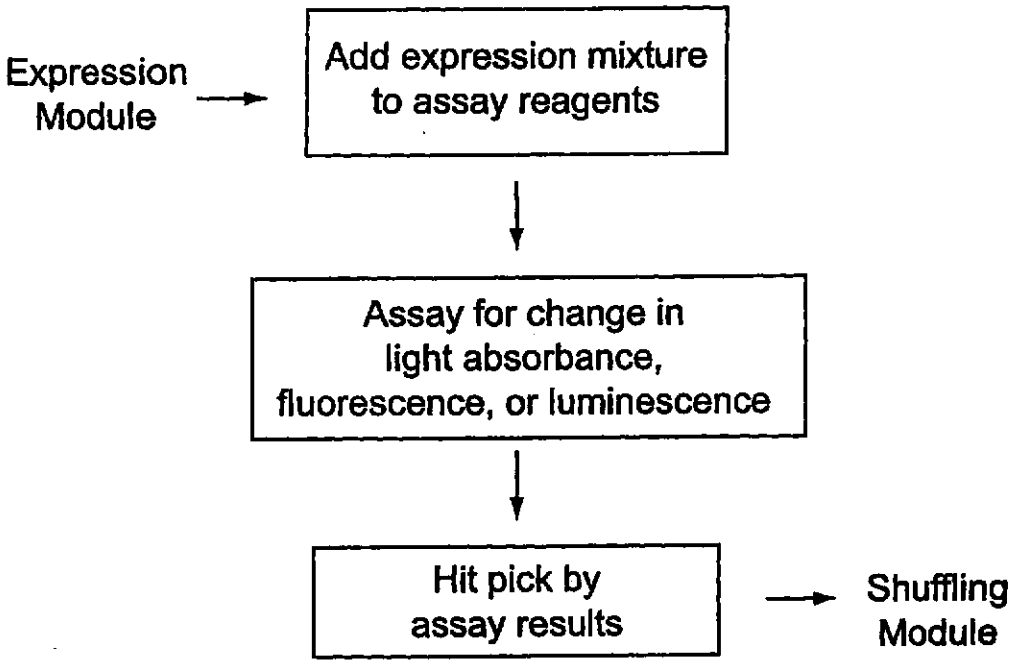

Expression Module
Add expression mixture to assay reagents
Assay for change in light absorbance, fluorescence, or luminescence
Hit pick by assay results
Shuffling Module

Fig. 7

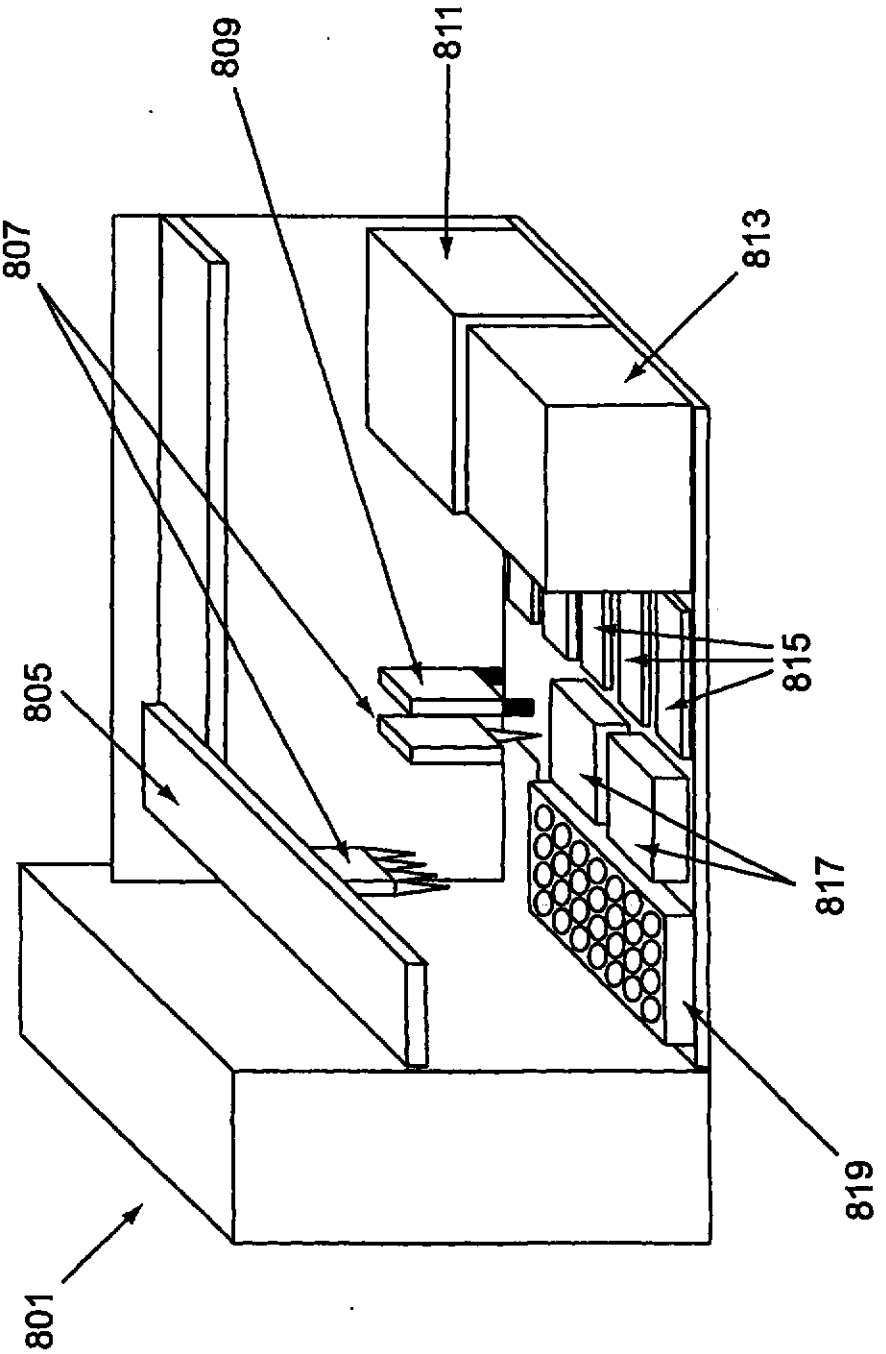


Fig. 8

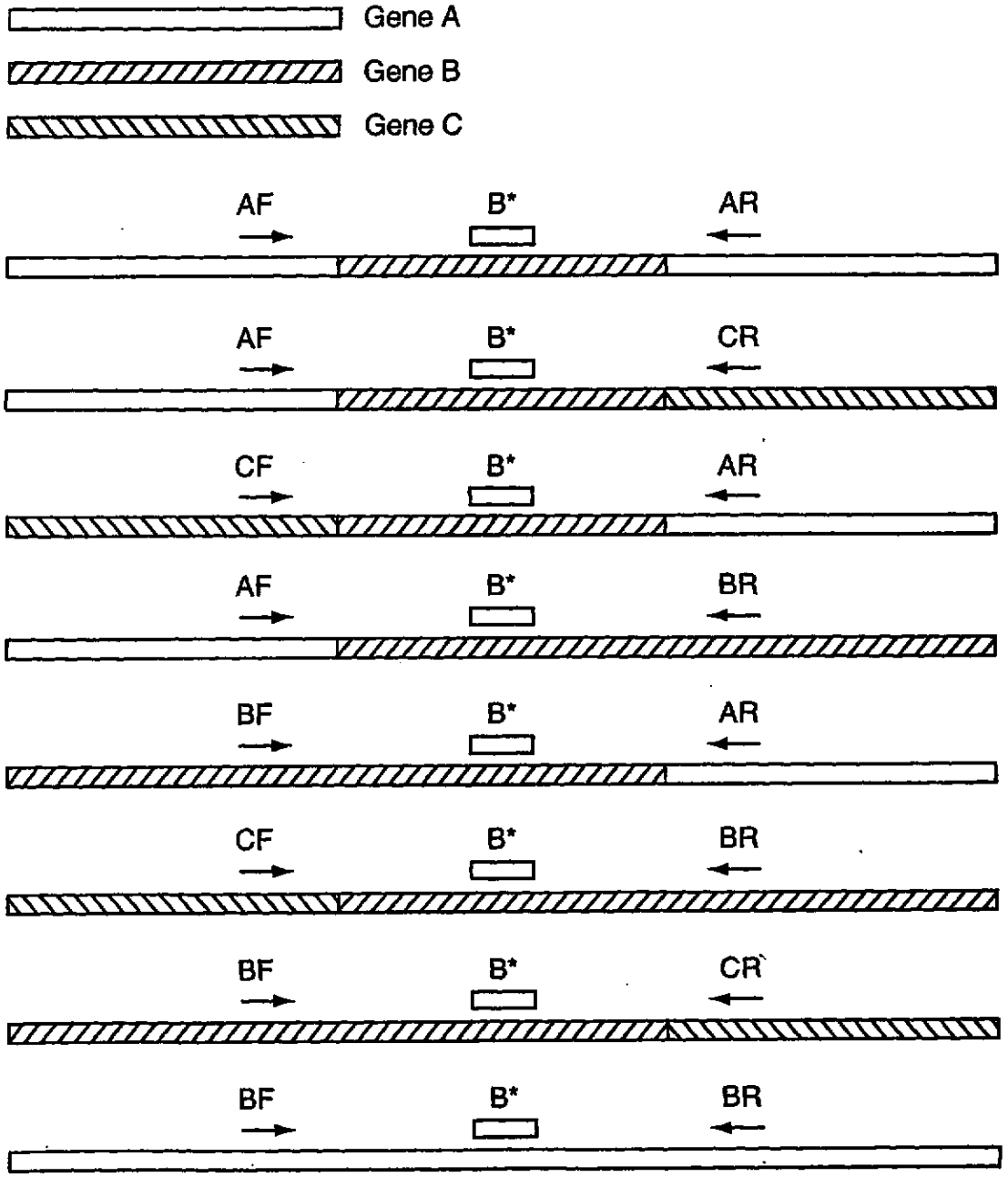
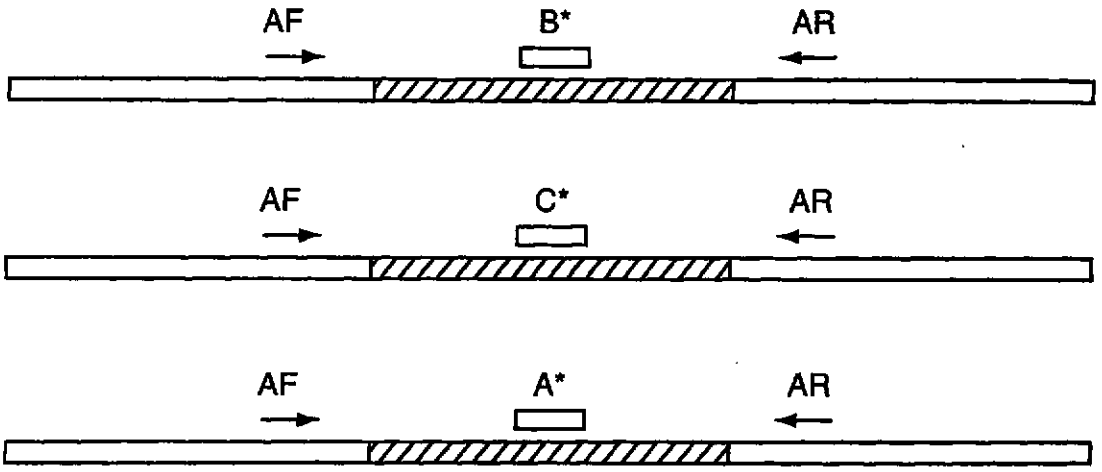

Gene A
Gene B
Gene C
AF
B*
AR
AF
B*
CR
CF
B*
AR
AF
B*
BR
BF
B*
AR
CF
B*
BR
BF
B*
CR
BF
B*
BR

Fig. 9A



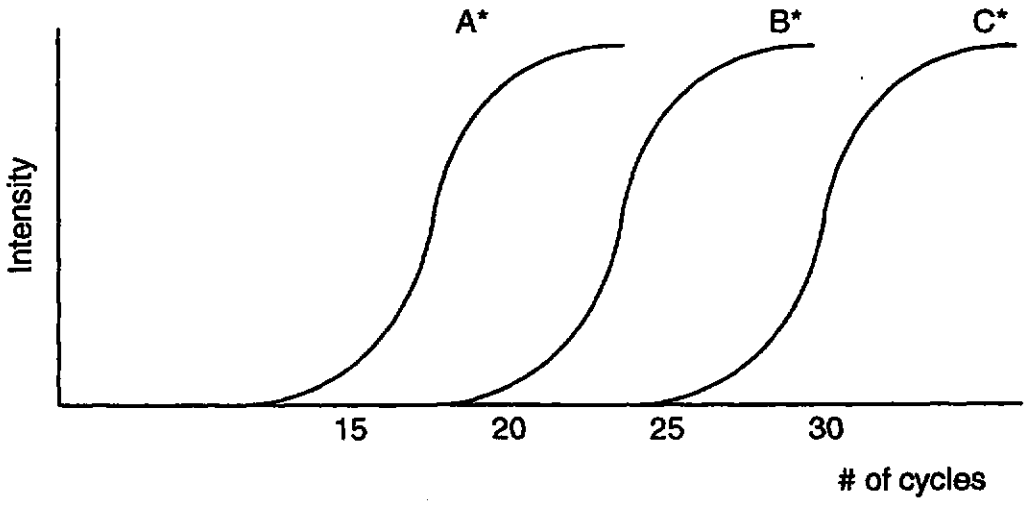


Fig. 9B

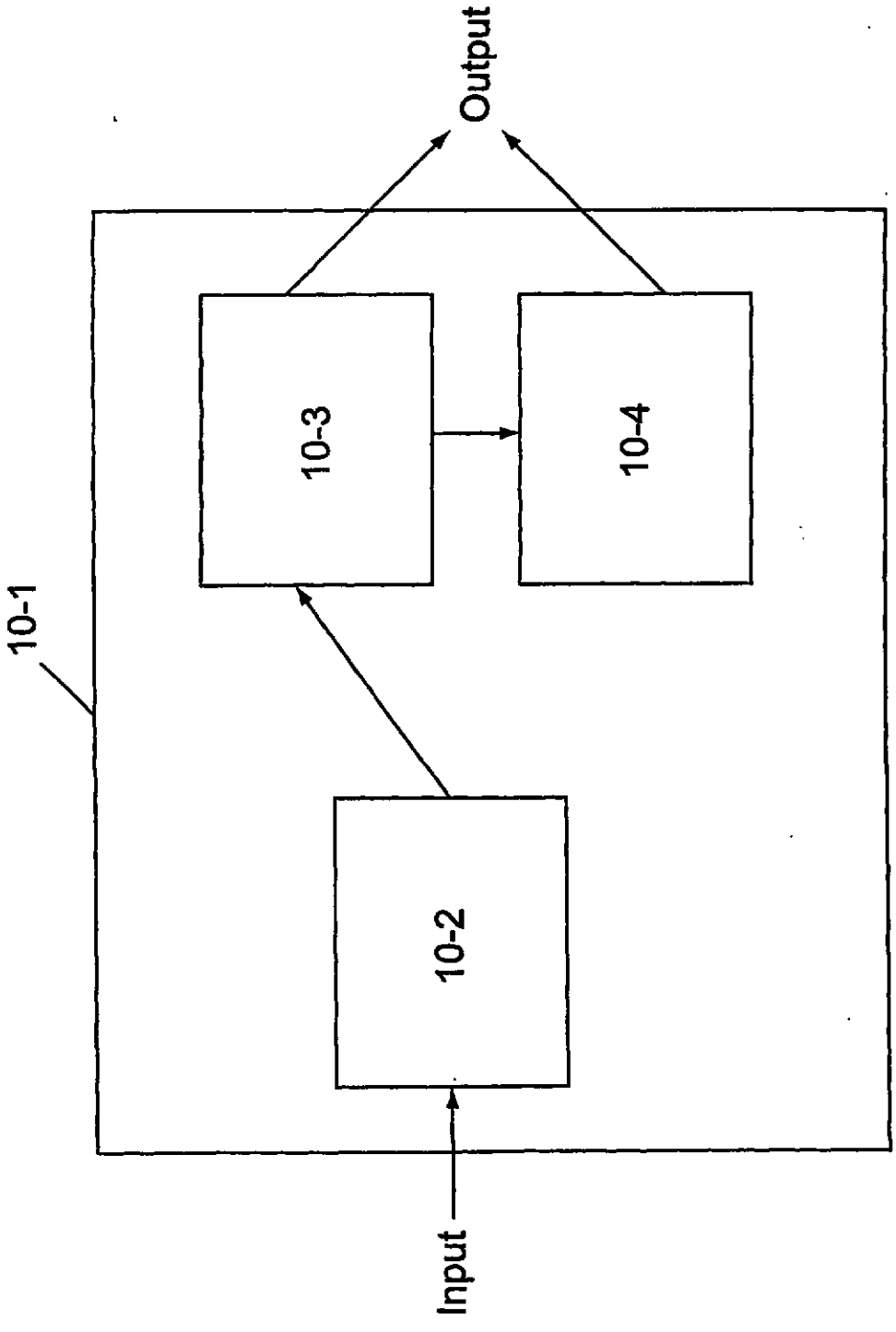


Fig. 10

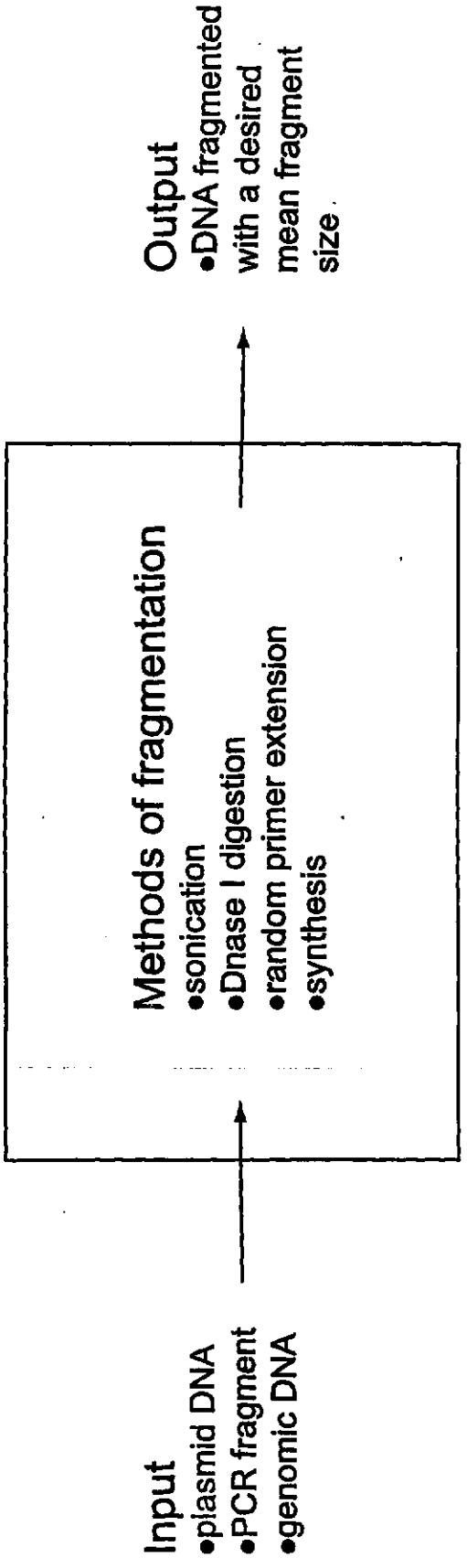

Input
•plasmid DNA
•PCR fragment
•genomic DNA
Methods of fragmentation
•sonication
•Dnase I digestion
•random primer extension
•synthesis
Output
•DNA fragmented with a desired mean fragment size

Fig. 11

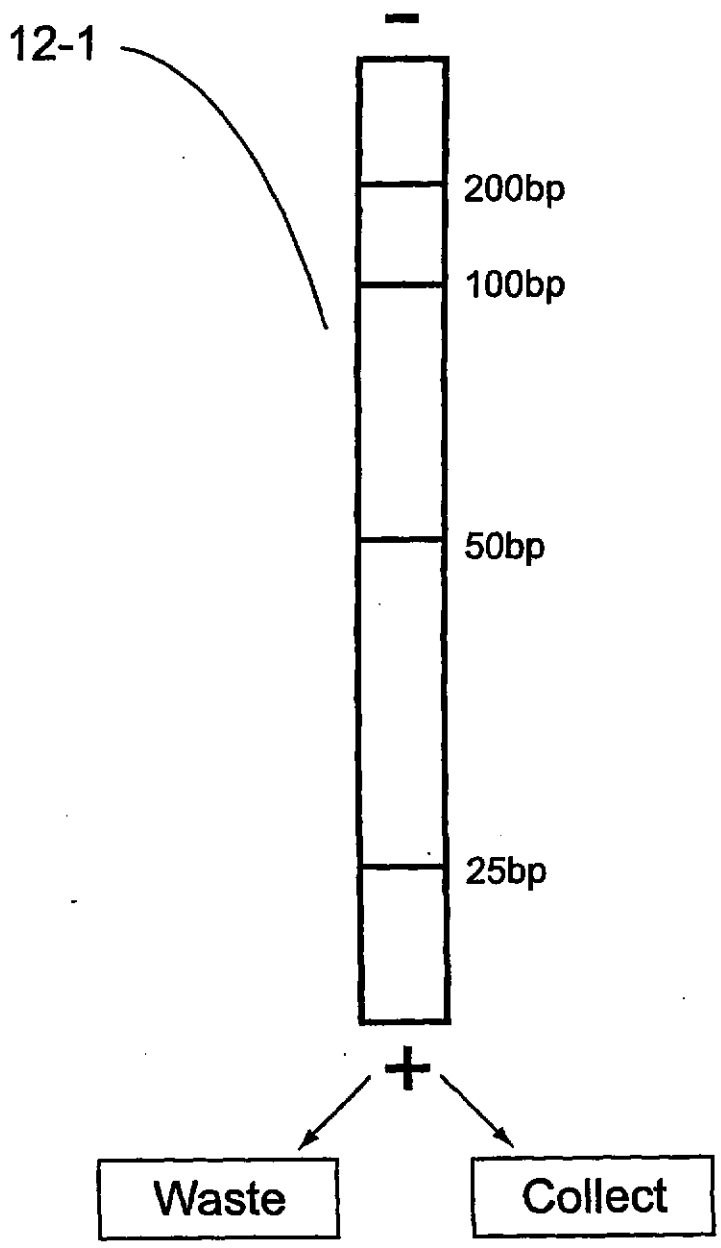


Fig. 12

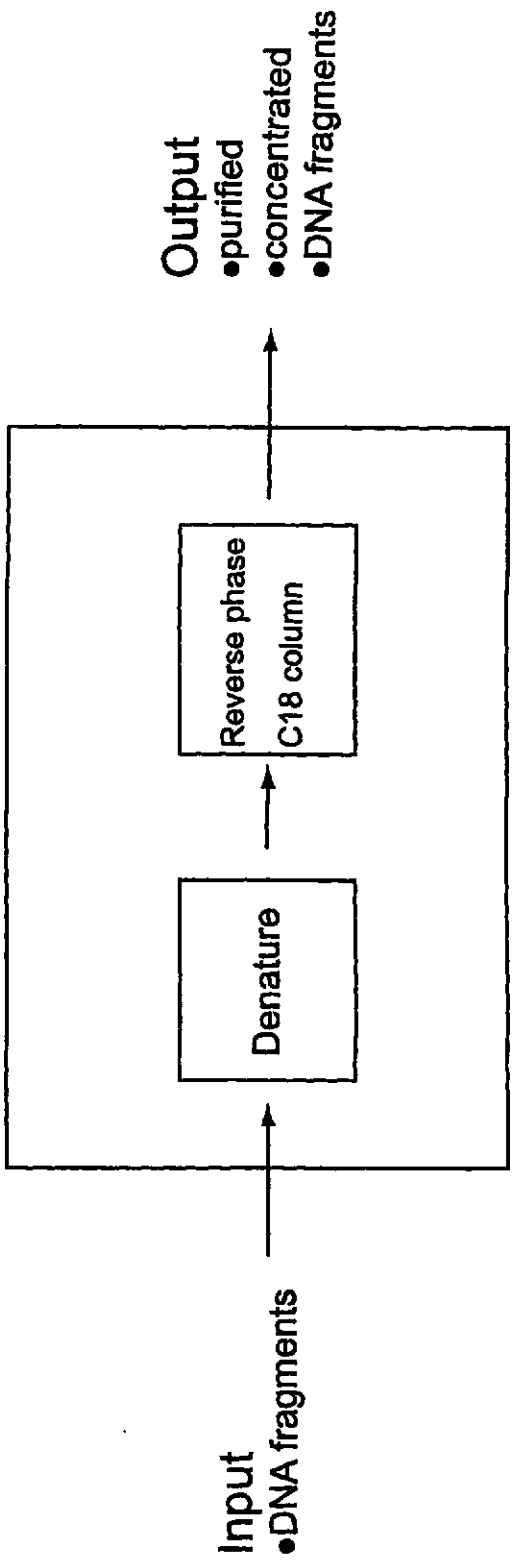

Output
•purified
•concentrated
•DNA fragments
Reverse phase
C18 column
Denature
Input
•DNA fragments

Fig. 13

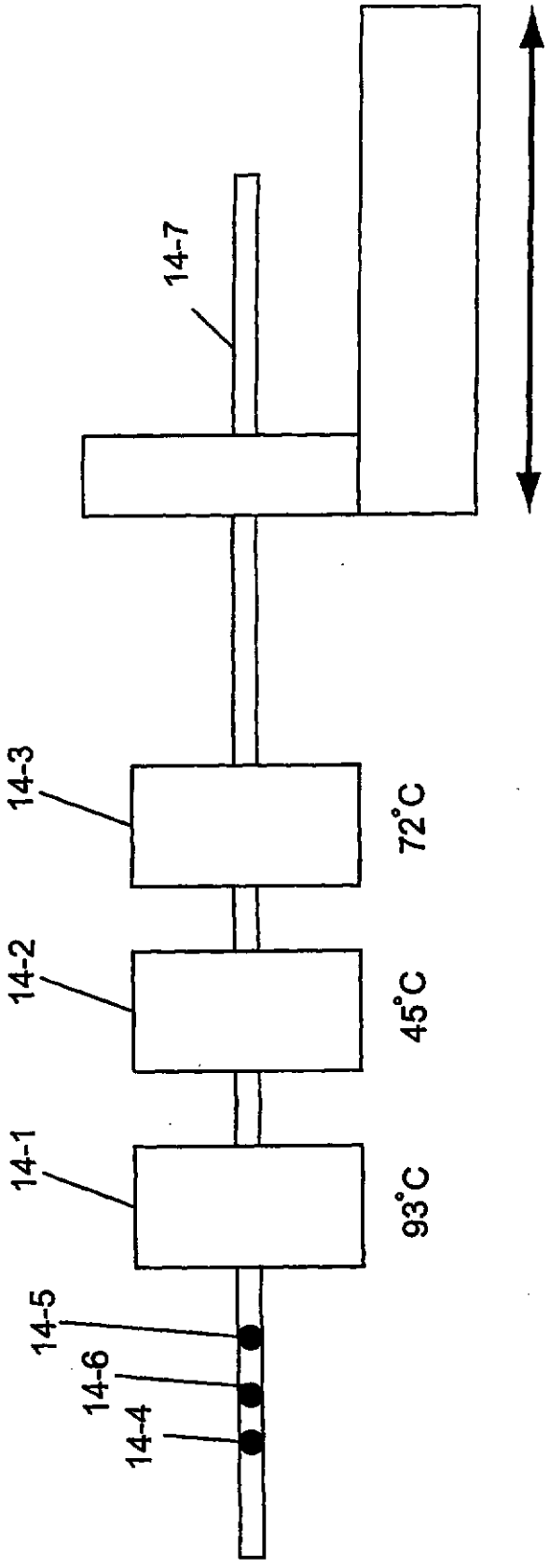


Fig. 14

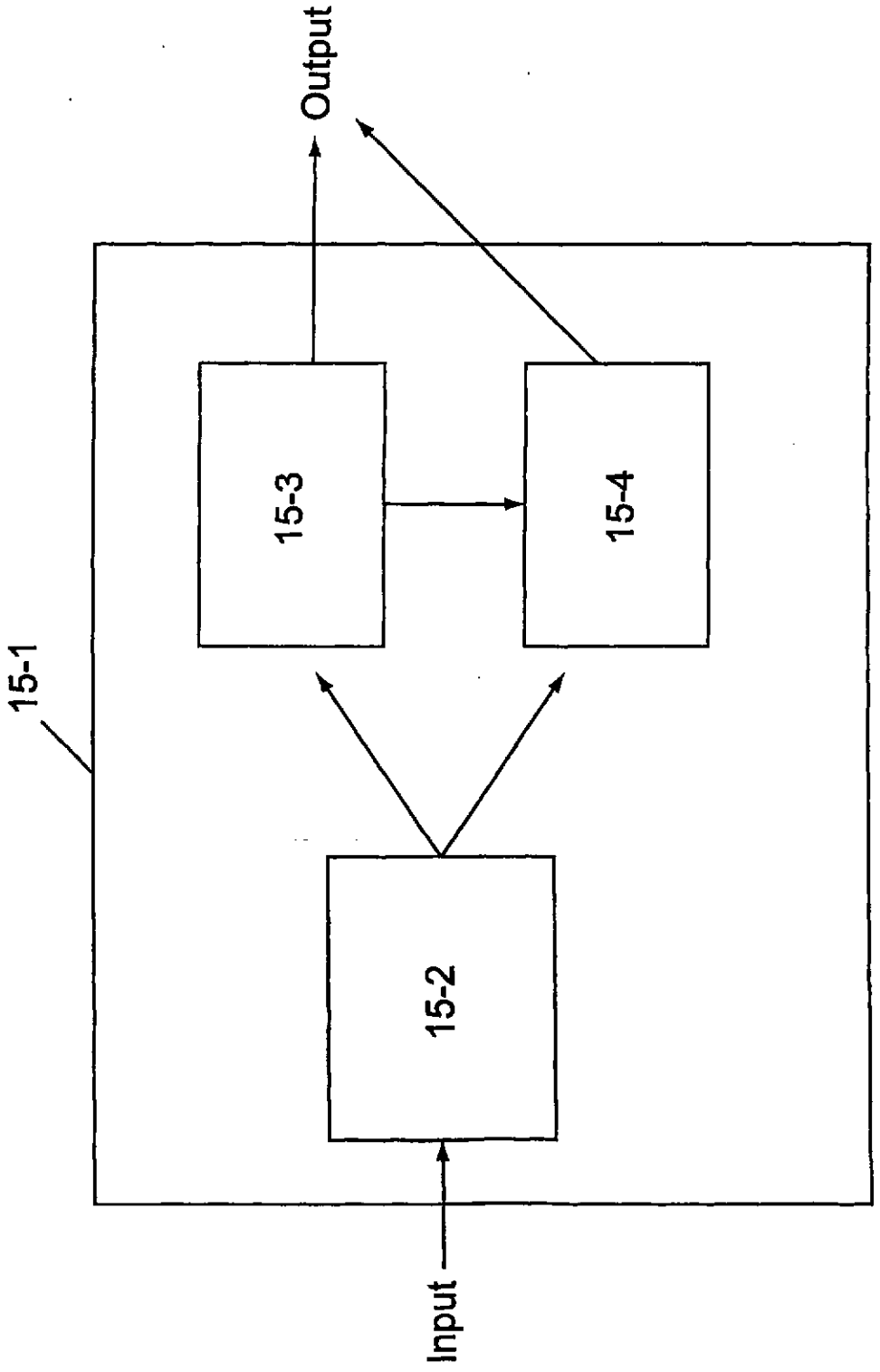


Fig. 15

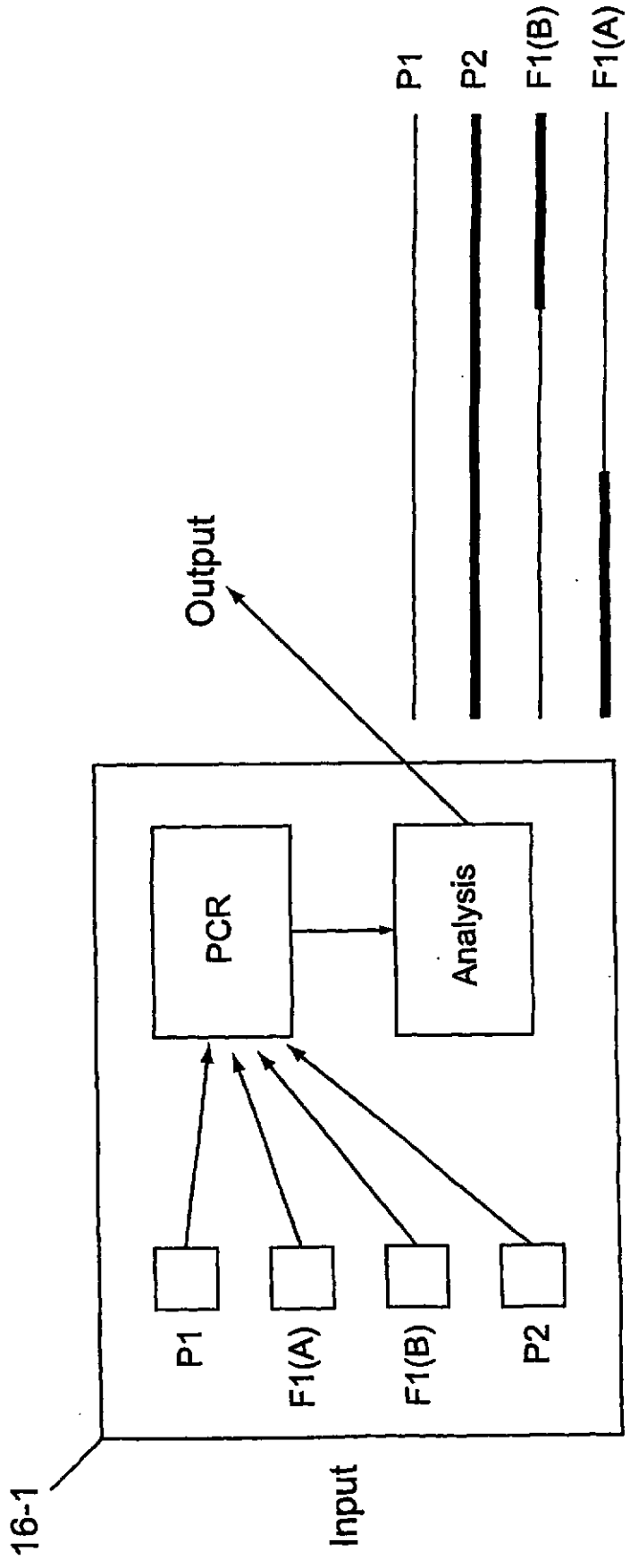


Fig. 16

11 OCT 2004

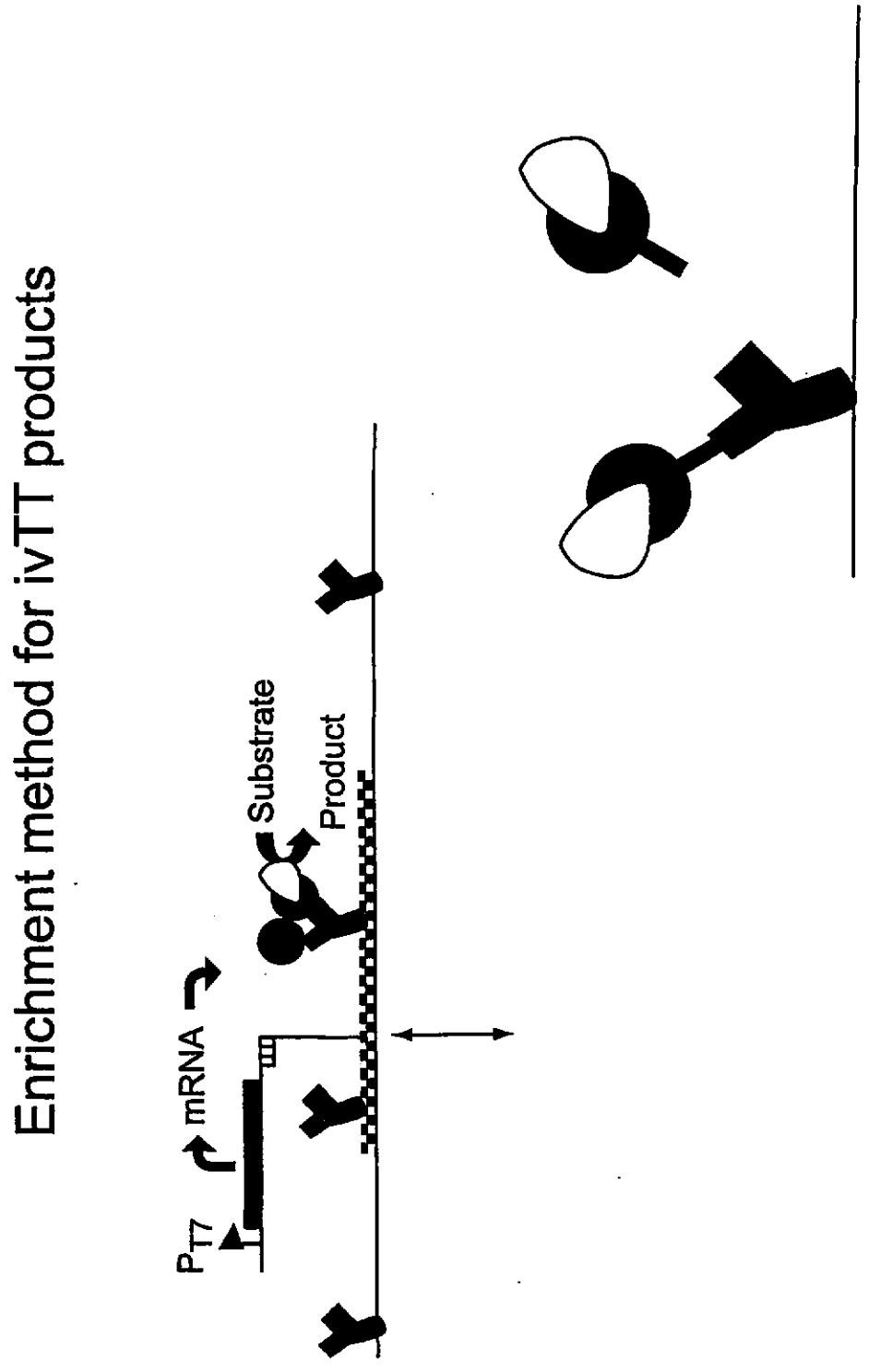


Fig. 17A

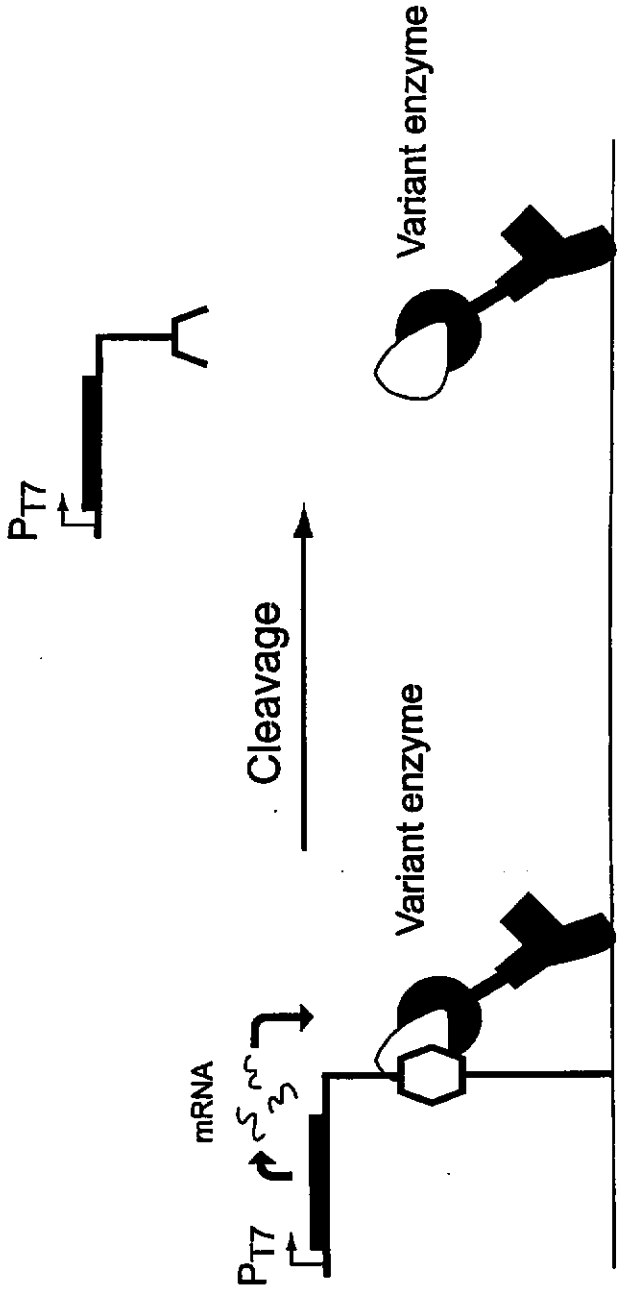


Fig. 17B

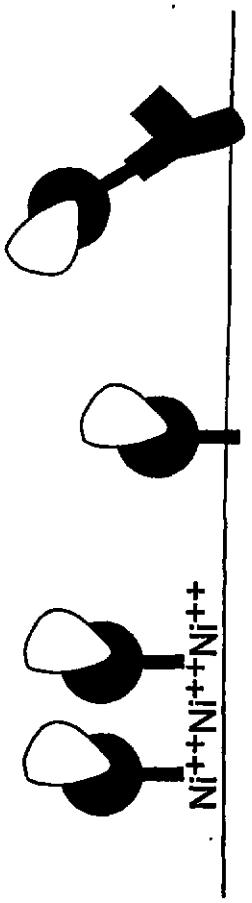


Fig. 17C

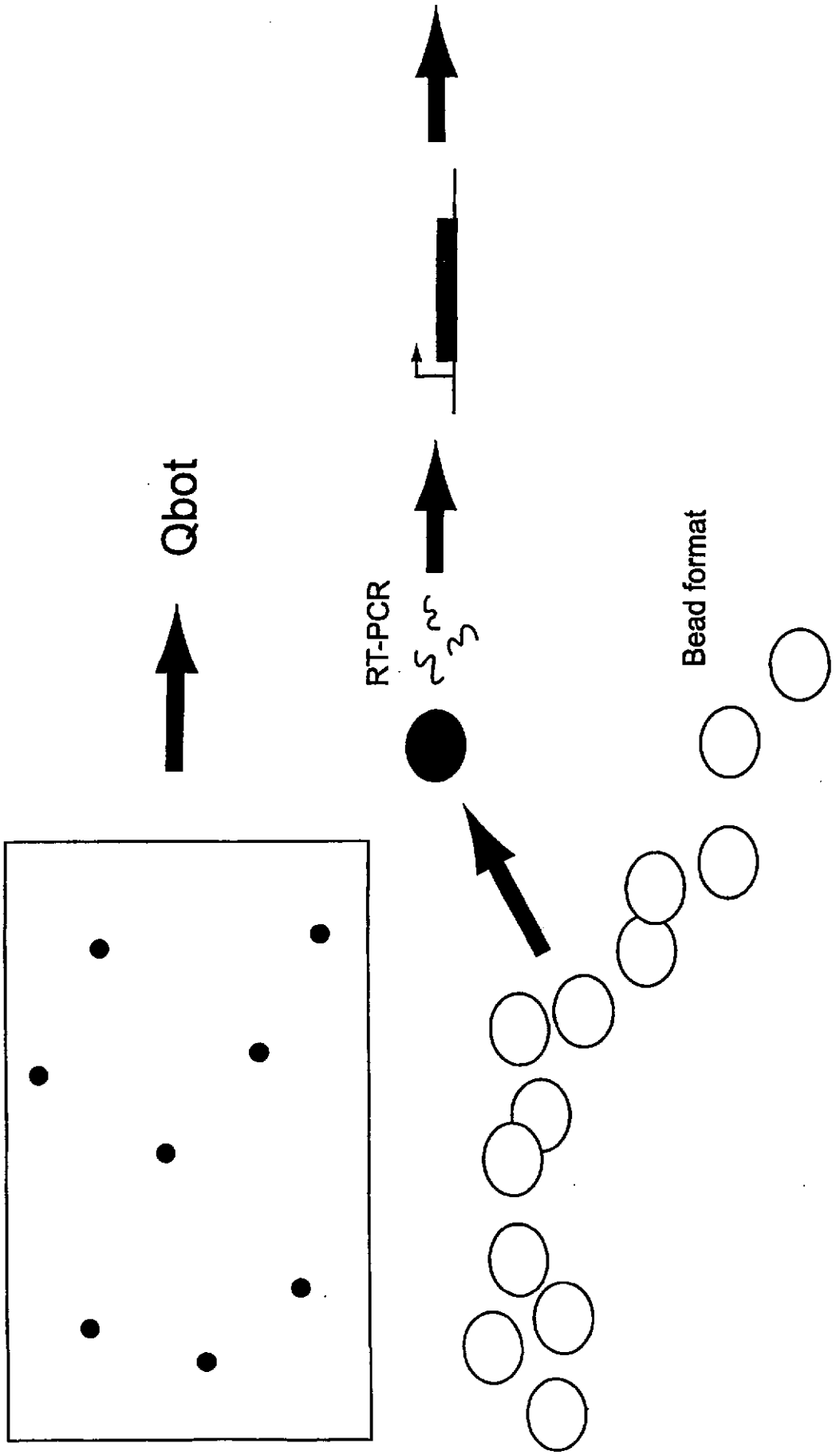


Fig. 17D

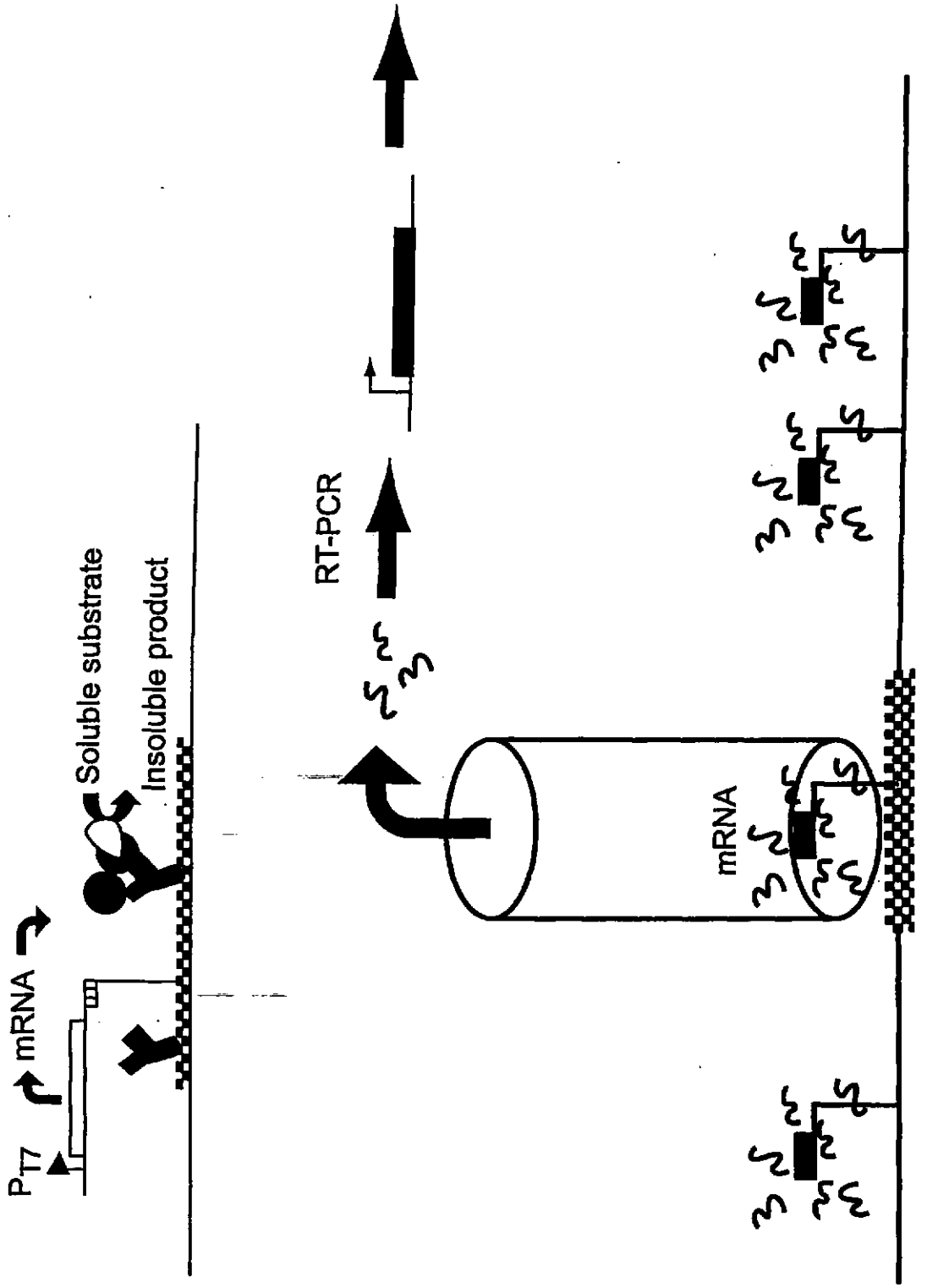


Fig. 17E

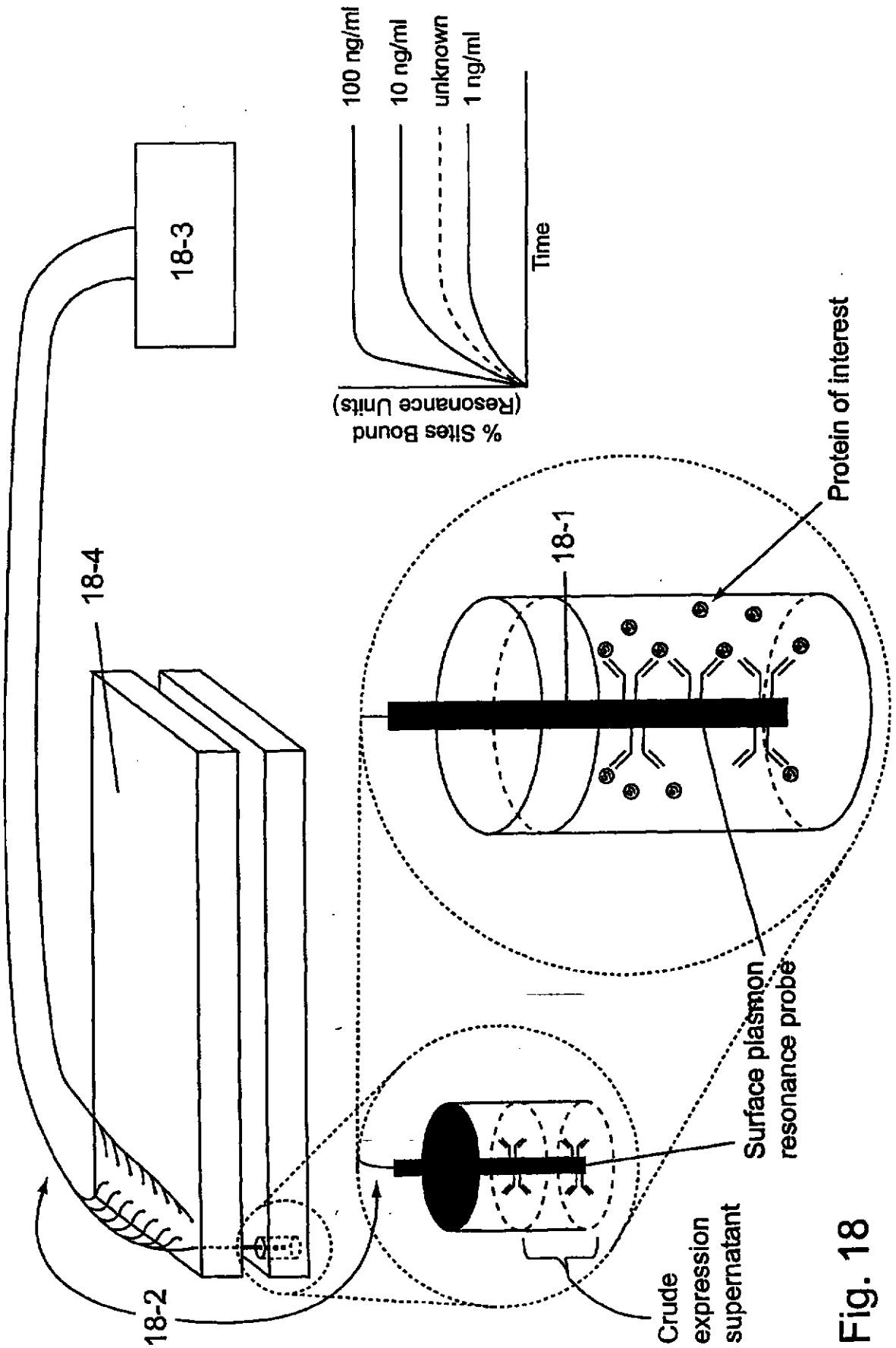

100 ng/ml
10 ng/ml
unknown
1 ng/ml
Time
% Sites Bound
(Resonance Units)
18-3
18-4
18-1
Protein of interest
Surface plasmon
resonance probe
Crude
expression
supernatant
18-2

Fig. 18

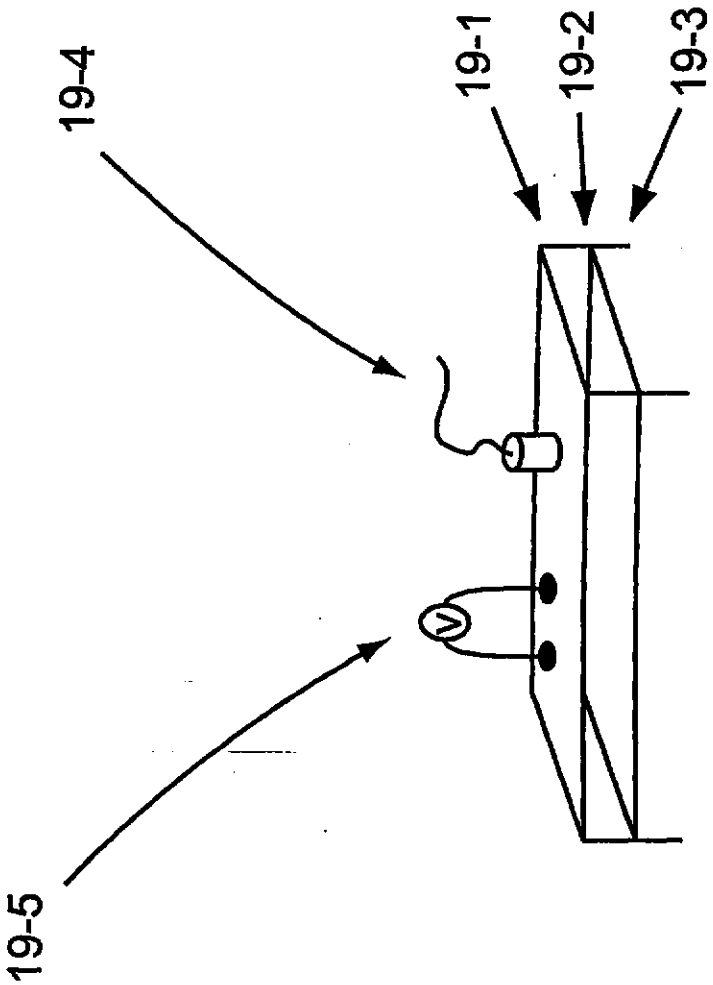


Fig. 19

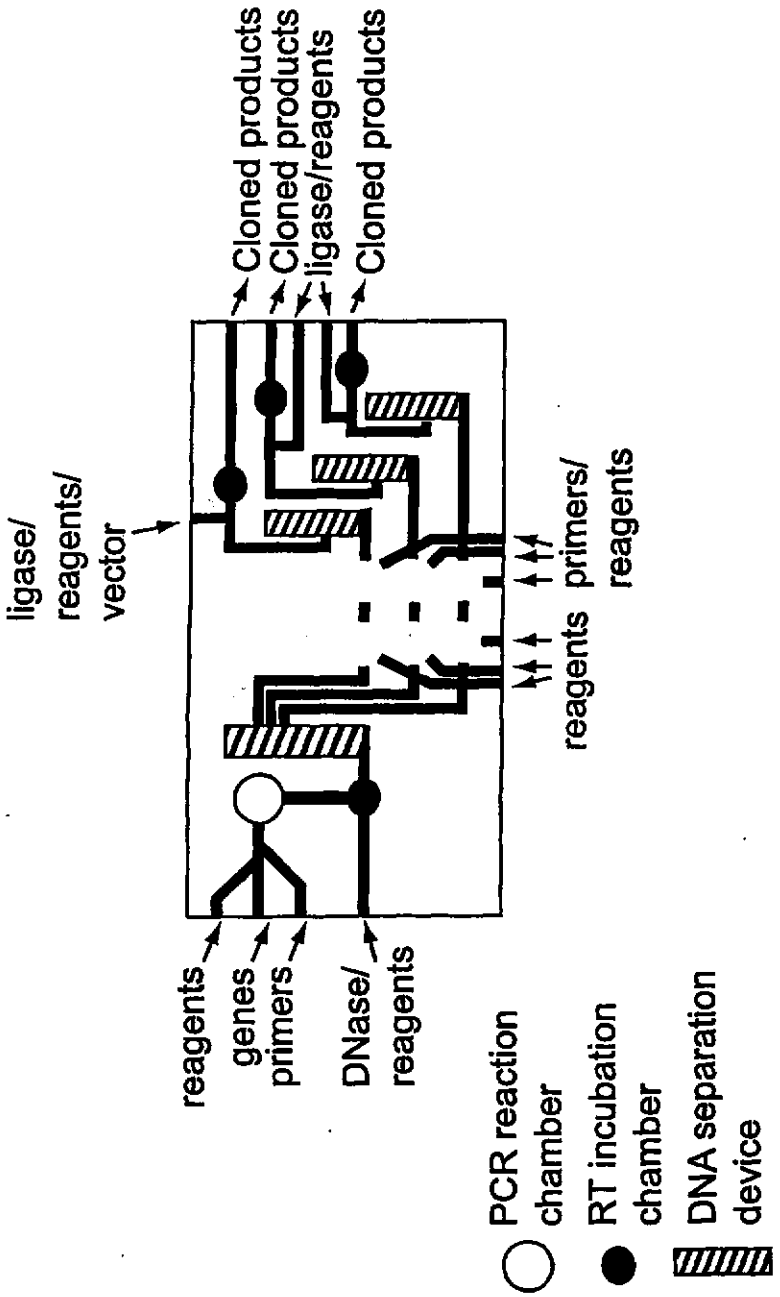

Cloned products
Cloned products
ligase/reagents
Cloned products
ligase/
reagents/
vector
primers/
reagents
reagents
genes
primers
DNase/
reagents
PCR reaction chamber
RT incubation chamber
DNA separation device

Fig. 20

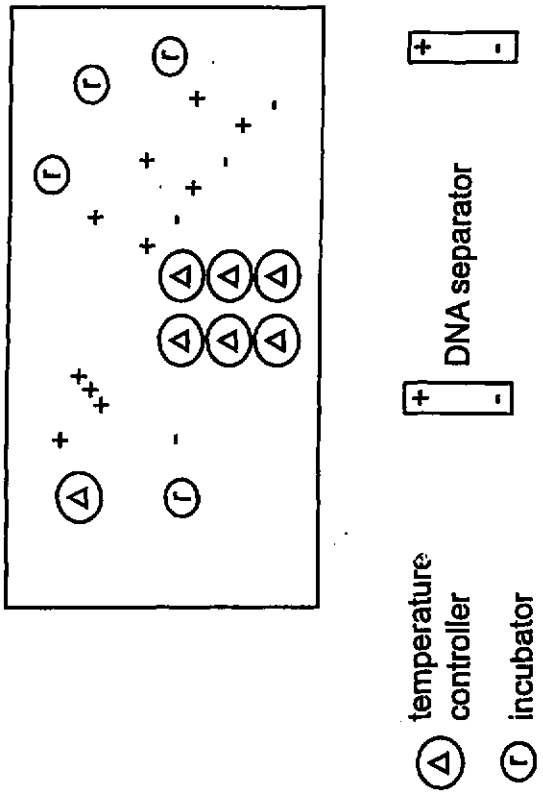

DNA separator, incl. fragment pruifier
DNA separator
temperature controller
incubator

Fig. 21

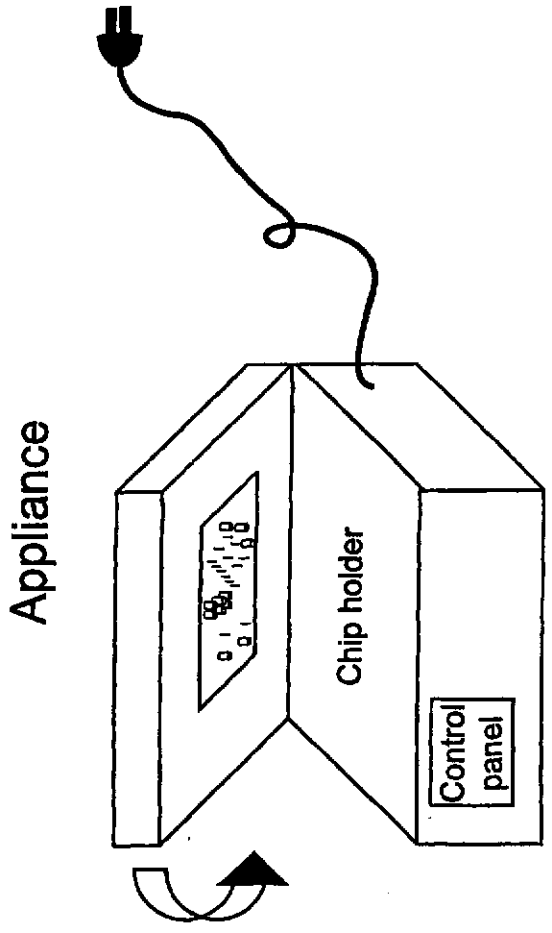


Fig. 22

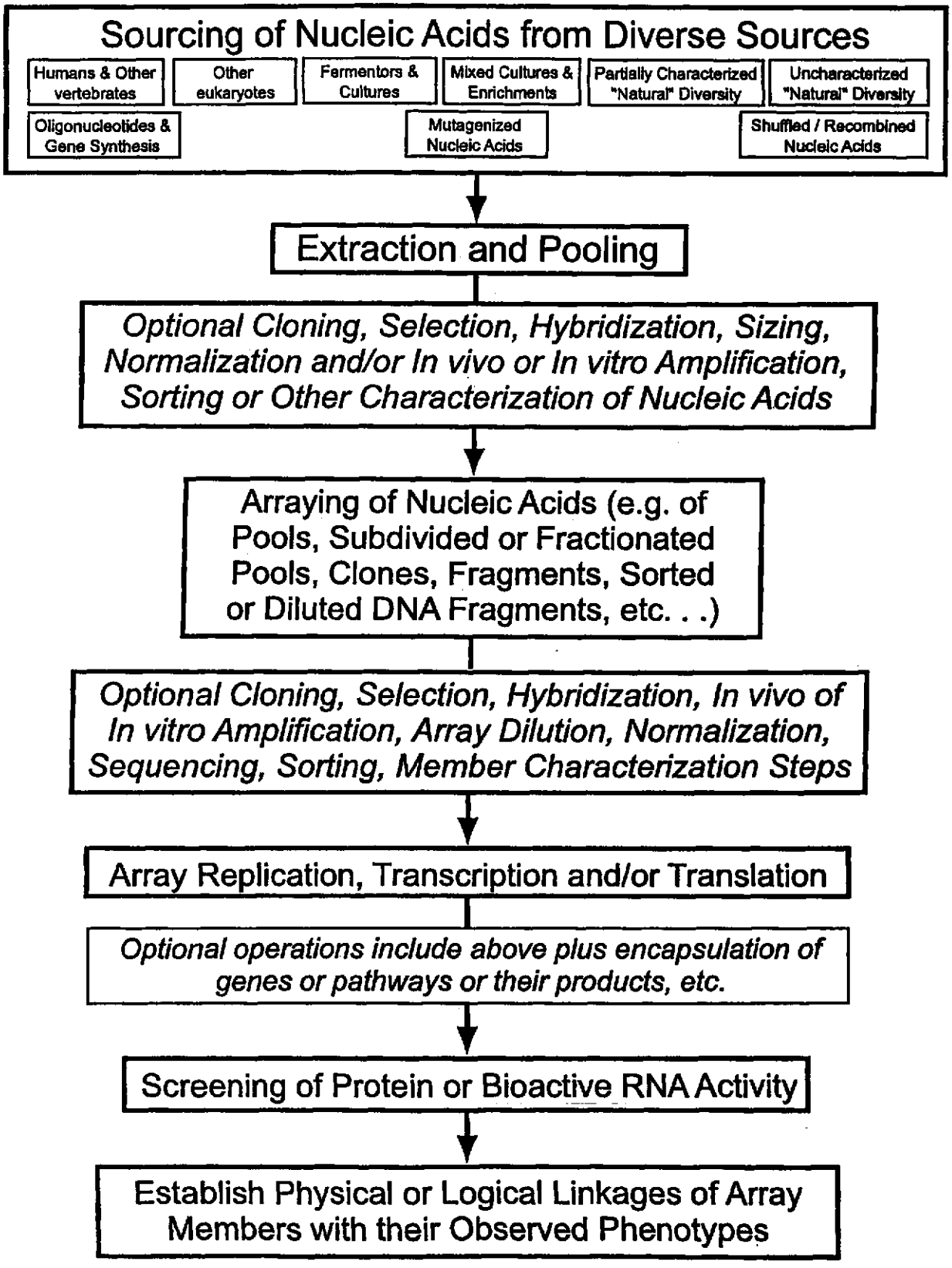

Sourcing of Nucleic Acids from Diverse Sources
Humans & Other vertebrates
Other eukaryotes
Fermentors & Cultures
Mixed Cultures & Enrichments
Partially Characterized "Natural" Diversity
Uncharacterized "Natural" Diversity
Oligonucleotides & Gene Synthesis
Mutagenized Nucleic Acids
Shuffled / Recombined Nucleic Acids
Extraction and Pooling
Optional Cloning, Selection, Hybridization, Sizing, Normalization and/or In vivo or In vitro Amplification, Sorting or Other Characterization of Nucleic Acids
Arraying of Nucleic Acids (e.g. of Pools, Subdivided or Fractionated Pools, Clones, Fragments, Sorted or Diluted DNA Fragments, etc. . .)
Optional Cloning, Selection, Hybridization, In vivo of In vitro Amplification, Array Dilution, Normalization, Sequencing, Sorting, Member Characterization Steps
Array Replication, Transcription and/or Translation
Optional operations include above plus encapsulation of genes or pathways or their products, etc.
Screening of Protein or Bioactive RNA Activity
Establish Physical or Logical Linkages of Array Members with their Observed Phenotypes

Fig. 23

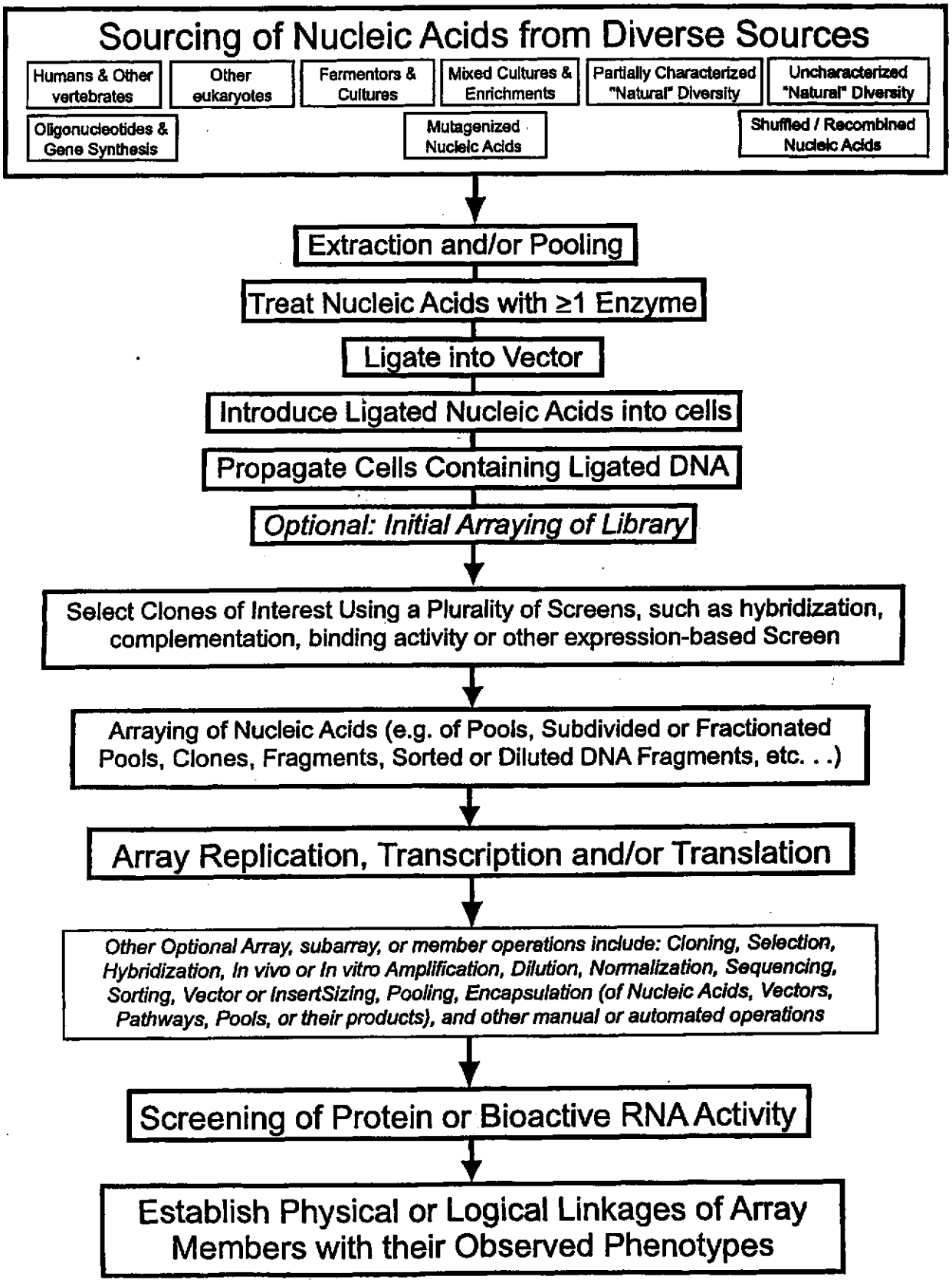

Sourcing of Nucleic Acids from Diverse Sources
Humans & Other vertebrates
Other eukaryotes
Fermentors & Cultures
Mixed Cultures & Enrichments
Partially Characterized "Natural" Diversity
Uncharacterized "Natural" Diversity
Oligonucleotides & Gene Synthesis
Mutagenized Nucleic Acids
Shuffled / Recombined Nucleic Acids
Extraction and/or Pooling
Treat Nucleic Acids with ≥1 Enzyme
Ligate into Vector
Introduce Ligated Nucleic Acids into cells
Propagate Cells Containing Ligated DNA
Optional: Initial Arraying of Library
Select Clones of Interest Using a Plurality of Screens, such as hybridization, complementation, binding activity or other expression-based Screen
Arraying of Nucleic Acids (e.g. of Pools, Subdivided or Fractionated Pools, Clones, Fragments, Sorted or Diluted DNA Fragments, etc. . .)
Array Replication, Transcription and/or Translation
Other Optional Array, subarray, or member operations include: Cloning, Selection, Hybridization, In vivo or In vitro Amplification, Dilution, Normalization, Sequencing, Sorting, Vector or InsertSizing, Pooling, Encapsulation (of Nucleic Acids, Vectors, Pathways, Pools, or their products), and other manual or automated operations
Screening of Protein or Bioactive RNA Activity
Establish Physical or Logical Linkages of Array Members with their Observed Phenotypes

Fig. 24

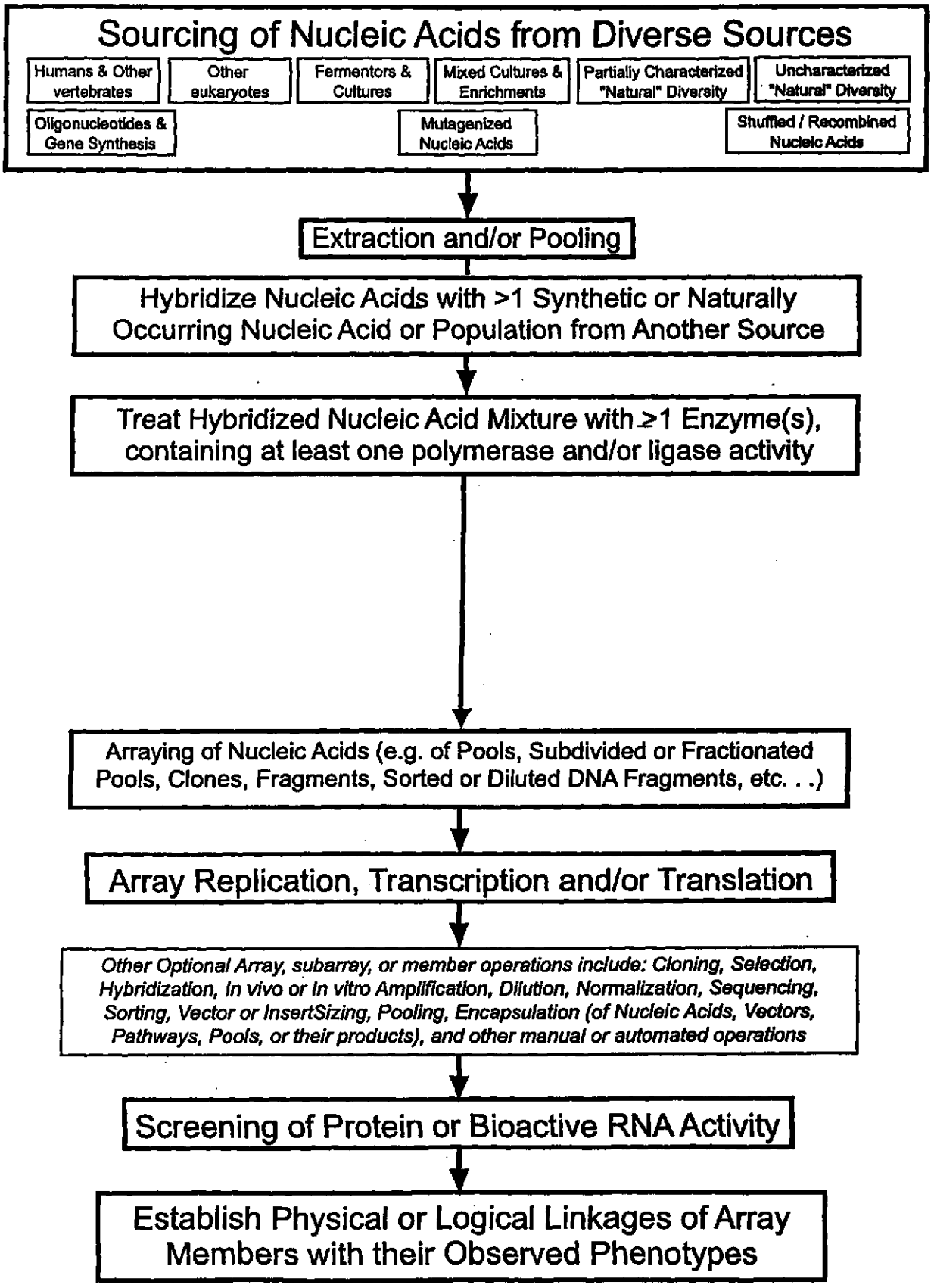

Sourcing of Nucleic Acids from Diverse Sources
Humans & Other vertebrates
Other eukaryotes
Fermentors & Cultures
Mixed Cultures & Enrichments
Partially Characterized "Natural" Diversity
Uncharacterized "Natural" Diversity
Oligonucleotides & Gene Synthesis
Mutagenized Nucleic Acids
Shuffled / Recombined Nucleic Acids
Extraction and/or Pooling
Hybridize Nucleic Acids with >1 Synthetic or Naturally Occurring Nucleic Acid or Population from Another Source
Treat Hybridized Nucleic Acid Mixture with ≥1 Enzyme(s), containing at least one polymerase and/or ligase activity
Arraying of Nucleic Acids (e.g. of Pools, Subdivided or Fractionated Pools, Clones, Fragments, Sorted or Diluted DNA Fragments, etc. . .)
Array Replication, Transcription and/or Translation
Other Optional Array, subarray, or member operations include: Cloning, Selection, Hybridization, In vivo or In vitro Amplification, Dilution, Normalization, Sequencing, Sorting, Vector or InsertSizing, Pooling, Encapsulation (of Nucleic Acids, Vectors, Pathways, Pools, or their products), and other manual or automated operations
Screening of Protein or Bioactive RNA Activity
Establish Physical or Logical Linkages of Array Members with their Observed Phenotypes

Fig. 25

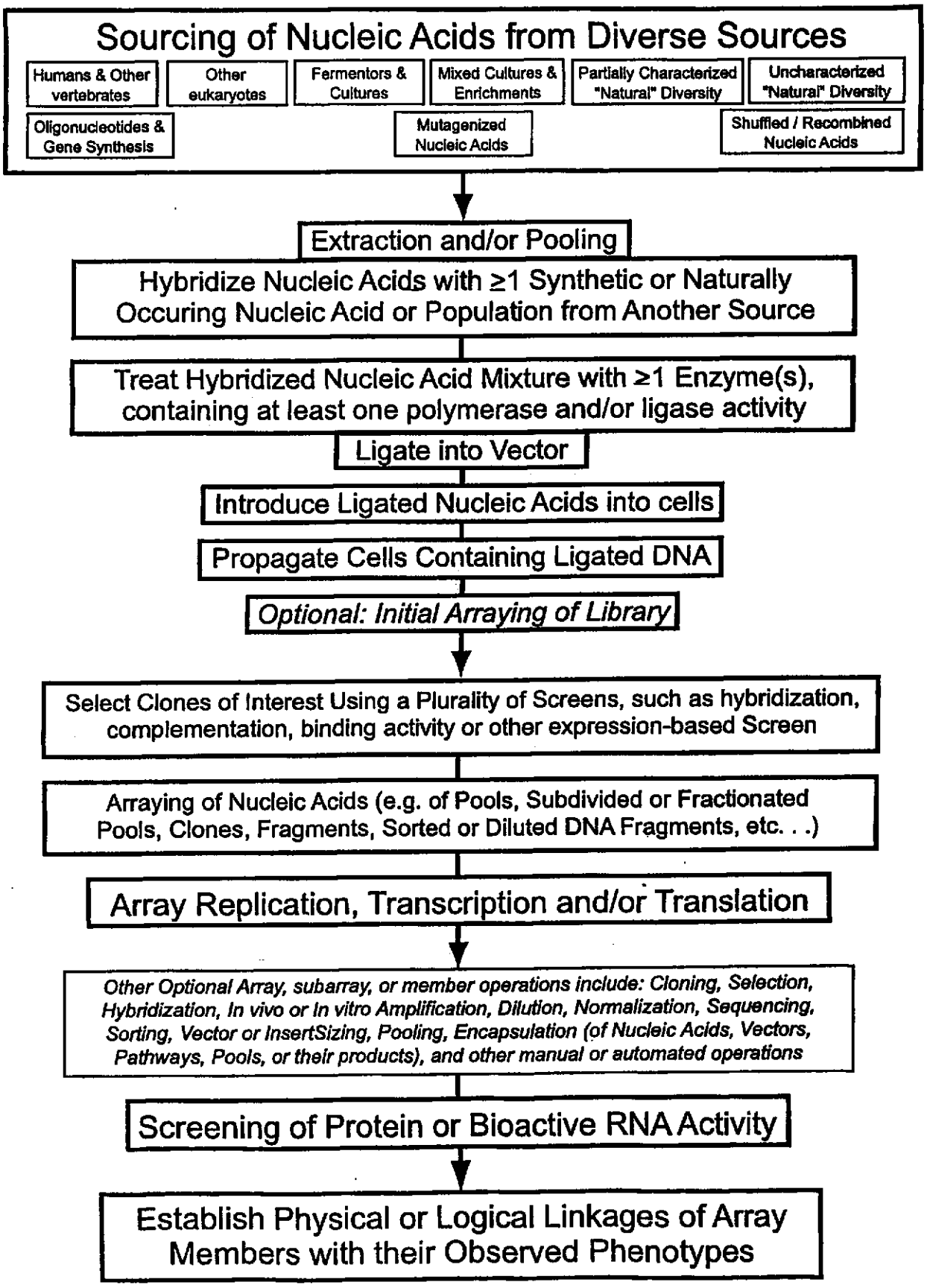

Sourcing of Nucleic Acids from Diverse Sources
Humans & Other vertebrates
Other eukaryotes
Fermentors & Cultures
Mixed Cultures & Enrichments
Partially Characterized "Natural" Diversity
Uncharacterized "Natural" Diversity
Oligonucleotides & Gene Synthesis
Mutagenized Nucleic Acids
Shuffled / Recombined Nucleic Acids
Extraction and/or Pooling
Hybridize Nucleic Acids with ≥1 Synthetic or Naturally Occuring Nucleic Acid or Population from Another Source
Treat Hybridized Nucleic Acid Mixture with ≥1 Enzyme(s), containing at least one polymerase and/or ligase activity
Ligate into Vector
Introduce Ligated Nucleic Acids into cells
Propagate Cells Containing Ligated DNA
Optional: Initial Arraying of Library
Select Clones of Interest Using a Plurality of Screens, such as hybridization, complementation, binding activity or other expression-based Screen
Arraying of Nucleic Acids (e.g. of Pools, Subdivided or Fractionated Pools, Clones, Fragments, Sorted or Diluted DNA Fragments, etc. . .)
Array Replication, Transcription and/or Translation
Other Optional Array, subarray, or member operations include: Cloning, Selection, Hybridization, In vivo or In vitro Amplification, Dilution, Normalization, Sequencing, Sorting, Vector or InsertSizing, Pooling, Encapsulation (of Nucleic Acids, Vectors, Pathways, Pools, or their products), and other manual or automated operations
Screening of Protein or Bioactive RNA Activity
Establish Physical or Logical Linkages of Array Members with their Observed Phenotypes

Fig. 26

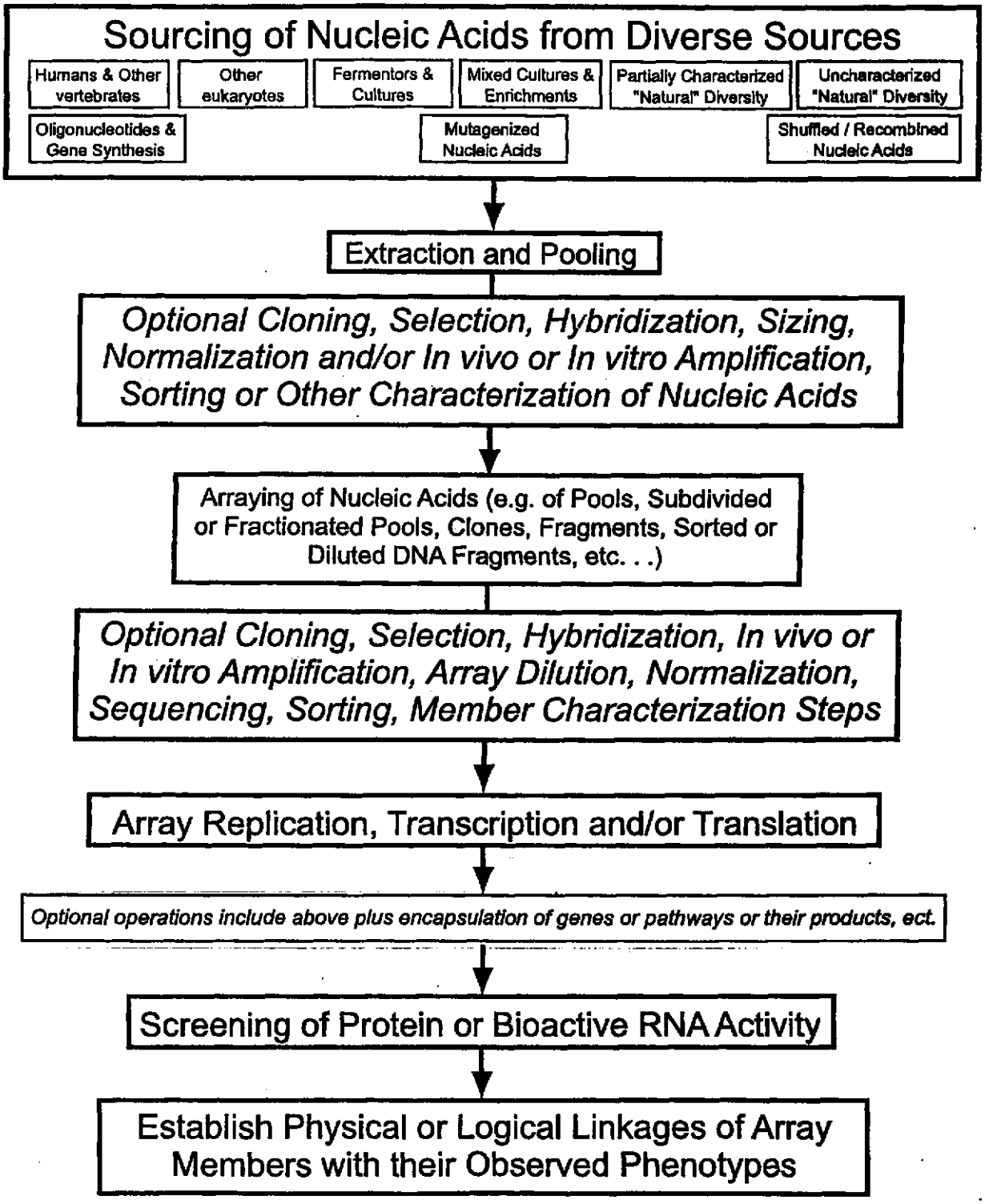

Sourcing of Nucleic Acids from Diverse Sources
Humans & Other vertebrates
Other eukaryotes
Fermentors & Cultures
Mixed Cultures & Enrichments
Partially Characterized "Natural" Diversity
Uncharacterized "Natural" Diversity
Oligonucleotides & Gene Synthesis
Mutagenized Nucleic Acids
Shuffled / Recombined Nucleic Acids
Extraction and Pooling
Optional Cloning, Selection, Hybridization, Sizing, Normalization and/or In vivo or In vitro Amplification, Sorting or Other Characterization of Nucleic Acids
Arraying of Nucleic Acids (e.g. of Pools, Subdivided or Fractionated Pools, Clones, Fragments, Sorted or Diluted DNA Fragments, etc. . .)
Optional Cloning, Selection, Hybridization, In vivo or In vitro Amplification, Array Dilution, Normalization, Sequencing, Sorting, Member Characterization Steps
Array Replication, Transcription and/or Translation
Optional operations include above plus encapsulation of genes or pathways or their products, ect.
Screening of Protein or Bioactive RNA Activity
Establish Physical or Logical Linkages of Array Members with their Observed Phenotypes

Fig. 27

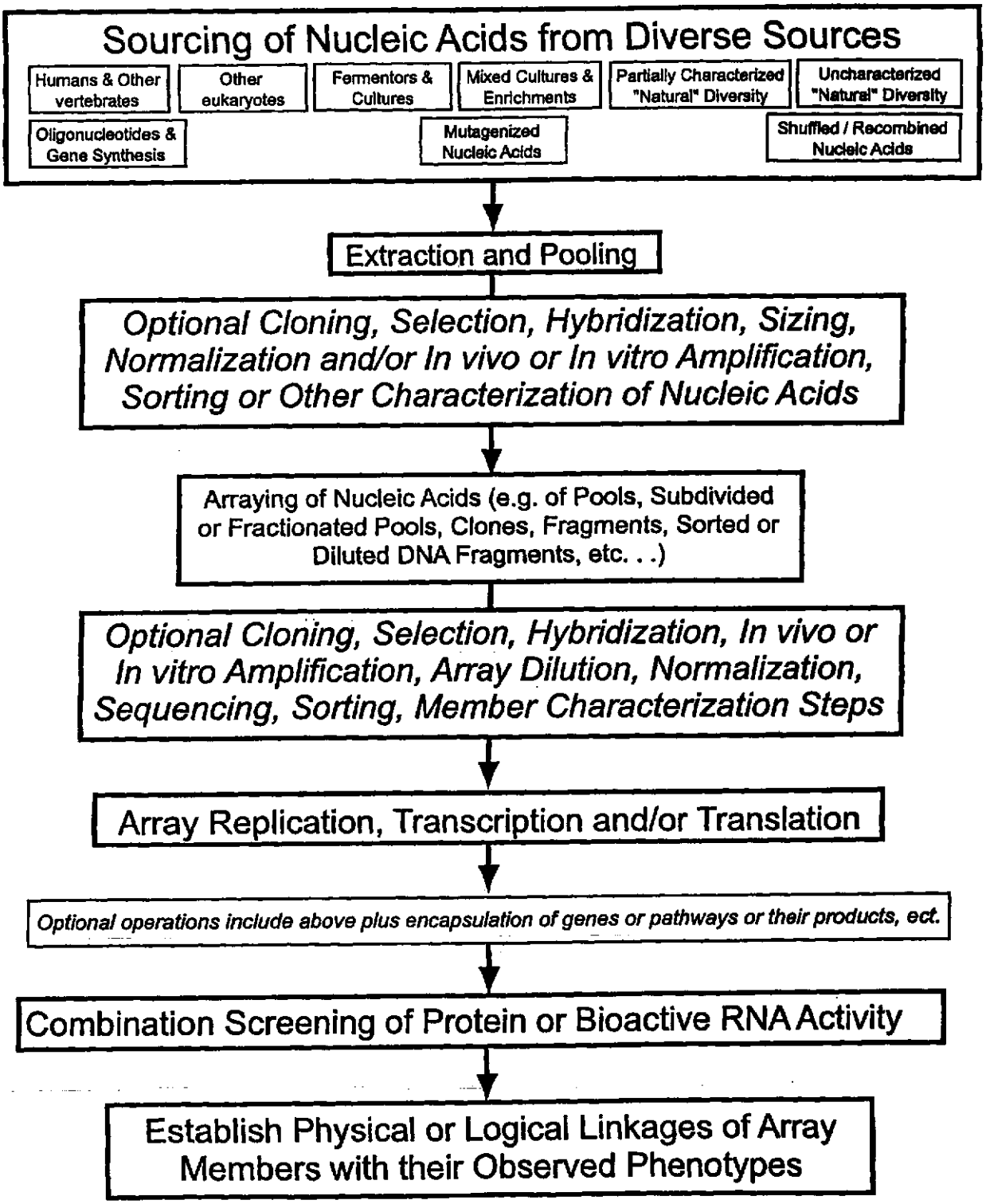

Sourcing of Nucleic Acids from Diverse Sources
Humans & Other vertebrates
Other eukaryotes
Fermentors & Cultures
Mixed Cultures & Enrichments
Partially Characterized "Natural" Diversity
Uncharacterized "Natural" Diversity
Oligonucleotides & Gene Synthesis
Mutagenized Nucleic Acids
Shuffled / Recombined Nucleic Acids
Extraction and Pooling
Optional Cloning, Selection, Hybridization, Sizing, Normalization and/or In vivo or In vitro Amplification, Sorting or Other Characterization of Nucleic Acids
Arraying of Nucleic Acids (e.g. of Pools, Subdivided or Fractionated Pools, Clones, Fragments, Sorted or Diluted DNA Fragments, etc. . .)
Optional Cloning, Selection, Hybridization, In vivo or In vitro Amplification, Array Dilution, Normalization, Sequencing, Sorting, Member Characterization Steps
Array Replication, Transcription and/or Translation
Optional operations include above plus encapsulation of genes or pathways or their products, ect.
Combination Screening of Protein or Bioactive RNA Activity
Establish Physical or Logical Linkages of Array Members with their Observed Phenotypes

Fig. 28

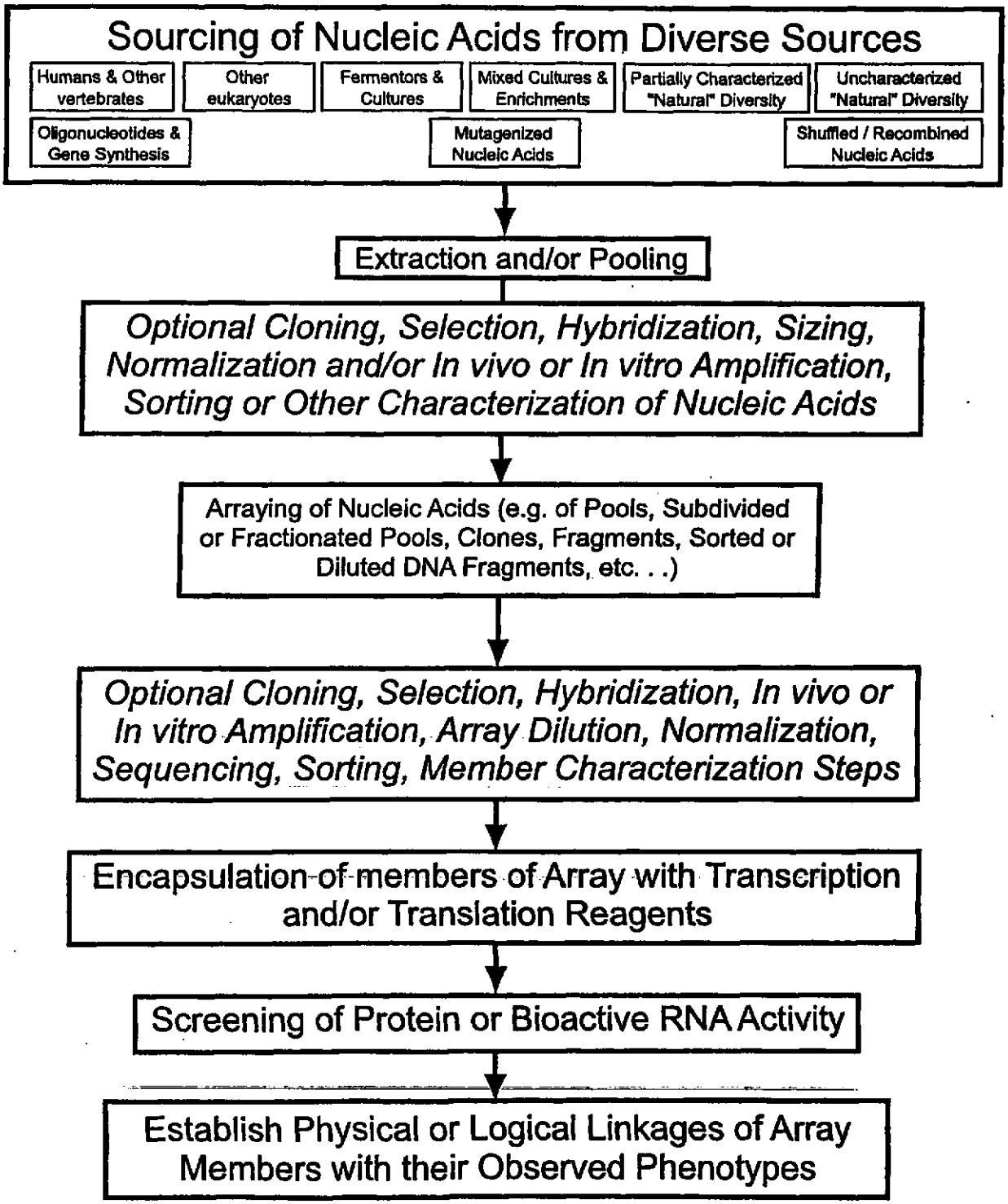

Sourcing of Nucleic Acids from Diverse Sources
Humans & Other vertebrates
Other eukaryotes
Fermentors & Cultures
Mixed Cultures & Enrichments
Partially Characterized "Natural" Diversity
Uncharacterized "Natural" Diversity
Oligonucleotides & Gene Synthesis
Mutagenized Nucleic Acids
Shuffled / Recombined Nucleic Acids
Extraction and/or Pooling
Optional Cloning, Selection, Hybridization, Sizing, Normalization and/or In vivo or In vitro Amplification, Sorting or Other Characterization of Nucleic Acids
Arraying of Nucleic Acids (e.g. of Pools, Subdivided or Fractionated Pools, Clones, Fragments, Sorted or Diluted DNA Fragments, etc. . .)
Optional Cloning, Selection, Hybridization, In vivo or In vitro Amplification, Array Dilution, Normalization, Sequencing, Sorting, Member Characterization Steps
Encapsulation of members of Array with Transcription and/or Translation Reagents
Screening of Protein or Bioactive RNA Activity
Establish Physical or Logical Linkages of Array Members with their Observed Phenotypes

Fig. 29

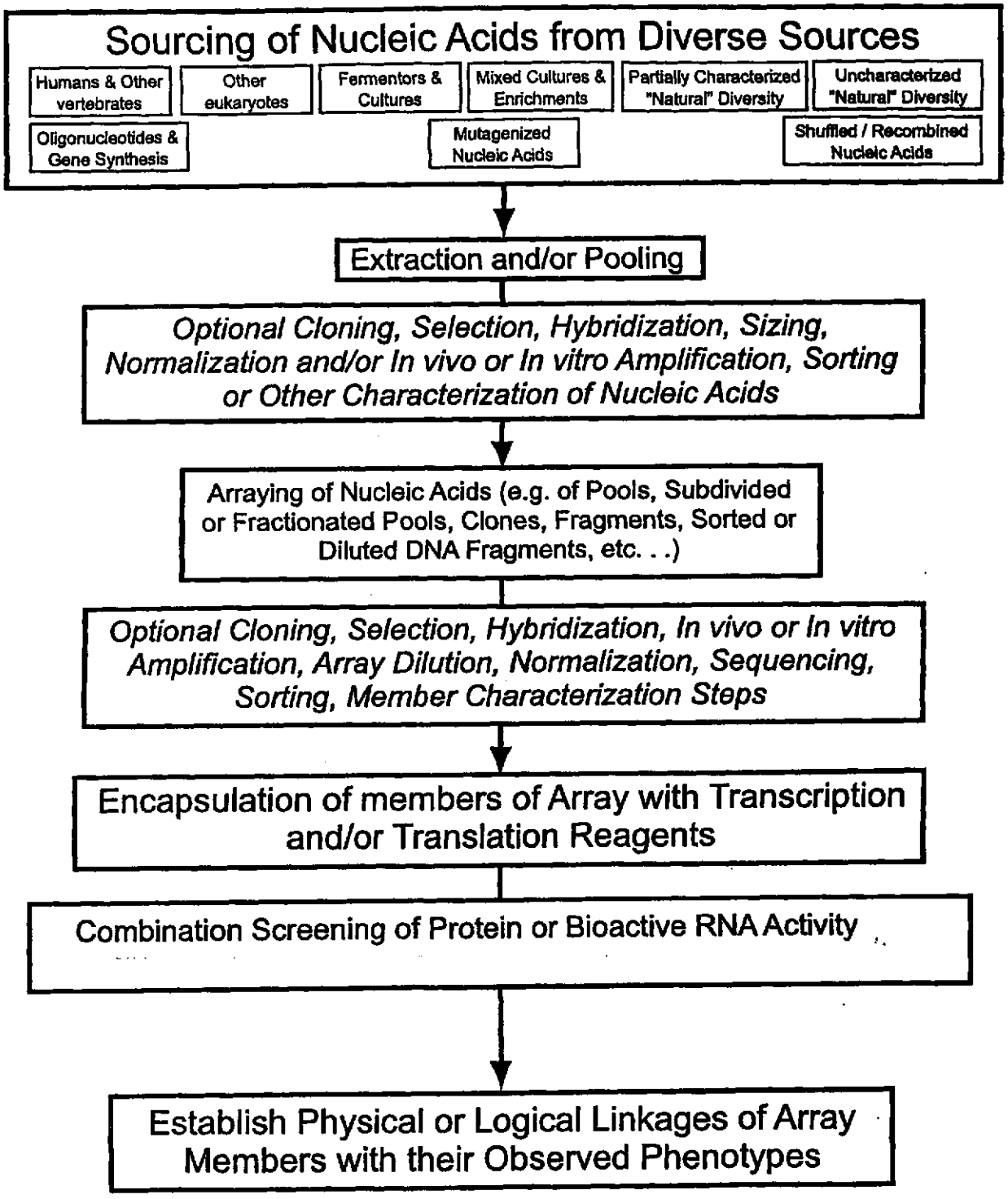

Sourcing of Nucleic Acids from Diverse Sources
Humans & Other vertebrates
Other eukaryotes
Fermentors & Cultures
Mixed Cultures & Enrichments
Partially Characterized "Natural" Diversity
Uncharacterized "Natural" Diversity
Oligonucleotides & Gene Synthesis
Mutagenized Nucleic Acids
Shuffled / Recombined Nucleic Acids
Extraction and/or Pooling
Optional Cloning, Selection, Hybridization, Sizing, Normalization and/or In vivo or In vitro Amplification, Sorting or Other Characterization of Nucleic Acids
Arraying of Nucleic Acids (e.g. of Pools, Subdivided or Fractionated Pools, Clones, Fragments, Sorted or Diluted DNA Fragments, etc. . .)
Optional Cloning, Selection, Hybridization, In vivo or In vitro Amplification, Array Dilution, Normalization, Sequencing, Sorting, Member Characterization Steps
Encapsulation of members of Array with Transcription and/or Translation Reagents
Combination Screening of Protein or Bioactive RNA Activity
Establish Physical or Logical Linkages of Array Members with their Observed Phenotypes

Fig. 30

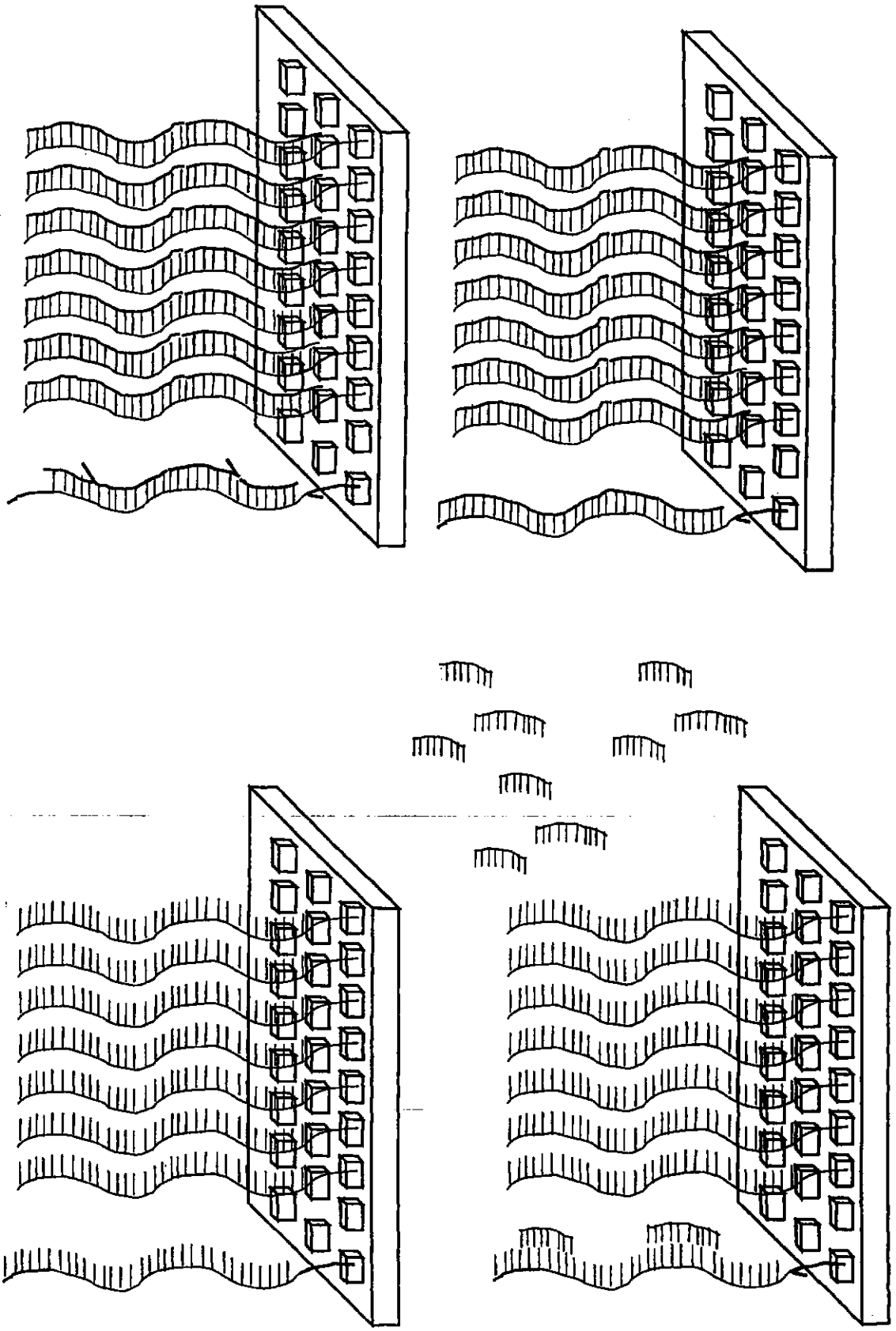


Fig. 31

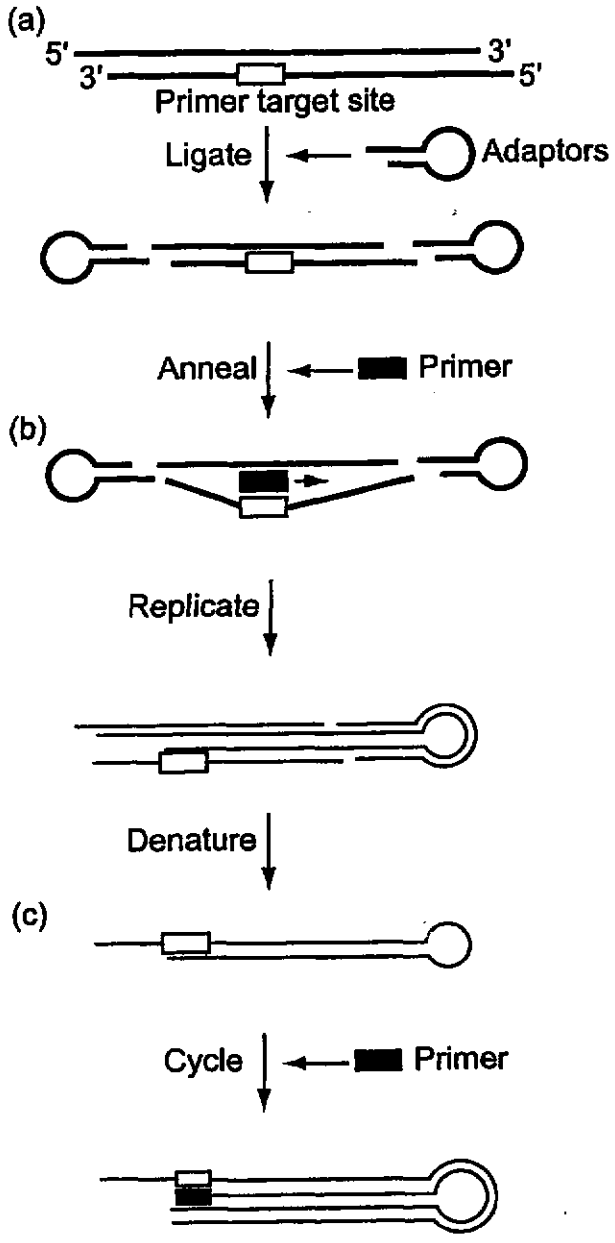

(a)
5'
3'
3'
5'
Primer target site
Ligate
Adaptors
Anneal
Primer
(b)
Replicate
Denature
(c)
Cycle
Primer

Fig. 32A

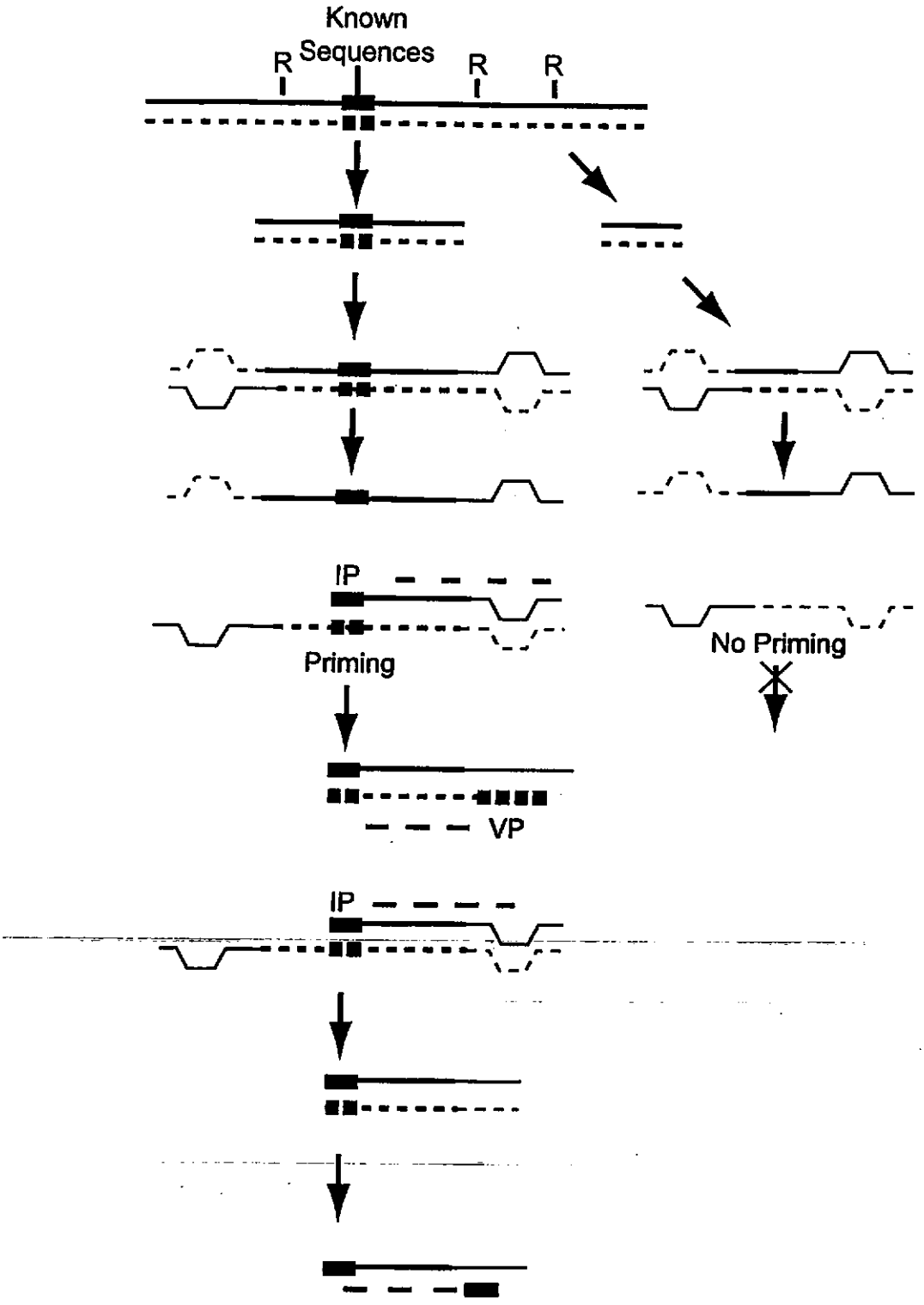


Fig. 32B

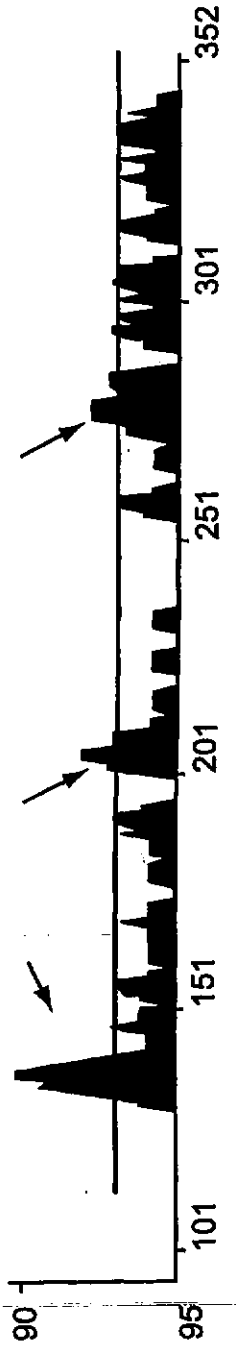


Fig. 33