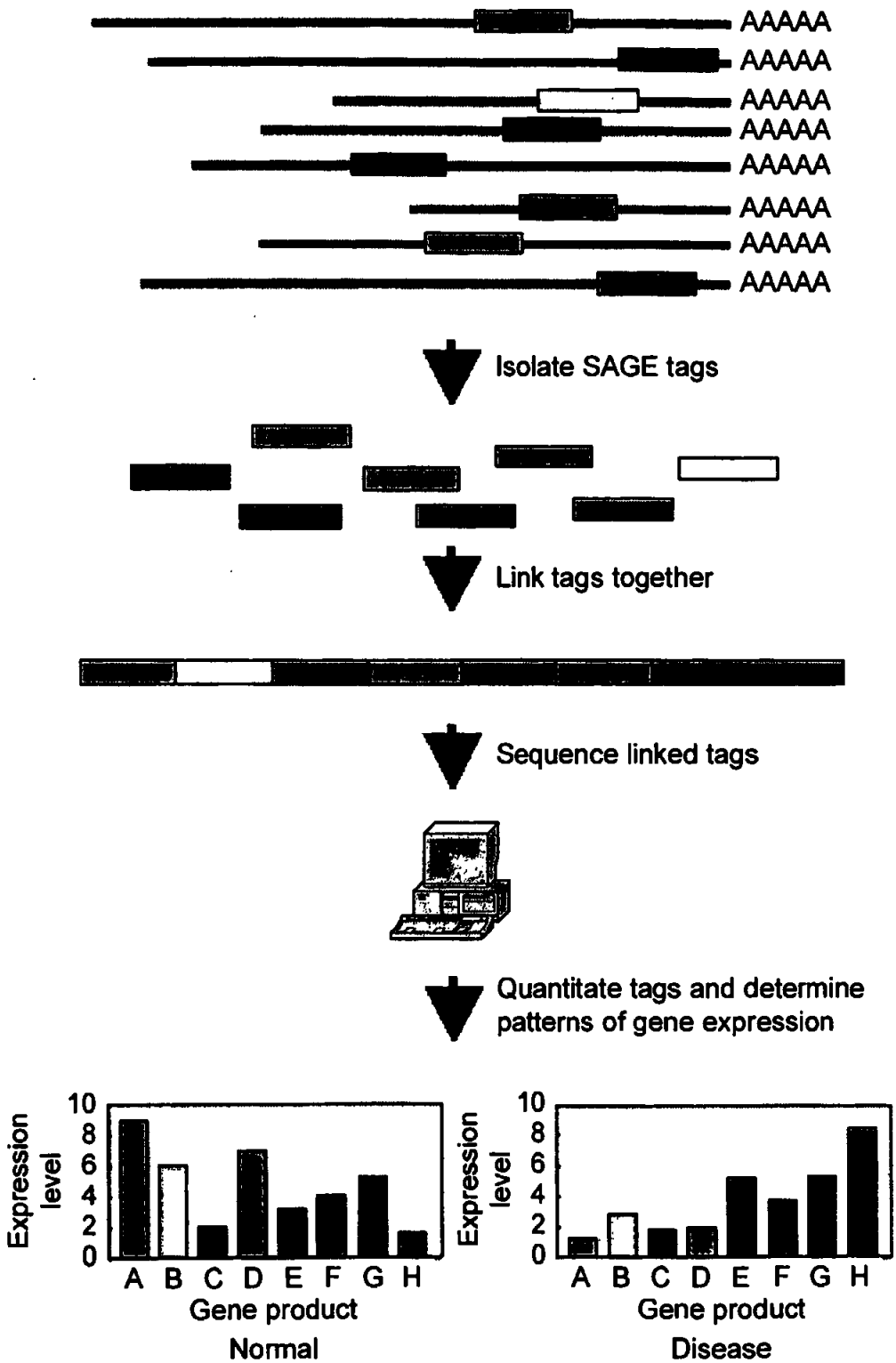

AAAAA
AAAAA
AAAAA
AAAAA
AAAAA
AAAAA
AAAAA
AAAAA
Isolate SAGE tags
Link tags together
Sequence linked tags
Quantitate tags and determine patterns of gene expression
Expression level
10
8
6
4
2
0
A B C D E F G H
Gene product
Normal
Expression level
10
8
6
4
2
0
A B C D E F G H
Gene product
Disease

FIG. 1A

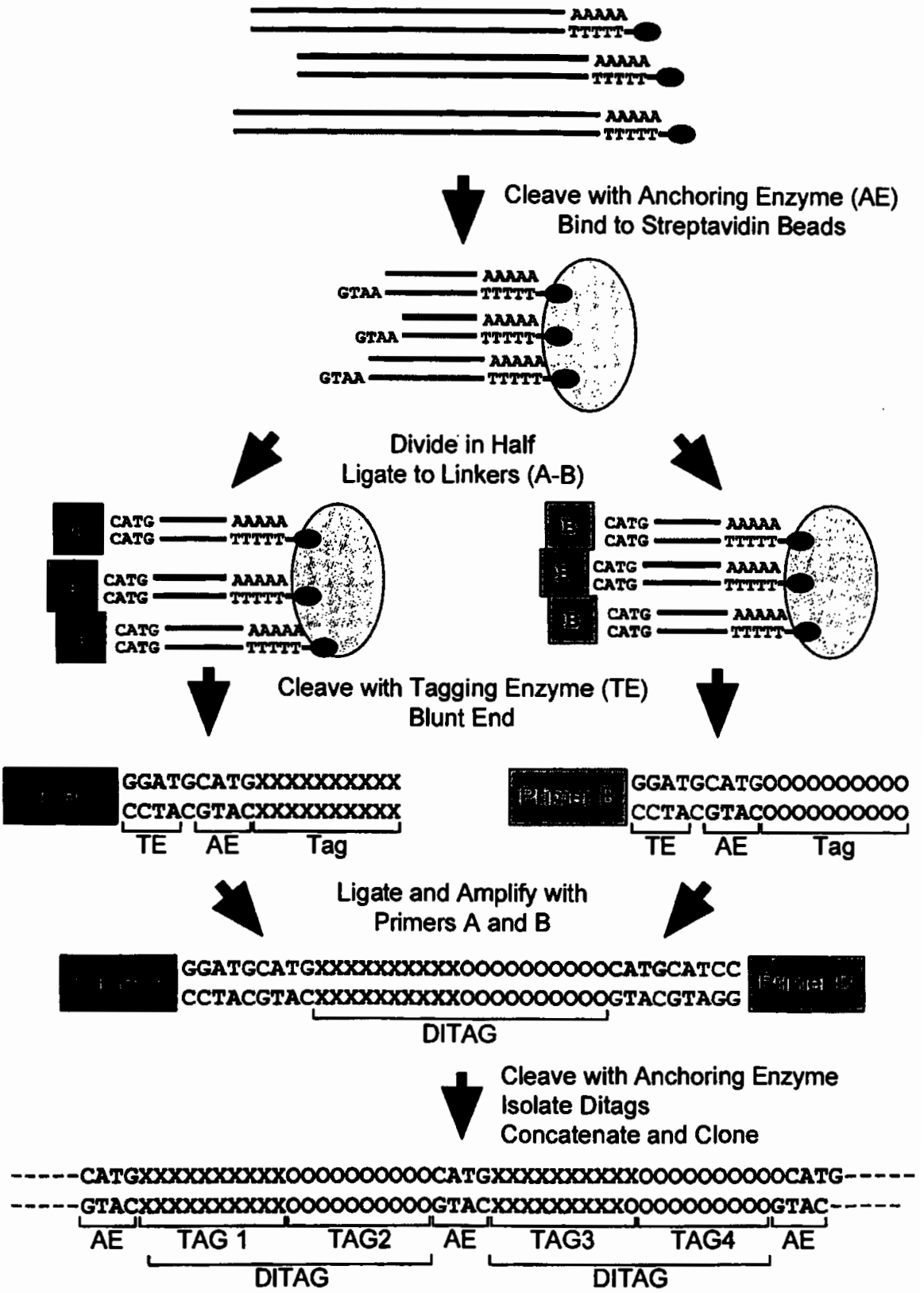

AAAAA
TTTTT
Cleave with Anchoring Enzyme (AE)
Bind to Streptavidin Beads
GTAA
Divide in Half
Ligate to Linkers (A-B)
CATG
Cleave with Tagging Enzyme (TE)
Blunt End
GGATGCATGXXXXXXXXXX
CCTACGTACXXXXXXXXXX
GGATGCATGOOOOOOOOOO
CCTACGTACOOOOOOOOOO
TE
AE
Tag
Ligate and Amplify with
Primers A and B
GGATGCATGXXXXXXXXXXOOOOOOOOOOCATGCATCC
CCTACGTACXXXXXXXXXXOOOOOOOOOOGTACGTAGG
DITAG
Cleave with Anchoring Enzyme
Isolate Ditags
Concatenate and Clone
-----CATGXXXXXXXXXXOOOOOOOOOOCATGXXXXXXXXXXOOOOOOOOOOCATG-----
-----GTACXXXXXXXXXXOOOOOOOOOOGTACXXXXXXXXXXOOOOOOOOOOGTAC-----
AE
TAG 1
TAG2
AE
TAG3
TAG4
AE
DITAG
DITAG

FIG. 1B

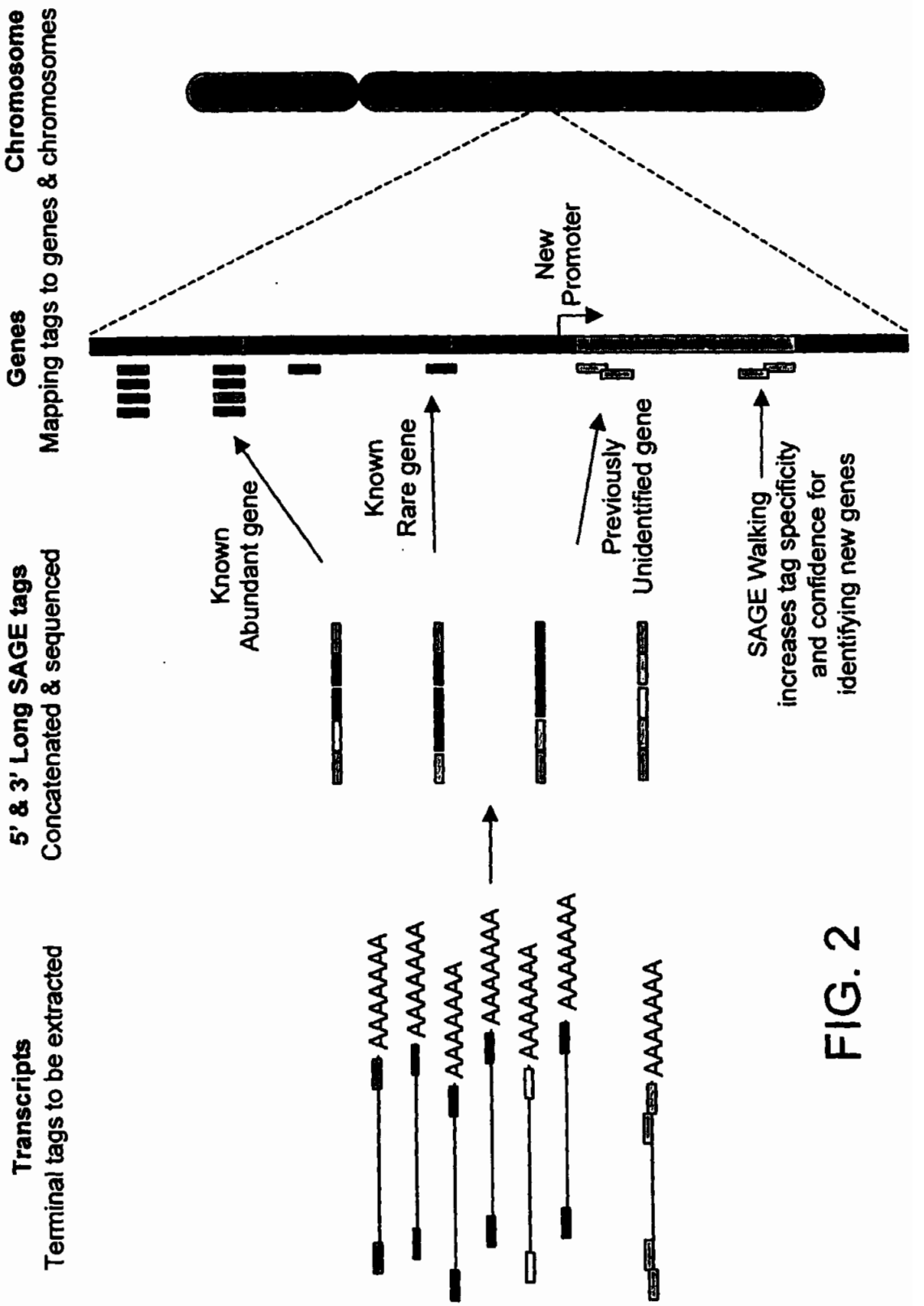
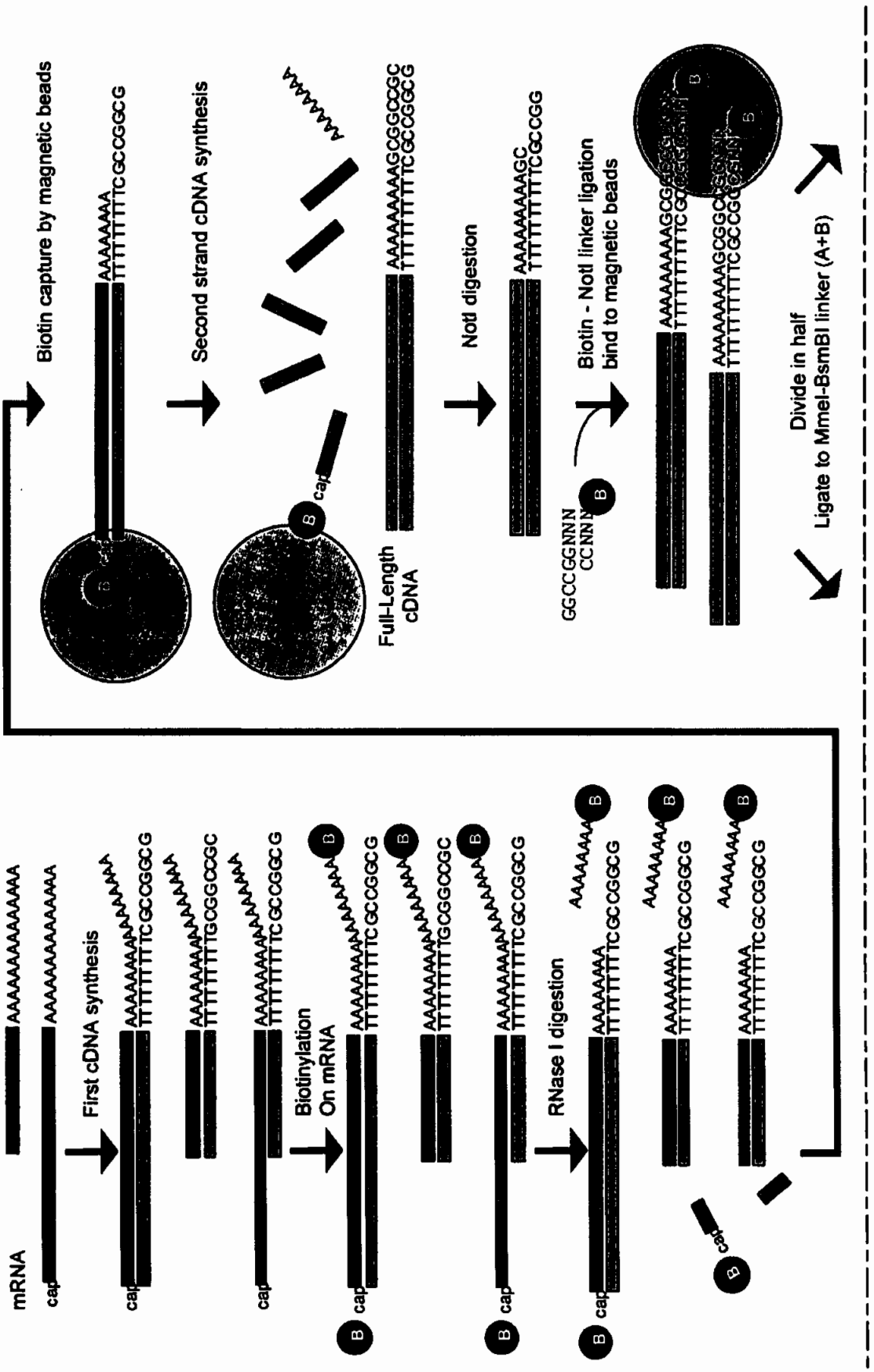

Transcripts
Terminal tags to be extracted
5' & 3' Long SAGE tags
Concatenated & sequenced
Genes
Mapping tags to genes & chromosomes
Chromosome
New Promoter
Known Abundant gene
Known Rare gene
Previously Unidentified gene
SAGE Walking increases tag specificity and confidence for identifying new genes
AAAAAAA

FIG. 2

FIG. 3


mRNA
First cDNA synthesis
Biotinylation
On mRNA
RNase I digestion
Biotin capture by magnetic beads
Second strand cDNA synthesis
Full-Length
cDNA
NotI digestion
Biotin - NotI linker ligation
bind to magnetic beads
GGCCGGNNN
CCNNN
Divide in half
Ligate to MmeI-BsmBI linker (A+B)

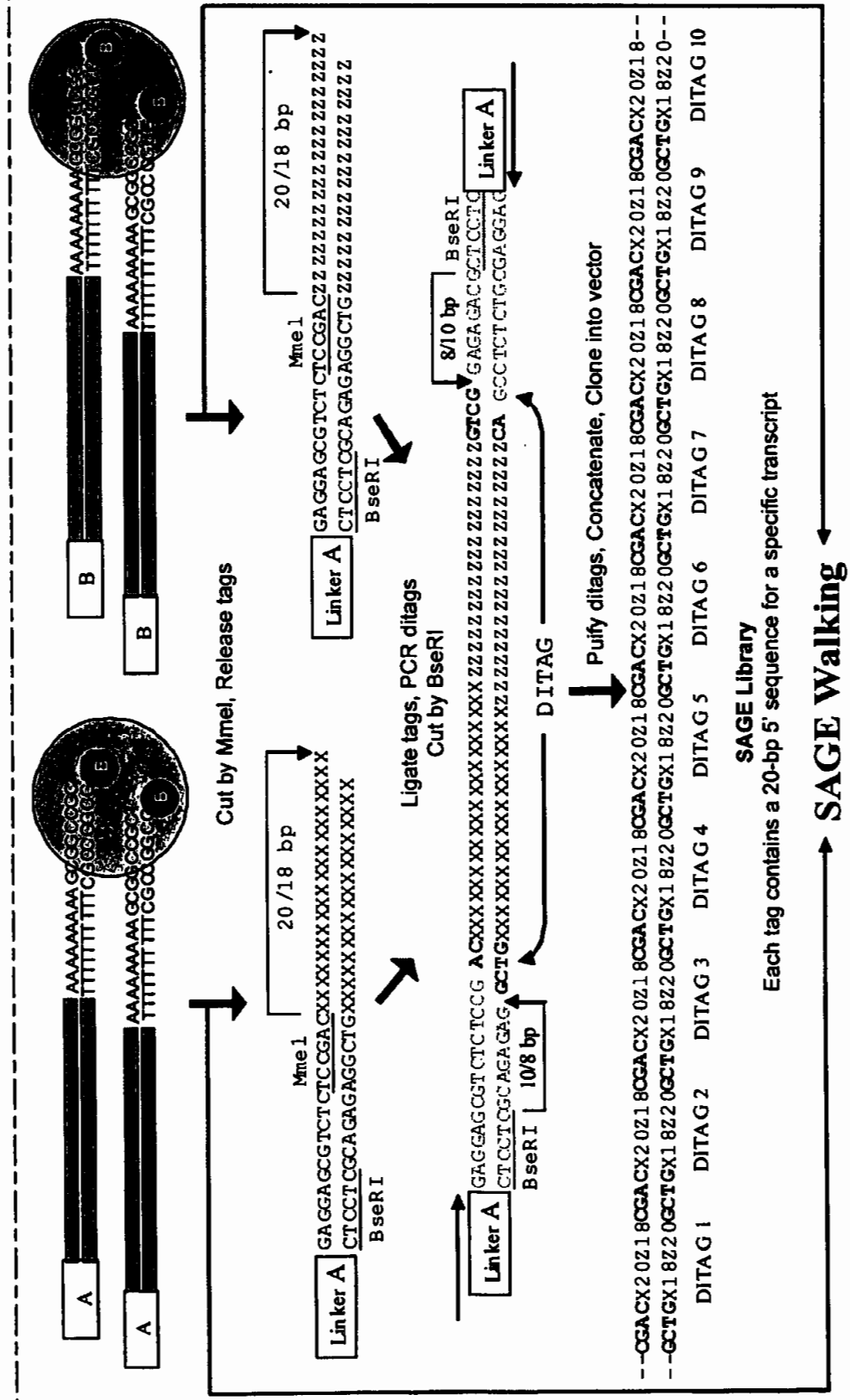
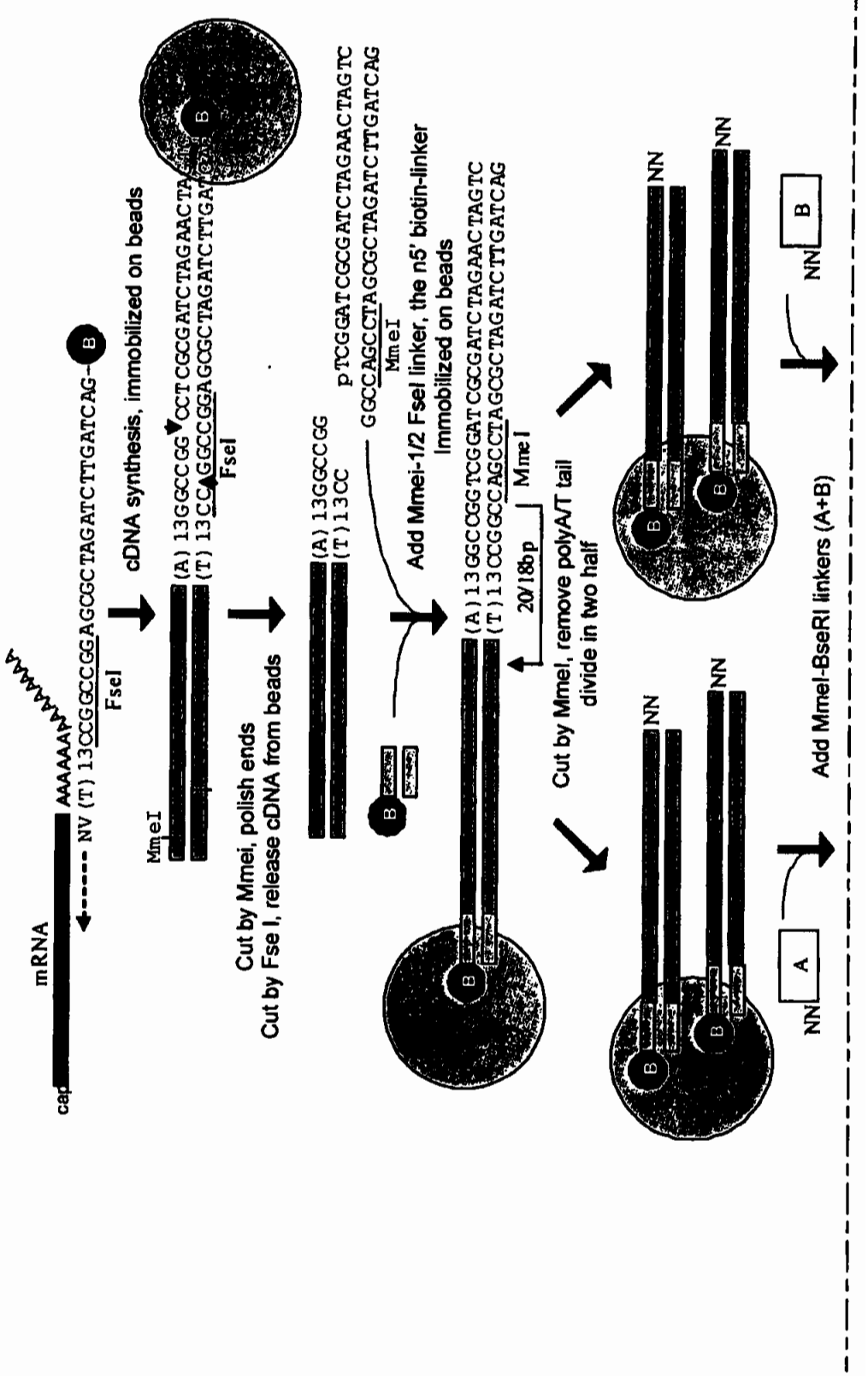

A
B
Cut by MmeI, Release tags
Mme I
20/18 bp
Linker A
BseRI
Ligate tags, PCR ditags
Cut by BseRI
8/10 bp
DITAG
Puify ditags, Concatenate, Clone into vector
SAGE Walking
SAGE Library
Each tag contains a 20-bp 5' sequence for a specific transcript
DITAG 1
DITAG 2
DITAG 3
DITAG 4
DITAG 5
DITAG 6
DITAG 7
DITAG 8
DITAG 9
DITAG 10
The second round of adding the Mme I-BsmBI linkers, releasing the tags,
Ligating and PCR the ditags, removing the arms, purifying ditags,
Concatenating and cloning→SAGE Walking Library

FIG. 3 CONT'D

FIG. 4


mRNA
AAAAAA
NV (T) 13CCGGCCGGAGCGCTAGATCTTGATCAG
FseI
B
cDNA synthesis, immobilized on beads
(A) 13GGCCGG CCTCGCGATCTAGAACTA
(T) 13CCAGGCCGGAGCGCTAGATCTTGAT
MmeI
FseI
Cut by Mmei, polish ends
Cut by Fse I, release cDNA from beads
(A) 13GGCCGG
(T) 13CC
pTCGGATCGCGATCTAGAACTAGTC
GCCCAGCCTAGCGCTAGATCTTGATCAG
MmeI
Add Mmei-1/2 FseI linker, the n5' biotin-linker
Immobilized on beads
(A) 13GGCCGGTCGGATCGCGATCTAGAACTAGTC
(T) 13CCGGCCAGCCTAGCGCTAGATCTTGATCAG
20/18bp
MmeI
Cut by MmeI, remove polyA/T tail
divide in two half
NN
A
B
Add MmeI-BseRI linkers (A+B)

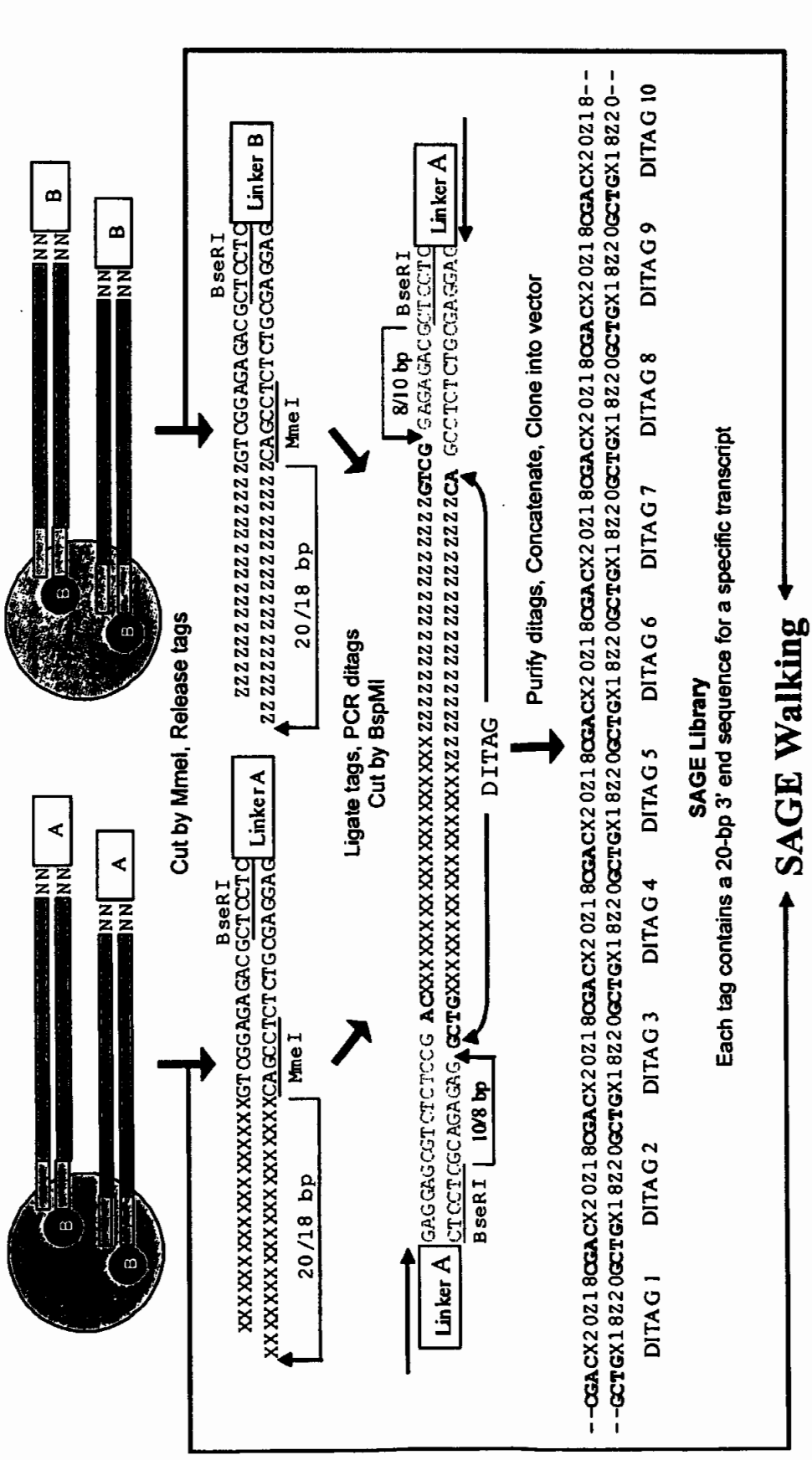

Cut by MmeI, Release tags
Ligate tags, PCR ditags
Cut by BspMI
Purify ditags, Concatenate, Clone into vector
SAGE Walking
Linker A
Linker B
BseRI
MmeI
20/18 bp
10/8 bp
8/10 bp
DITAG
DITAG 1
DITAG 2
DITAG 3
DITAG 4
DITAG 5
DITAG 6
DITAG 7
DITAG 8
DITAG 9
DITAG 10
SAGE Library
Each tag contains a 20-bp 3' end sequence for a specific transcript
The second round of adding the Mme I-BsmBI linkers, releasing the tags,
Ligating and PCR the ditags, removing the arms, purifying ditags,
Concatenating and cloning → SAGE Walking Library

FIG. 4 CONT'D