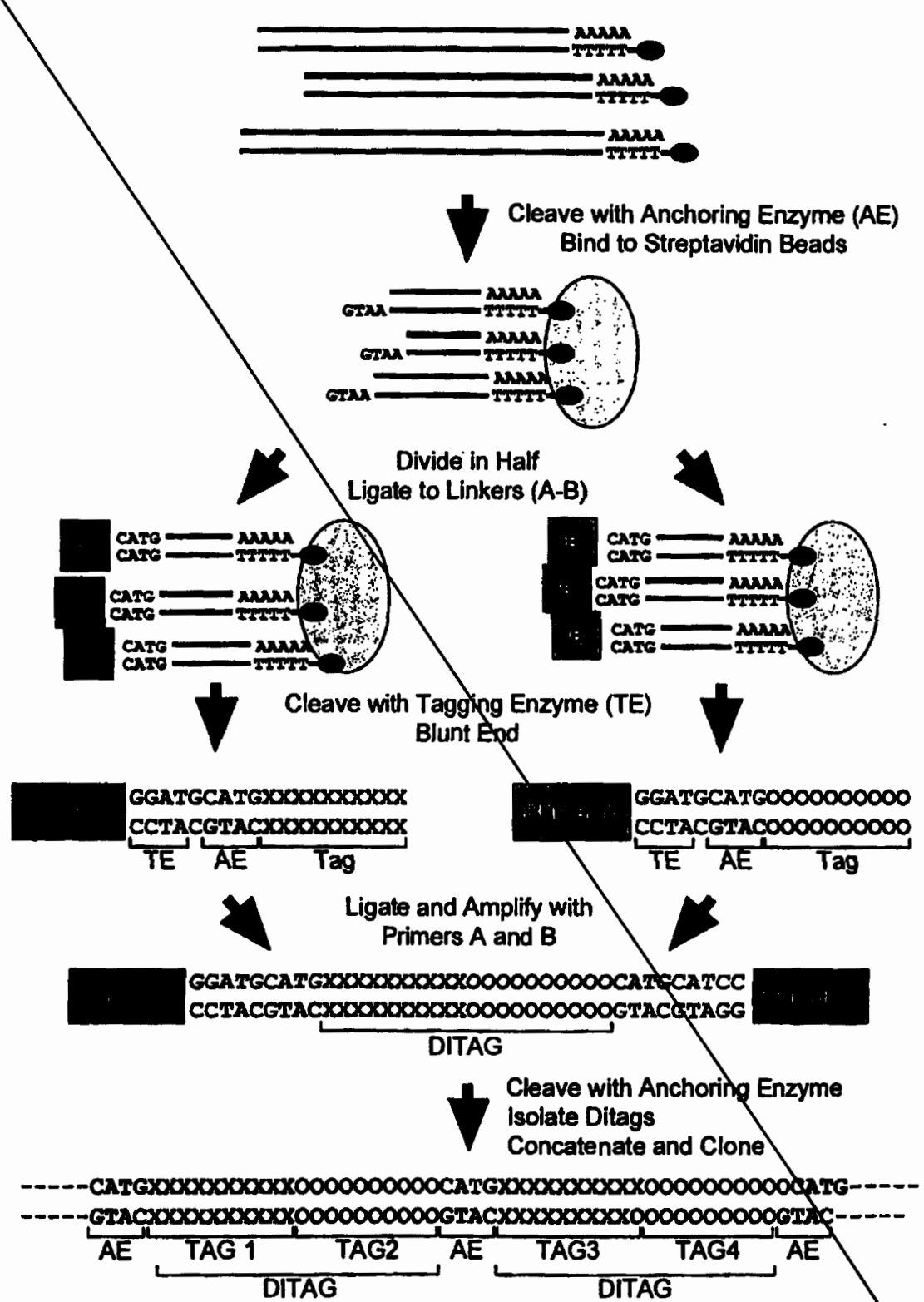

AAAAA
TTTTT
Cleave with Anchoring Enzyme (AE)
Bind to Streptavidin Beads
GTAA
Divide in Half
Ligate to Linkers (A-B)
CATG
Cleave with Tagging Enzyme (TE)
Blunt End
GGATGCATGXXXXXXXXXX
CCTACGTACXXXXXXXXXX
GGATGCATGOOOOOOOOOO
CCTACGTACOOOOOOOOOO
TE
AE
Tag
Ligate and Amplify with
Primers A and B
GGATGCATGXXXXXXXXXXOOOOOOOOOOCATGCATCC
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

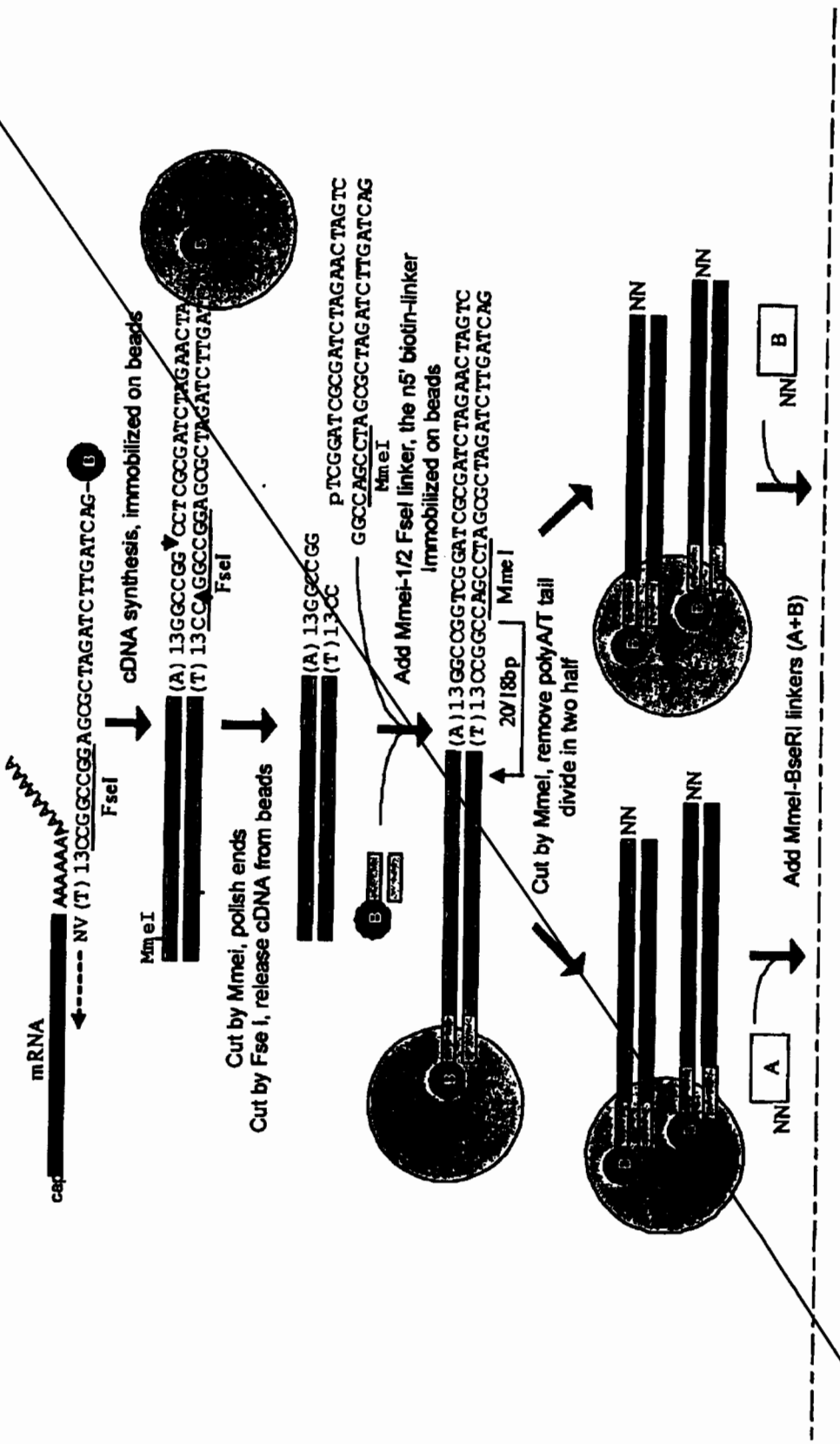
FIG. 3

mRNA
cap
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
20/18 bp
MmeI
Linker A
BseRI
Ligate tags, PCR ditags
Cut by BseRI
8/10 bp
DITAG
Purify ditags, Concatenate, Clone into vector
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
SAGE Walking
The second round of adding the MmeI-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
cap
NV (T) 13CCGGCCGGAGCGCTAGATCTTGATCAG-B
FseI
cDNA synthesis, immobilized on beads
(A) 13GGCCGG CCTCGCGATCTAGAACTA
(T) 13CCAGGCCGGAGCGCTAGATCTTGAT
FseI
MmeI
Cut by Mmei, polish ends
Cut by Fse I, release cDNA from beads
(A) 13GGCCGG
(T) 13CC
pTCGGATCGCGATCTAGAACTAGTC
GGCCAGCCTAGCGCTAGATCTTGATCAG
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
NN
NN
NN
A
B
Add MmeI-BseRI linkers (A+B)

Linker A
Linker B
BseRI
MmeI
20/18 bp
10/8 bp
Cut by MmeI, Release tags
Ligate tags, PCR ditags
Cut by BspMI
Purify ditags, Concatenate, Clone into vector
DITAG
SAGE Walking
SAGE Library
Each tag contains a 20-bp 3' end 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 MmeI-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

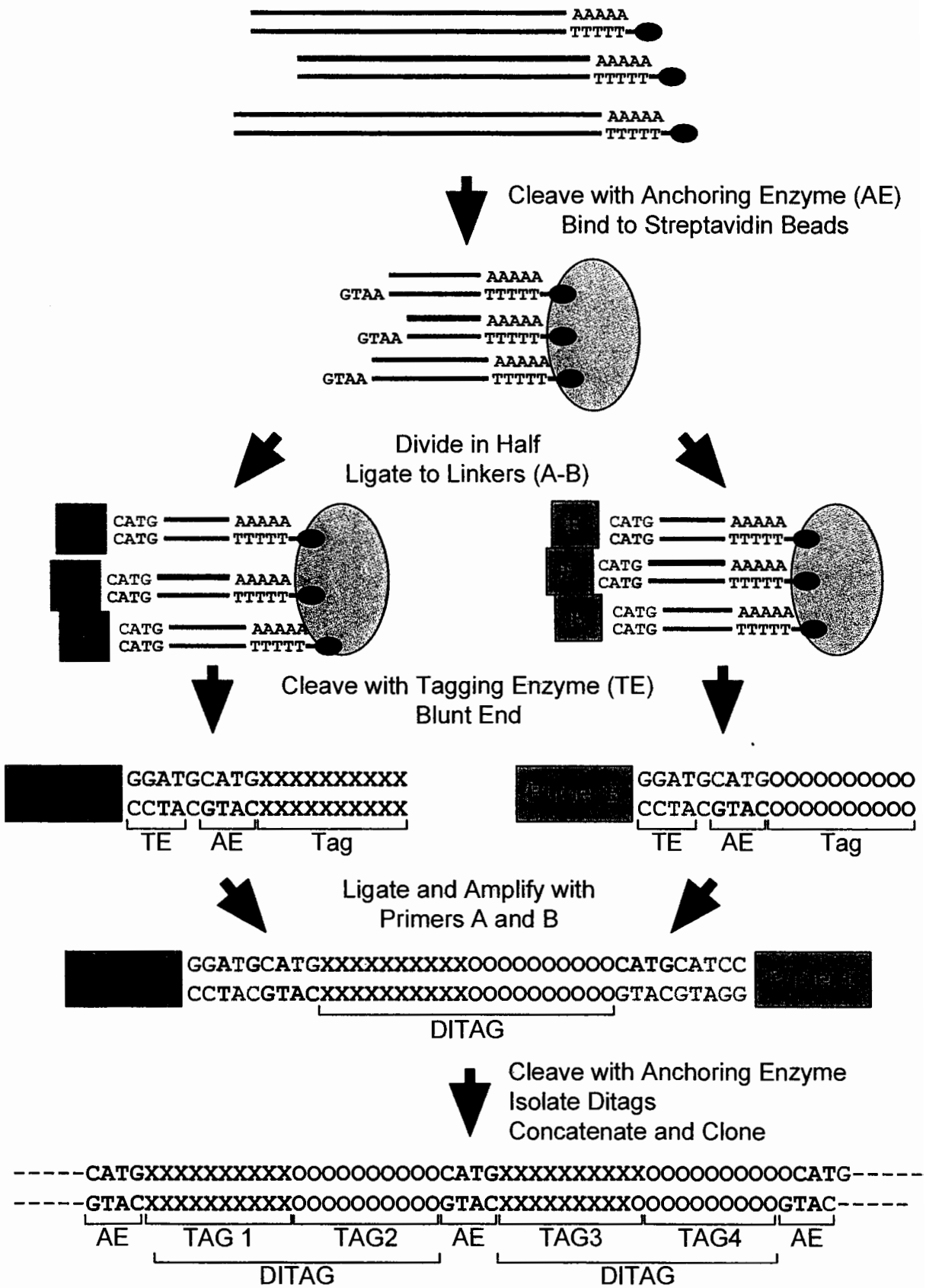
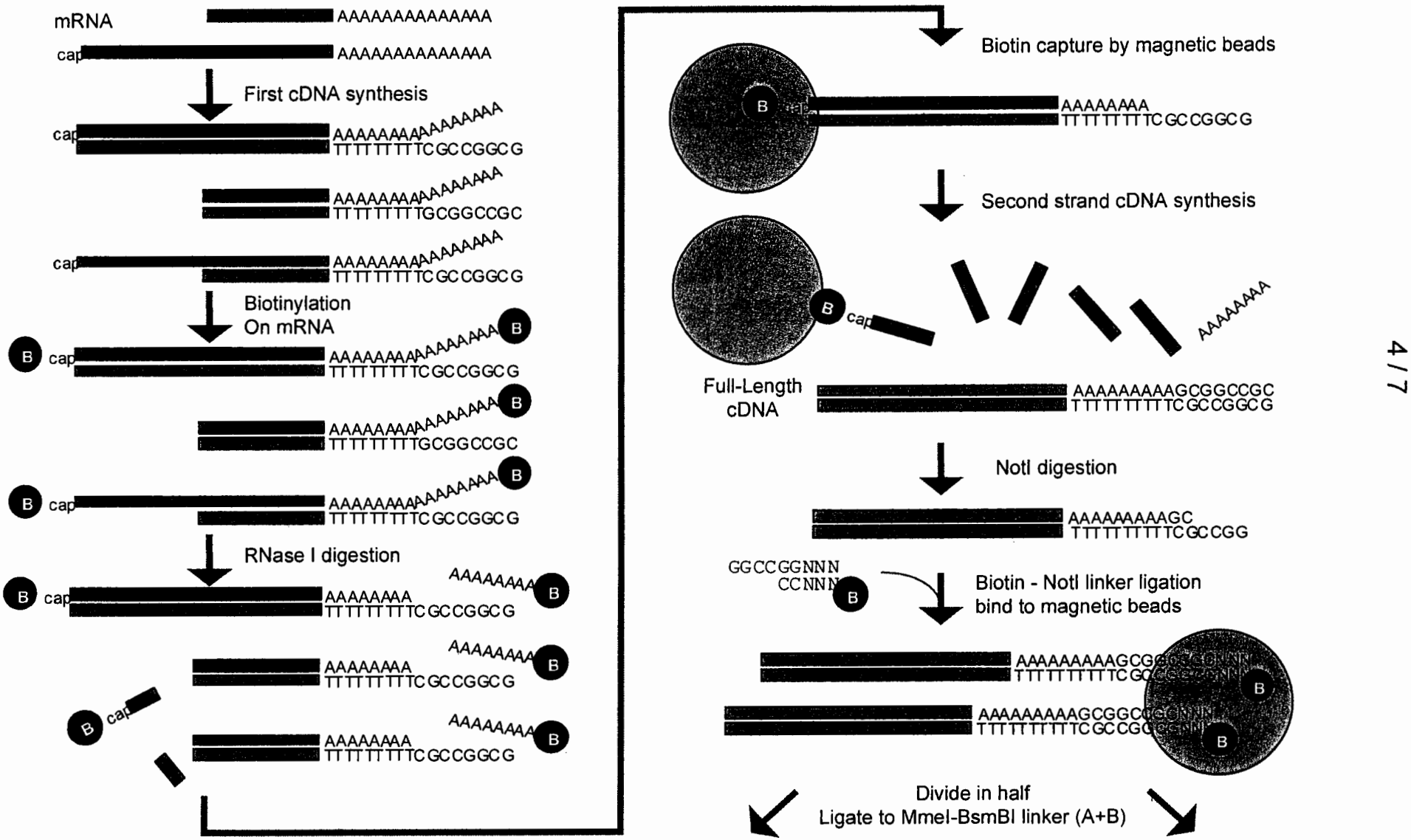
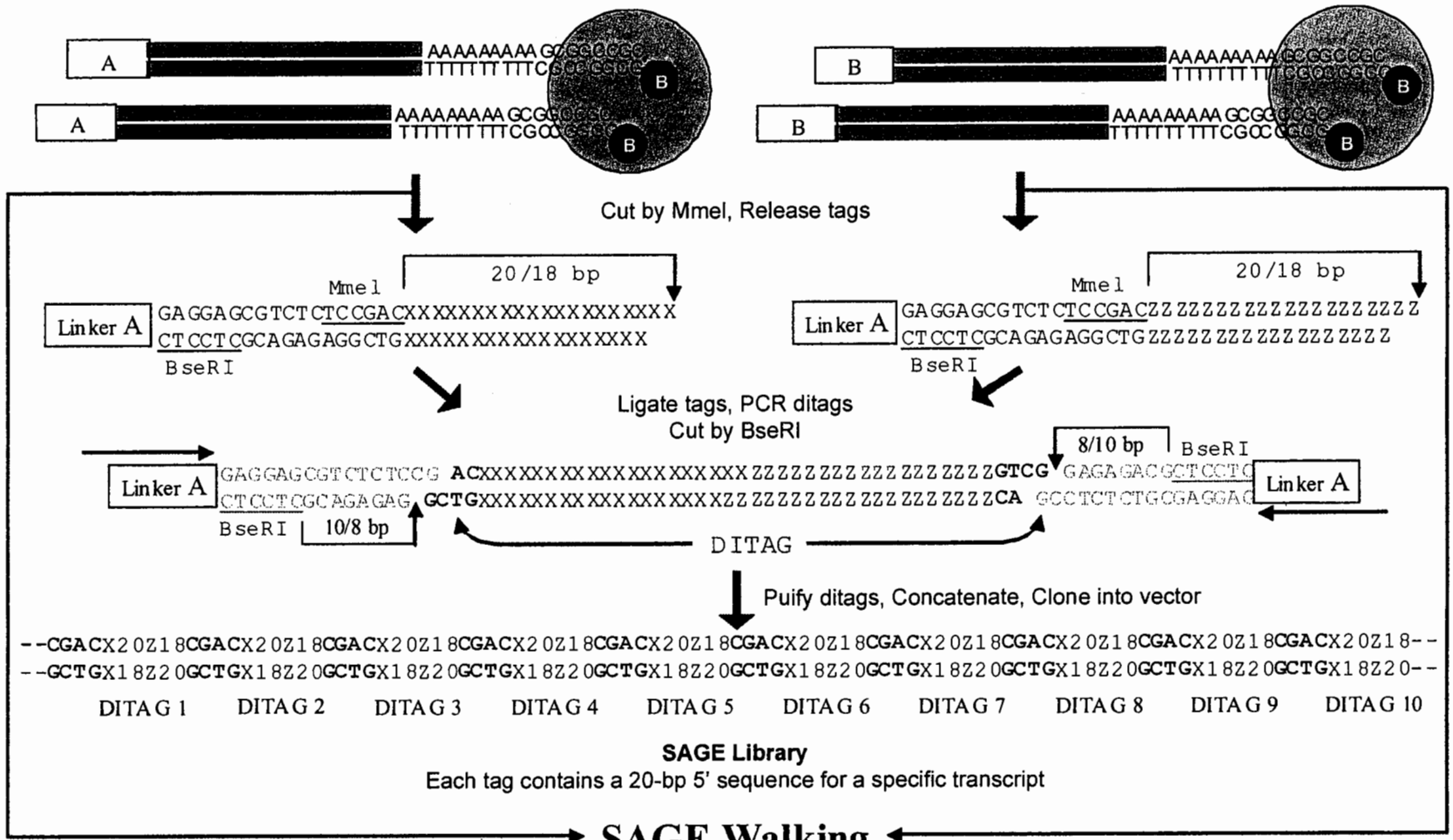

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GGATGCATGOOOOOOOOOO
CCTACGTACOOOOOOOOOO
TE
AE
Tag
Ligate and Amplify with
Primers A and B
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DITAG
Cleave with Anchoring Enzyme
Isolate Ditags
Concatenate and Clone
-----CATGXXXXXXXXXXOOOOOOOOOOCATGXXXXXXXXXXOOOOOOOOOOCATG-----
-----GTACXXXXXXXXXXOOOOOOOOOOGTACXXXXXXXXXXOOOOOOOOOOGTAC-----
AE
TAG 1
TAG2
AE
TAG3
TAG4
AE
DITAG
DITAG

FIG. 1B

FIG. 3

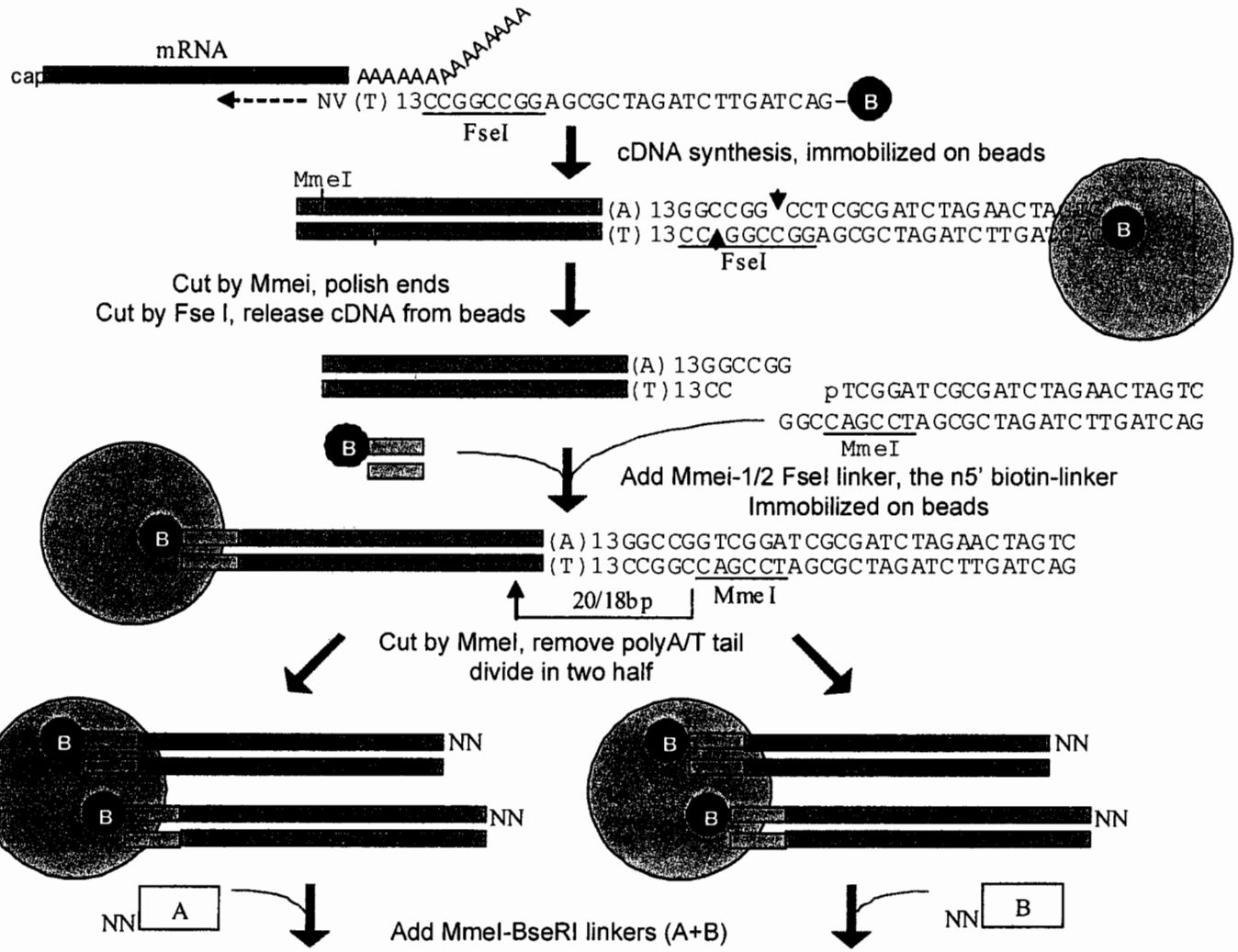

mRNA
cap
AAAAAAAAAAAAAA
First cDNA synthesis
TTTTTTTTCGCCGGCG
TTTTTTTTGCGGCCGC
Biotinylation
On mRNA
B
RNase I digestion
AAAAAAAA
Biotin capture by magnetic beads
Second strand cDNA synthesis
Full-Length
cDNA
AAAAAAAAAGCGGCCGC
NotI digestion
AAAAAAAAAGC
TTTTTTTTTCGCCGG
GGCCGGNNN
CCNNN
Biotin - NotI linker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

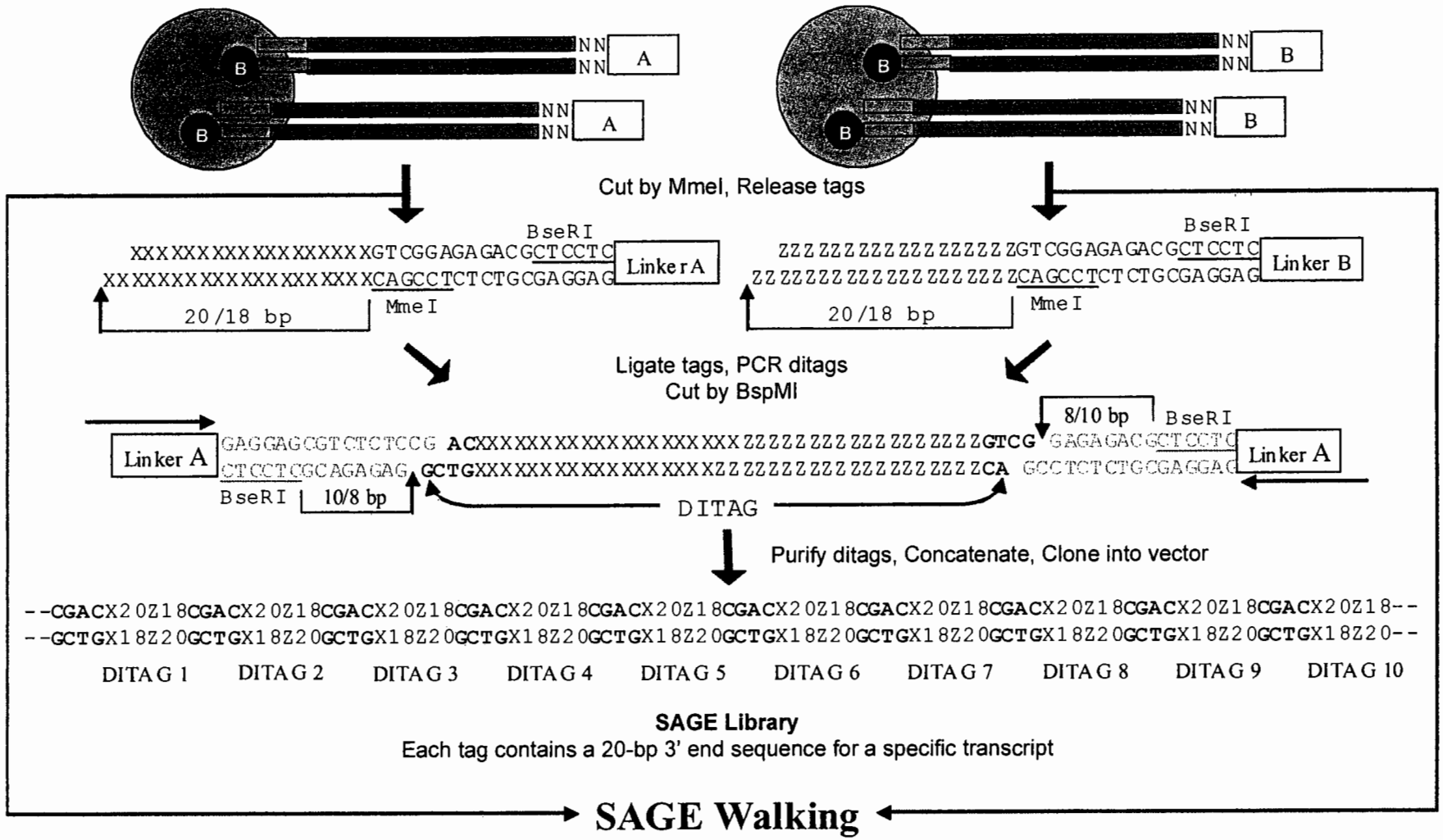


The second round of adding the Mme I-BsmBI linkers, releasing the tags, Ligating and PCR the ditags, removing the arms, purifying ditags, Concatenating and cloning → SAGE Walking Library

FIG. 3 CONT'D

FIG. 4


mRNA
cap
AAAAAA
NV(T)13CCGGCCGGAGCGCTAGATCTTGATCAG-B
FseI
cDNA synthesis, immobilized on beads
MmeI
(A)13GGCCGG
(T)13CC
FseI
B
Cut by Mmei, polish ends
Cut by Fse I, release cDNA from beads
(A)13GGCCGG
(T)13CC
pTCGGATCGCGATCTAGAACTAGTC
GGCCAGCCTAGCGCTAGATCTTGATCAG
MmeI
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Immobilized on beads
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20/18bp
MmeI
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divide in two half
NN
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Add MmeI-BseRI linkers (A+B)



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