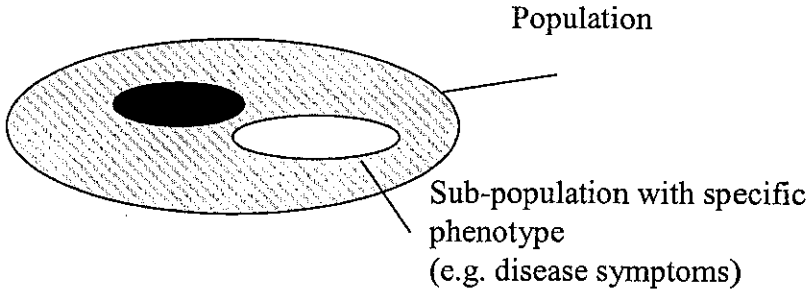
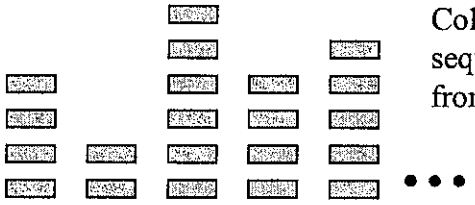
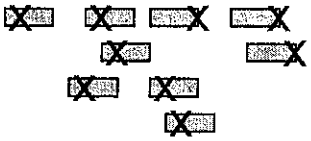
1. Cluster population in sub-populations according to specific phenotypes and collect documented biological samples



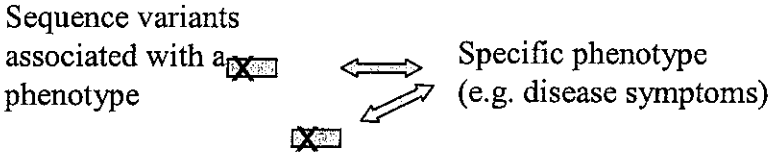
2. Generate restriction sequence tags for each sample using one of the methods of the invention



3. Compare tags between samples or with reference data to identify sequence variants



4. Identify sequence variants associated with a sub-population, i.e. variants associated with a specific phenotype



5. Map these sequence variants on the genomic DNA and identify genomic regions associated with phenotype

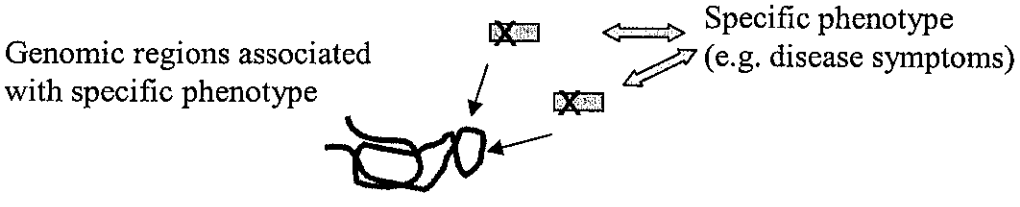
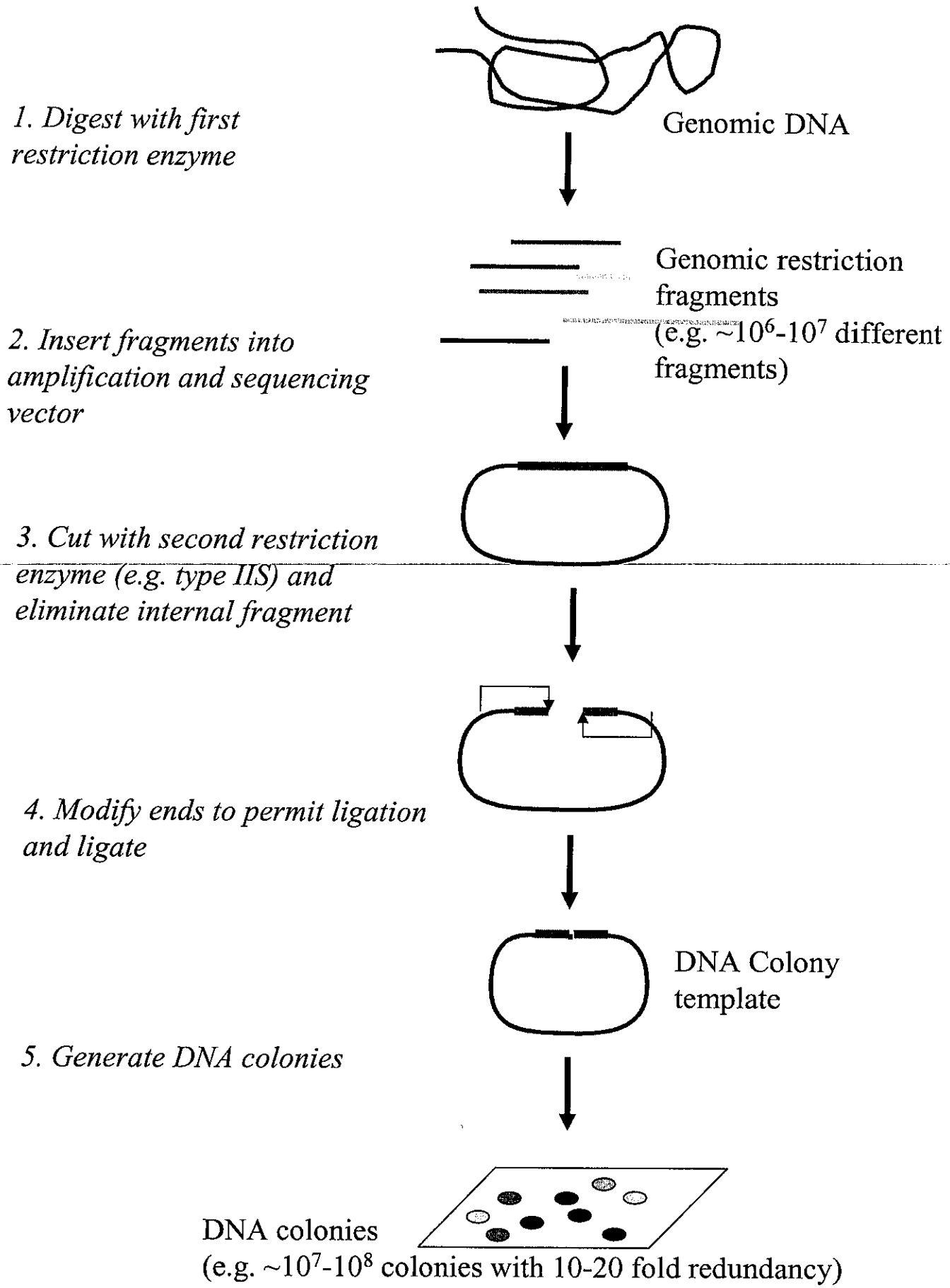
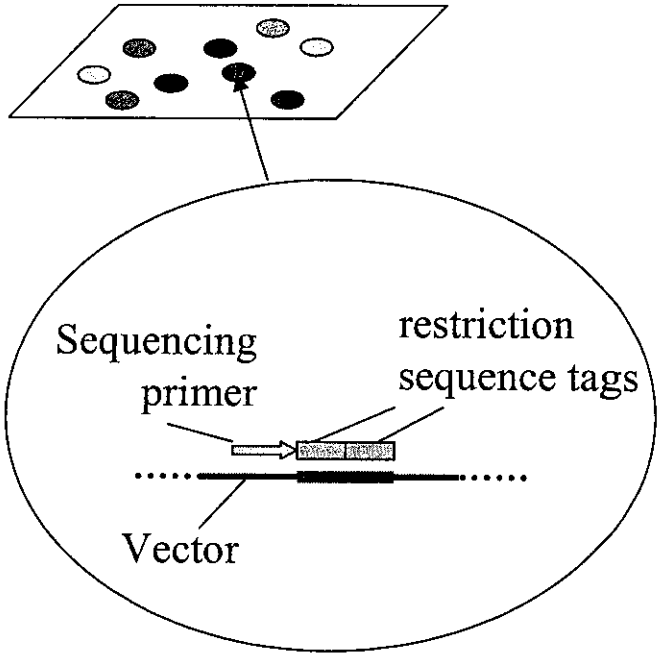


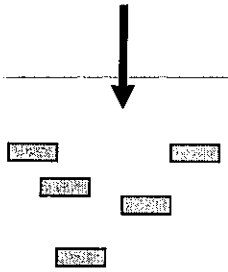
FIG. 2A


1. Digest with first restriction enzyme
Genomic DNA
Genomic restriction fragments
(e.g. ~10^6-10^7 different fragments)
2. Insert fragments into amplification and sequencing vector
3. Cut with second restriction enzyme (e.g. type IIS) and eliminate internal fragment
4. Modify ends to permit ligation and ligate
DNA Colony template
5. Generate DNA colonies
DNA colonies
(e.g. ~10^7-10^8 colonies with 10-20 fold redundancy)

6. Sequence DNA colonies

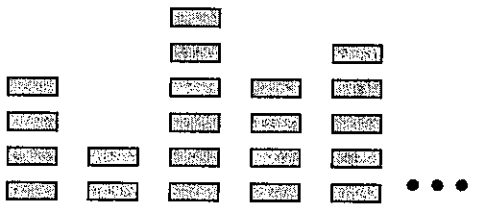


7. Generate restriction sequence tags



Two tags obtained from each DNA colony (e.g. ~$2x10^6$-$2x10^7$ unique tags)

8. Order restriction sequence tags



Collection of ordered restriction sequence tags

1. Digest with first restriction enzyme that has the particularity to cut DNA on both sides outside of its recognition site

2. Insert fragments into amplification and sequencing vector

3. Generate DNA colonies

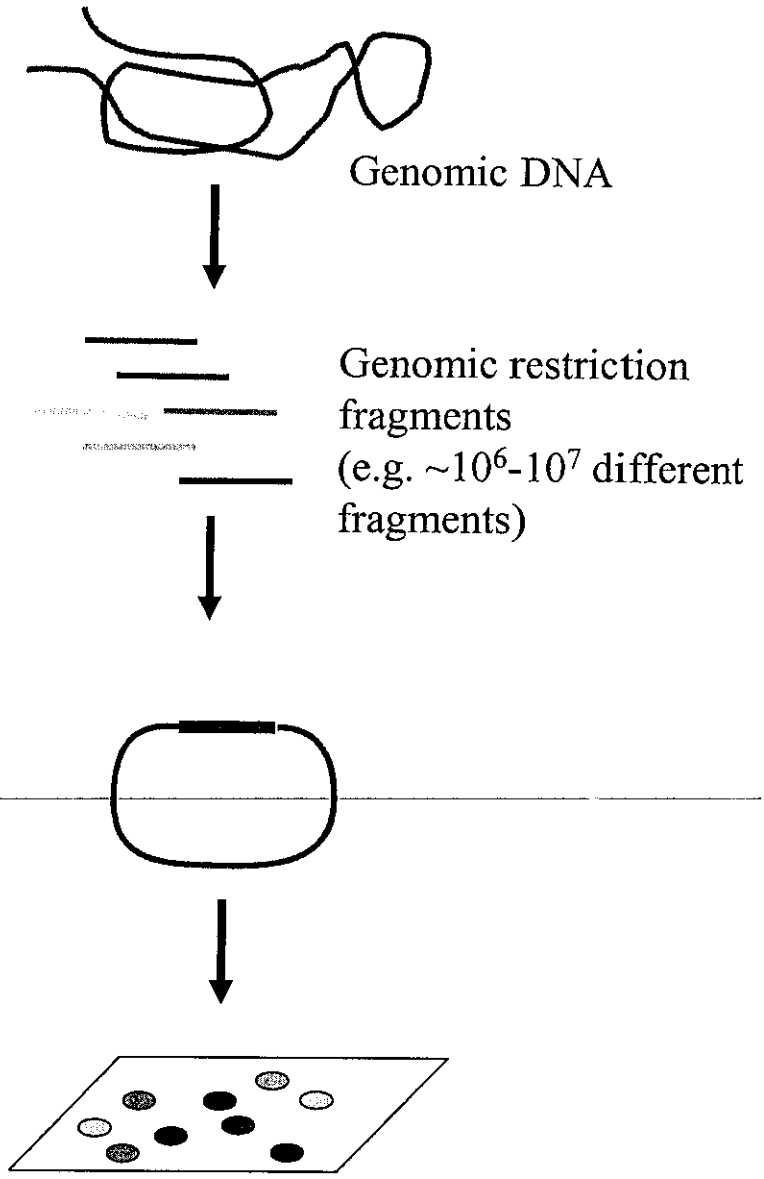
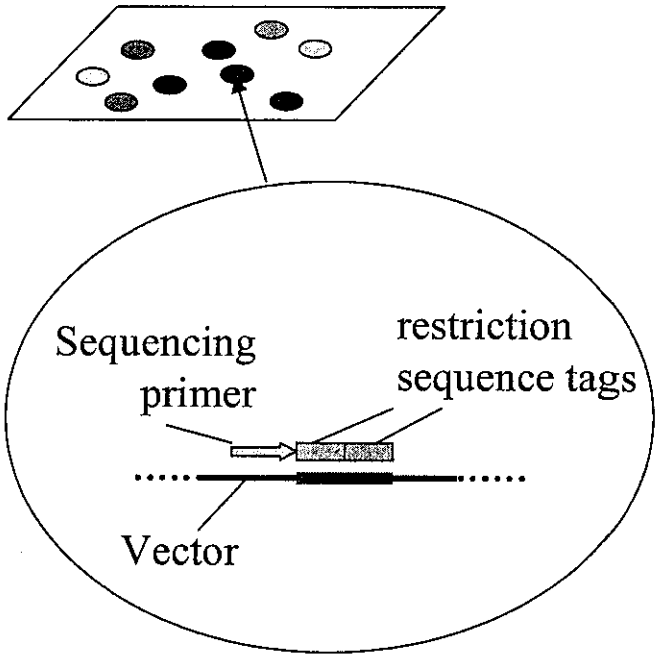
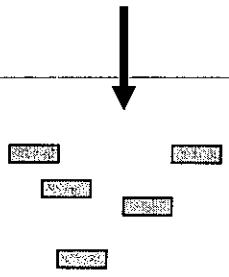


FIG. 3B

4. Sequence DNA colonies

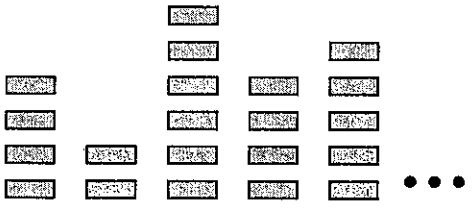


5. Generate restriction sequence tags



Two tags obtained from each DNA colony (e.g. ~$2x10^6$-$2x10^7$ unique tags)

6. Order restriction sequence tags



Collection of ordered restriction sequence tags

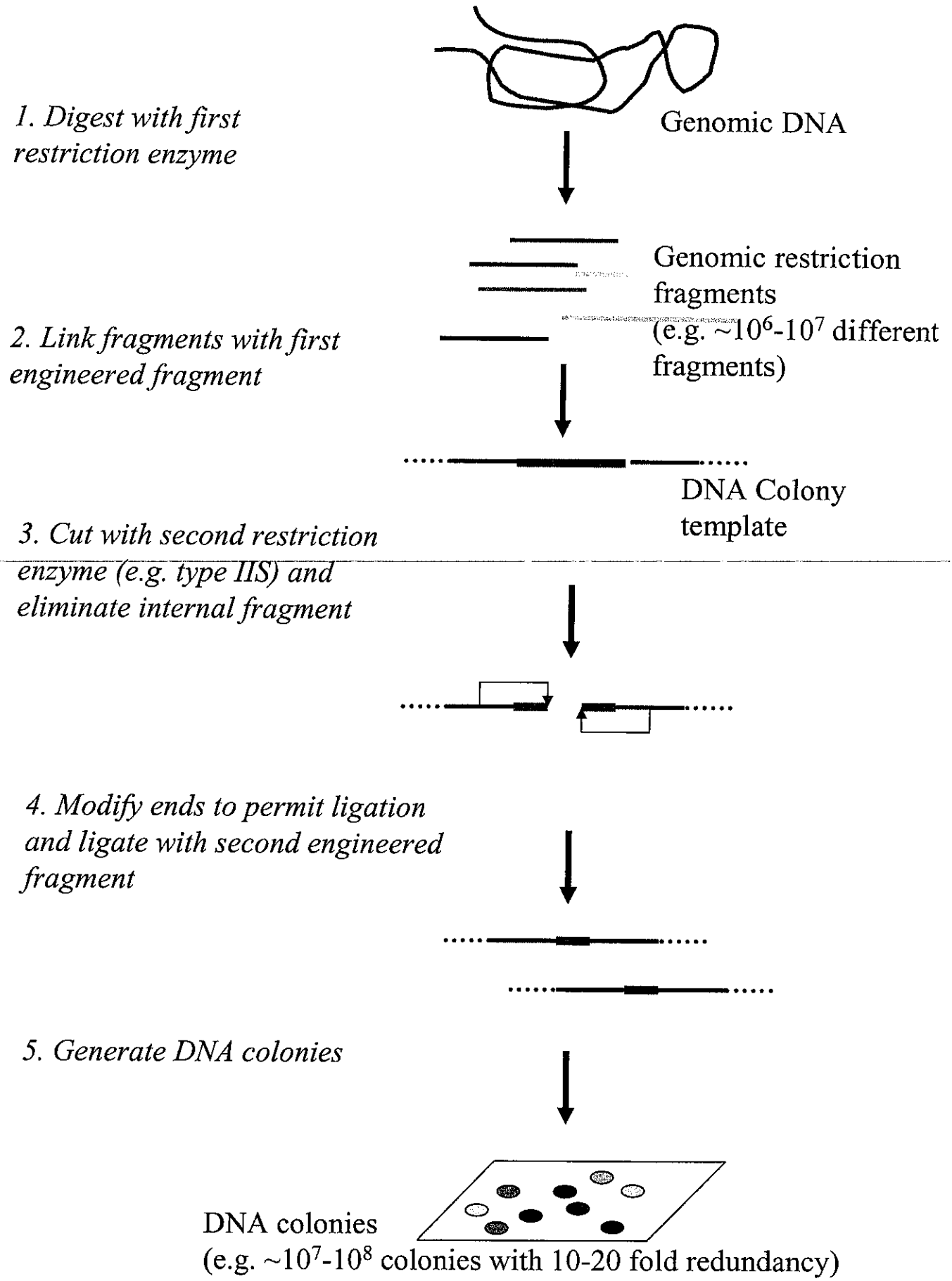
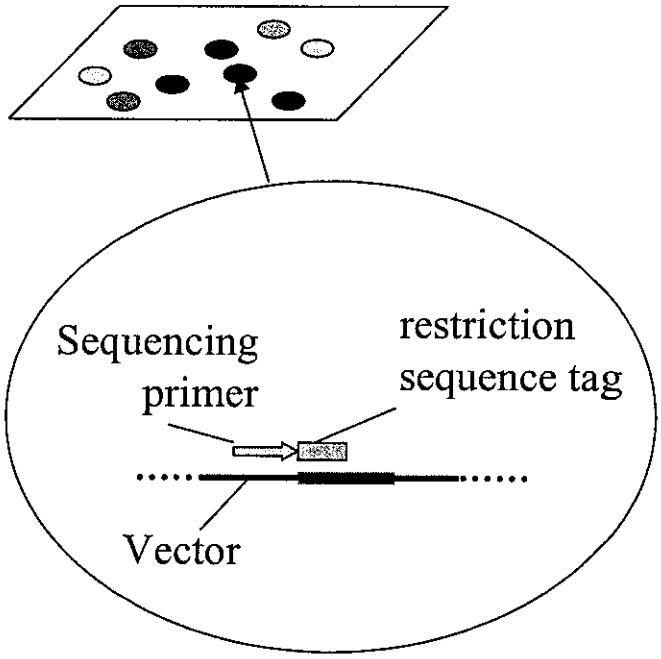
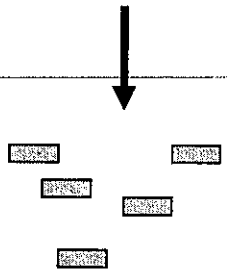

1. Digest with first restriction enzyme
Genomic DNA
Genomic restriction fragments
(e.g. ~10^6-10^7 different fragments)
2. Link fragments with first engineered fragment
DNA Colony template
3. Cut with second restriction enzyme (e.g. type IIS) and eliminate internal fragment
4. Modify ends to permit ligation and ligate with second engineered fragment
5. Generate DNA colonies
DNA colonies
(e.g. ~10^7-10^8 colonies with 10-20 fold redundancy)

FIG. 4B

6. Sequence DNA colonies

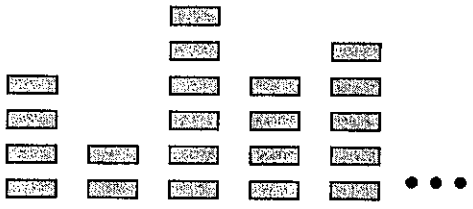


7. Generate restriction sequence tags

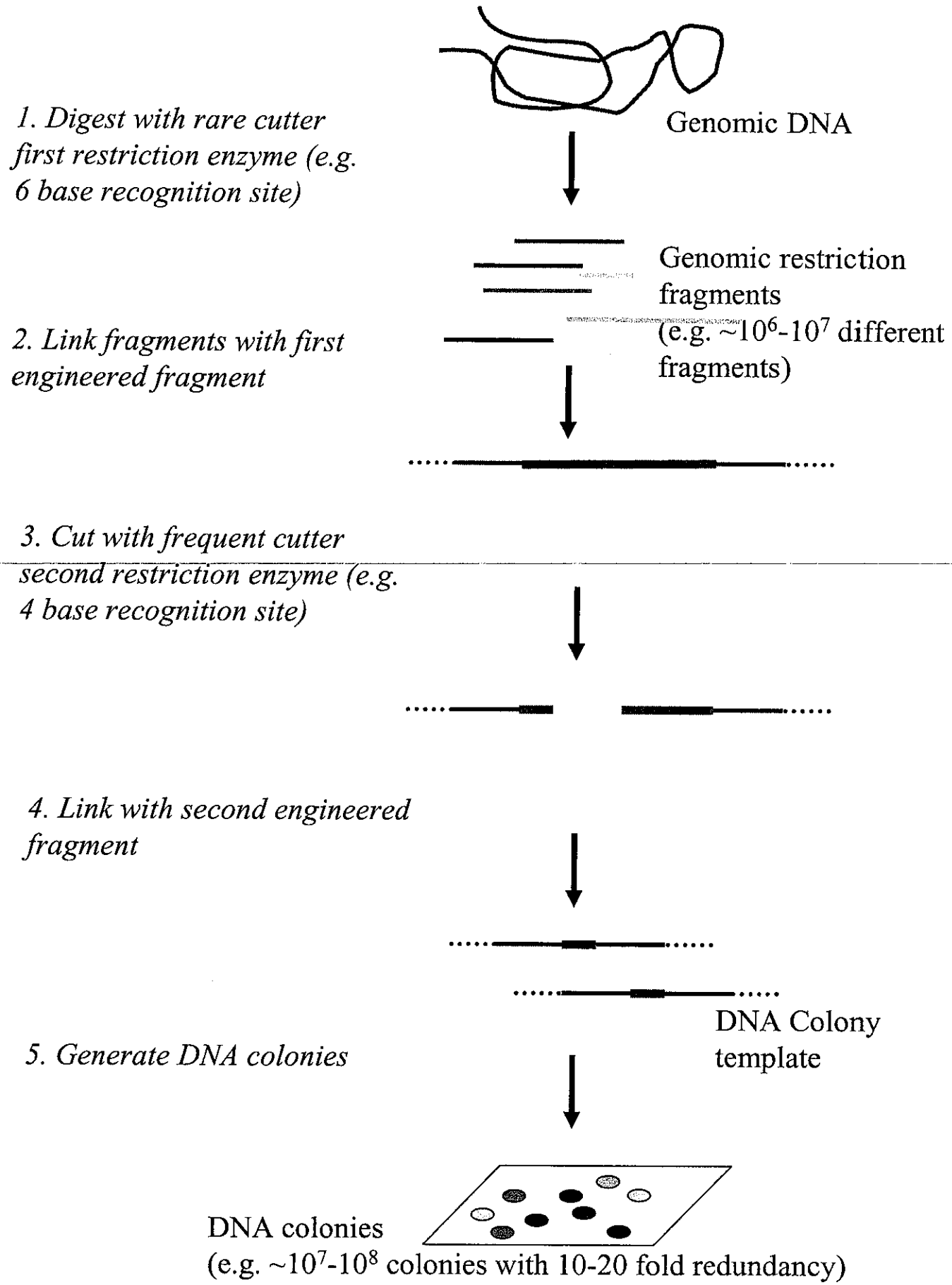


One tag obtained from each DNA colony (e.g. ~$2x10^6$-$2x10^7$ unique tags)

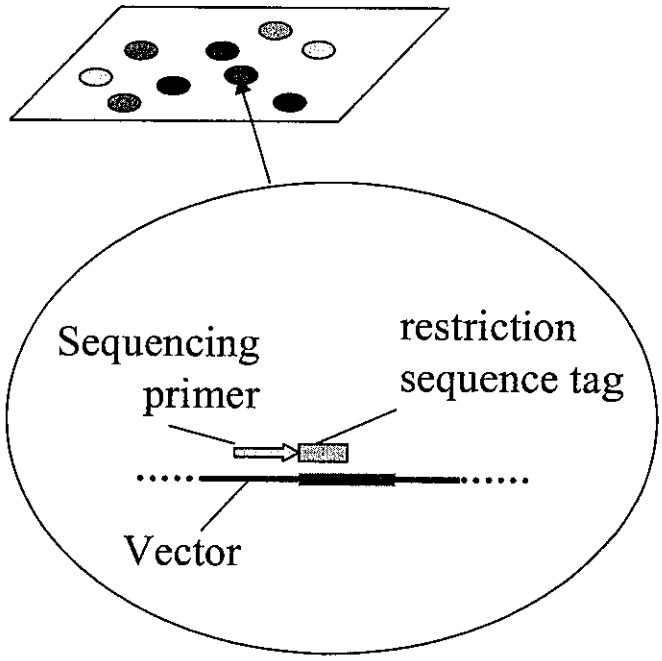
8. Order restriction sequence tags



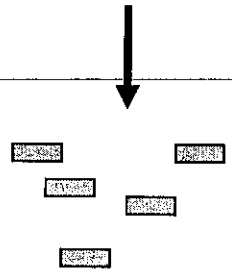
Collection of ordered restriction sequence tags


1. Digest with rare cutter first restriction enzyme (e.g. 6 base recognition site)
Genomic DNA
Genomic restriction fragments
(e.g. ~10^6-10^7 different fragments)
2. Link fragments with first engineered fragment
3. Cut with frequent cutter second restriction enzyme (e.g. 4 base recognition site)
4. Link with second engineered fragment
DNA Colony template
5. Generate DNA colonies
DNA colonies
(e.g. ~10^7-10^8 colonies with 10-20 fold redundancy)

6. Sequence DNA colonies

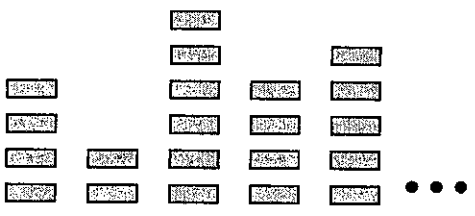


7. Generate restriction sequence tags

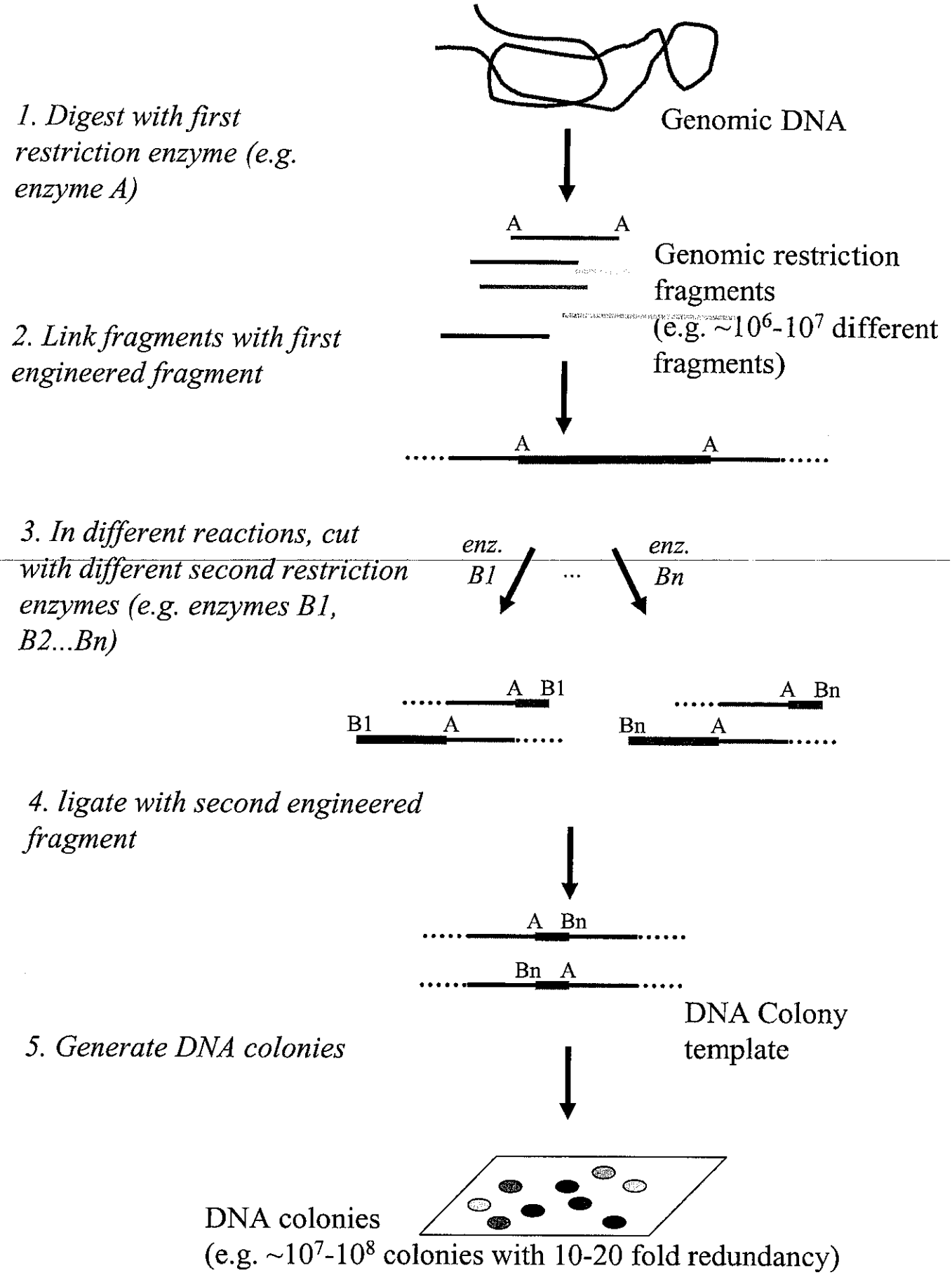


One tag obtained from each DNA colony (e.g. ~$2x10^6$-$2x10^7$ unique tags)

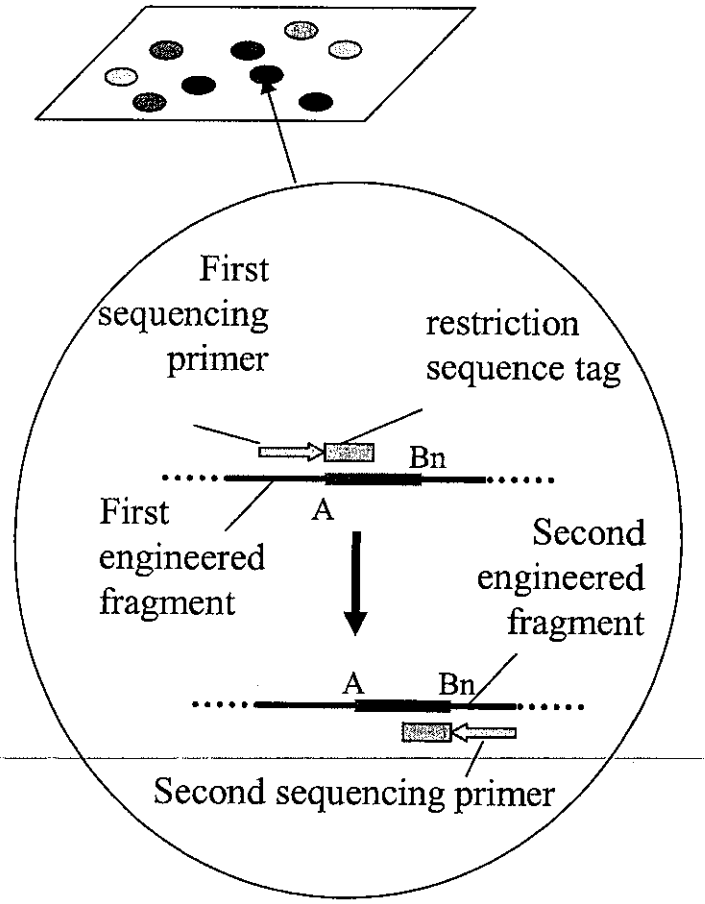
8. Order restriction sequence tags



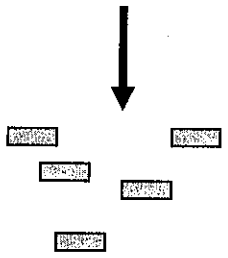
Collection of ordered restriction sequence tags


1. Digest with first restriction enzyme (e.g. enzyme A)
Genomic DNA
A A
Genomic restriction fragments
(e.g. ~10^6-10^7 different fragments)
2. Link fragments with first engineered fragment
A A
3. In different reactions, cut with different second restriction enzymes (e.g. enzymes B1, B2...Bn)
enz. B1
enz. Bn
A B1
B1 A
A Bn
Bn A
4. ligate with second engineered fragment
A Bn
Bn A
DNA Colony template
5. Generate DNA colonies
DNA colonies
(e.g. ~10^7-10^8 colonies with 10-20 fold redundancy)

6. Sequence DNA colonies sequentially with first and second sequencing primers

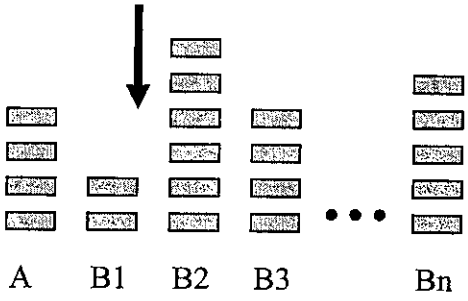


7. Generate restriction sequence tags

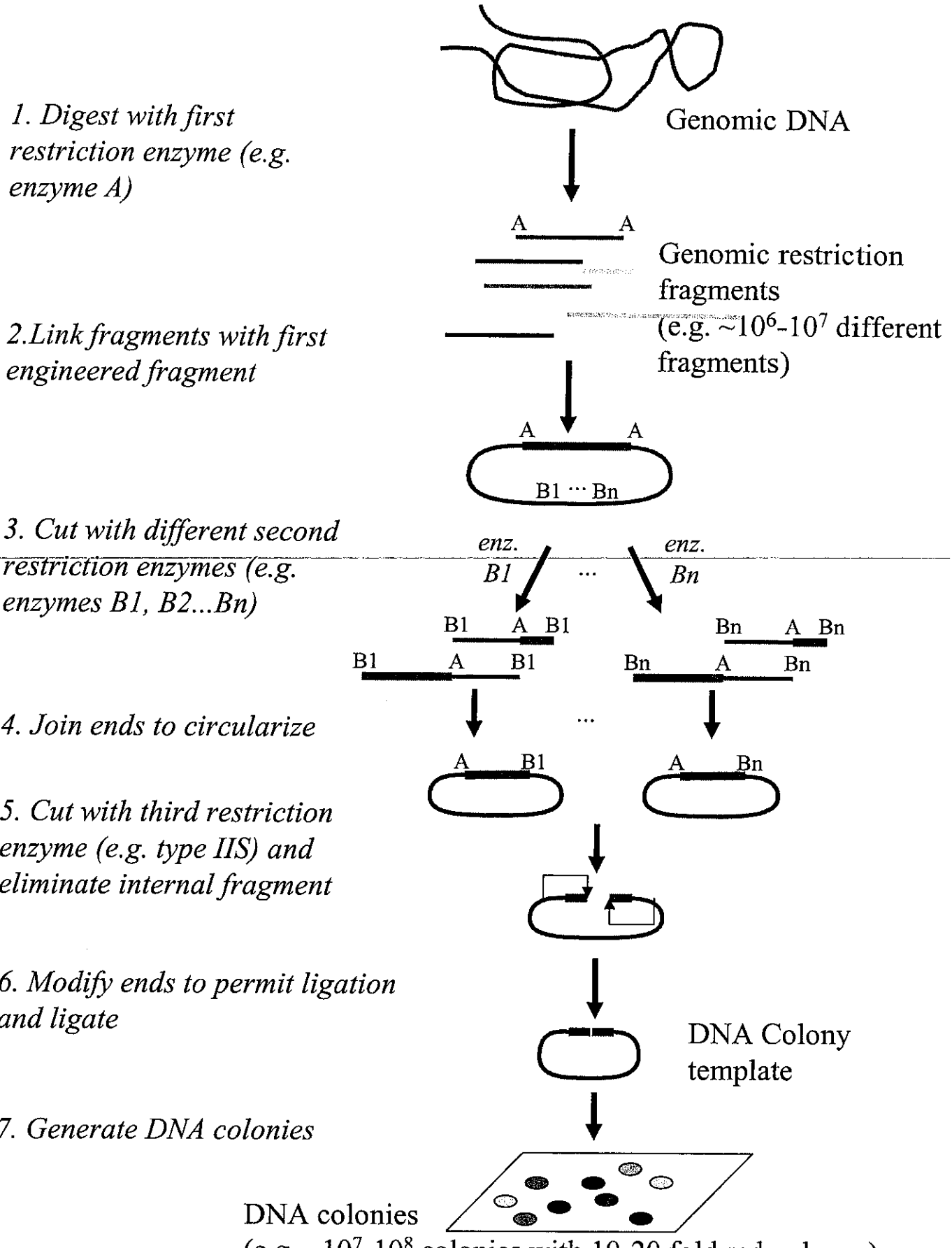


One tag obtained from each DNA colony (e.g. ~$2x10^6$-$2x10^7$ unique tags)

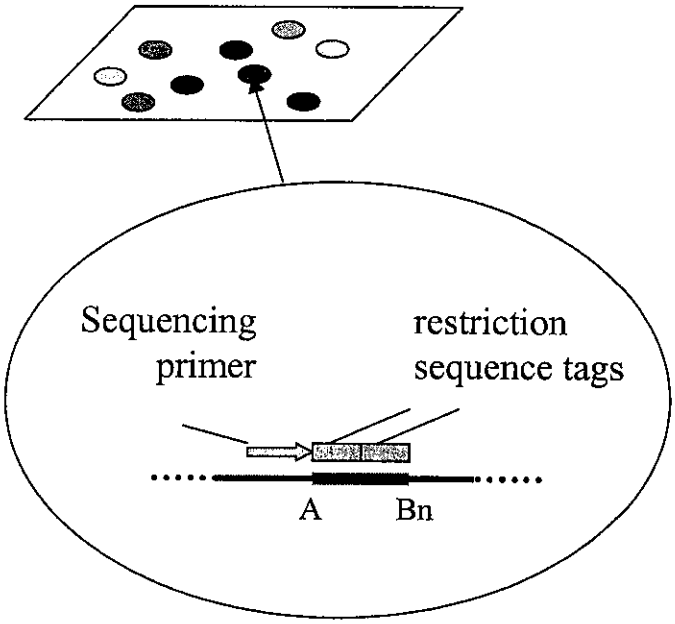
8. Order restriction sequence tags, e.g. second tags (B1 to Bn) that are paired with the same first tag (A)



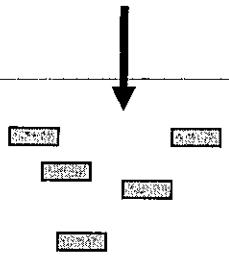
Collection of ordered restriction sequence tags


1. Digest with first restriction enzyme (e.g. enzyme A)
Genomic DNA
A
A
Genomic restriction fragments
(e.g. ~10^6-10^7 different fragments)
2.Link fragments with first engineered fragment
A
A
B1 ··· Bn
3. Cut with different second restriction enzymes (e.g. enzymes B1, B2...Bn)
enz. B1
enz. Bn
B1 A B1
B1 A B1
Bn A Bn
Bn A Bn
4. Join ends to circularize
A B1
A Bn
5. Cut with third restriction enzyme (e.g. type IIS) and eliminate internal fragment
6. Modify ends to permit ligation and ligate
DNA Colony template
7. Generate DNA colonies
DNA colonies
(e.g. ~10^7-10^8 colonies with 10-20 fold redundancy)

8. Sequence DNA colonies using sequencing primer

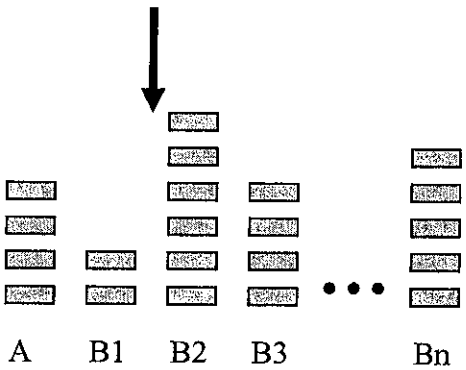


9. Process sequence to identify restriction sequence tags

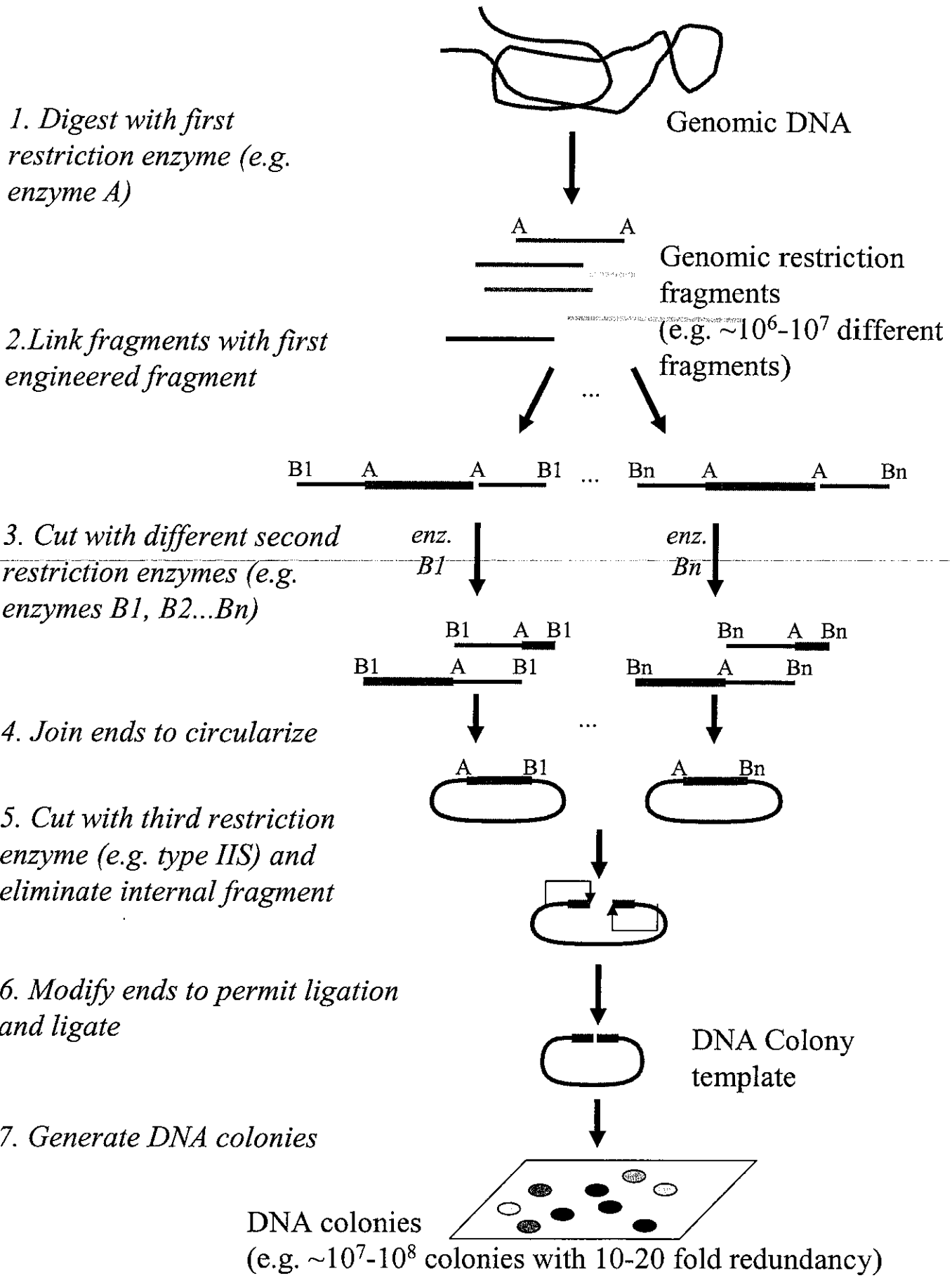


Two tags obtained from each DNA colony (e.g. ~$2x10^6$-$2x10^7$ unique tags)

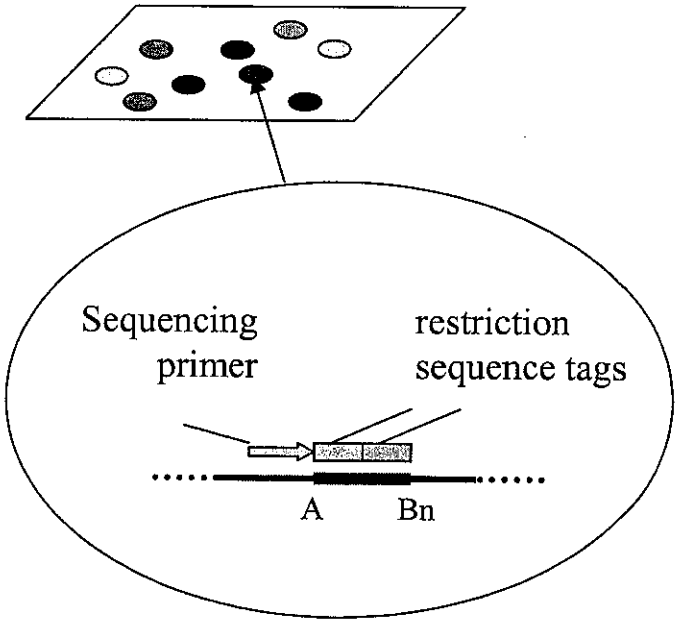
10. Order restriction sequence tags, e.g. second tags (B1 to Bn) that are paired with the same first tag (A)



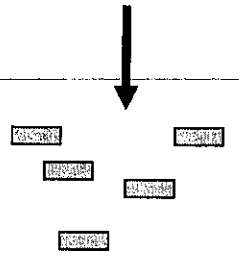
Collection of ordered restriction sequence tags


1. Digest with first restriction enzyme (e.g. enzyme A)
Genomic DNA
A A
Genomic restriction fragments
(e.g. ~10^6-10^7 different fragments)
2.Link fragments with first engineered fragment
B1 A A B1 ... Bn A A Bn
3. Cut with different second restriction enzymes (e.g. enzymes B1, B2...Bn)
enz. B1
enz. Bn
B1 A B1
B1 A B1
Bn A Bn
Bn A Bn
4. Join ends to circularize
A B1
A Bn
5. Cut with third restriction enzyme (e.g. type IIS) and eliminate internal fragment
6. Modify ends to permit ligation and ligate
DNA Colony template
7. Generate DNA colonies
DNA colonies
(e.g. ~10^7-10^8 colonies with 10-20 fold redundancy)

8. Sequence DNA colonies using sequencing primer

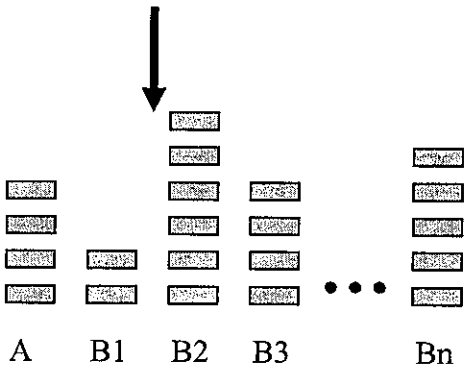


9. Process sequence to identify restriction sequence tags



Two tags obtained from each DNA colony (e.g. ~$2x10^6$-$2x10^7$ unique tags)

10. Order restriction sequence tags, e.g. second tags (B1 to Bn) that are paired with the same first tag (A)



Collection of ordered restriction sequence tags

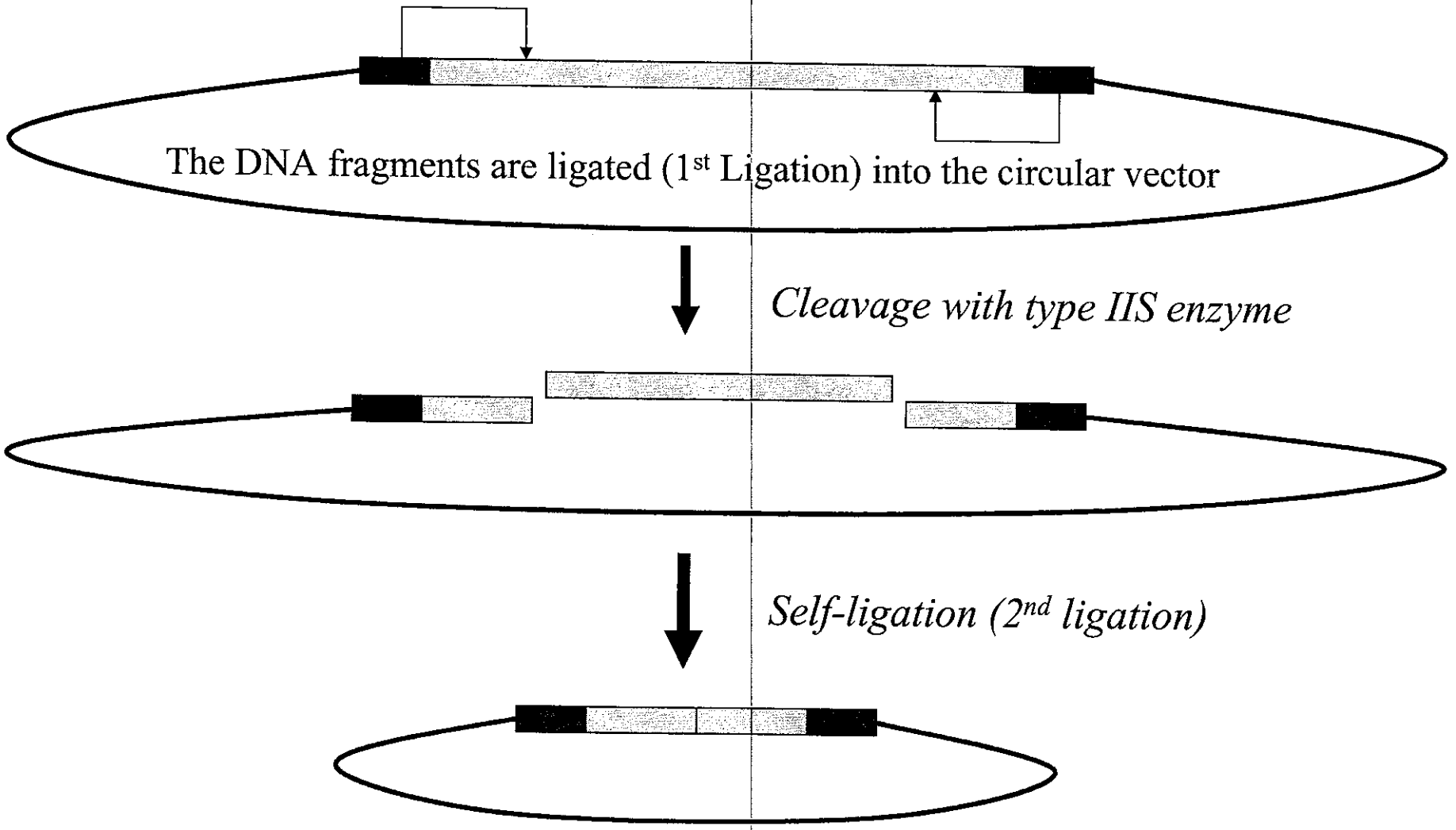

The DNA fragments are ligated (1st Ligation) into the circular vector
Cleavage with type IIS enzyme
Self-ligation (2nd ligation)

FIG. 9A

FIG. 9B

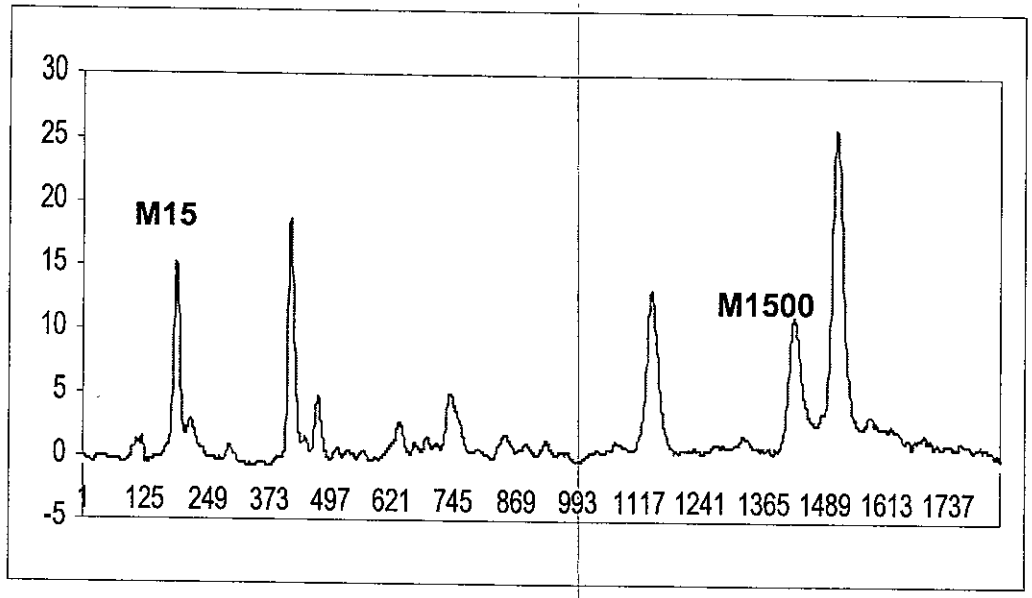
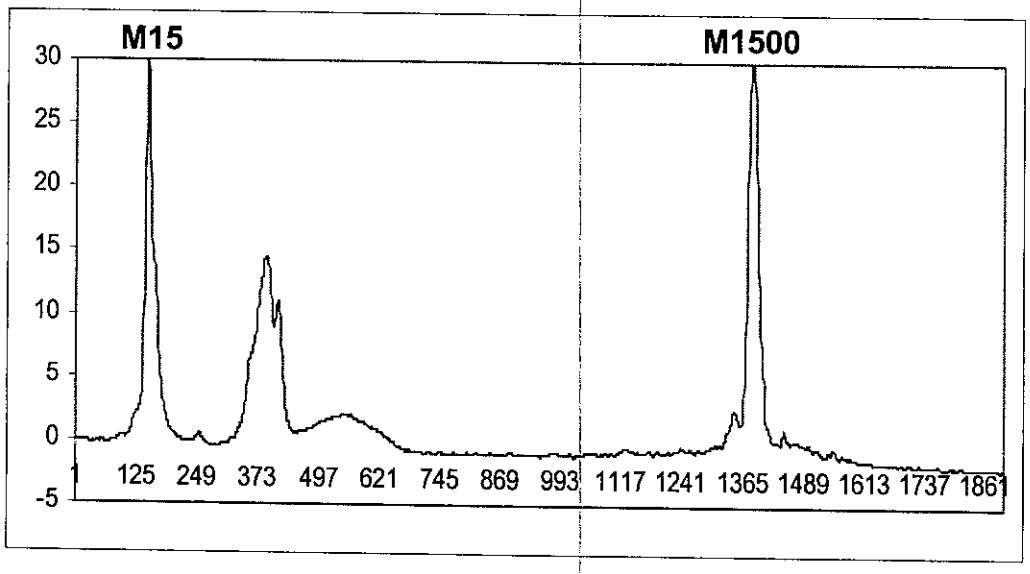
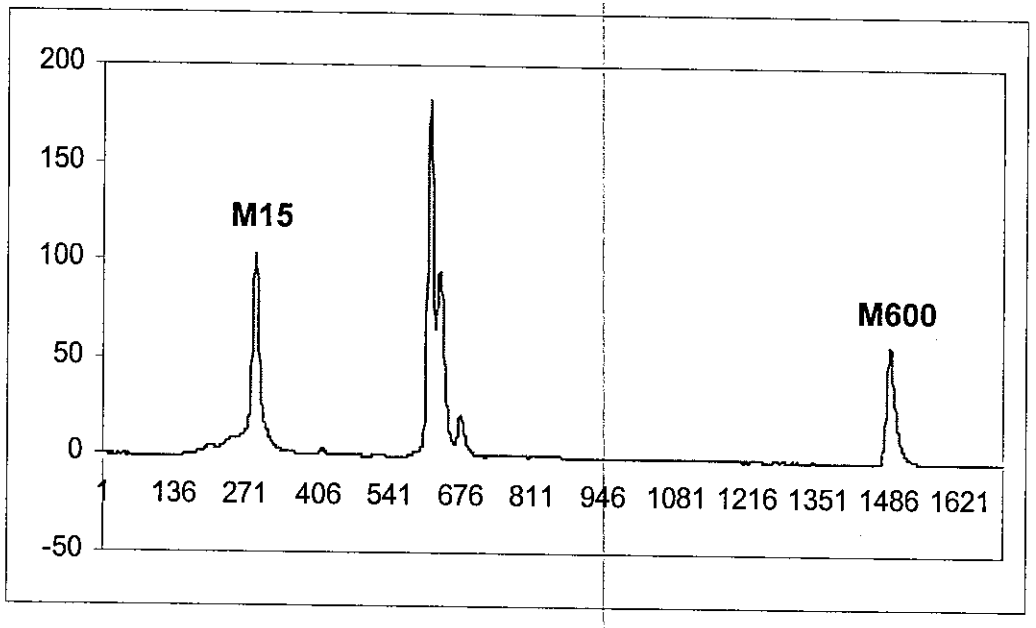


FIG. 9C



M15: Size marker 15 bp
M1500: Size marker 1500 bp



M15: Size marker 15 bp
M600: Size marker 600 bp

Peak	Mig.Time(secs)	Corr.Area	Size(bp)	Conc.(ng/ul)	Molarity(nmol/l)	Marker
1	40.15	18.1				
2	44.35	271.86	15	4.2	424.24	Lower
3	60.8	217.65	133	9.1	104.48	
4	61.75	146.13	140	6.1	65.59	
5	63.7	28.96	156	1.2	11.47	
6	104.25	54.62	600	2.1	5.3	Upper

FIG. 9D

Vector

*Sph*I *Acc*I

GGGAC GCATGC -------1000 bp----GTCGAC **GTCCC** (SEQ ID NO:5)

CCCTG CGTACG -------1000 bp----CAGCTG **CAGGG** (SEQ ID NO:6)

*Bsm*FI *Bsm*FI

Vector opened with *Sph*I and *Acc*I:

GGGACGCATG CGACGTCCC (SEQ ID NO:7)

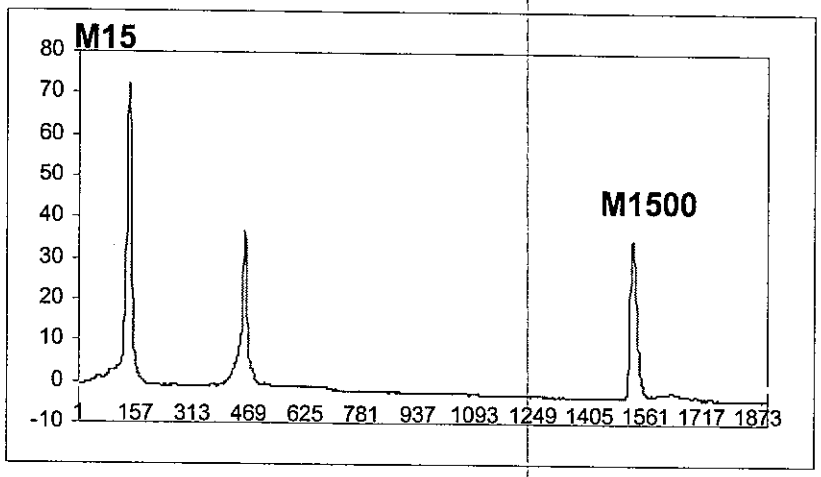
CCCTGC TGCAGGG (SEQ ID NO:8)

Can be ligated to:

4-base cutter
*Nla*III
Degenerated cutter
*Nsp*I « 5 base »

4-base cutters
HpyCH4IV, *Msp*I, *Hpa*II
*Hin*P1I, *Taq*I
6-base cutters:
*Cla*I, *Bst*BI, *Nar*I, *Sal*I

FIG. 10A



M15: Size marker 15 bp
M1500: Size marker 1500 bp

Peak			Size bp			
1	41.8	152	15	4.2	424	Lower
2	**57.8**	**67.8**	**134**	**3.6**	**41.1**	
3	111	37.6	1500	2.1	2.12	Upper

FIG. 10B

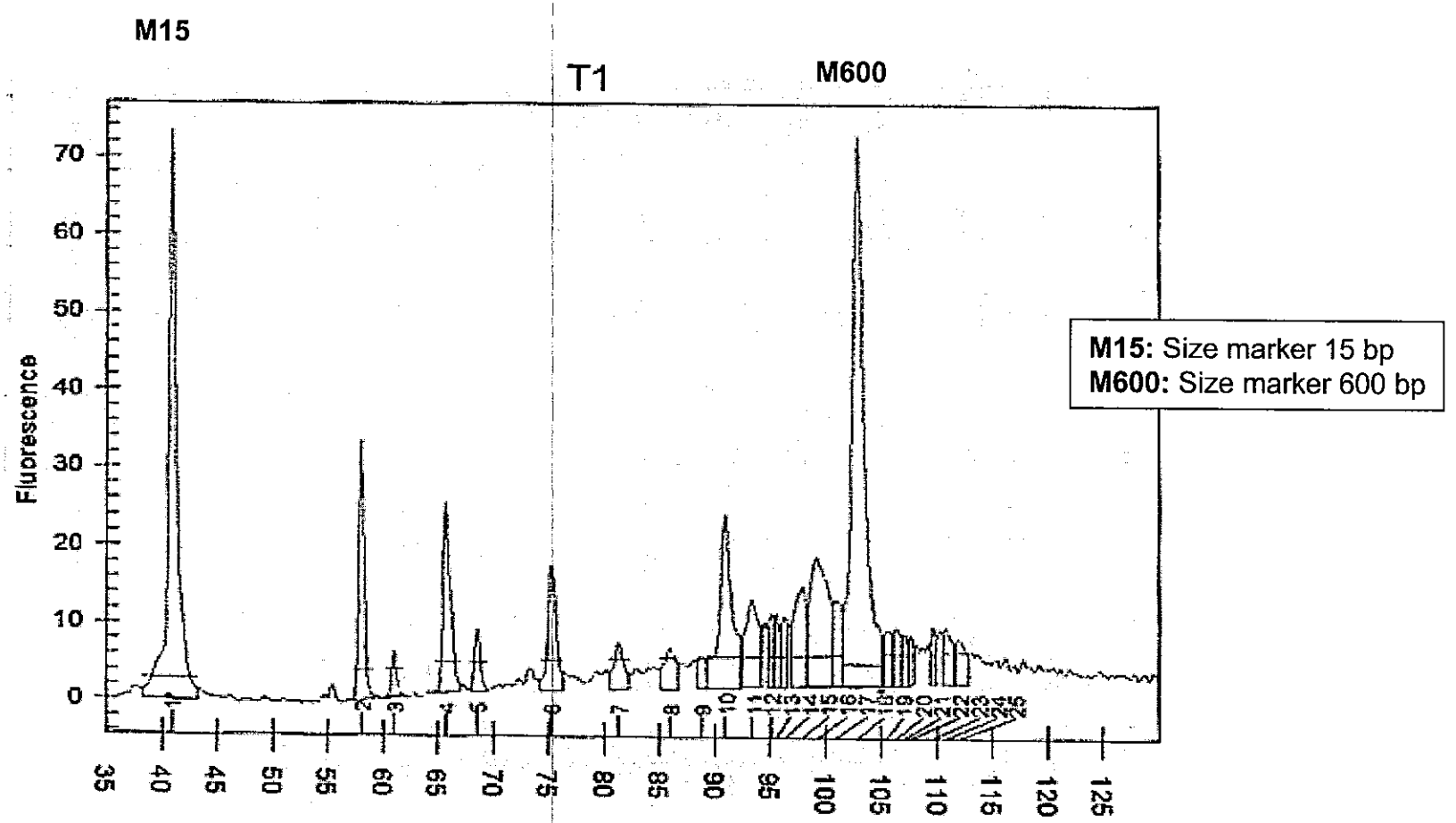
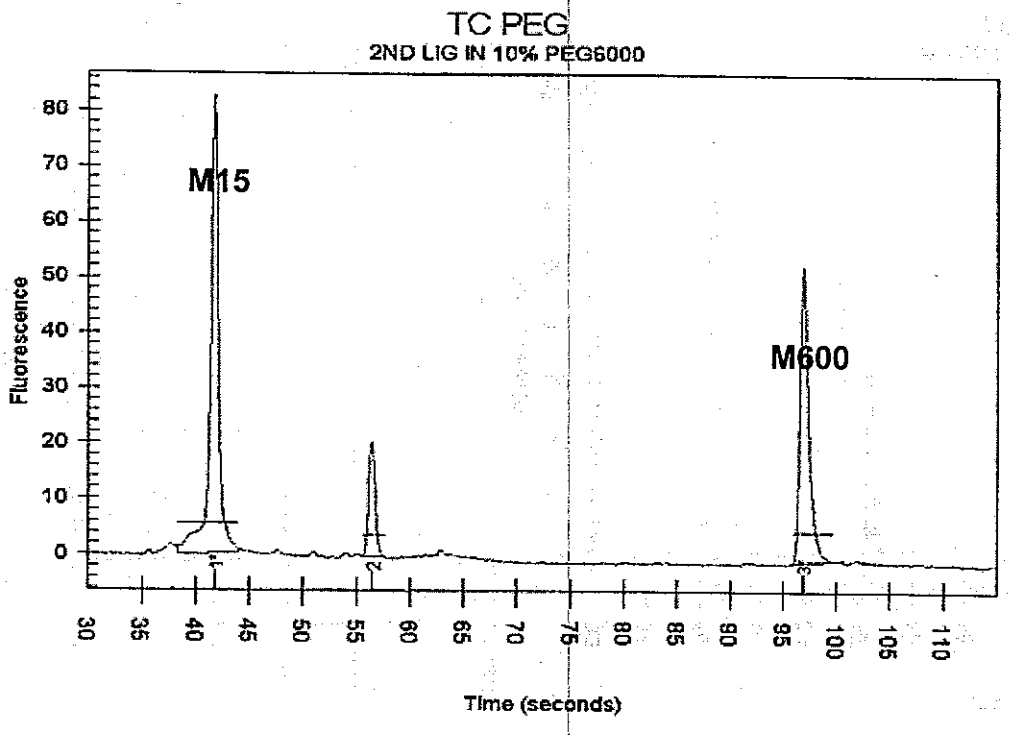

M15
T1
M600
Fluorescence
70
60
50
40
30
20
10
0
35
40
45
50
55
60
65
70
75
80
85
90
95
100
105
110
115
120
125
M15: Size marker 15 bp
M600: Size marker 600 bp

FIG. 10C



M15: Size marker 15 bp
M600: Size marker 600 bp

Peak	Mig.Time (secs)	Corr.Area	Size (bp)	Conc. (ng/ul)	Molarity (nmol/l)	Observations
1*	41.75	173.42	15	4.2	424.24	Lower Marker
2	56.45	25.05	126	1.1	13.59	
3*	96.85	51.37	600	2.1	5.30	Upper Marker

FIG. 10D

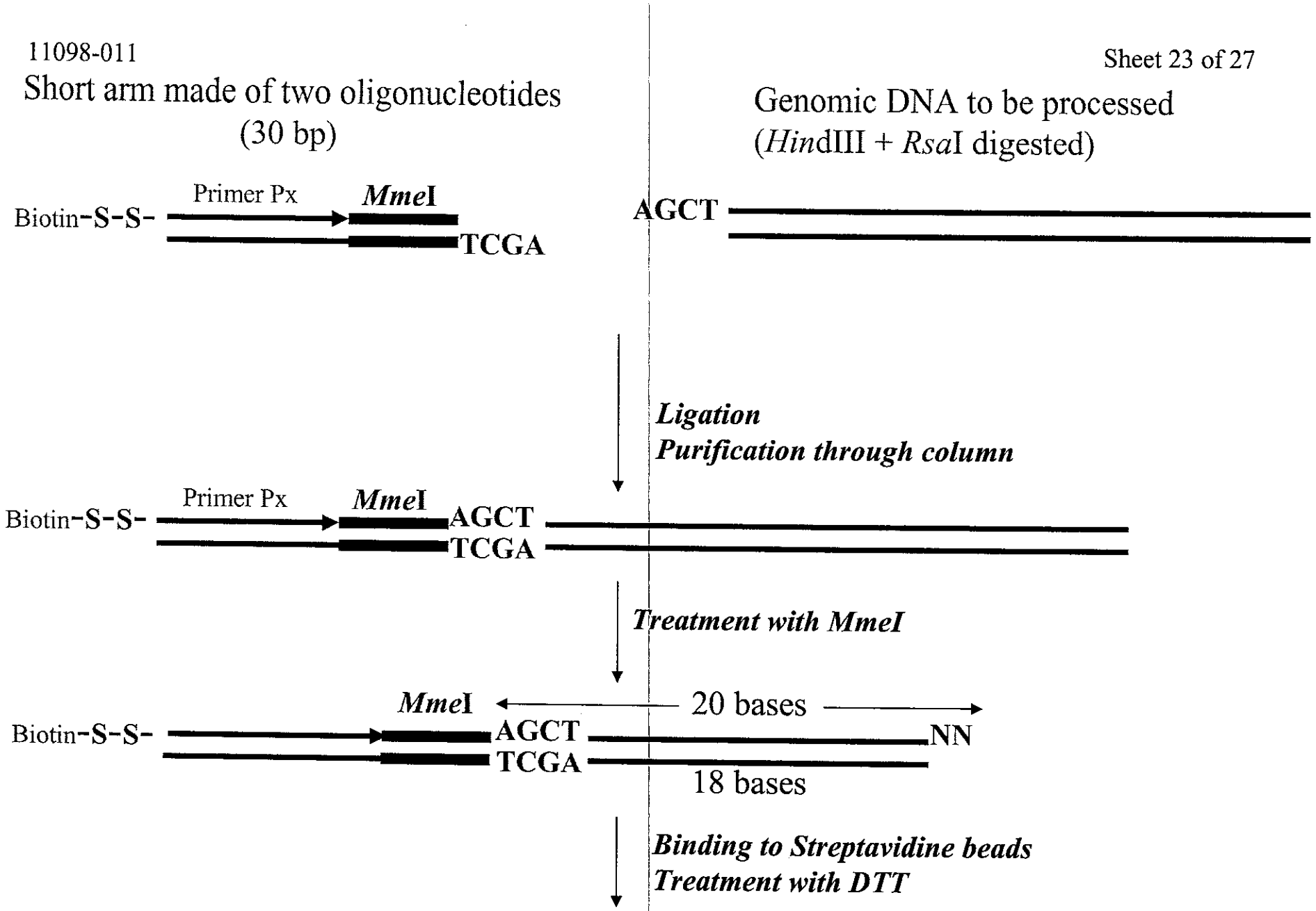
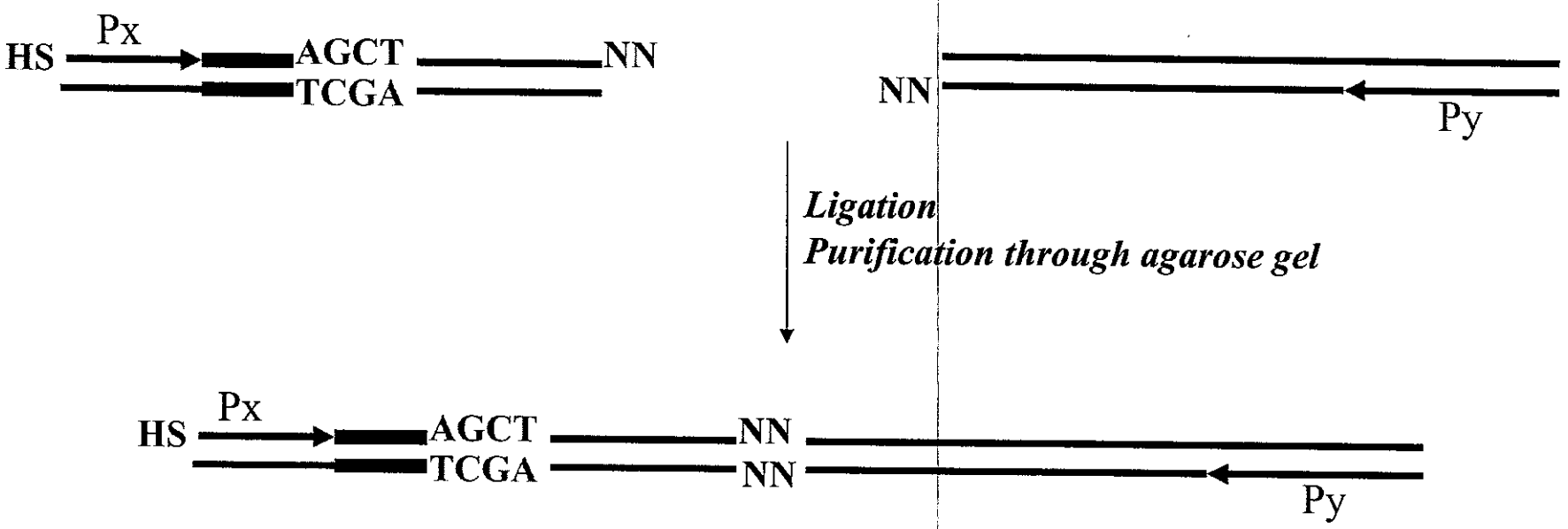

Short arm made of two oligonucleotides
(30 bp)
Primer Px
MmeI
Biotin-S-S-
TCGA
Genomic DNA to be processed
(HindIII + RsaI digested)
AGCT
Ligation
Purification through column
Primer Px
MmeI
Biotin-S-S-
AGCT
TCGA
Treatment with MmeI
MmeI
20 bases
Biotin-S-S-
AGCT
NN
TCGA
18 bases
Binding to Streptavidine beads
Treatment with DTT

FIG. 11A



DNA Colony Template 350 bp

FIG. 11B

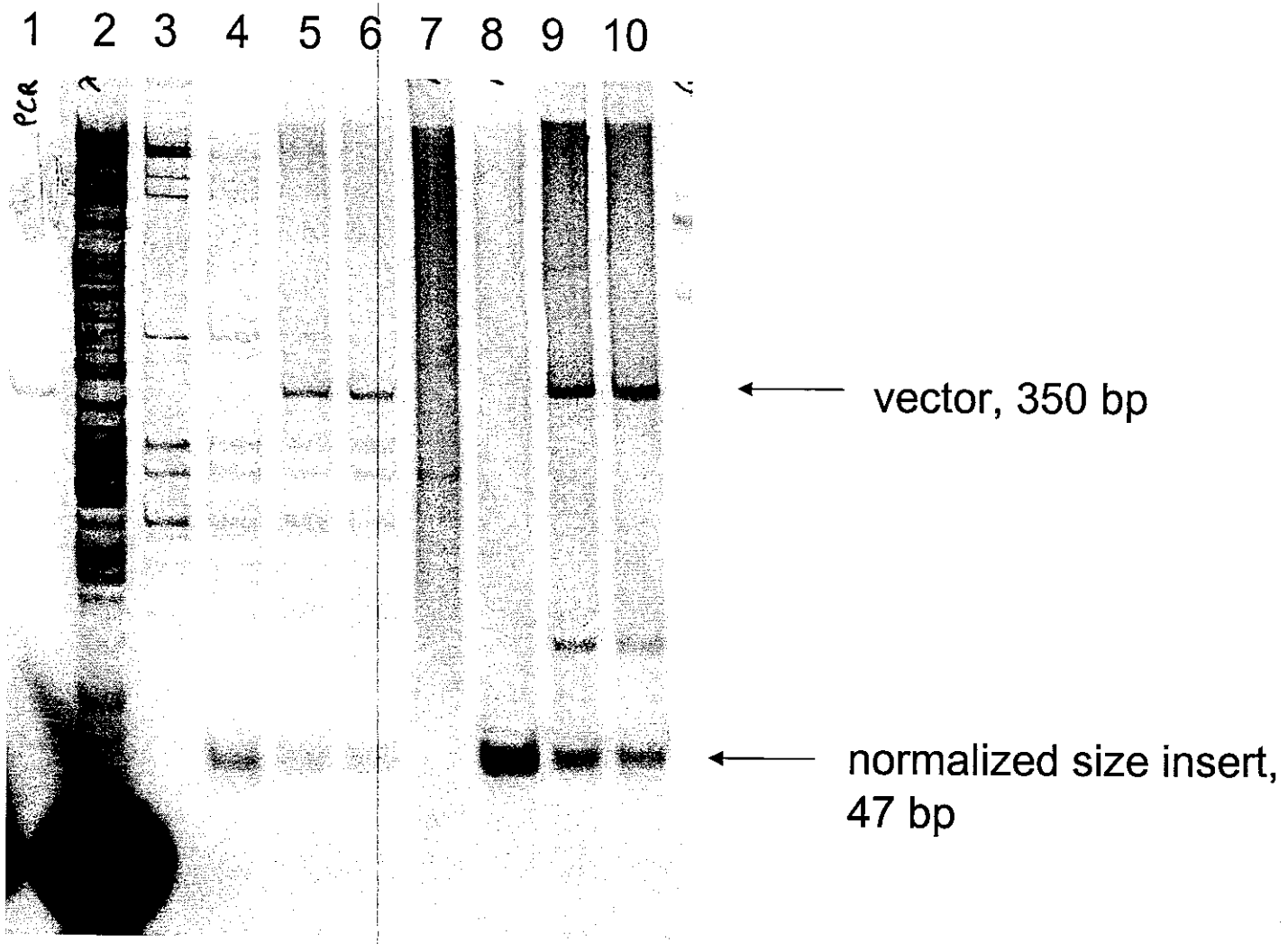
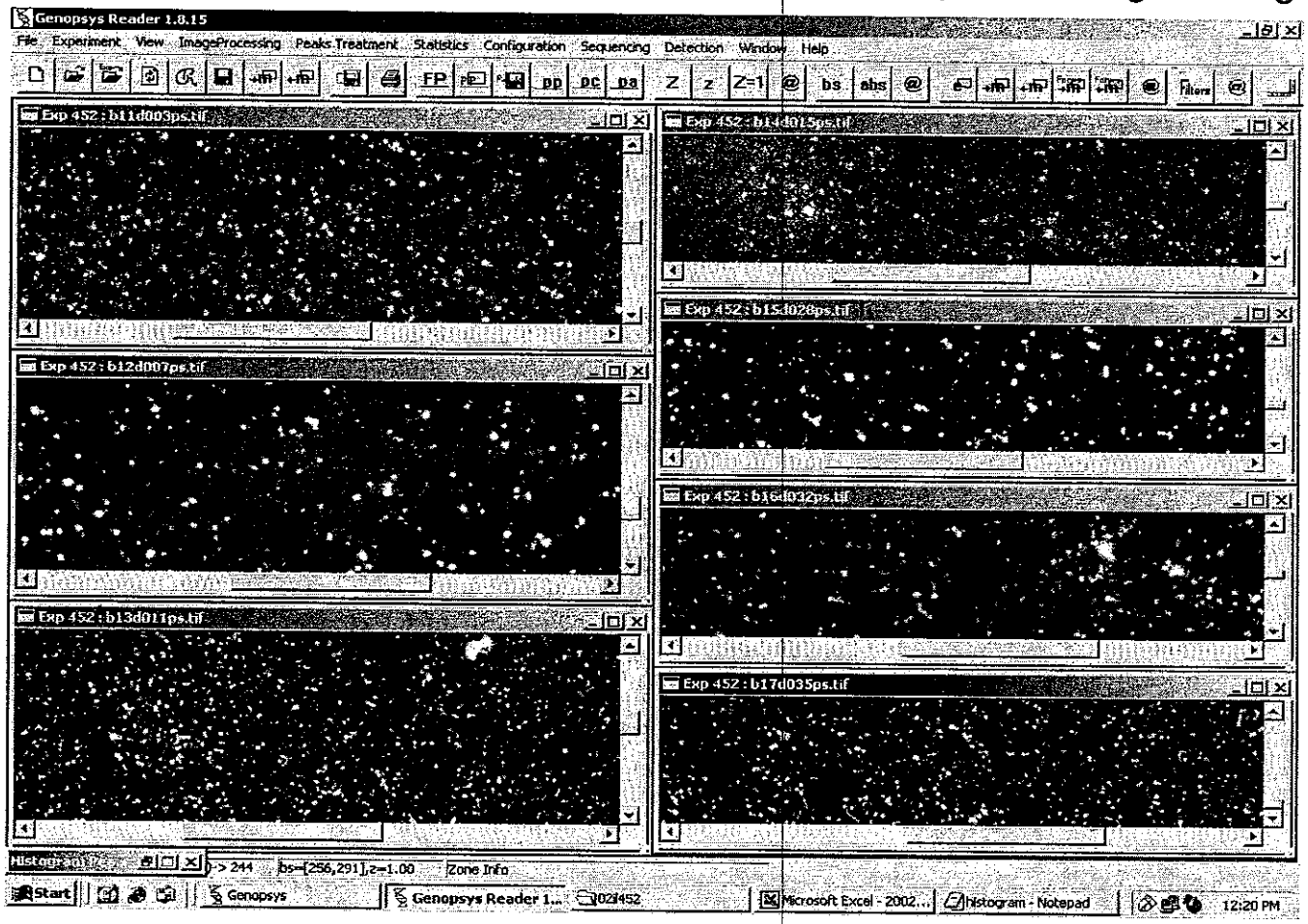


FIG. 11C

FIG. 11D Lambda DNA



FIG. 11E Lambda DNA (left column) or Human DNA (first 3 images of right column)



PstI	XXXXCTGCA	→	Klenow	→	XXXXC
end	XXXXG		+ dCTP		XXXXG
MspI	XXXXC	→	Klenow	→	XXXXCC
end	XXXXCGC		+ dCTP		XXXXCGC

FIG. 12