Figure 1

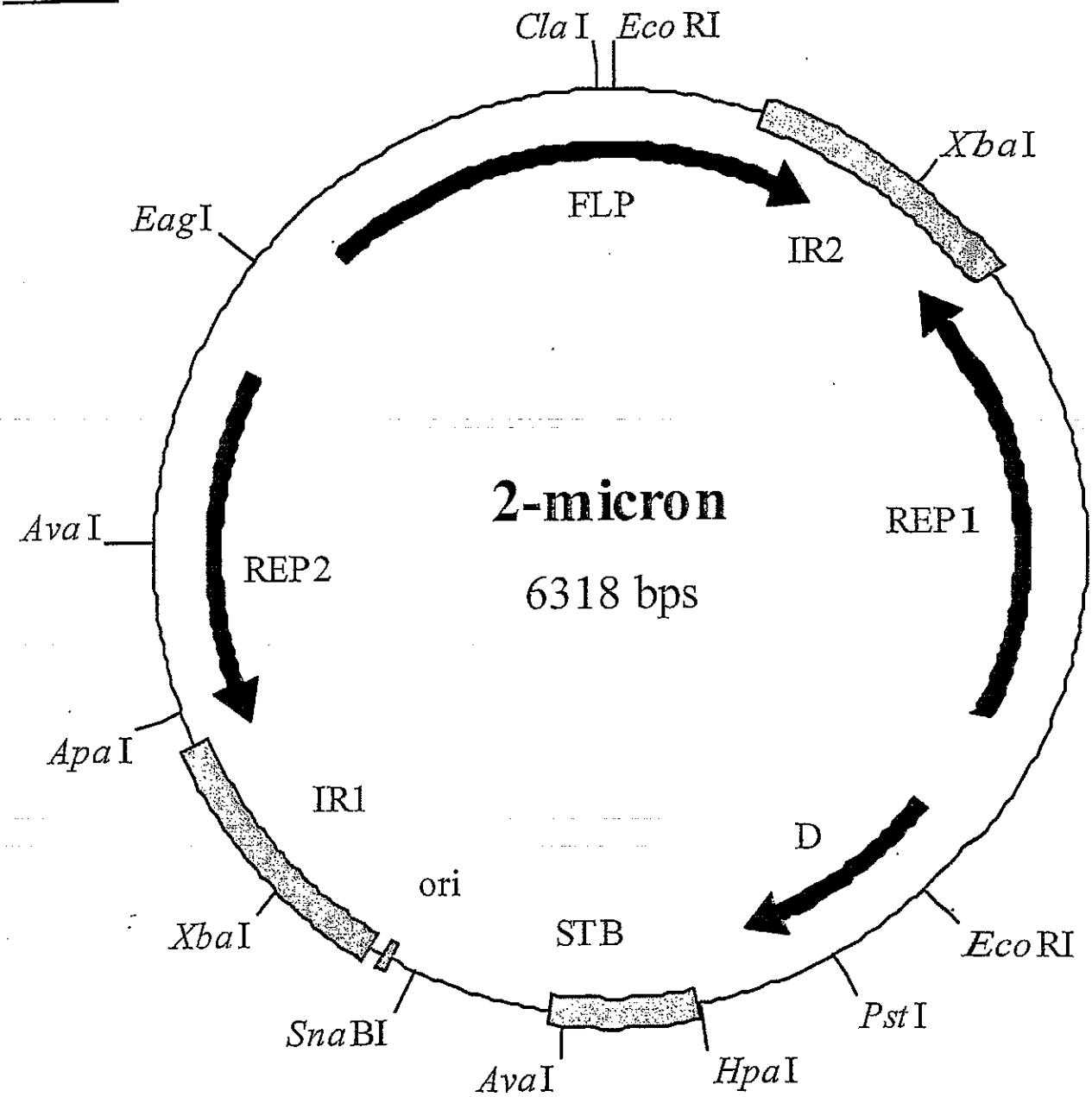

ClaI
EcoRI
XbaI
FLP
IR2
EagI
2-micron
6318 bps
REP1
AvaI
REP2
ApaI
IR1
D
ori
XbaI
STB
EcoRI
SnaBI
PstI
AvaI
HpaI

Figure 2

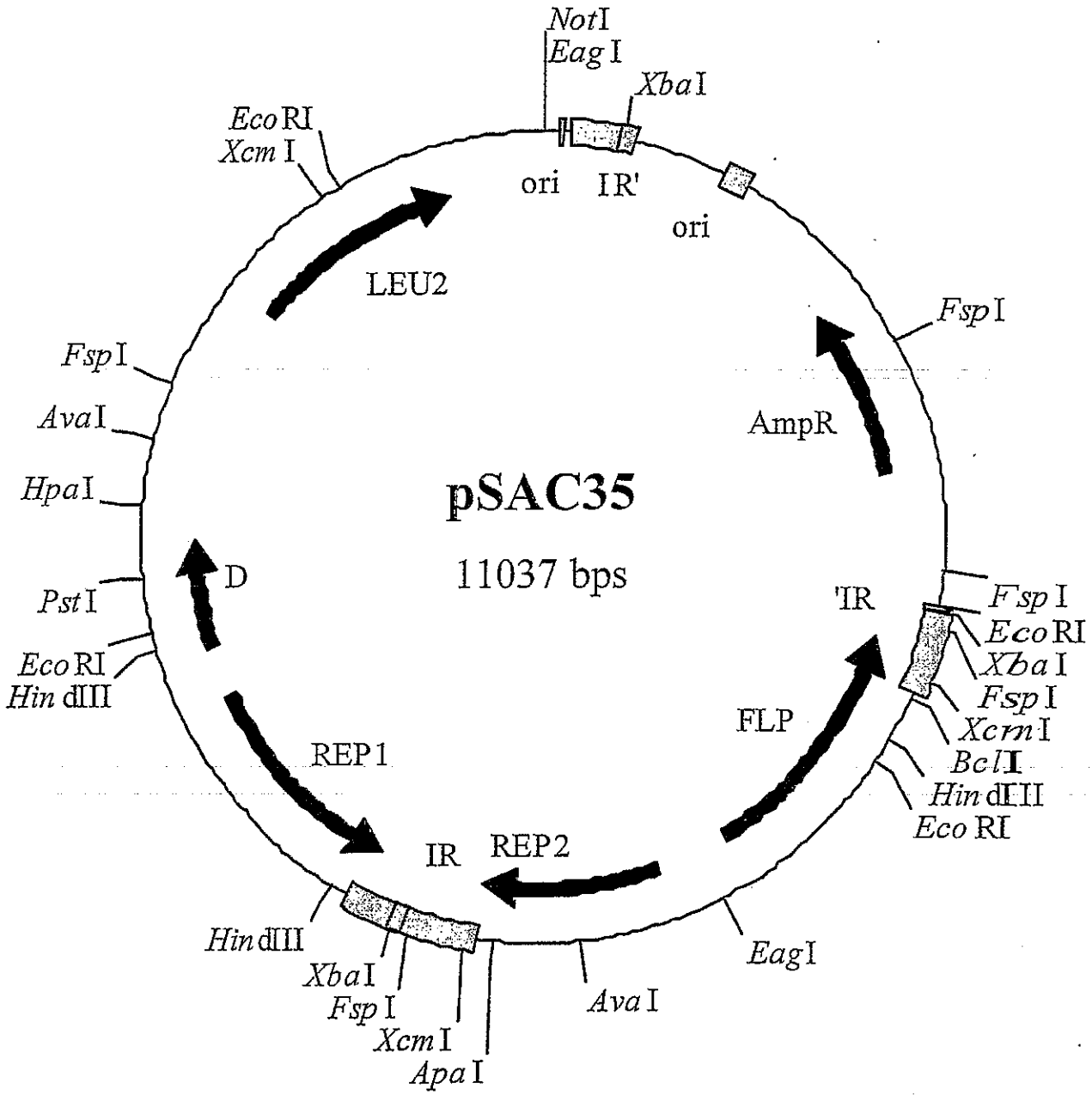

NotI
Eag I
XbaI
EcoRI
Xcm I
ori
IR'
ori
LEU2
FspI
FspI
AvaI
AmpR
HpaI
pSAC35
11037 bps
D
IR
FspI
PstI
EcoRI
XbaI
EcoRI
FspI
Hin dIII
FLP
XcmI
BclI
Hin dIII
EcoRI
REP1
IR
REP2
Hin dIII
EagI
XbaI
AvaI
FspI
XcmI
ApaI

Figure 3

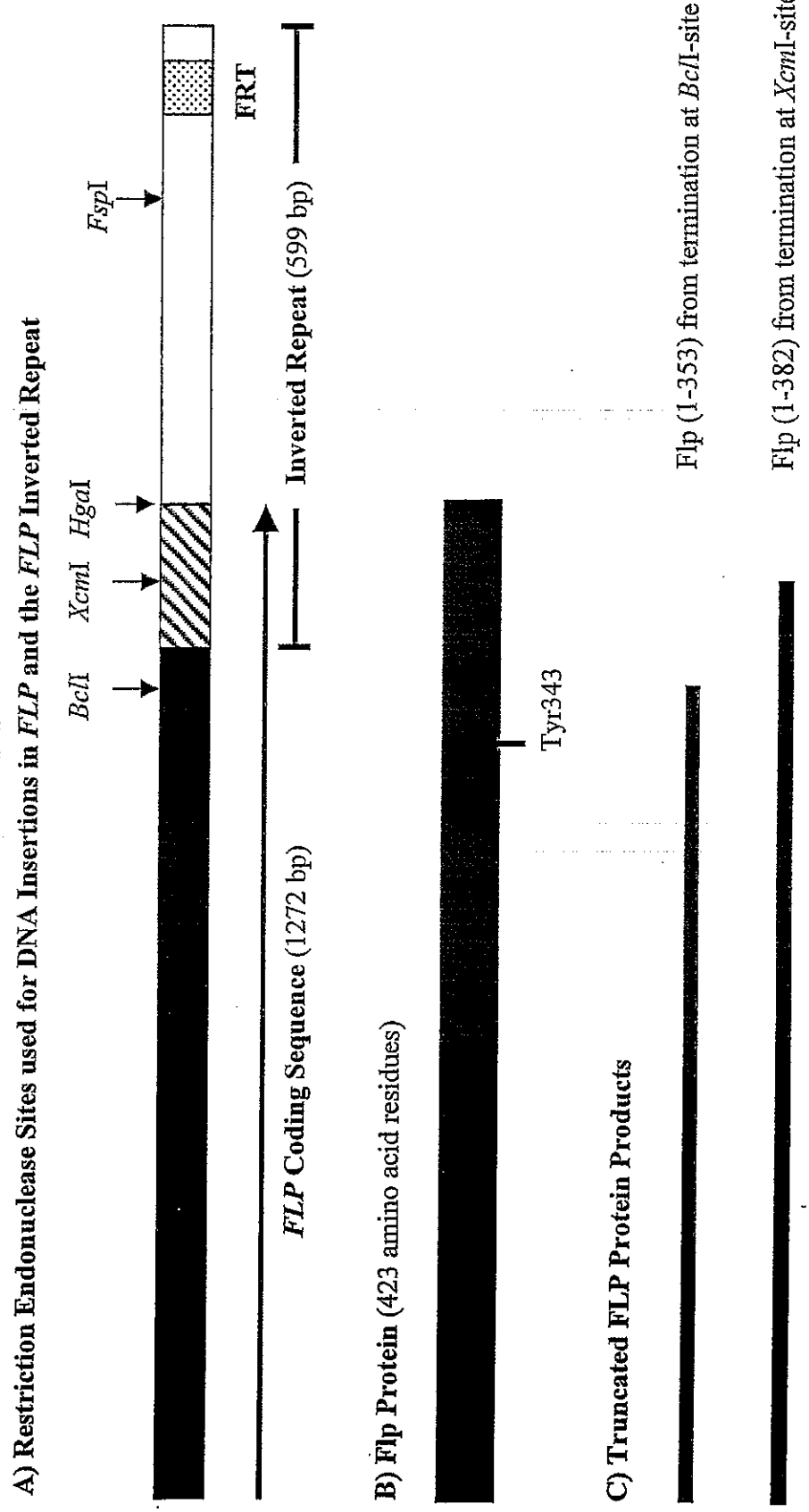

A) Restriction Endonuclease Sites used for DNA Insertions in FLP and the FLP Inverted Repeat
BclI
XcmI
HgaI
FspI
FRT
Inverted Repeat (599 bp)
FLP Coding Sequence (1272 bp)
B) Flp Protein (423 amino acid residues)
Tyr343
C) Truncated FLP Protein Products
Flp (1-353) from termination at BclI-site
Flp (1-382) from termination at XcmI-site

Figure 4

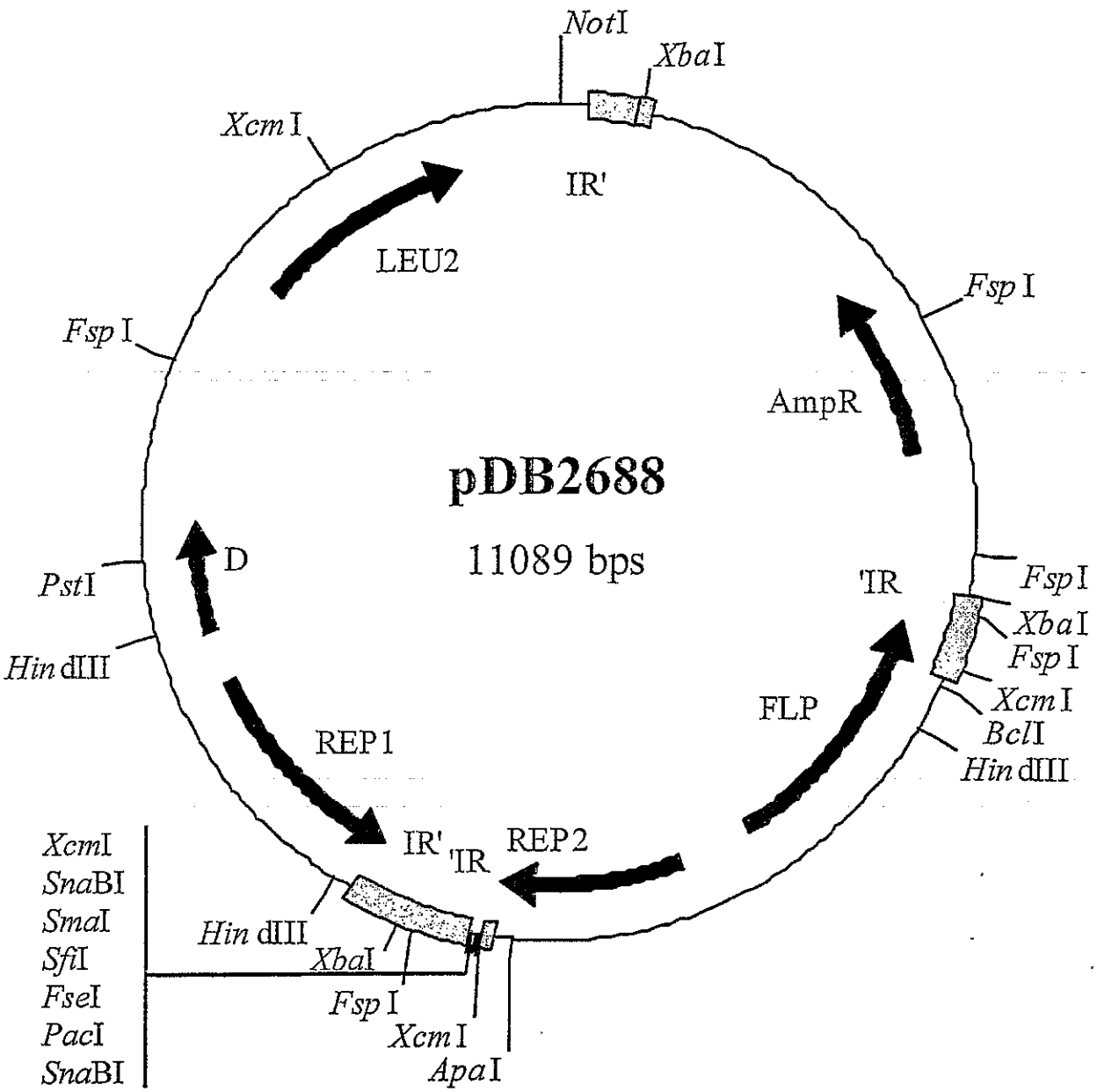

NotI
XbaI
XcmI
IR'
LEU2
FspI
FspI
AmpR
pDB2688
11089 bps
PstI
D
'IR
FspI
XbaI
FspI
HindIII
FLP
XcmI
BclI
HindIII
REP1
IR'
'IR
REP2
XcmI
SnaBI
SmaI
SfiI
FseI
PacI
SnaBI
HindIII
XbaI
FspI
XcmI
ApaI

Figure 5

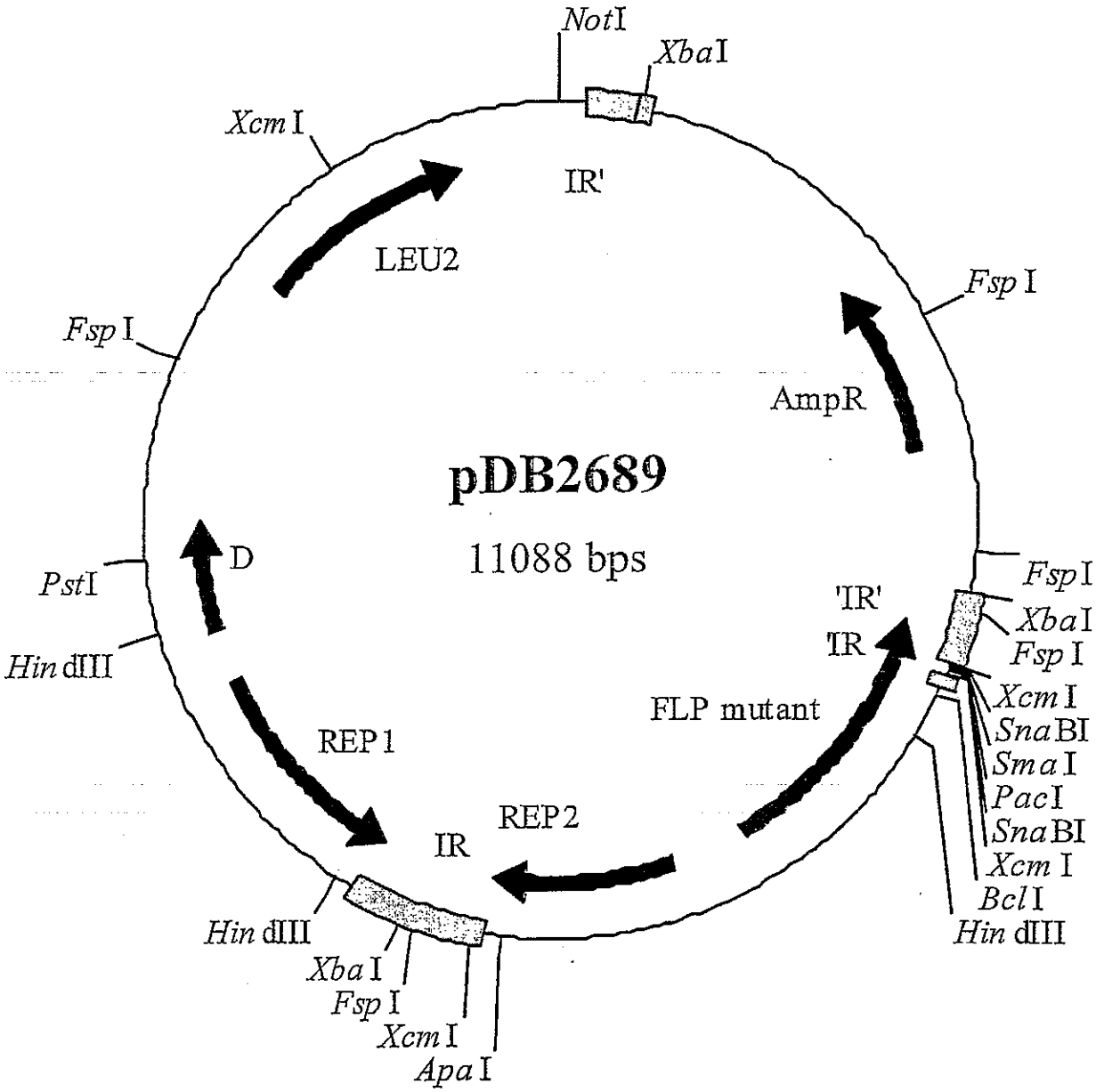

NotI
XbaI
Xcm I
IR'
LEU2
Fsp I
Fsp I
AmpR
pDB2689
11088 bps
D
PstI
Hin dIII
'IR'
IR
FLP mutant
REP1
REP2
IR
Fsp I
Xba I
Fsp I
Xcm I
Sna BI
Sma I
Pac I
Sna BI
Xcm I
Bcl I
Hin dIII
Hin dIII
Xba I
Fsp I
Xcm I
Apa I

Figure 6

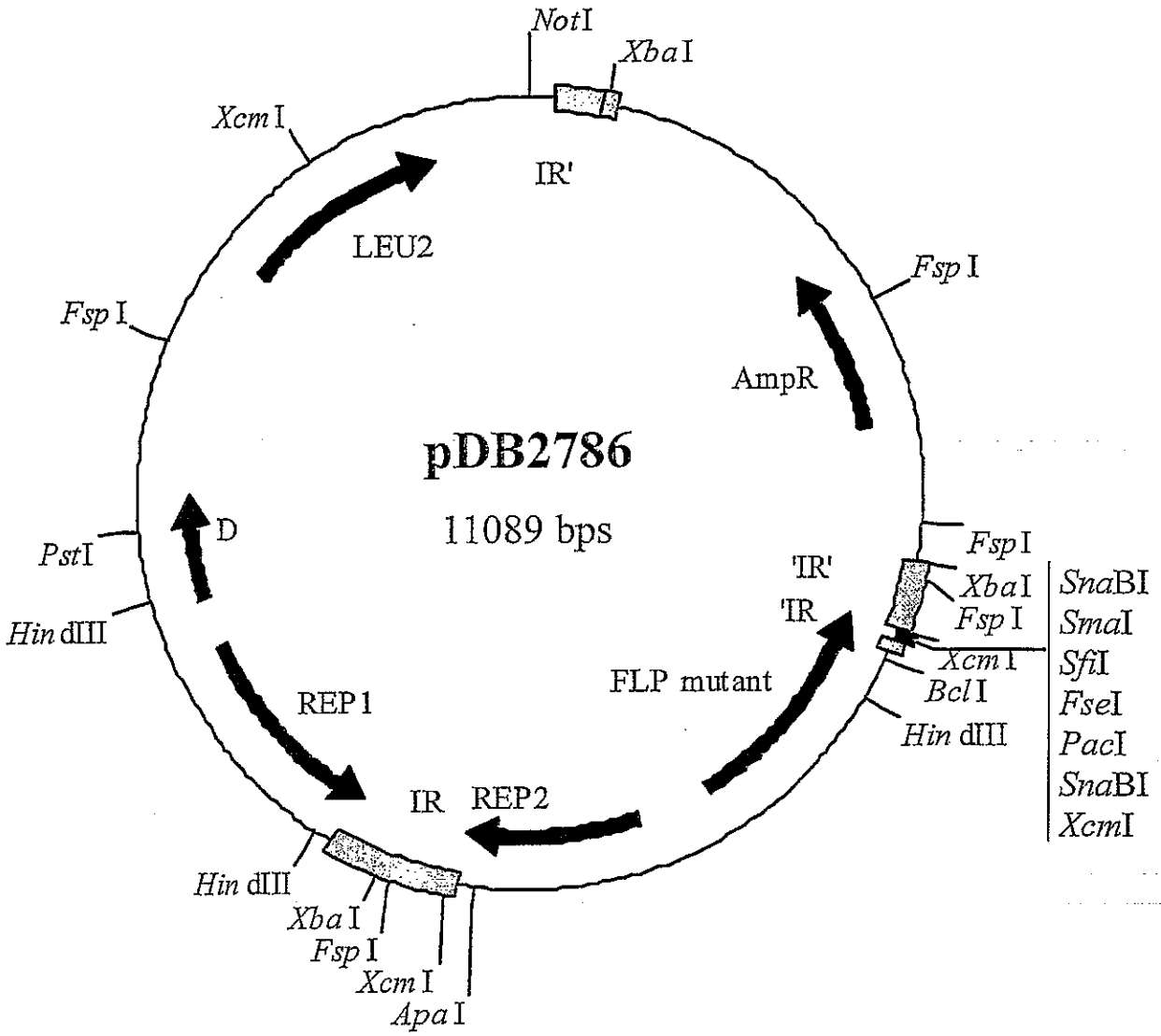

NotI
XbaI
XcmI
IR'
LEU2
FspI
FspI
AmpR
pDB2786
11089 bps
PstI
D
'IR'
'IR
FspI
XbaI
FspI
XcmI
BclI
HindIII
SnaBI
SmaI
SfiI
FseI
PacI
SnaBI
XcmI
HindIII
REP1
FLP mutant
IR
REP2
HindIII
XbaI
FspI
XcmI
ApaI

Figure 7

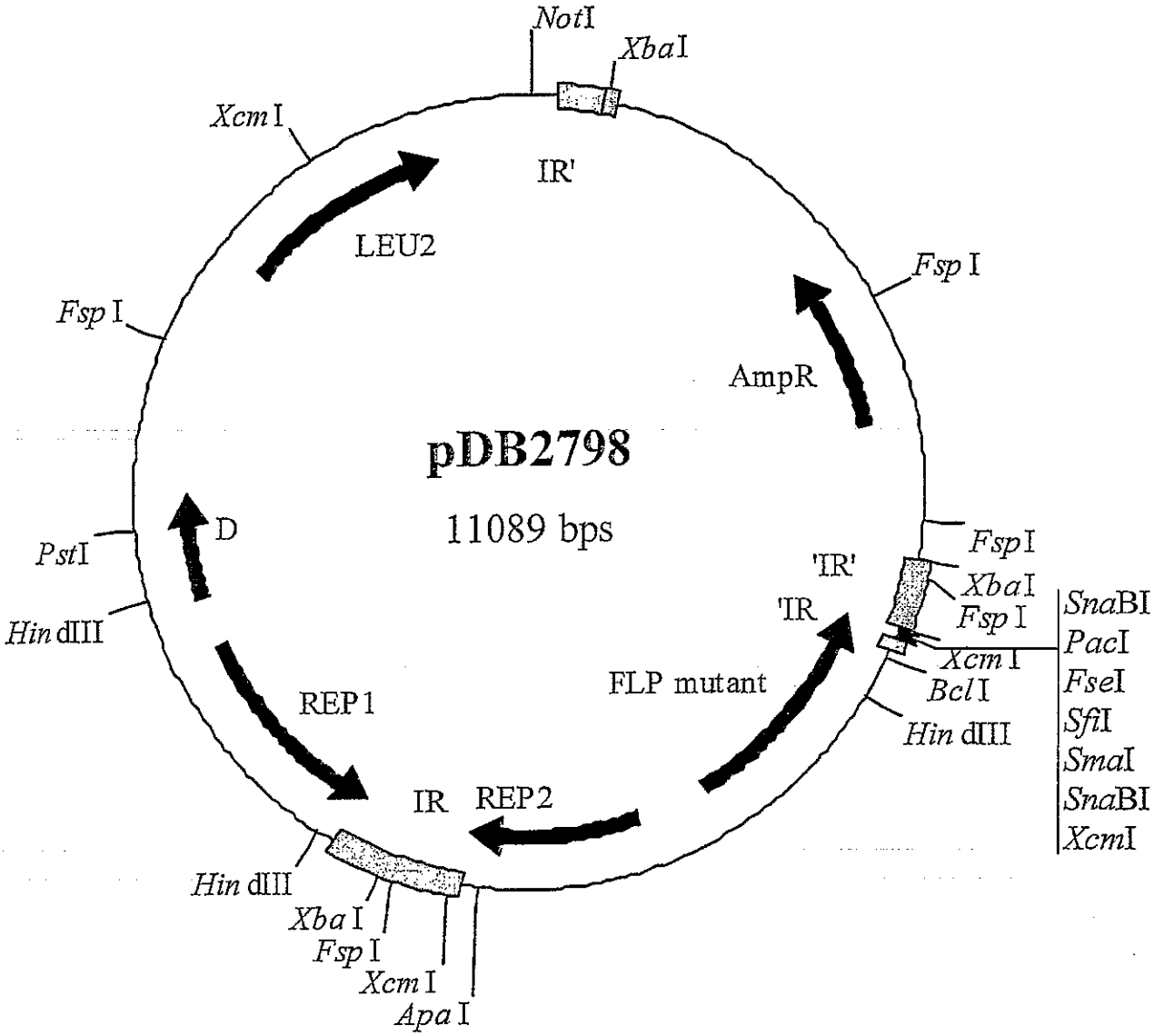

NotI
XbaI
XcmI
IR'
LEU2
FspI
FspI
AmpR
pDB2798
11089 bps
PstI
D
HindIII
'IR'
'IR
FspI
XbaI
FspI
XcmI
BclI
HindIII
SnaBI
PacI
FseI
SfiI
SmaI
SnaBI
XcmI
FLP mutant
REP1
IR
REP2
HindIII
XbaI
FspI
XcmI
ApaI

Figure 8

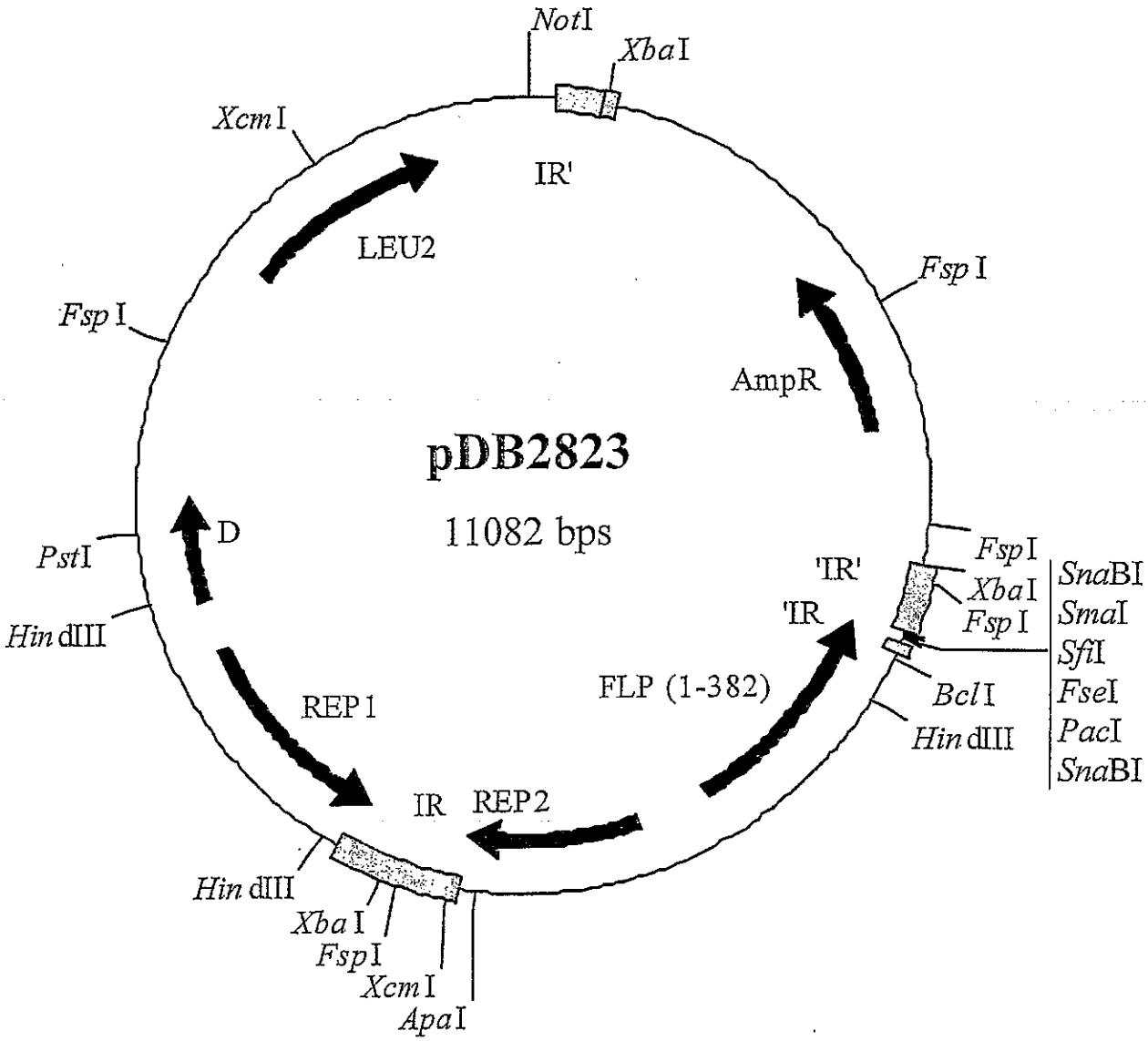

NotI
XbaI
XcmI
IR'
LEU2
FspI
FspI
AmpR
pDB2823
11082 bps
D
PstI
HindIII
'IR'
'IR
FspI
XbaI
FspI
SnaBI
SmaI
SfiI
FseI
PacI
SnaBI
BclI
HindIII
FLP (1-382)
REP1
IR REP2
HindIII
XbaI
FspI
XcmI
ApaI

Figure 9

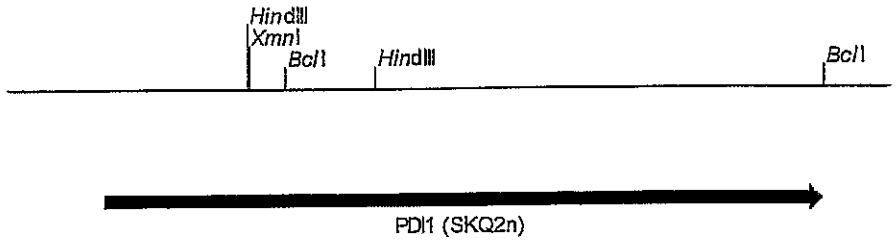


Figure 10

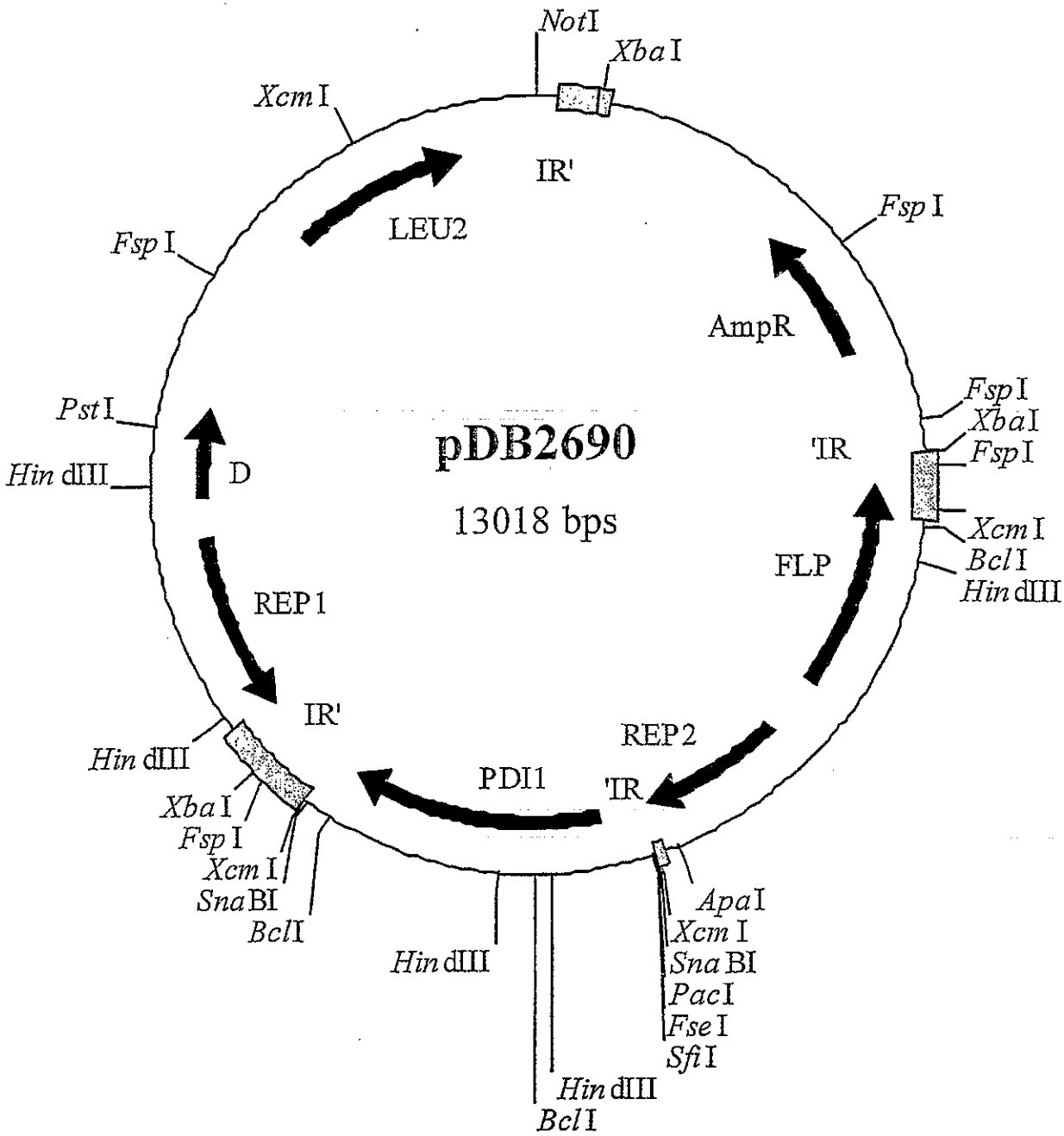
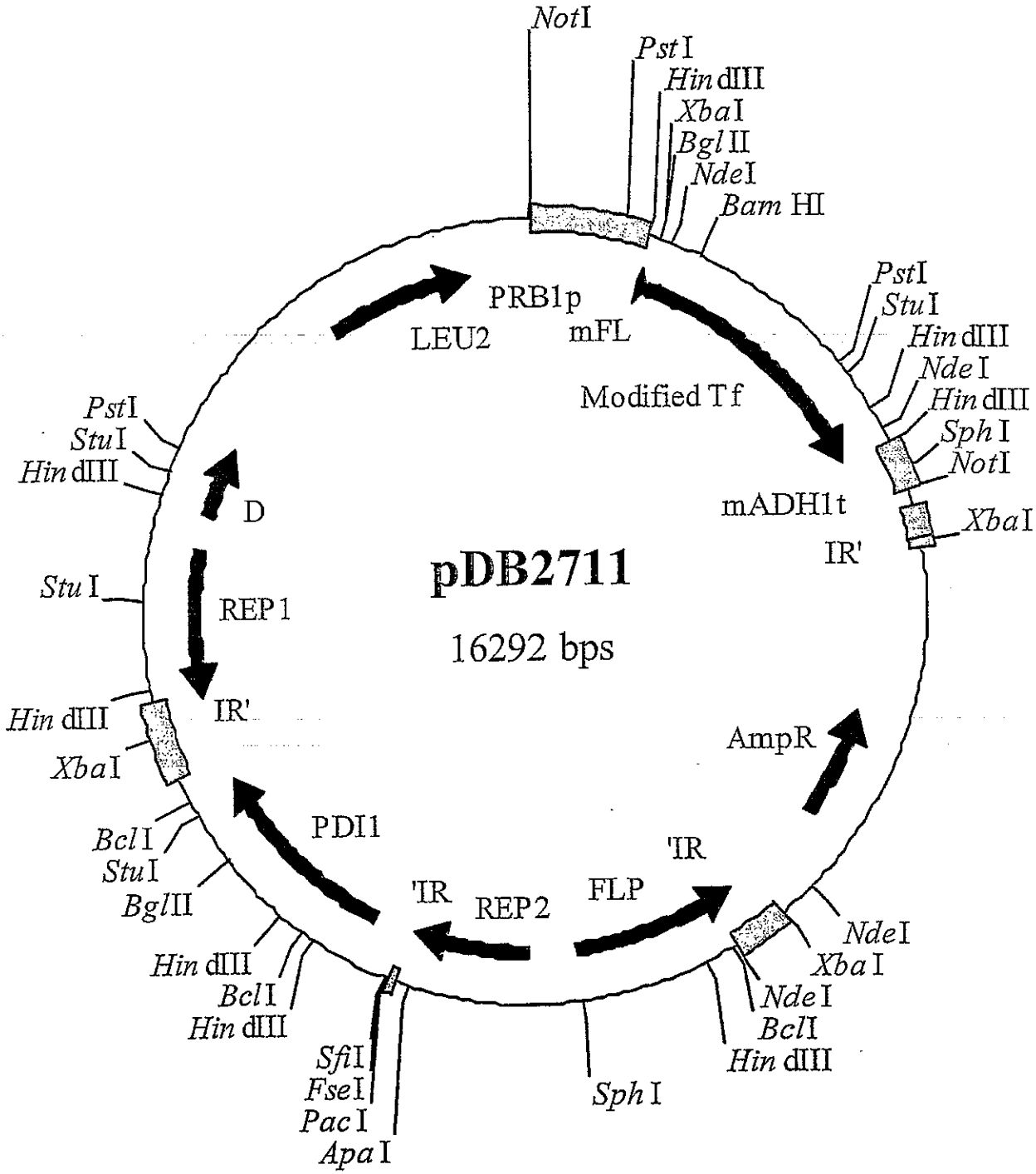

NotI
XbaI
XcmI
IR'
LEU2
FspI
FspI
AmpR
PstI
pDB2690
FspI
XbaI
FspI
HindIII
D
13018 bps
'IR
XcmI
BclI
HindIII
FLP
REP1
IR'
REP2
HindIII
PDI1
'IR
XbaI
FspI
XcmI
SnaBI
BclI
ApaI
XcmI
SnaBI
PacI
FseI
SfiI
HindIII
HindIII
BclI

Figure 11

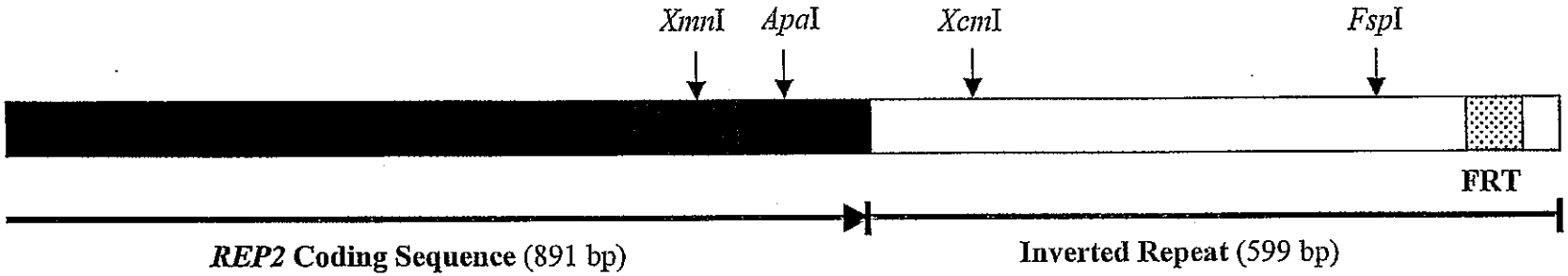
mFL = modified HSA(pre)/MFα1(pro) fusion leader sequence



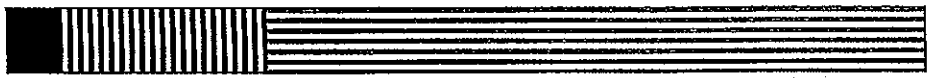
5

Figure 12

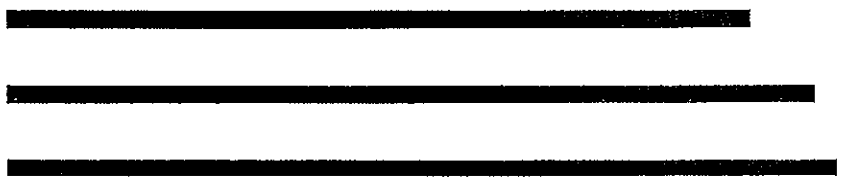
A) Restriction Endonuclease Sites used for DNA Insertions in *REP2* and the *REP2* Inverted Repeat



B) Rep2 Protein (296 amino acid residues)



C) Truncated Rep2 Protein Products



Rep2 (1-244) from termination at *Xmn*I-site

Rep2 (1-269) from termination at *Apa*I-site digested by T4 DNA polymerase

Rep2 (1-271) from termination at *Apa*I-site

Figure 13

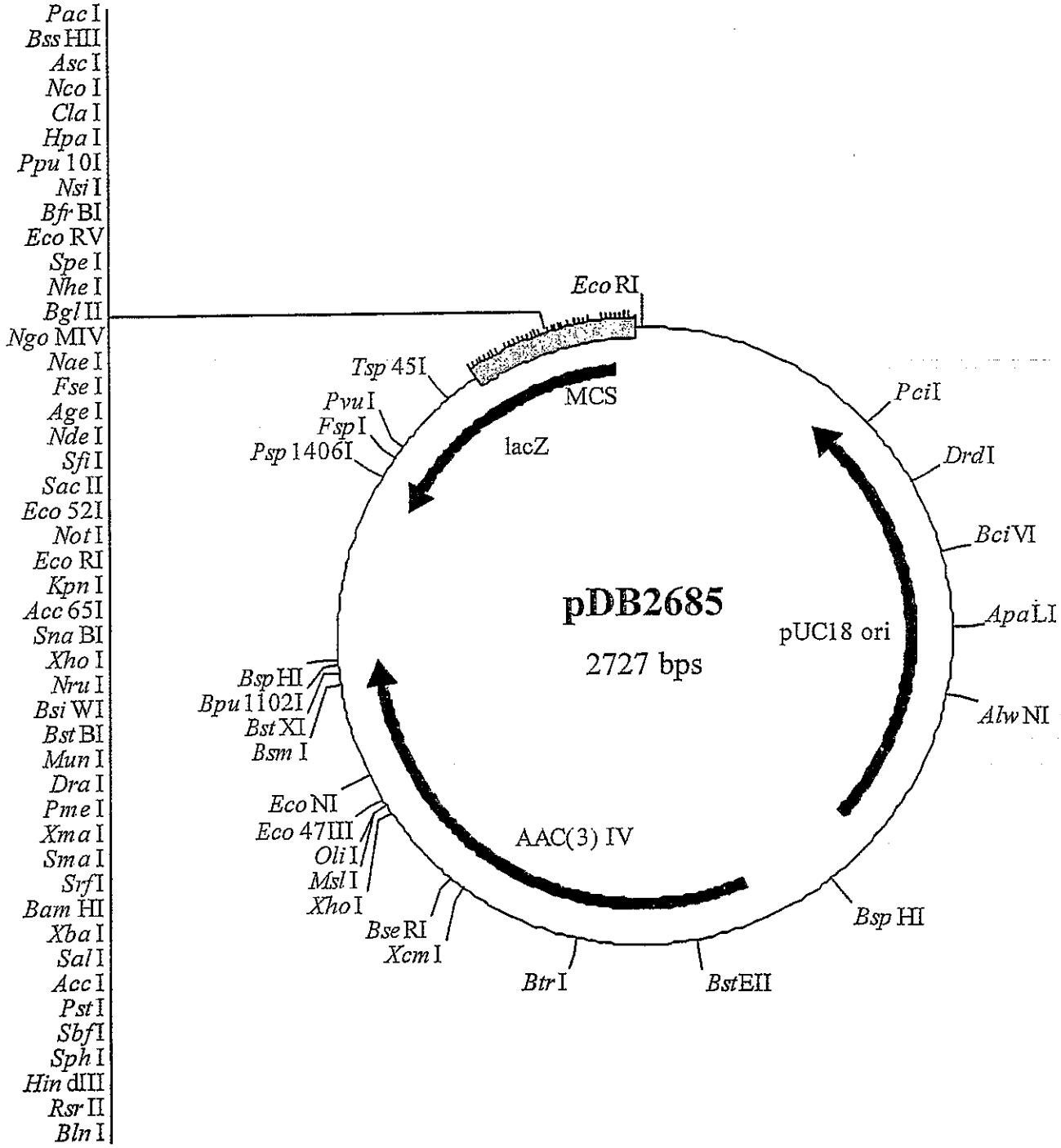

Pac I
Bss HII
Asc I
Nco I
Cla I
Hpa I
Ppu 10I
Nsi I
Bfr BI
Eco RV
Spe I
Nhe I
Bgl II
Ngo MIV
Nae I
Fse I
Age I
Nde I
Sfi I
Sac II
Eco 52I
Not I
Eco RI
Kpn I
Acc 65I
Sna BI
Xho I
Nru I
Bsi WI
Bst BI
Mun I
Dra I
Pme I
Xma I
Sma I
Srf I
Bam HI
Xba I
Sal I
Acc I
Pst I
Sbf I
Sph I
Hin dIII
Rsr II
Bln I
Eco RI
Tsp 45I
Pvu I
Fsp I
Psp 1406I
MCS
lacZ
Pci I
Drd I
Bci VI
Apa LI
Alw NI
Bsp HI
Btr I
Bst EII
Bse RI
Xcm I
Xho I
Msl I
Oli I
Eco 47III
Eco NI
Bsm I
Bst XI
Bpu 1102I
Bsp HI
pDB2685
2727 bps
pUC18 ori
AAC(3) IV

Figure 14

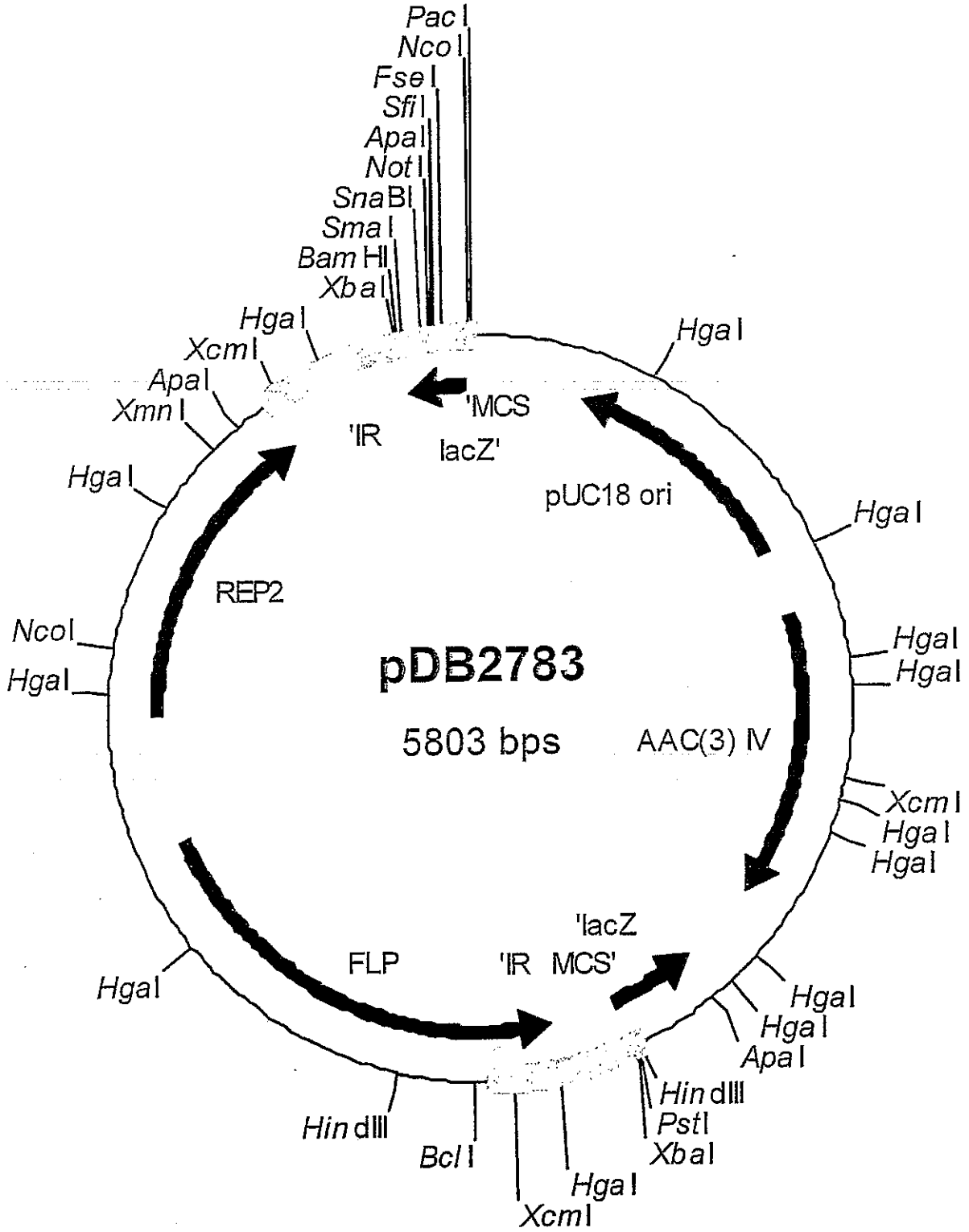

pDB2783
5803 bps
REP2
'IR
'MCS
lacZ'
pUC18 ori
AAC(3) IV
FLP
'IR
MCS'
'lacZ
Pac I
Nco I
Fse I
Sfi I
Apa I
Not I
Sna BI
Sma I
Bam HI
Xba I
Hga I
Xcm I
Apa I
Xmn I
Hga I
Nco I
Hga I
Hga I
Hga I
Hga I
Xcm I
Hga I
Hga I
Hga I
Apa I
Hin dIII
Pst I
Xba I
Hga I
Xcm I
Bcl I
Hin dIII
Hga I
Hga I

Figure 15

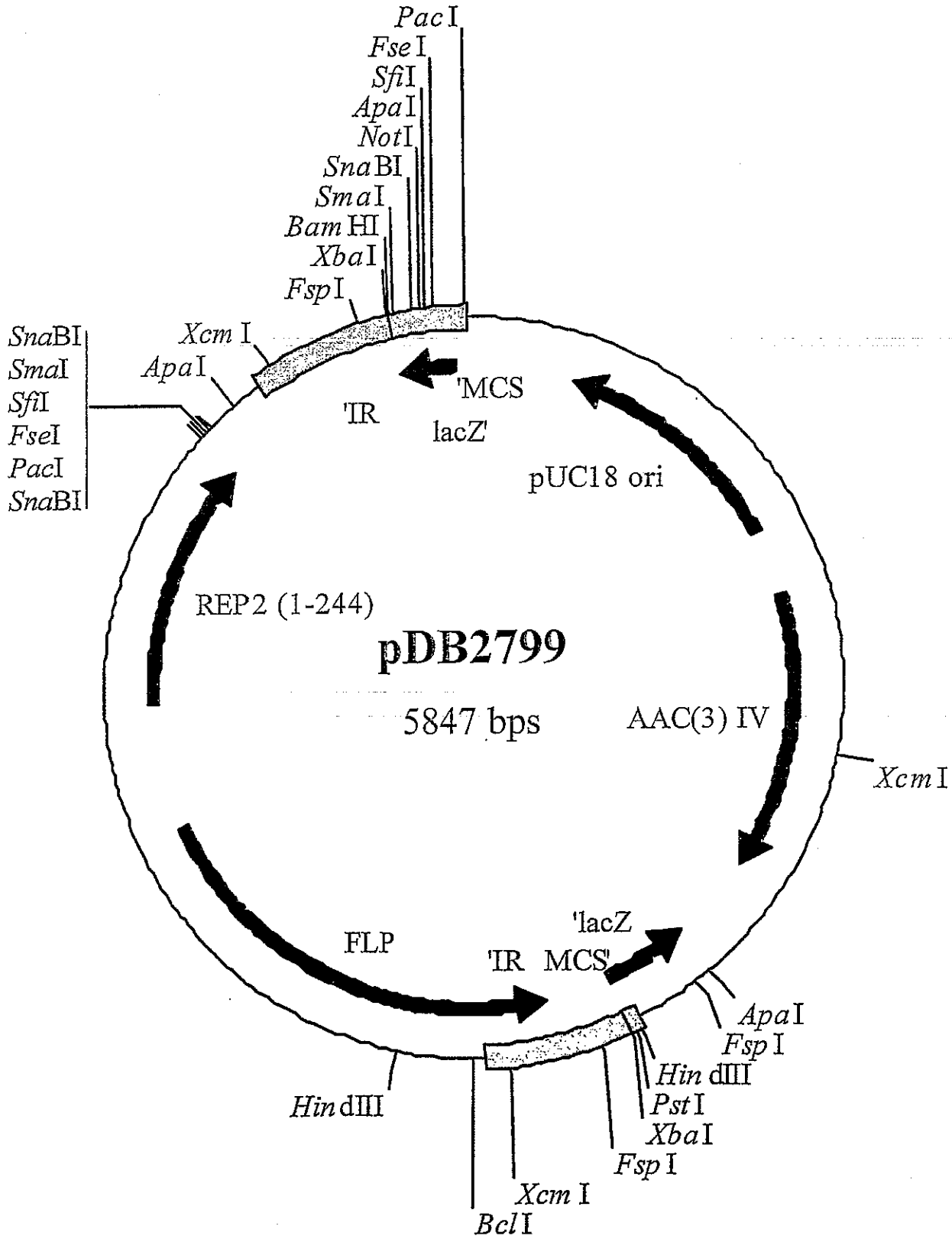

PacI
FseI
SfiI
ApaI
NotI
SnaBI
SmaI
BamHI
XbaI
FspI
SnaBI
SmaI
SfiI
FseI
PacI
SnaBI
XcmI
ApaI
'IR
'MCS
lacZ'
pUC18 ori
REP2 (1-244)
pDB2799
5847 bps
AAC(3) IV
XcmI
FLP
'lacZ
'IR MCS'
ApaI
FspI
HindIII
PstI
XbaI
FspI
HindIII
XcmI
BclI

Figure 16

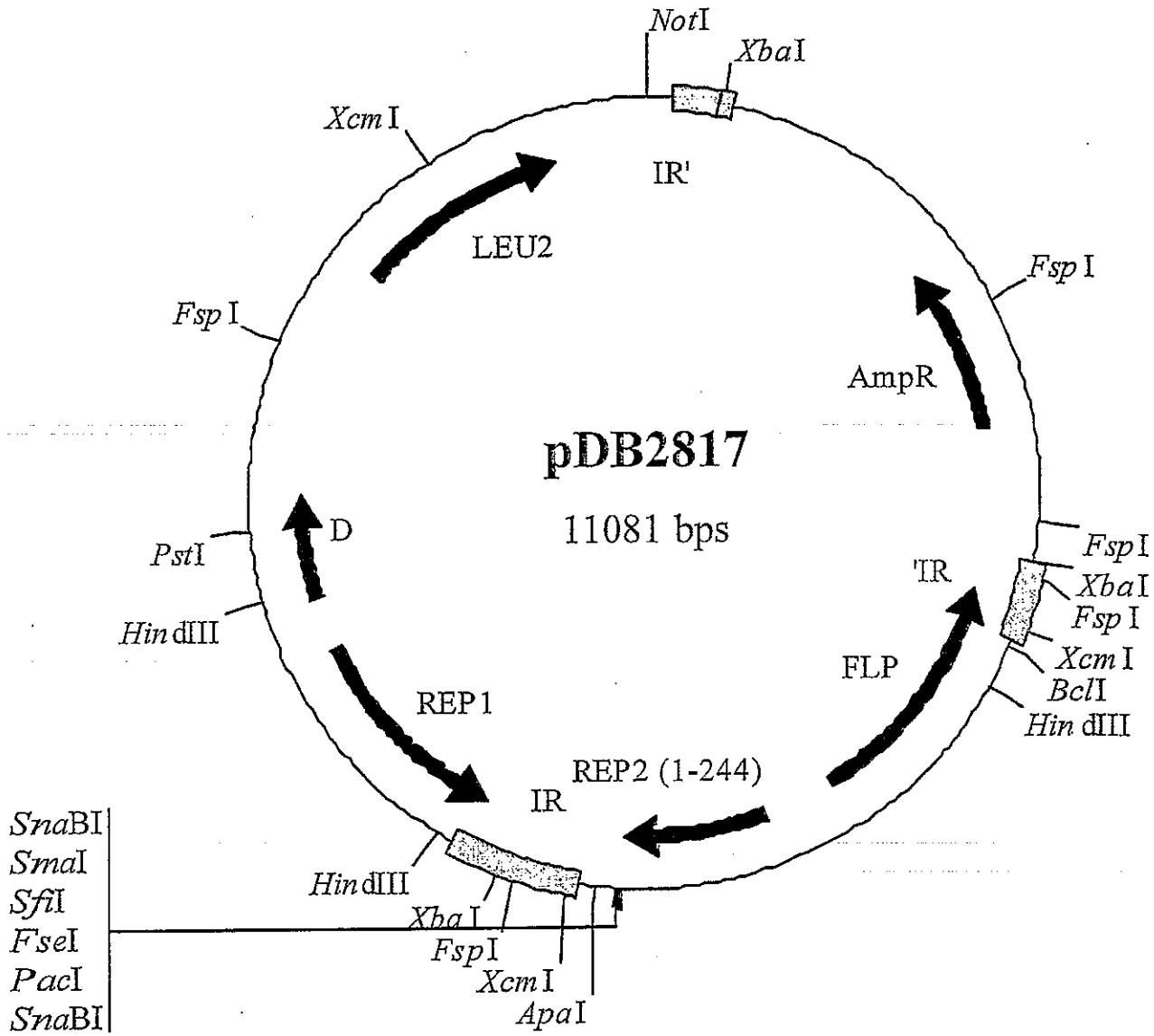

NotI
XbaI
XcmI
IR'
LEU2
FspI
FspI
AmpR
pDB2817
11081 bps
D
PstI
HindIII
'IR
FspI
XbaI
FspI
XcmI
BclI
HindIII
FLP
REP1
REP2 (1-244)
IR
SnaBI
SmaI
SfiI
FseI
PacI
SnaBI
HindIII
XbaI
FspI
XcmI
ApaI

Figure 17

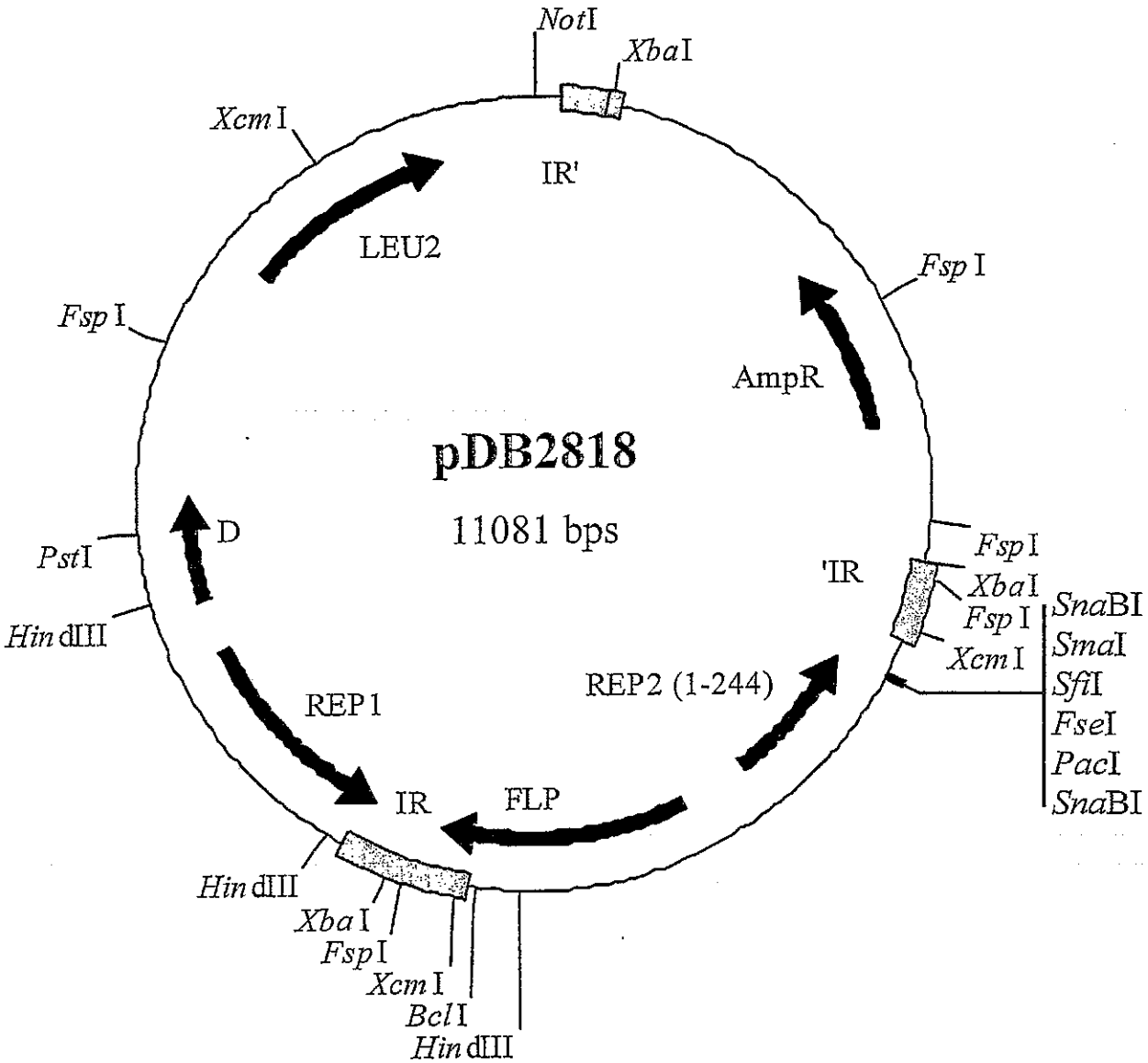

NotI
XbaI
XcmI
IR'
LEU2
FspI
FspI
AmpR
pDB2818
11081 bps
PstI
D
'IR
FspI
XbaI
FspI
SnaBI
SmaI
XcmI
SfiI
FseI
PacI
SnaBI
HindIII
REP1
REP2 (1-244)
IR
FLP
HindIII
XbaI
FspI
XcmI
BclI
HindIII

Figure 18

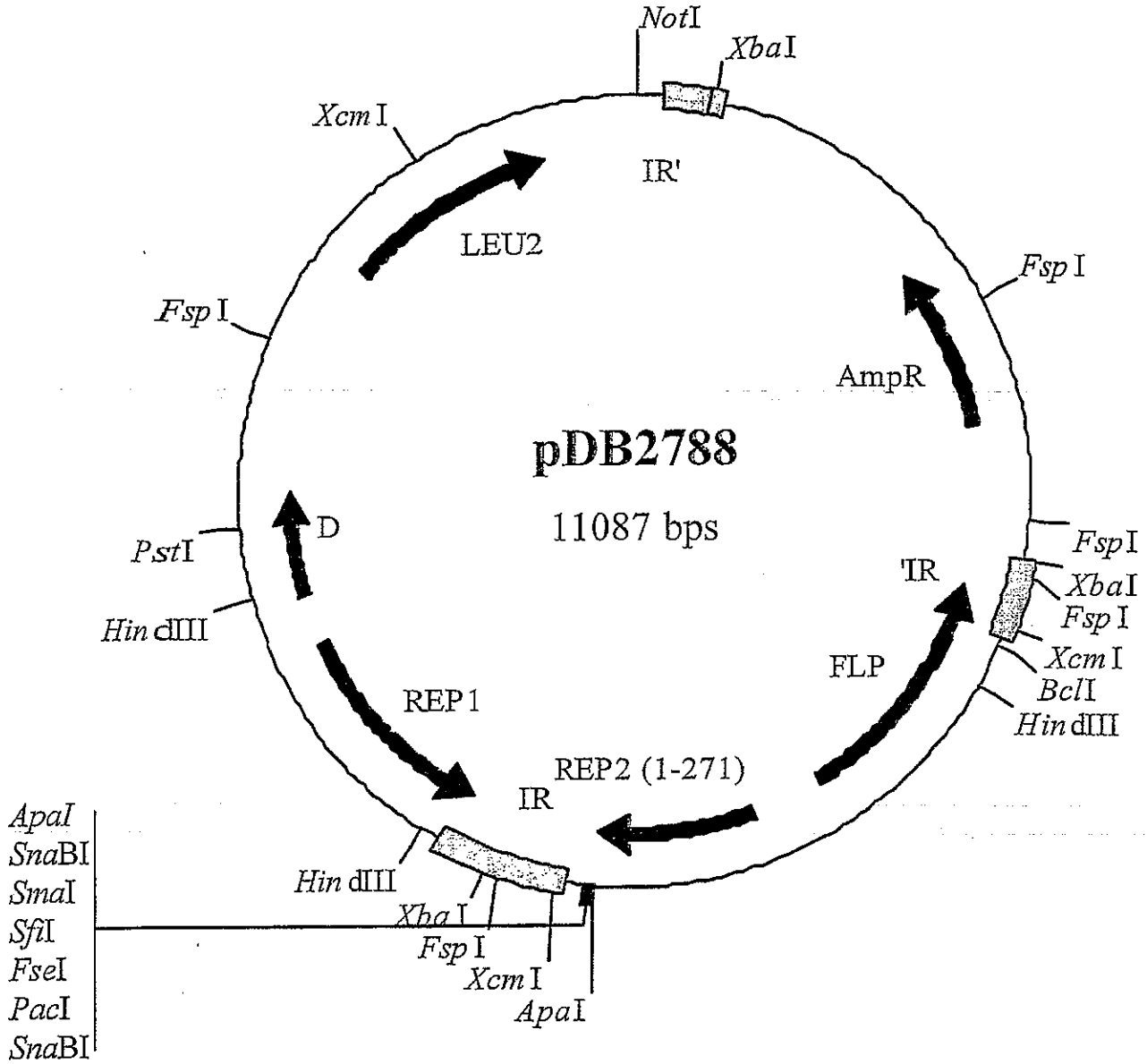

NotI
XbaI
XcmI
IR'
LEU2
FspI
FspI
AmpR
pDB2788
11087 bps
D
PstI
HindIII
FspI
XbaI
FspI
XcmI
BclI
HindIII
IR
FLP
REP1
REP2 (1-271)
IR
ApaI
SnaBI
SmaI
SfiI
FseI
PacI
SnaBI
HindIII
XbaI
FspI
XcmI
ApaI

Figure 19

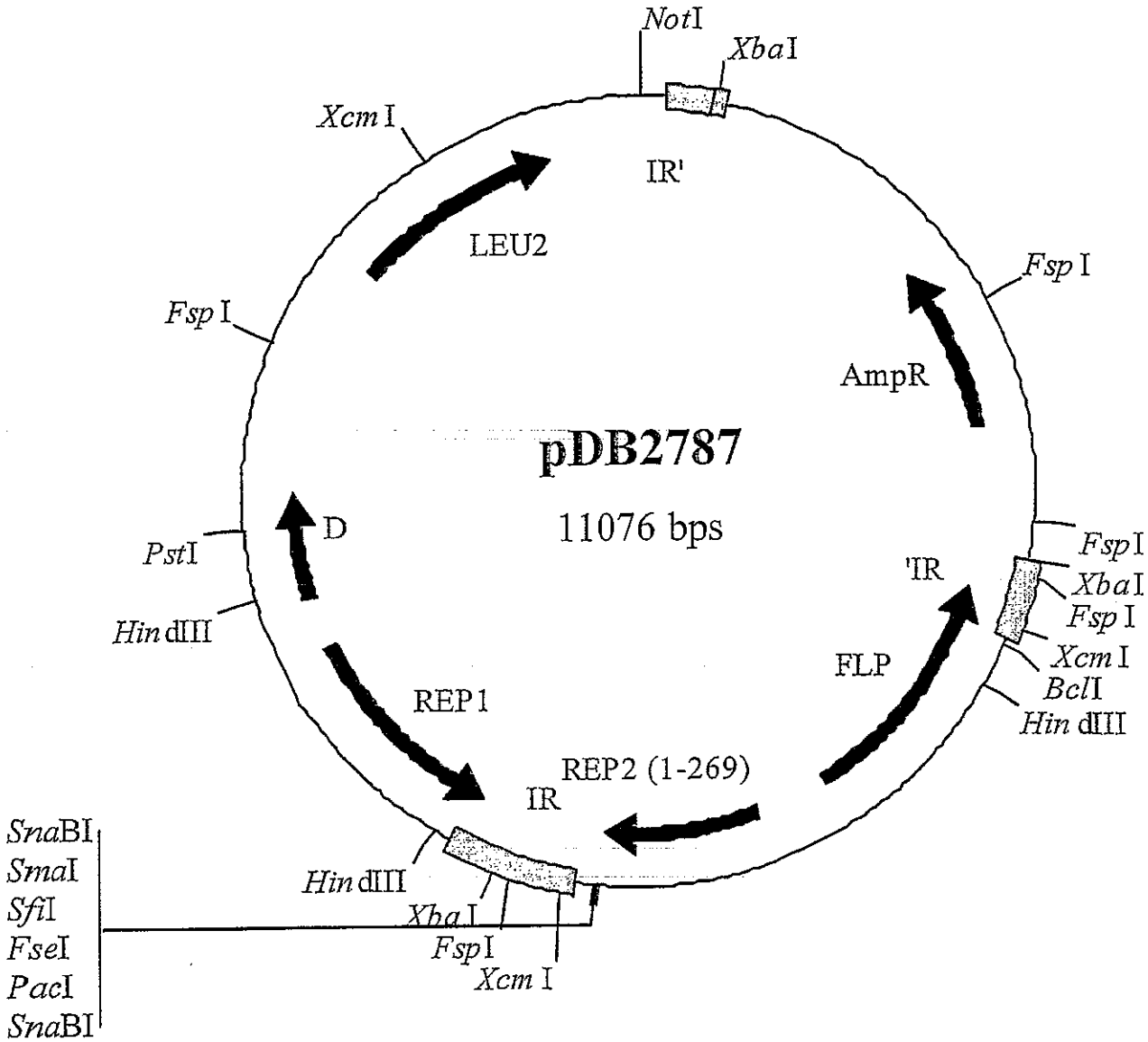

NotI
XbaI
XcmI
IR'
LEU2
FspI
FspI
AmpR
pDB2787
11076 bps
D
PstI
HindIII
'IR
FspI
XbaI
FspI
XcmI
BclI
HindIII
FLP
REP1
REP2 (1-269)
IR
SnaBI
SmaI
SfiI
FseI
PacI
SnaBI
HindIII
XbaI
FspI
XcmI

Figure 20

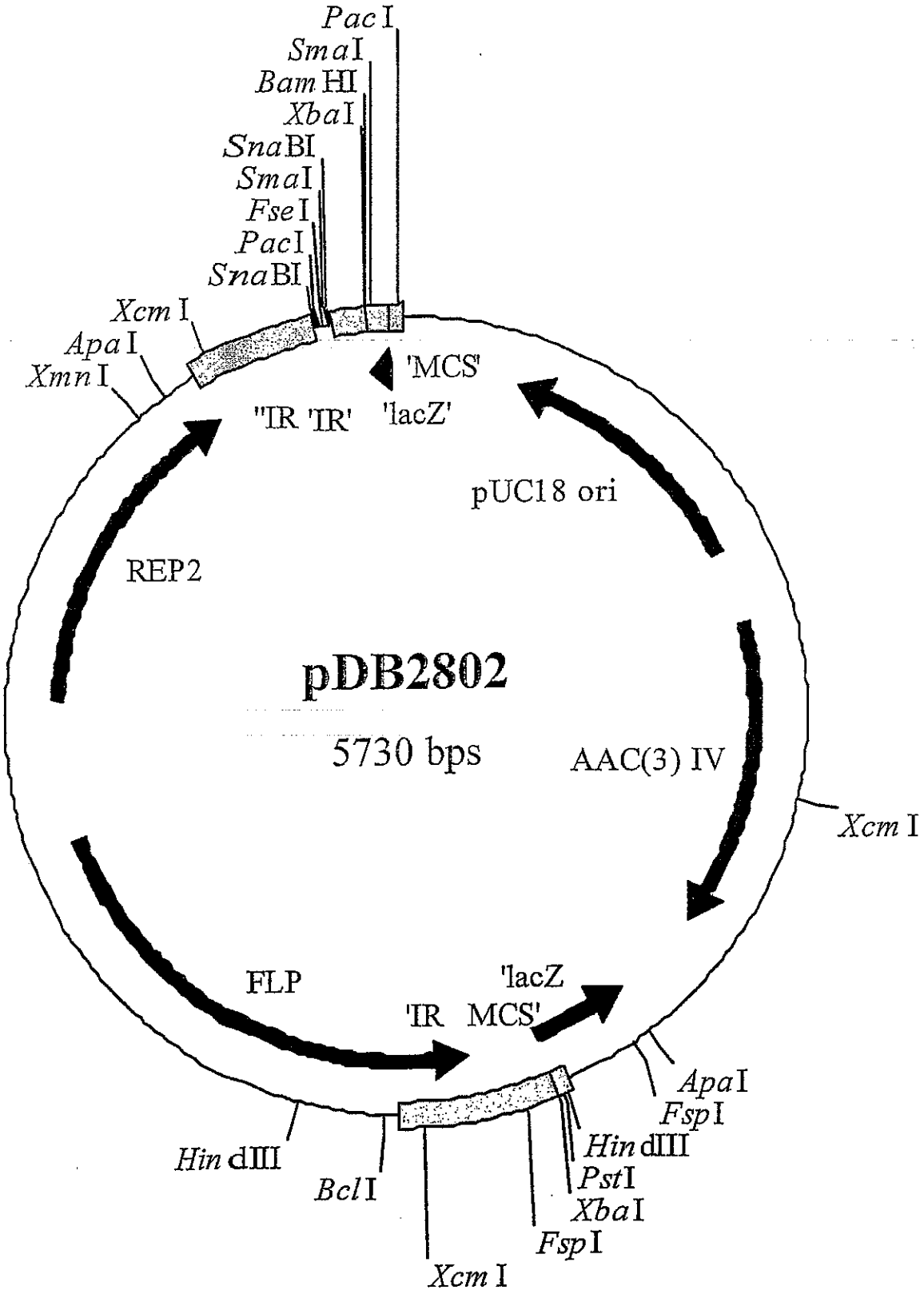

PacI
SmaI
BamHI
XbaI
SnaBI
SmaI
FseI
PacI
SnaBI
XcmI
ApaI
XmnI
'MCS'
"IR 'IR'
'lacZ'
pUC18 ori
REP2
pDB2802
5730 bps
AAC(3) IV
XcmI
FLP
'lacZ
'IR MCS'
ApaI
FspI
HindIII
PstI
XbaI
FspI
HindIII
BclI
XcmI

Figure 21

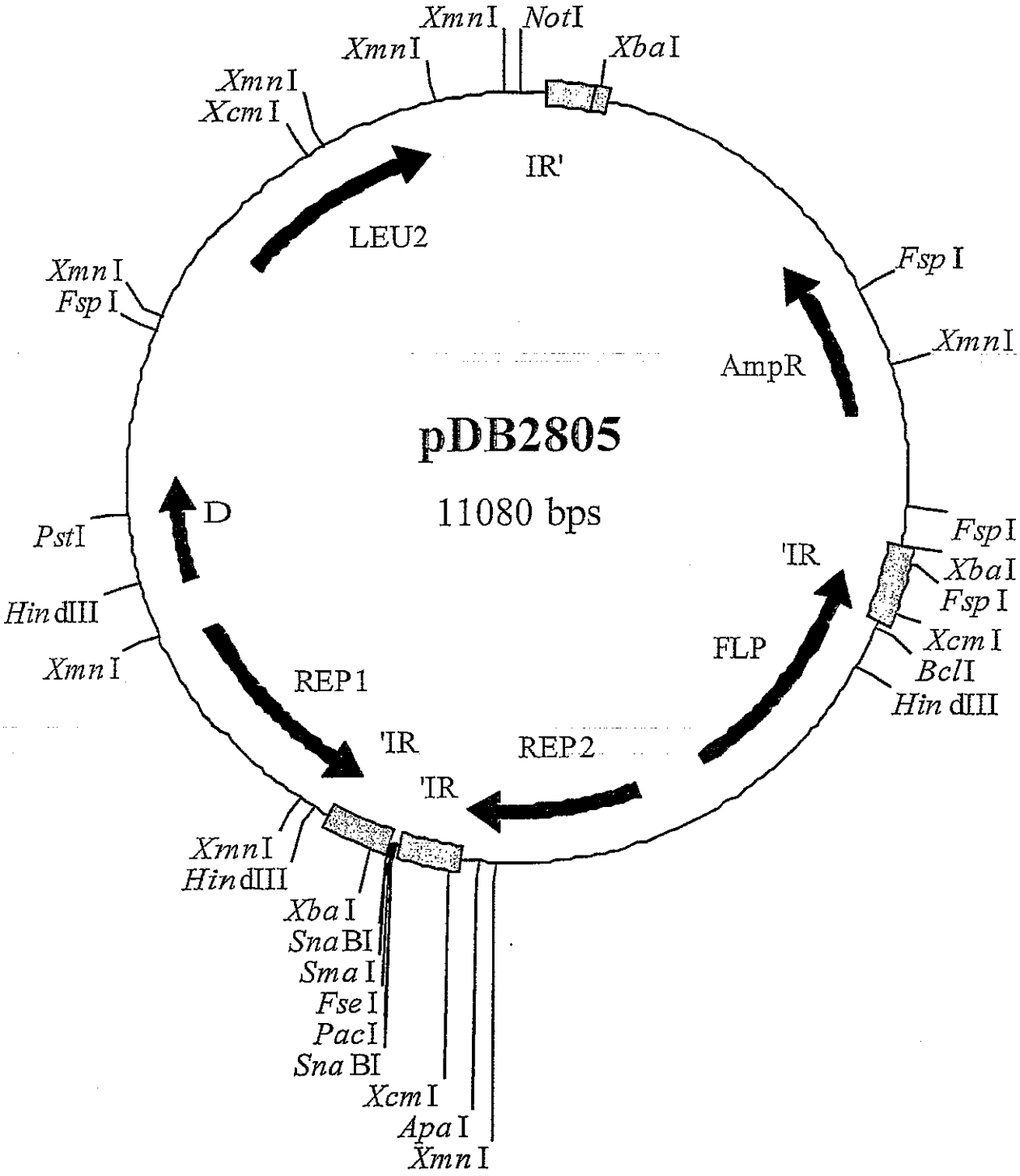

pDB2805
11080 bps
LEU2
IR'
AmpR
FLP
REP1
REP2
D
'IR
'IR
'IR
XmnI
NotI
XbaI
XmnI
XmnI
XcmI
XmnI
FspI
FspI
XmnI
FspI
XbaI
FspI
XcmI
BclI
HindIII
PstI
HindIII
XmnI
XmnI
HindIII
XbaI
SnaBI
SmaI
FseI
PacI
SnaBI
XcmI
ApaI
XmnI

Figure 22

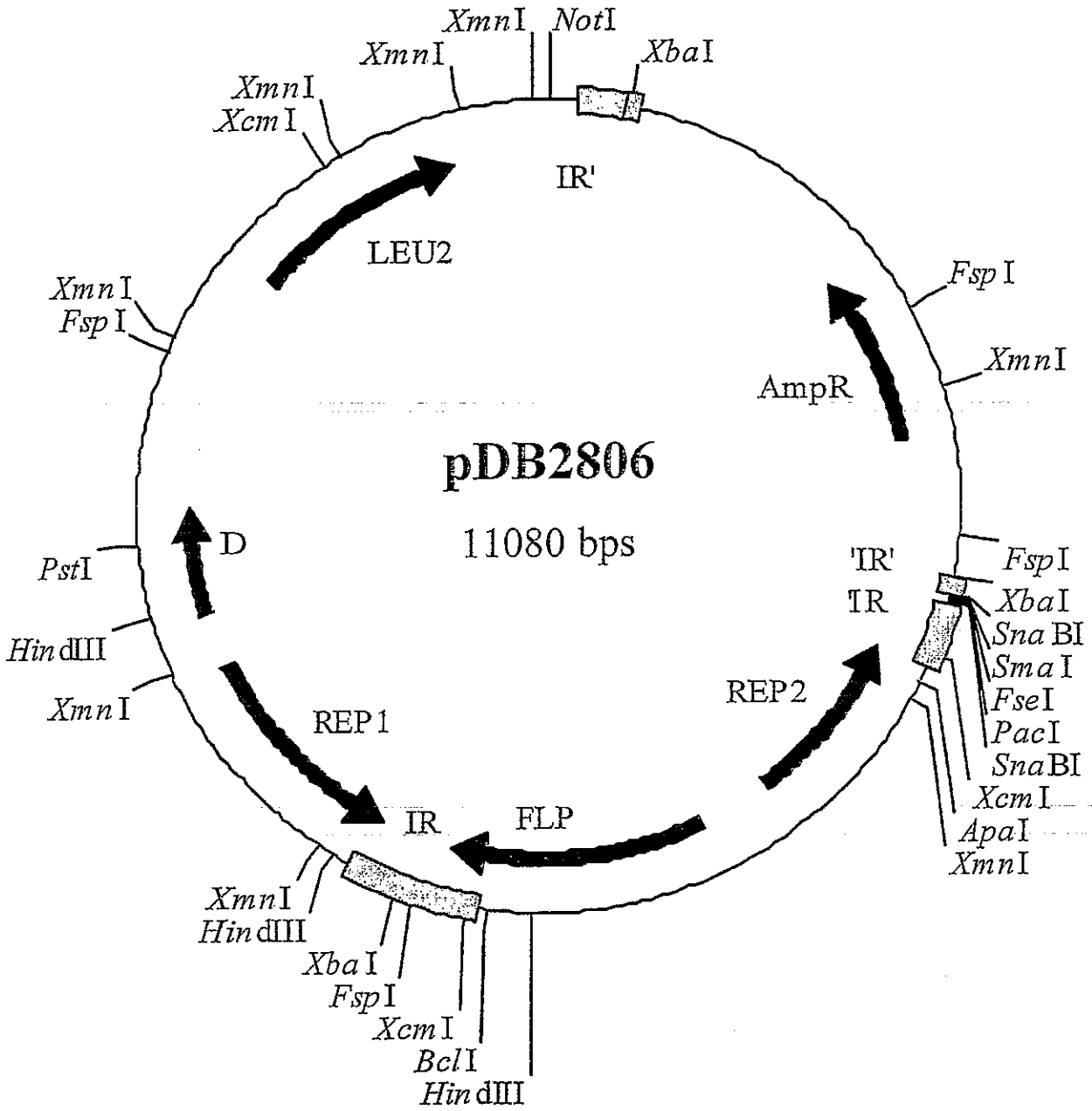

pDB2806
11080 bps
XmnI
NotI
XbaI
XmnI
XmnI
XcmI
IR'
LEU2
XmnI
FspI
FspI
XmnI
AmpR
D
PstI
HindIII
XmnI
REP1
REP2
'IR'
IR
FspI
XbaI
SnaBI
SmaI
FseI
PacI
SnaBI
XcmI
ApaI
XmnI
IR
FLP
XmnI
HindIII
XbaI
FspI
XcmI
BclI
HindIII

Figure 23

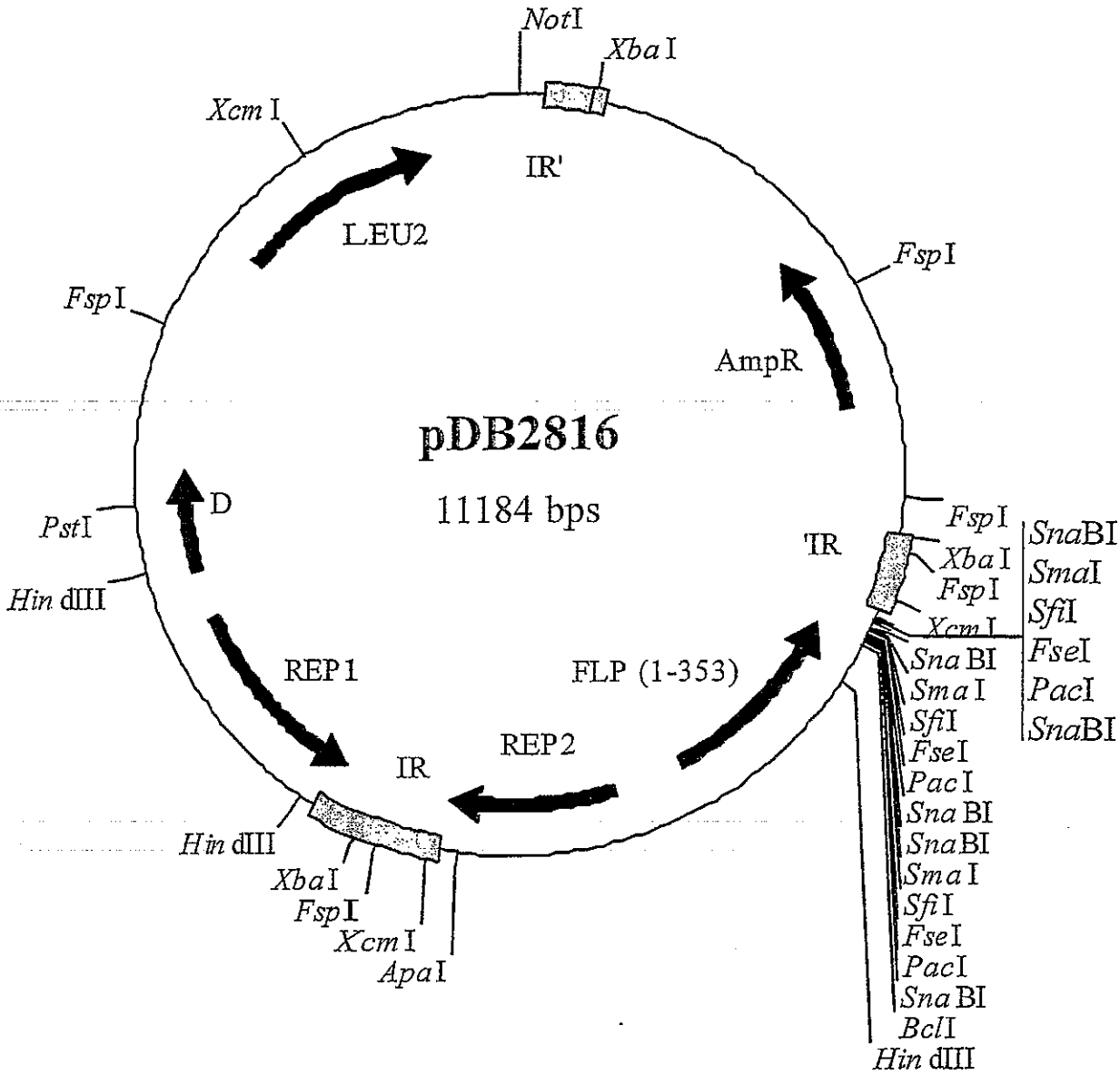

NotI
XbaI
XcmI
IR'
LEU2
FspI
FspI
AmpR
pDB2816
11184 bps
D
PstI
HindIII
IR
FspI
XbaI
FspI
XcmI
SnaBI
SmaI
SfiI
FseI
PacI
SnaBI
REP1
FLP (1-353)
REP2
IR
HindIII
XbaI
FspI
XcmI
ApaI
SnaBI
SmaI
SfiI
FseI
PacI
SnaBI
SnaBI
SmaI
SfiI
FseI
PacI
SnaBI
BclI
HindIII

Figure 24

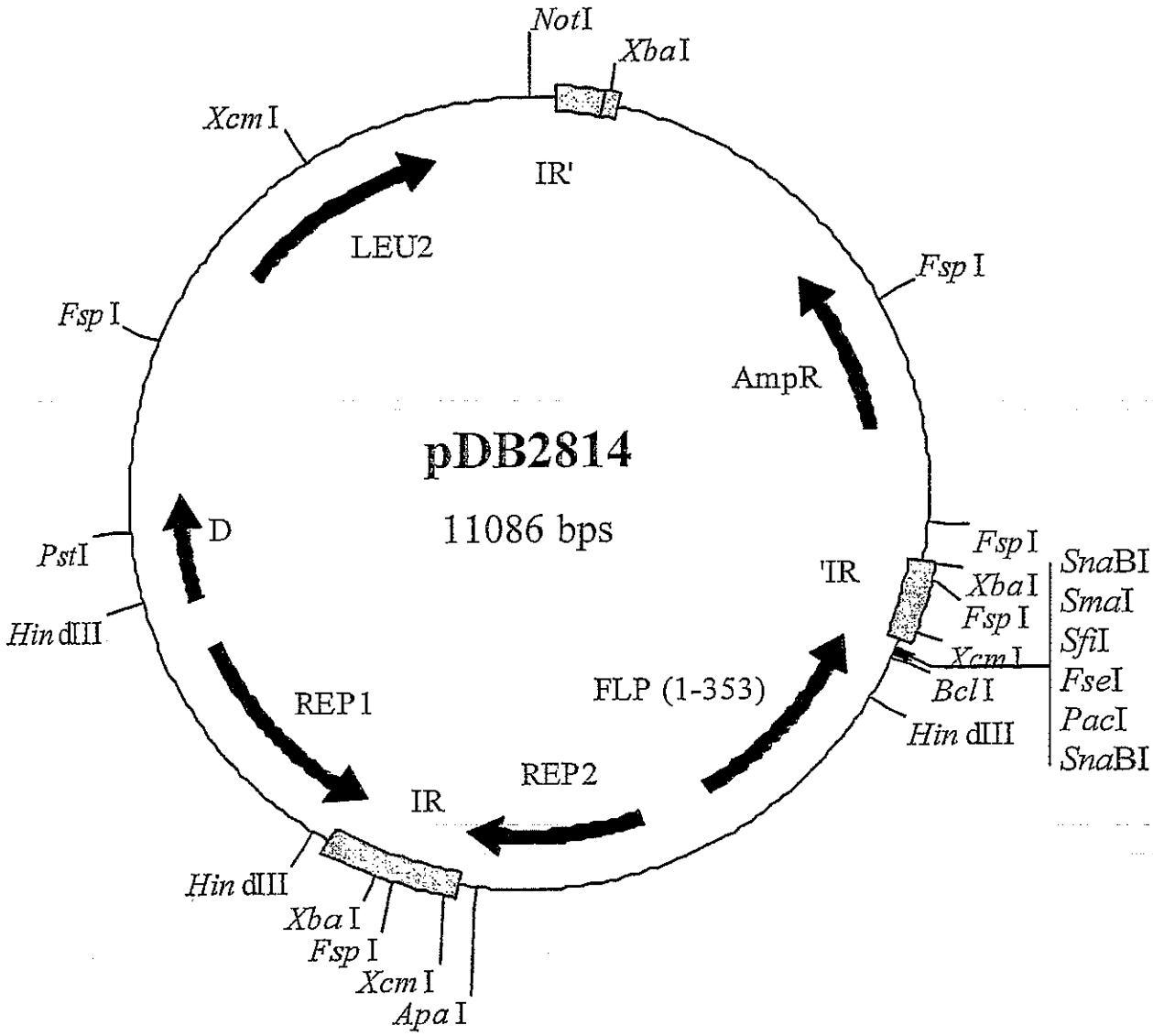

NotI
XbaI
XcmI
IR'
LEU2
FspI
FspI
AmpR
pDB2814
11086 bps
D
PstI
HindIII
'IR
FspI
XbaI
FspI
XcmI
BclI
HindIII
SnaBI
SmaI
SfiI
FseI
PacI
SnaBI
REP1
FLP (1-353)
REP2
IR
HindIII
XbaI
FspI
XcmI
ApaI

Figure 25

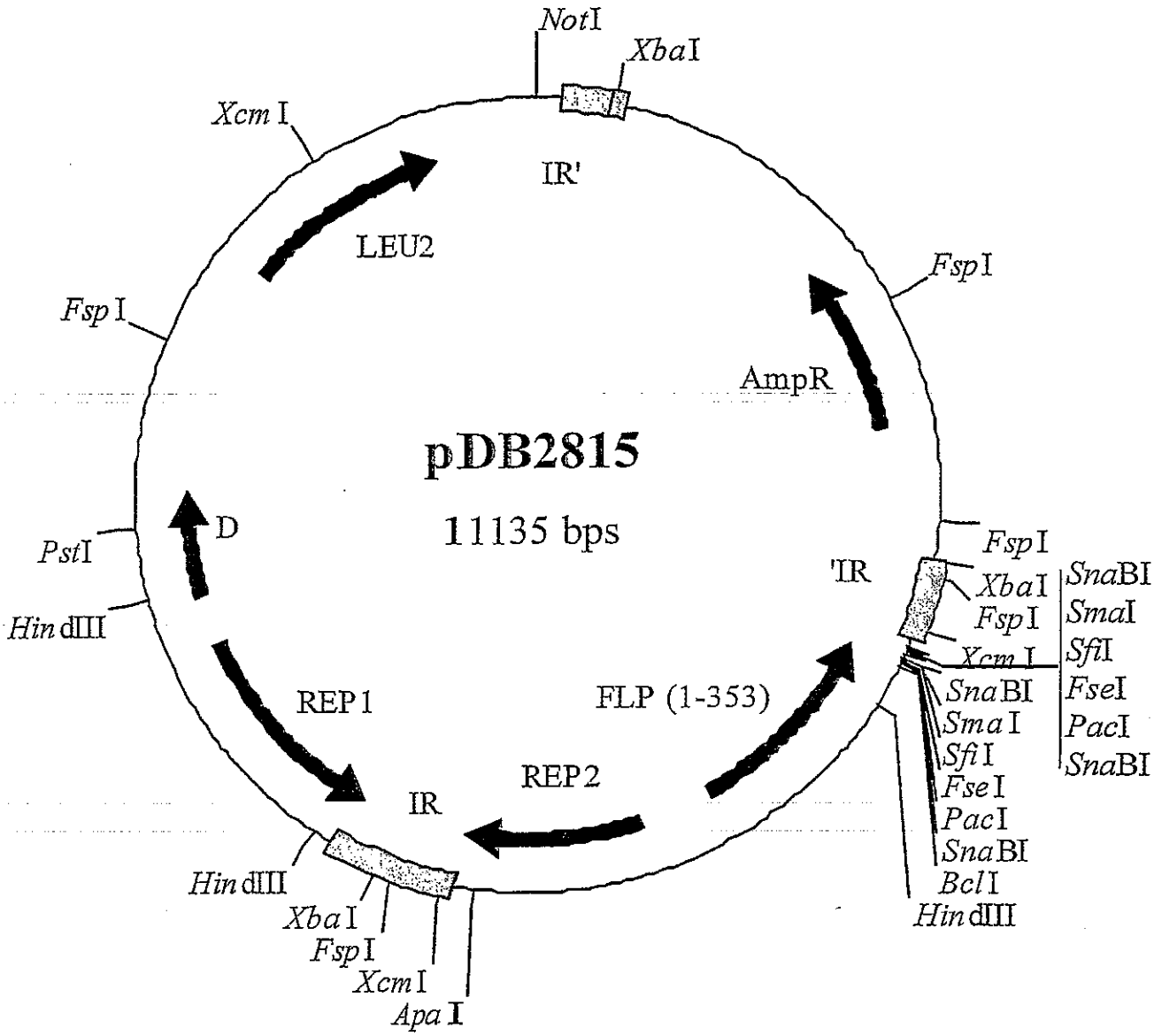

NotI
XbaI
Xcm I
IR'
LEU2
Fsp I
FspI
AmpR
pDB2815
11135 bps
PstI
D
'IR
Hin dIII
FspI
XbaI
FspI
Xcm I
SnaBI
SmaI
SfiI
FseI
PacI
SnaBI
REP1
FLP (1-353)
SnaBI
SmaI
SfiI
FseI
PacI
SnaBI
BclI
Hin dIII
REP2
IR
Hin dIII
XbaI
FspI
Xcm I
Apa I

Figure 26

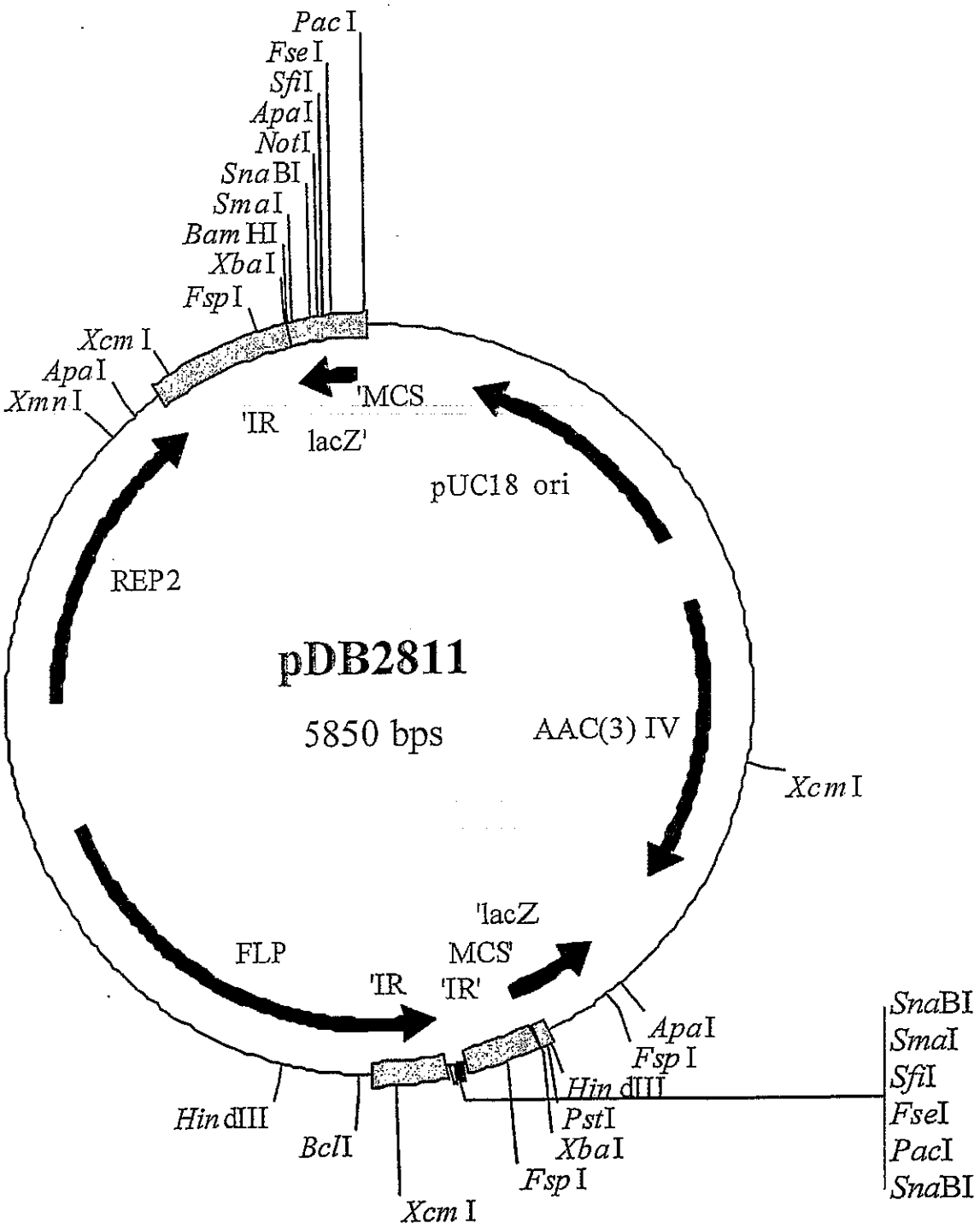

Pac I
Fse I
Sfi I
Apa I
Not I
Sna BI
Sma I
Bam HI
Xba I
Fsp I
Xcm I
Apa I
Xmn I
'IR
'MCS
lacZ'
pUC18 ori
REP2
pDB2811
5850 bps
AAC(3) IV
Xcm I
'lacZ
FLP
MCS'
'IR
'IR
Apa I
Fsp I
Hin dIII
Pst I
Xba I
Fsp I
Hin dIII
Bcl I
Xcm I
Sna BI
Sma I
Sfi I
Fse I
Pac I
Sna BI

Figure 27

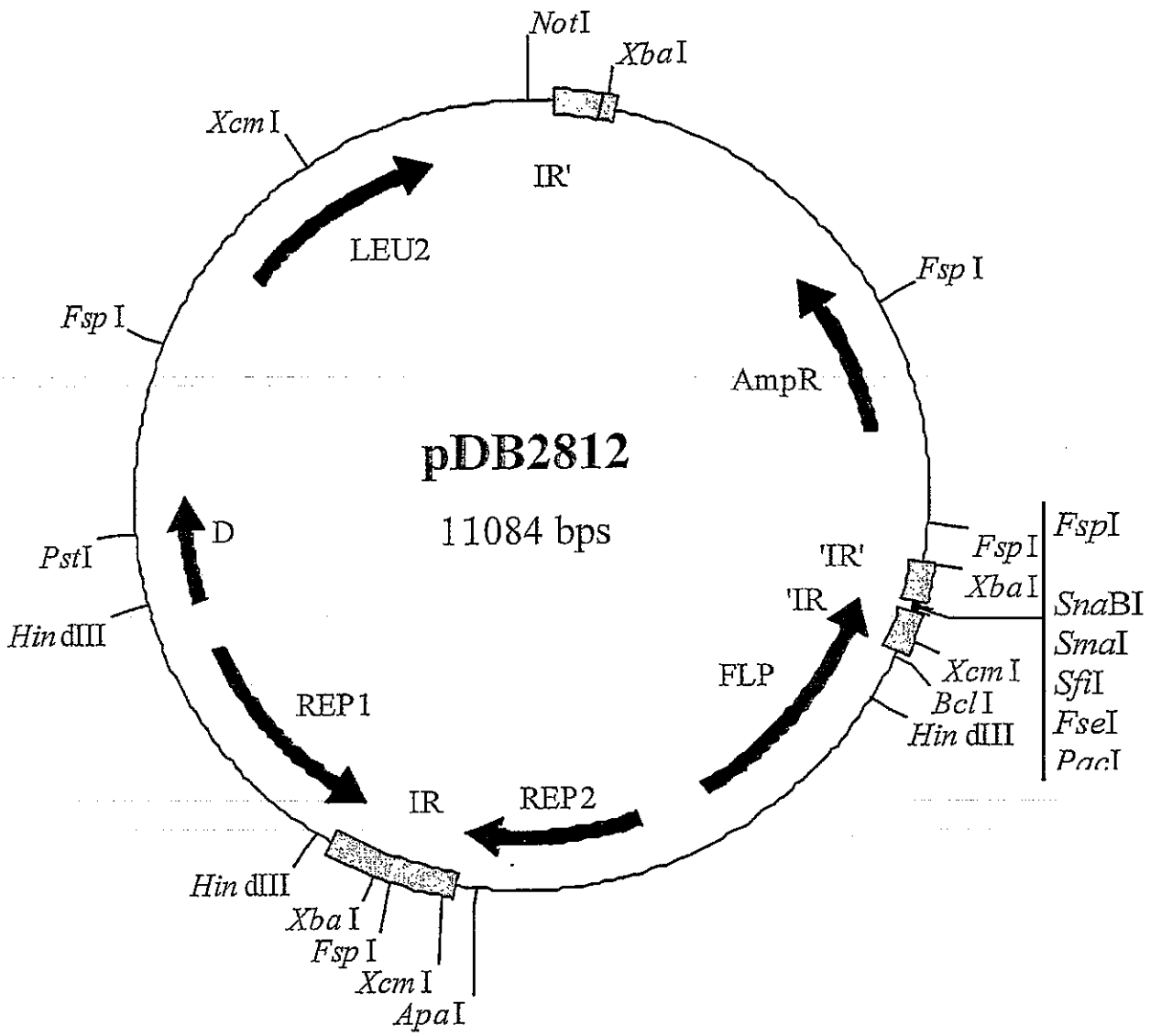

NotI
XbaI
XcmI
IR'
LEU2
FspI
FspI
AmpR
pDB2812
11084 bps
PstI
D
'IR'
FspI
XbaI
FspI
SnaBI
SmaI
SfiI
FseI
PacI
'IR
HindIII
FLP
XcmI
BclI
HindIII
REP1
IR
REP2
HindIII
XbaI
FspI
XcmI
ApaI

Figure 28

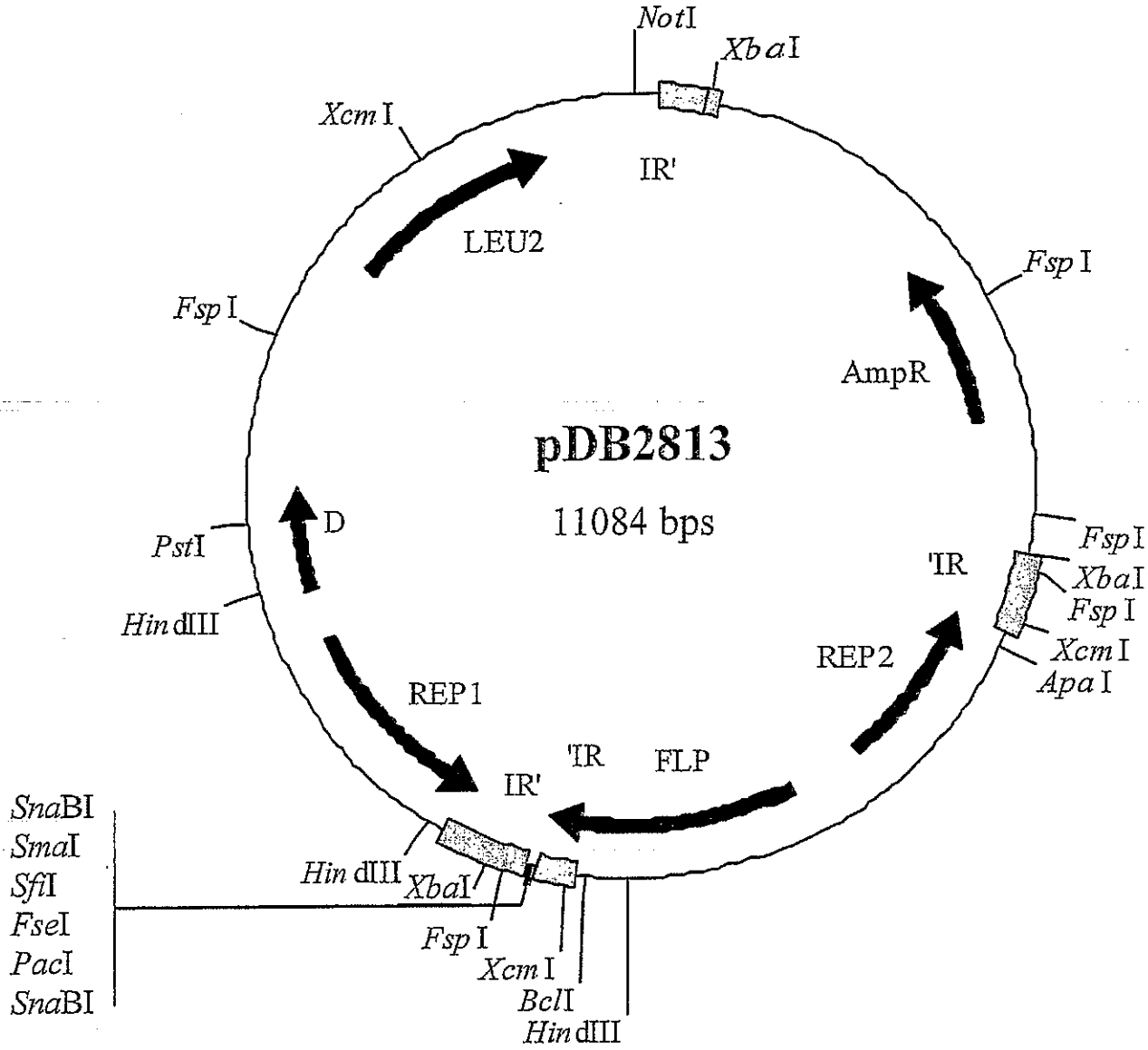

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XbaI
XcmI
IR'
LEU2
FspI
FspI
AmpR
pDB2813
11084 bps
PstI
D
FspI
XbaI
FspI
XcmI
ApaI
HindIII
'IR
REP2
REP1
'IR
FLP
IR'
SnaBI
SmaI
SfiI
FseI
PacI
SnaBI
HindIII
XbaI
FspI
XcmI
BclI
HindIII

Figure 29

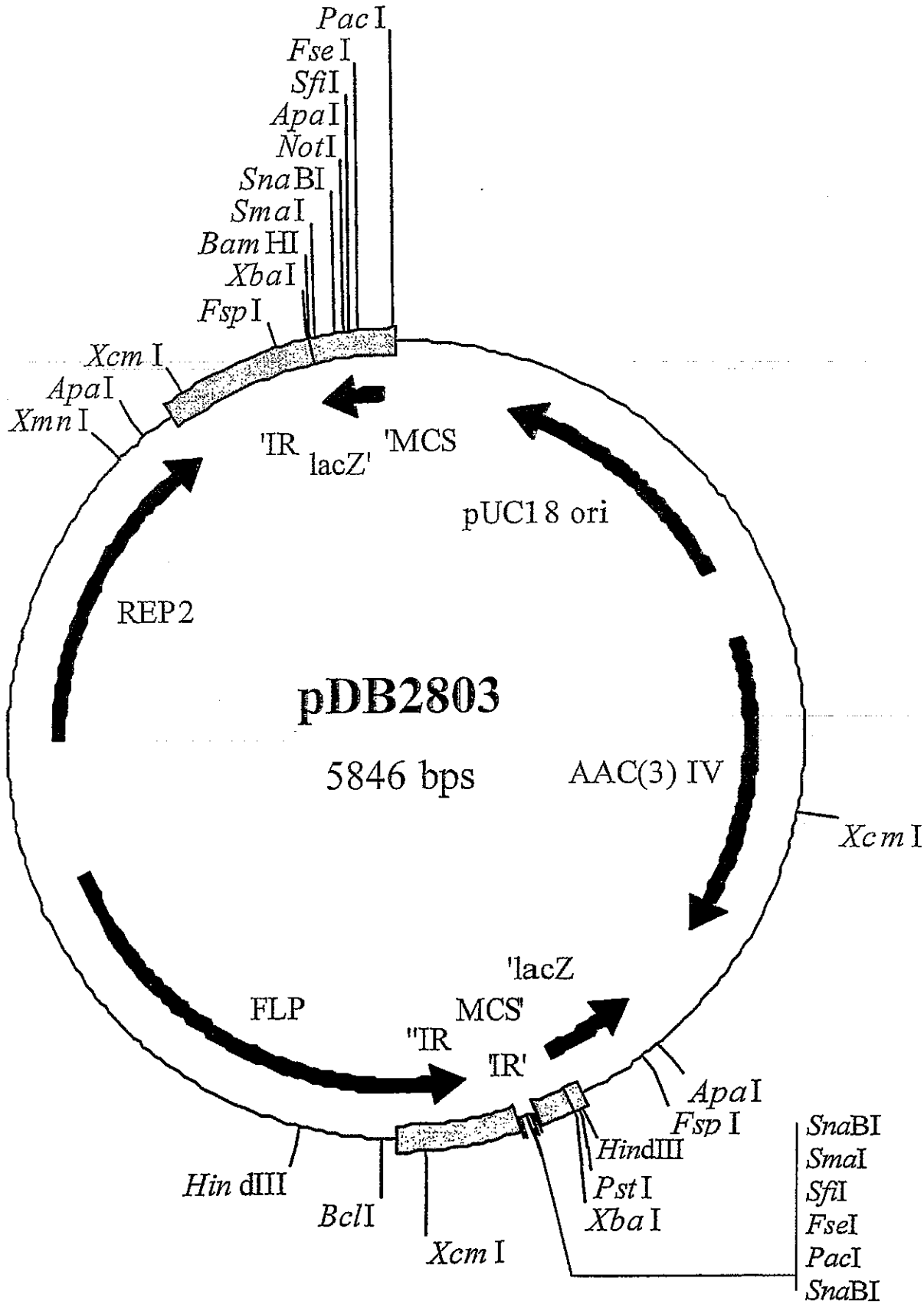

Pac I
Fse I
Sfi I
Apa I
Not I
Sna BI
Sma I
Bam HI
Xba I
Fsp I
Xcm I
Apa I
Xmn I
'IR
lacZ'
'MCS
pUC18 ori
REP2
pDB2803
5846 bps
AAC(3) IV
Xcm I
'lacZ
FLP
MCS'
"IR
'IR'
Apa I
Fsp I
Hin dIII
Bcl I
Xcm I
Hind III
Pst I
Xba I
Sna BI
Sma I
Sfi I
Fse I
Pac I
Sna BI

Figure 30

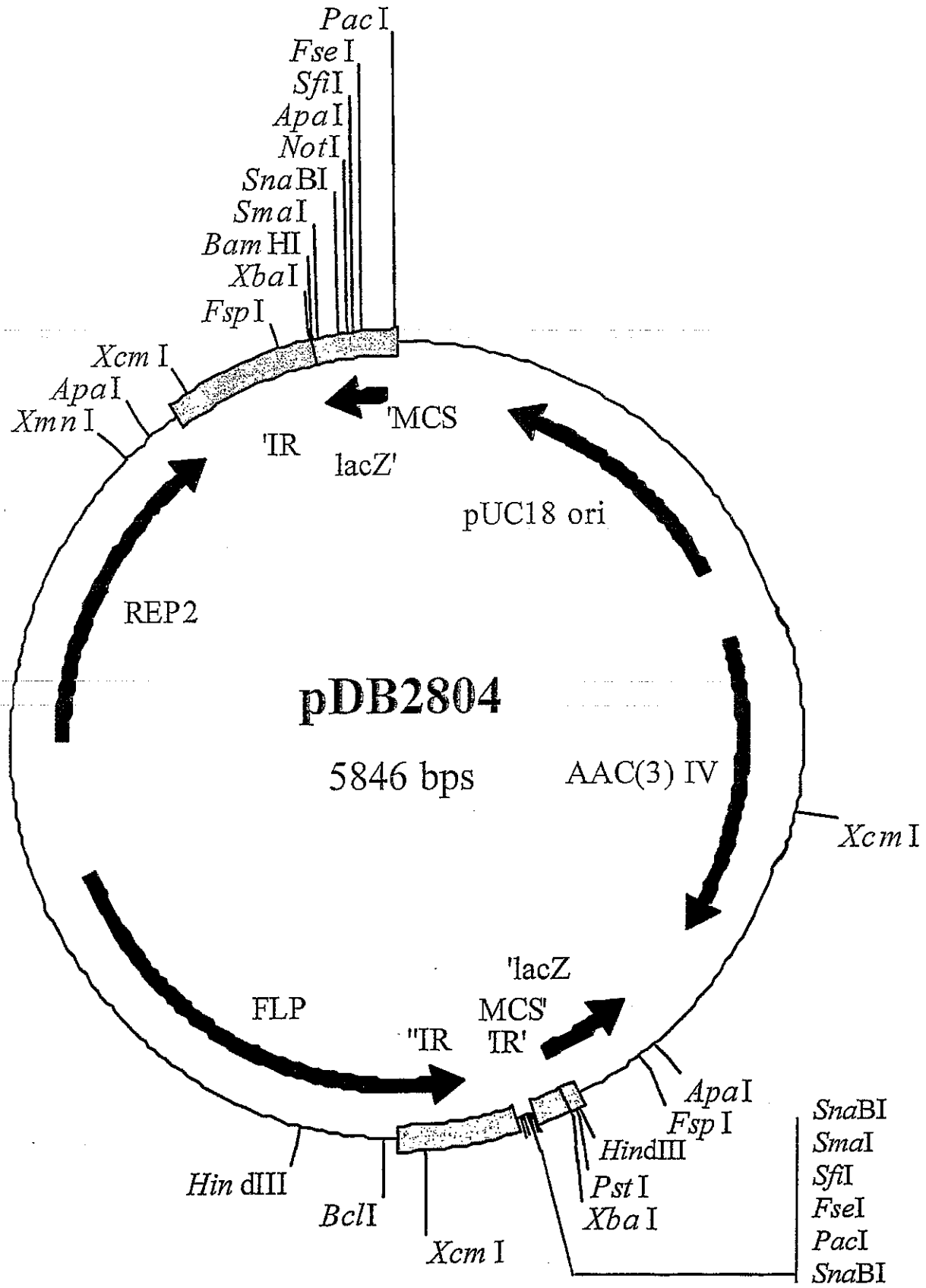

Pac I
Fse I
Sfi I
Apa I
Not I
Sna BI
Sma I
Bam HI
Xba I
Fsp I
Xcm I
Apa I
Xmn I
'IR
'MCS
lacZ'
pUC18 ori
REP2
pDB2804
5846 bps
AAC(3) IV
Xcm I
'lacZ
FLP
MCS'
"IR
'IR'
Apa I
Fsp I
Hin dIII
Bcl I
Hind III
Pst I
Xba I
Xcm I
Sna BI
Sma I
Sfi I
Fse I
Pac I
Sna BI

Figure 31

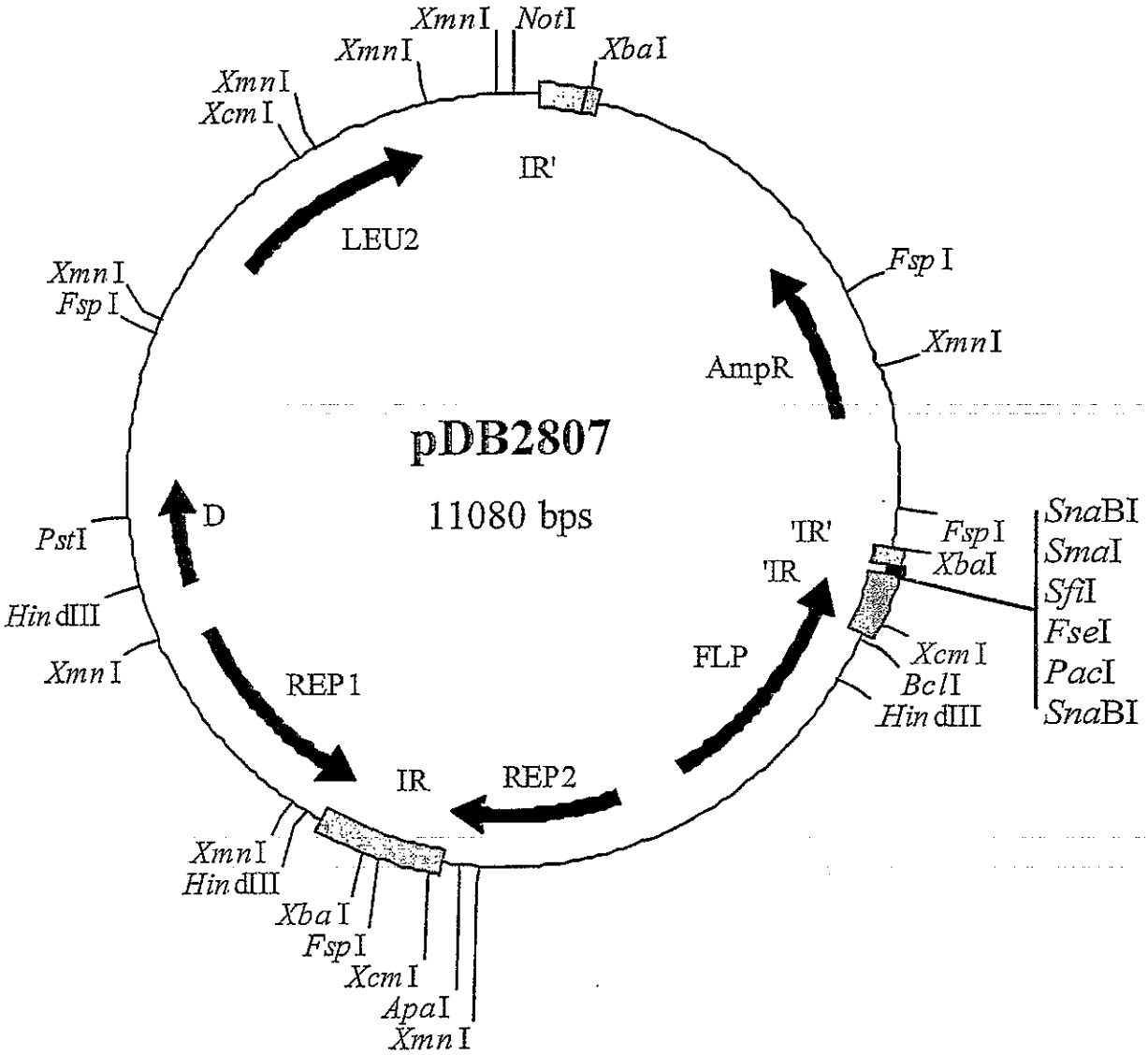

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11080 bps
XmnI
NotI
XbaI
XmnI
XmnI
XcmI
IR'
LEU2
XmnI
FspI
FspI
XmnI
AmpR
D
PstI
HindIII
XmnI
REP1
IR
REP2
FLP
'IR'
'IR
FspI
XbaI
XcmI
BclI
HindIII
SnaBI
SmaI
SfiI
FseI
PacI
SnaBI
XmnI
HindIII
XbaI
FspI
XcmI
ApaI
XmnI

Figure 32

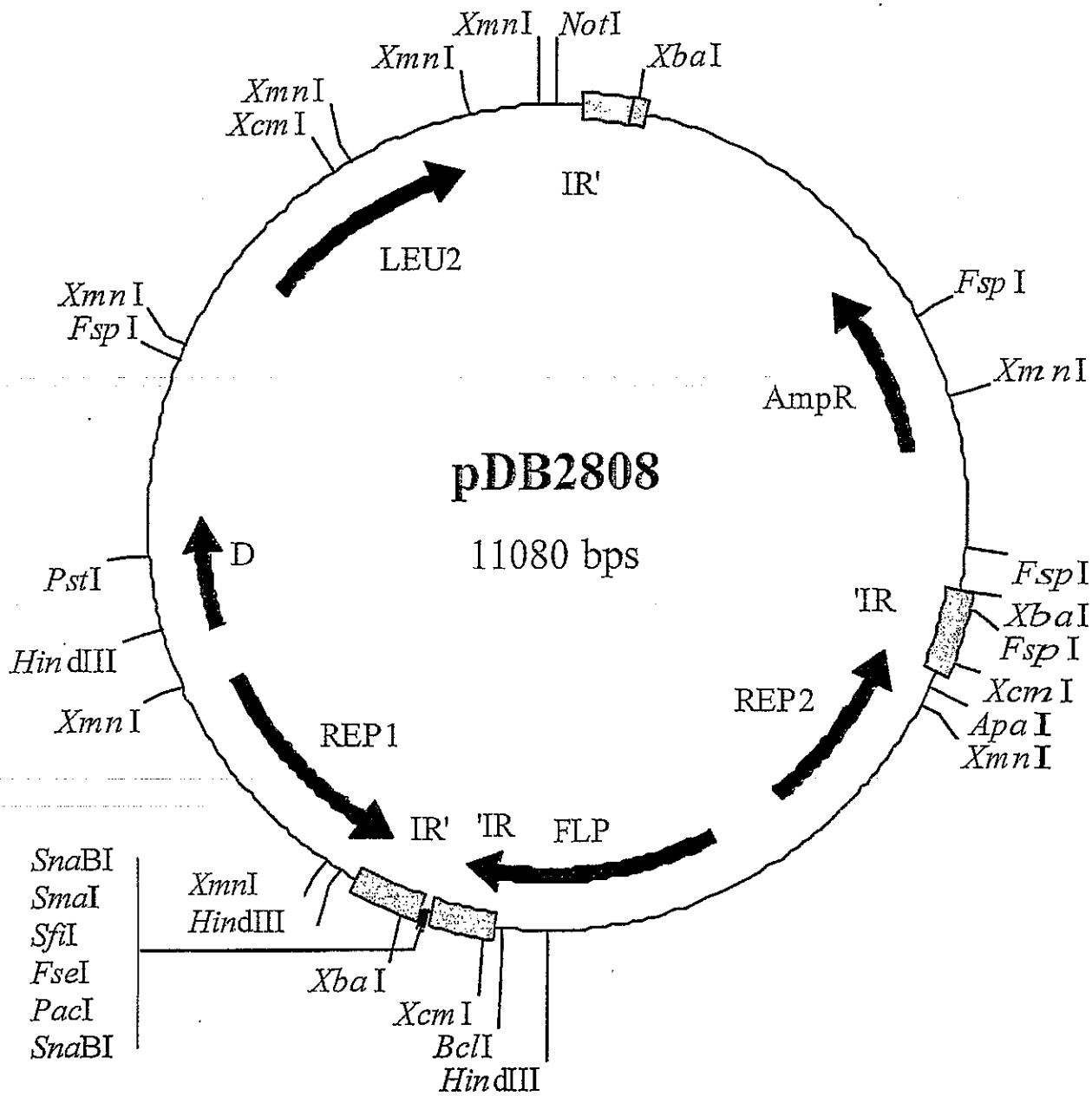

pDB2808
11080 bps
XmnI
NotI
XmnI
XbaI
XmnI
XcmI
IR'
LEU2
XmnI
FspI
FspI
XmnI
AmpR
D
PstI
FspI
'IR
XbaI
HindIII
FspI
REP2
XcmI
XmnI
ApaI
XmnI
REP1
IR'
'IR
FLP
SnaBI
SmaI
SfiI
FseI
PacI
SnaBI
XmnI
HindIII
XbaI
XcmI
BclI
HindIII

Figure 33A

Table 3 (part 1)

Plasmid	Insertion Site	Insertion Site Details	2µm Form	Initial OD_{600}	Leucine Prototrophs		
					Number	%	Average
pSAC35	-	-	B	0.074	100/100	100%	99%
pSAC35	-	-	B	0.068	95/98	97%	
pDB2818	*Xmn*I	REP2 (1-244)	A	0.062	32/100	32%	42%
pDB2818	*Xmn*I	REP2 (1-244)	A	0.059	52/100	52%	
pDB2787	*Apa*I/T4 pol.	REP2 (1-269)	B	0.054	34/100	34%	45%
pDB2787	*Apa*I/T4 pol.	REP2 (1-269)	B	0.084	45/100	45%	
pDB2788	*Apa*I	REP2 (1-271)	B	0.066	23/100	23%	33%
pDB2788	*Apa*I	REP2 (1-271)	B	0.051	43/100	43%	

Figure 33B

Table 3 (part 2)

Plasmid	Insertion Site	Insertion Site Details	2μm Form	Initial OD_{600}	Leucine Prototrophs		
					Number	%	Average
pDB2688	*Xcm*I	Inverted Repeat	B	0.055	100/100	100%	100%
PDB2688	*Xcm*I	Inverted Repeat	B	0.066	100/100	100%	
pDB2806	*Fsp*I	Inverted Repeat	A	0.073	100/100	100%	100%
pDB2806	*Fsp*I	Inverted Repeat	A	0.070	100/100	100%	
pDB2817	*Xmn*I	REP2 (1-244)	B	0.063	36/100	36%	35%
pDB2817	*Xmn*I	REP2 (1-244)	B	0.082	34/100	34%	
pDB2805	*Fsp*I	Inverted Repeat	B	0.069	100/100	100%	100%
pDB2805	*Fsp*I	Inverted Repeat	B	0.078	100/100	100%	
pDB2814	*Bcl*I	FLP (1-353), 1× Insert	B	0.080	69/100	69%	67%
pDB2814	*Bcl*I	FLP (1-353), 1× Insert	B	0.057	64/100	64%	
pDB2815	*Bcl*I	FLP (1-353), 2× Insert	B	0.067	70/100	70%	67%
pDB2815	*Bcl*I	FLP (1-353), 2× Insert	B	0.068	64/100	64%	

Figure 33C

Table 3 (part 3)

Plasmid	Insertion Site	Insertion Site Details	2μm Form	Initial OD_{600}	Leucine Prototrophs		
					Number	%	Average
pDB2816	*Bcl*I	FLP (1-353), 3× Insert	B	0.069	67/100	67%	74%
pDB2816	*Bcl*I	FLP (1-353), 3× Insert	B	0.056	81/100	81%	
pDB2689	*Xcm*I	C-terminal FLP Mutant (FLP 1-384, plus 56 other residues)	B	0.054	73/100	73%	75%
pDB2689	*Xcm*I	C-terminal FLP Mutant (FLP 1-384, plus 56 other residues)	B	0.056	77/100	77%	
pDB2786	*Xcm*I	C-terminal FLP Mutant (FLP 1-384, plus 14 other residues)	B	0.079	73/100	73%	67%
pDB2786	*Xcm*I	C-terminal FLP Mutant (FLP 1-384, plus 14 other residues)	B	0.052	61/100	61%	
pDB2823	*Xcm*I	FLP (1-382)	B	0.071	70/100	70%	64%
pDB2823	*Xcm*I	FLP (1-382)	B	0.055	57/100	57%	

Figure 33D

Table 3 (part 4)

Plasmid	Insertion Site	Insertion Site Details	2μm Form	Initial OD_{600}	Leucine Prototrophs		
					Number	%	Average
pDB2813	*Hga*I	Inverted Repeat	A	0.057	100/100	100%	100%
pDB2813	*Hga*I	Inverted Repeat	A	0.076	100/100	100%	
pDB2808	*Fsp*I	Inverted Repeat	A	0.058	100/100	100%	100%
pDB2808	*Fsp*I	Inverted Repeat	A	0.060	100/100	100%	
pDB2812	*Hga*I	Inverted Repeat	B	0.062	100/100	100%	100%
pDB2812	*Hga*I	Inverted Repeat	B	0.071	100/100	100%	

Figure 34

SEQ ID NO:1

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 101  ATGTAGATAT TGATATAGAG AGTAAACGTA AGTCTGATGA GCTACTCTTT CCAGGATATG TCATAAGGCC CATGGAATCT CTCACAACCG GTAGGCCGTA
 201  TGGTCTTGAT TCTAGCGCAG AAGATTCCAG CGTATCTTCT GACTCCAGTG CTGAGGTAAT TTTGCCTGCT GCGAAGATGG TTAAGGAAAG GTTTGATTCG
 301  ATTGGAAATG GTATGCTCTC TTCACAAGAA GCAAGTCAGG CTGCCATAGA TTTGATGCTA CAGAATAACA AGCTGTTAGA CAATAGAAAG CAACTATACA
 401  AATCTATTGC TATAATAATA GGAAGATTGC CCGAGAAAGA CAAGAAGAGA GCTACCGAAA TGCTCATGAG AAAAATGGAT TGTACACAGT TATTAGTCCC
 501  ACCAGCTCCA ACGGAAGAAG ATGTTATGAA GCTCGTAAGC GTCGTTACCC AATTGCTTAC TTTAGTTCCA CCAGATCGTC AAGCTGCTTT AATAGGTGAT
 601  TTATTCATCC CGGAATCTCT AAAGGATATA TTCAATAGTT TCAATGAACT GGCGGCAGAG AATCGTTTAC AGCAAAAAAA GAGTGAGTTG GAAGGAAGGA
 701  CTGAAGTGAA CCATGCTAAT ACAAATGAAG AAGTTCCCTC CAGGCGAACA AGAAGTAGAG ACACAAATGC AAGAGGAGCA TATAAATTAC AAAACACCAT
 801  CACTGAGGGC CCTAAAGCGG TTCCCACGAA AAAAAGGAGA GTAGCAACGA GGGTAAGGGG CAGAAAATCA CGTAATACTT CTAGGGTATG ATCCAATATC
 901  AAAGGAAATG ATAGCATTGA AGGATGAGAC TAATCCAATT GAGGAGTGGC AGCATATAGA ACAGCTAAAG GGTAGTGCTG AAGGAAGCAT ACGATACCCC
1001  GCATGGAATG GGATAATATC ACAGGAGGTA CTAGACTACC TTTCATCCTA CATAAATAGA CGCATATAAG TACGCATTTA AGCATAAACA CGCACTATGC
1101  CGTTCTTCTC ATGTATATAT ATATACAGGC AACACGCAGA TATAGGTGCG ACGTGAACAG TGAGCTGTAT GTGCGCAGCT CGCGTTGCAT TTTCGGAAAC
1201  GCTCGTTTTC GGAAACGCTT TGAAGTTCCT ATTCCGAAGT TCCTATTCTC TAGAAAGTAT AGGAACTTCA GAGCGCTTTT GAAAACCAAA AGCGCTCTGA
1301  AGACGCACTT TCAAAAAACC AAAAACGCAC CGGACTGTAA CGAGCTACTA AAATATTGCG AATACCGCTT CCACAAACAT TGCTCAAAAG TATCTCTTTG
1401  CTATATATCT CTGTGCTATA TCCCTATATA ACCTACCCAT CCACCTTTCG CTCCTTGAAC TTGCATCTAA ACTCGACCTC TACAT
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Figure 35

SEQ ID NO:2

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 101  GTGCTGCTGA ACTAACCTAT TTATGTTGGA TGATTACACA TAACGGAACA GCAATCAAGA GAGCCACATT CATGAGCTAT AATACTATCA TAAGCAATTC
 201  GCTGAGTTTC GATATTGTCA ATAAATCACT CCAGTTTAAA TACAAGACGC AAAAAGCAAC AATTCTGGAA GCCTCATTAA AGAAATTGAT TCCTGCTTGG
 301  GAATTTACAA TTATTCCTTA CTATGGACAA AAACATCAAT CTGATATCAC TGATATTGTA AGTAGTTTGC AATTACAGTT CGAATCATCG GAAGAAGCAG
 401  ATAAGGGAAA TAGCCACAGT AAAAAAATGC TTAAAGCACT TCTAAGTGAG GGTGAAAGCA TCTGGGAGAT CACTGAGAAA ATACTAAATT CGTTTGAGTA
 501  TACTTCGAGA TTTACAAAAA CAAAAACTTT ATACCAATTC CTCTTCCTAG CTACTTTCAT CAATTGTGGA AGATTCAGCG ATATTAAGAA CGTTGATCCG
 601  AAATCATTTA AATTAGTCCA AAATAAGTAT CTGGGAGTAA TAATCCAGTG TTTAGTGACA GAGACAAAGA CAAGCGTTAG TAGGCACATA TACTTCTTTA
 701  GCGCAAGGGG TAGGATCGAT CCACTTGTAT ATTTGGATGA ATTTTTGAGG AATTCTGAAC CAGTCCTAAA ACGAGTAAAT AGGACCGGCA ATTCTTCAAG
 801  CAATAAACAG GAATACCAAT TATTAAAAGA TAACTTAGTC AGATCGTACA ATAAAGCTTT GAAGAAAAAT GCGCCTTATT CAATCTTTGC TATAAAAAAT
 901  GGCCCAAAAT CTCACATTGG AAGACATTTG ATGACCTCAT TTCTTTCAAT GAAGGGCCTA ACGGAGTTGA CTAATGTTGT GGGAAATTGG AGCGATAAGC
1001  GTGCTTCTGC CGTGGCCAGG ACAACGTATA CTCATCAGAT AACAGCAATA CCTGATCACT ACTTCGCACT AGTTTCTCGG TACTATGCAT ATGATCCAAT
1101  ATCAAAGGAA ATGATAGCAT TGAAGGATGA GACTAATCCA ATTGAGGAGT GGCAGCATAT AGAACAGCTA AAGGGTAGTG CTGAAGGAAG CATACGATAC
1201  CCCGCATGGA ATGGGATAAT ATCACAGGAG GTACTAGACT ACCTTTCATC CTACATAAAT AGACGCATAT AAGTACGCAT TTAAGCATAA ACACGCACTA
1301  TGCCGTTCTT CTCATGTATA TATATATACA GGCAACACGC AGATATAGGT GCGACGTGAA CAGTGAGCTG TATGTGCGCA GCTCGCGTTG CATTTTCGGA
1401  AGCGCTCGTT TTCGGAAACG CTTTGAAGTT CCTATTCCGA AGTTCCTATT CTCTAGAAAG TATAGGAACT TCAGAGCGCT TTTGAAAACC AAAAGCGCTC
1501  TGAAGACGCA CTTTCAAAAA ACCAAAAACG CACCGGACTG TAACGAGCTA CTAAAATATT GCGAATACCG CTTCCACAAA CATTGCTCAA AAGTATCTCT
1601  TTGCTATATA TCTCTGTGCT ATATCCCTAT ATAACCTACC CATCCACCTT TCGCTCCTTG AACTTGCATC TAAACTCGAC CTCTACAT
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Figure 36

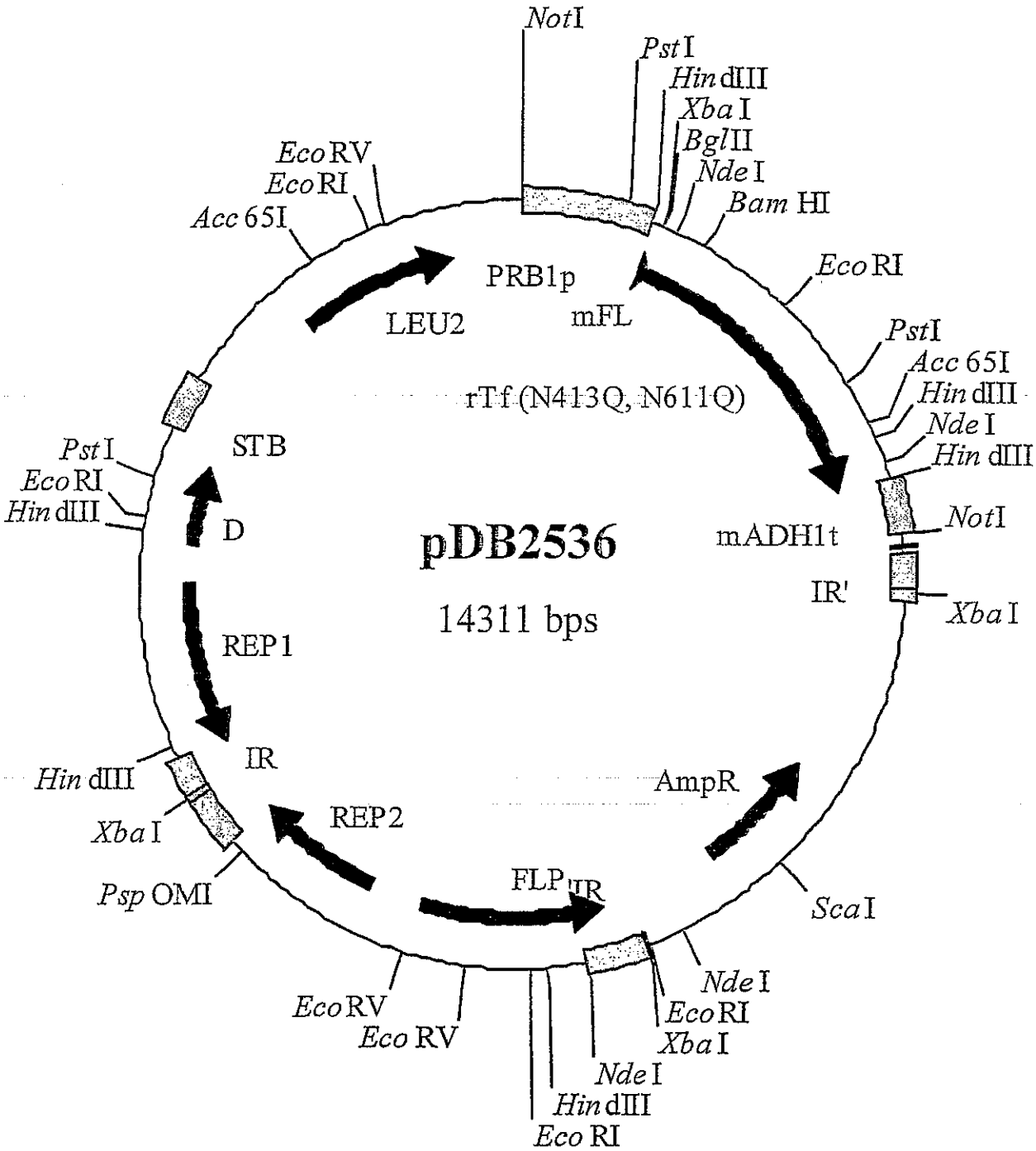

NotI
PstI
HindIII
XbaI
BglII
NdeI
BamHI
EcoRI
PstI
Acc65I
HindIII
NdeI
HindIII
NotI
XbaI
IR'
mADH1t
AmpR
ScaI
NdeI
EcoRI
XbaI
NdeI
HindIII
EcoRI
EcoRV
EcoRV
FLPIR
REP2
IR
HindIII
XbaI
PspOMI
REP1
D
STB
PstI
EcoRI
HindIII
EcoRV
EcoRI
Acc65I
LEU2
PRB1p
mFL
rTf (N413Q, N611Q)
pDB2536
14311 bps

Figure 37

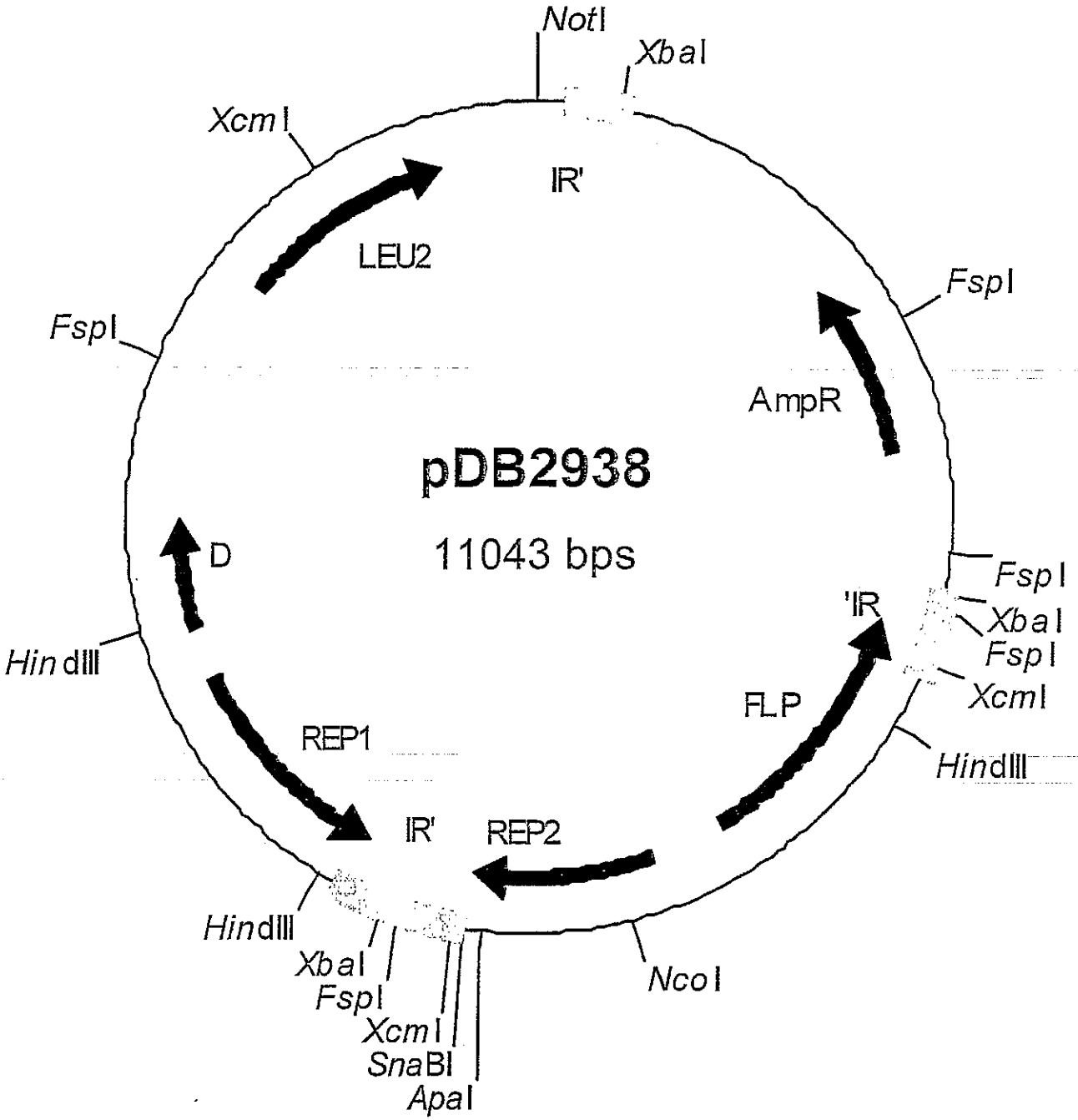

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XcmI
IR'
LEU2
FspI
FspI
AmpR
pDB2938
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D
FspI
'IR
XbaI
FspI
HindIII
XcmI
FLP
REP1
HindIII
IR'
REP2
XbaI
FspI
XcmI
SnaBI
ApaI
NcoI

Figure 38

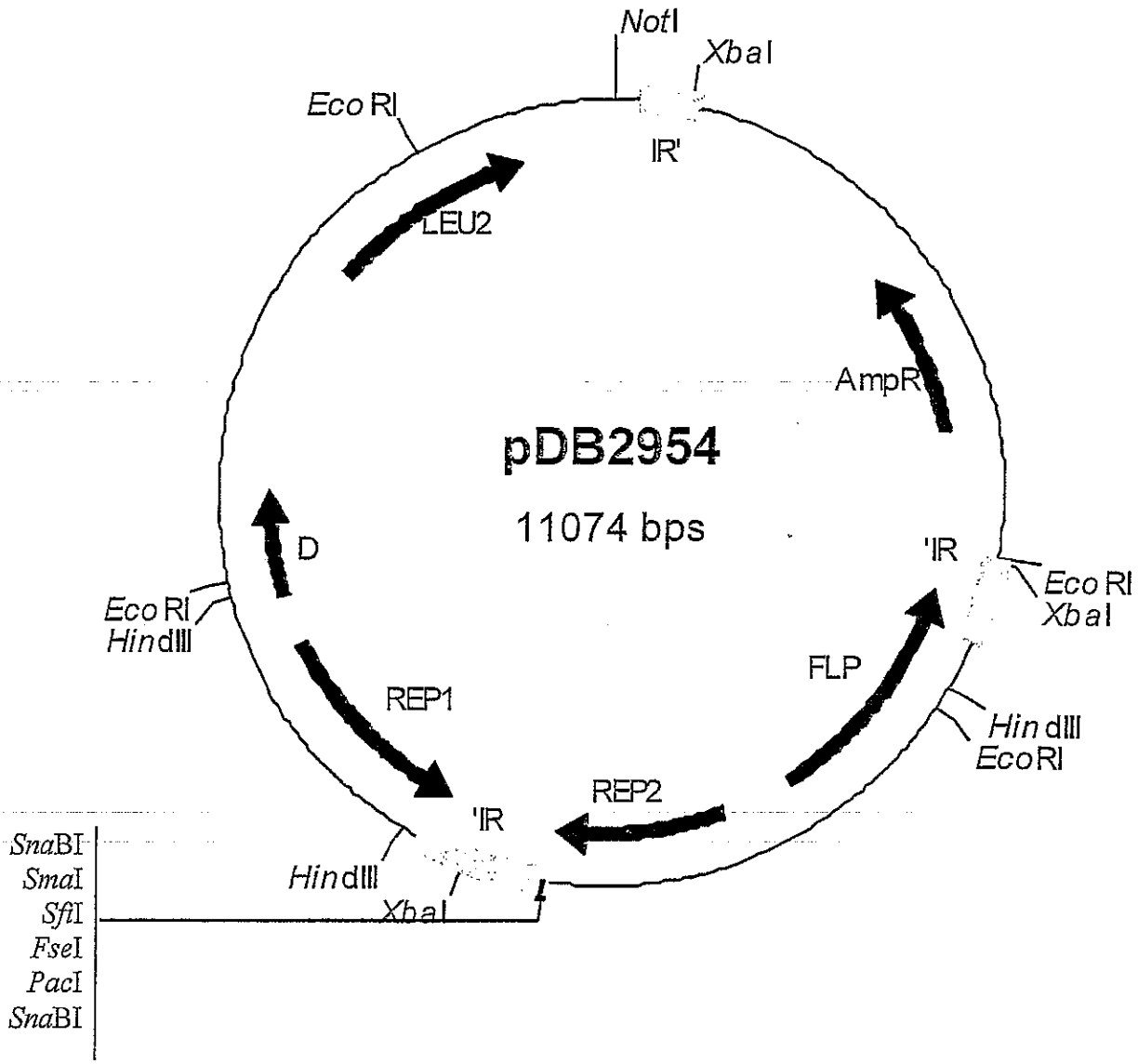

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IR'
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D
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XbaI
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FLP
REP1
HindIII
EcoRI
REP2
'IR
SnaBI
SmaI
SfiI
FseI
PacI
SnaBI
HindIII
XbaI

Figure 39

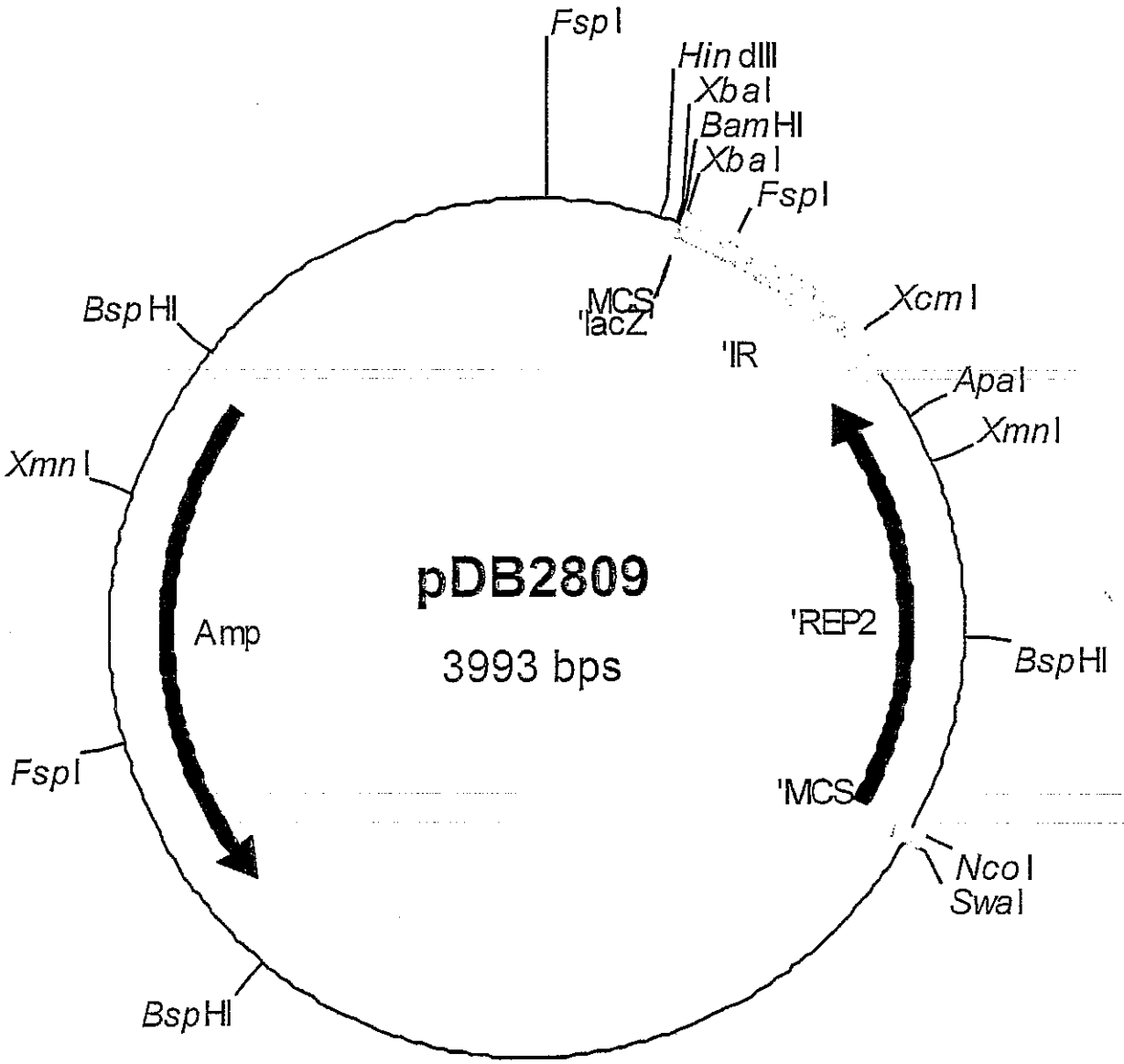

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FspI
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XcmI
ApaI
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XmnI
pDB2809
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Amp
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FspI
'MCS
NcoI
SwaI
BspHI

Figure 40

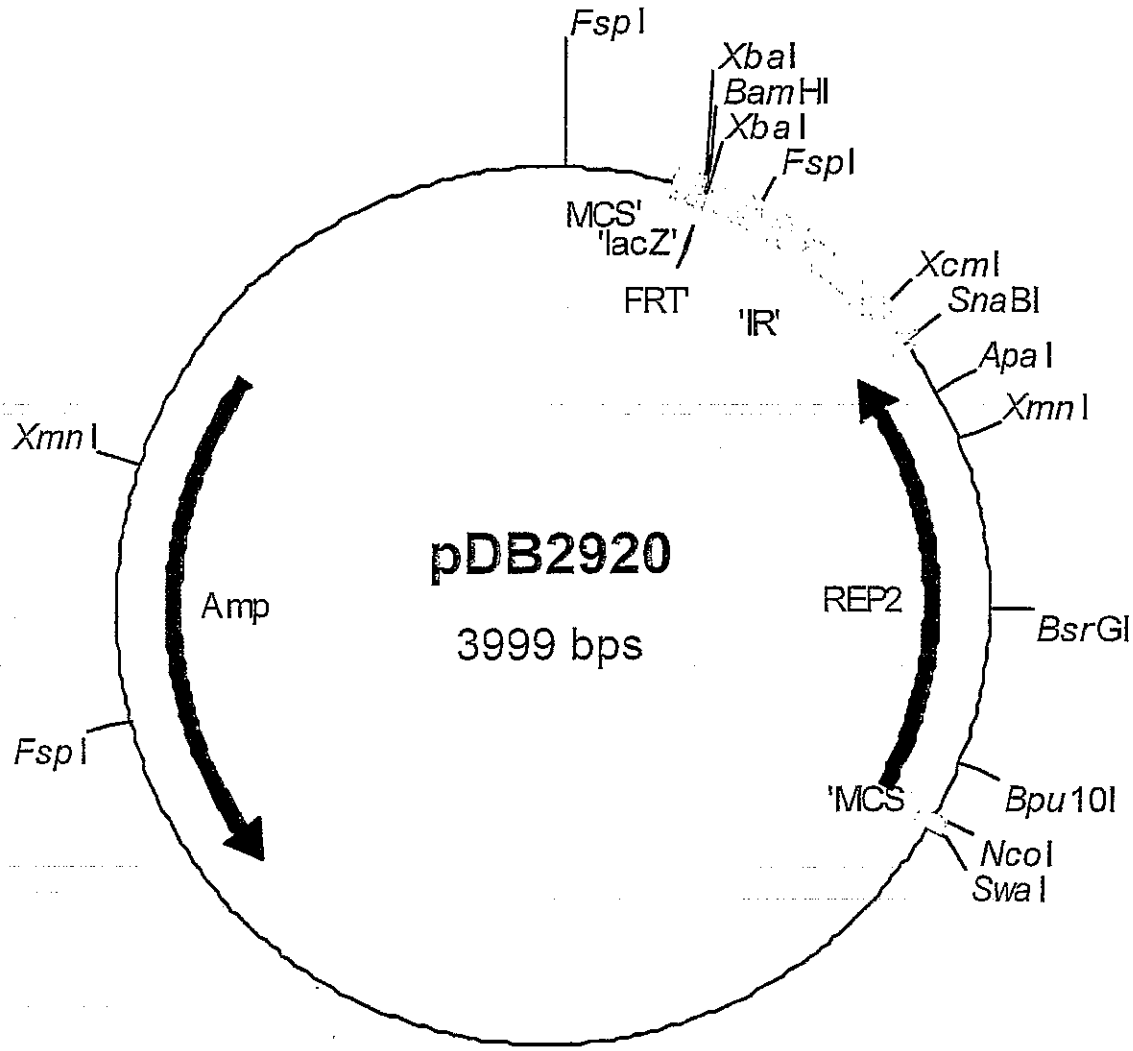

Fsp I
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BamHI
Xba I
Fsp I
MCS'
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'IR'
Xcm I
SnaBI
Apa I
Xmn I
Xmn I
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Bpu10I
Nco I
Swa I

Figure 41

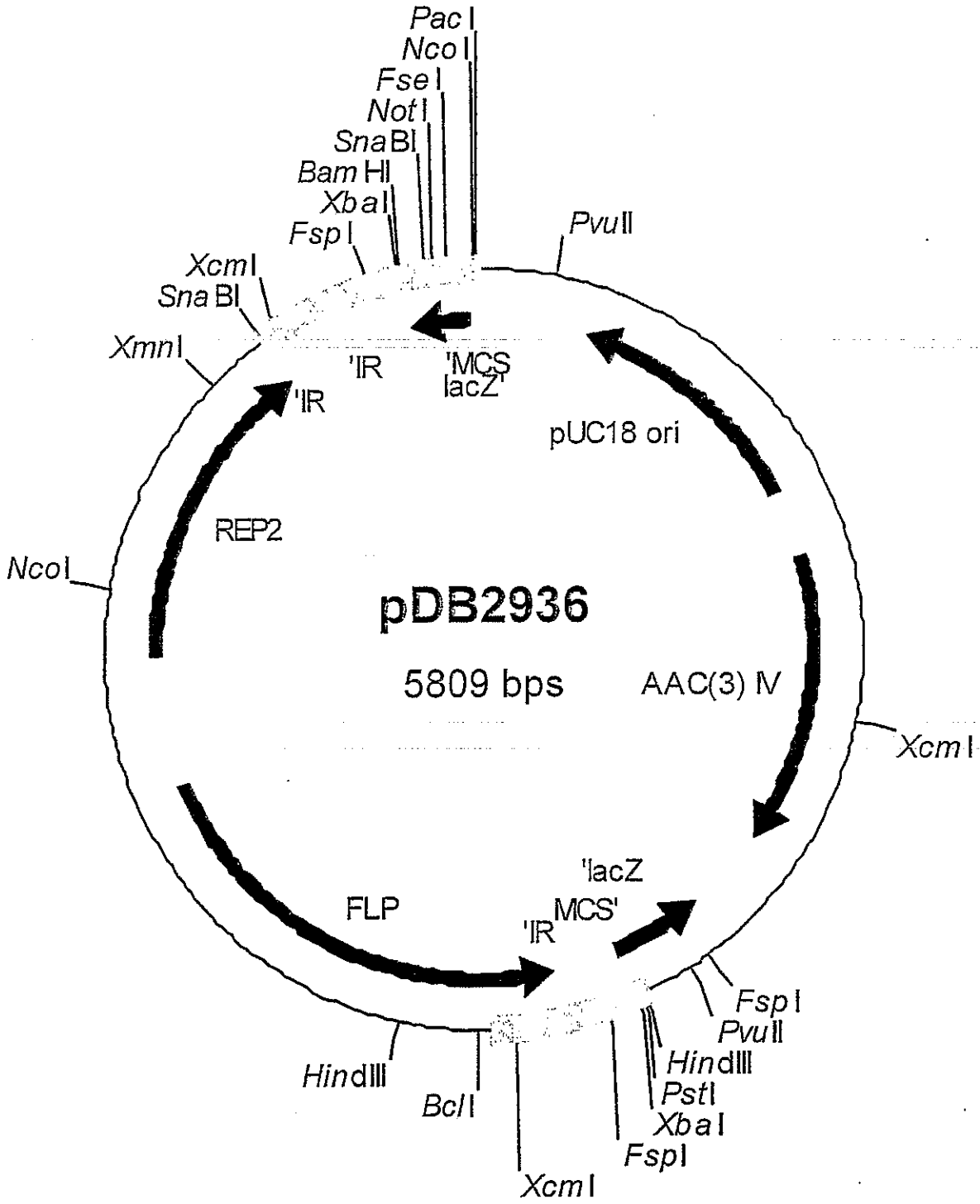

Pac I
Nco I
Fse I
Not I
Sna BI
Bam HI
Xba I
Fsp I
Pvu II
Xcm I
Sna BI
Xmn I
'IR
'MCS
lacZ'
'IR
pUC18 ori
REP2
Nco I
pDB2936
5809 bps
AAC(3) IV
Xcm I
'lacZ
FLP
'IR
MCS'
Fsp I
Pvu II
Hind III
Pst I
Xba I
Fsp I
Hind III
Bcl I
Xcm I

Figure 42

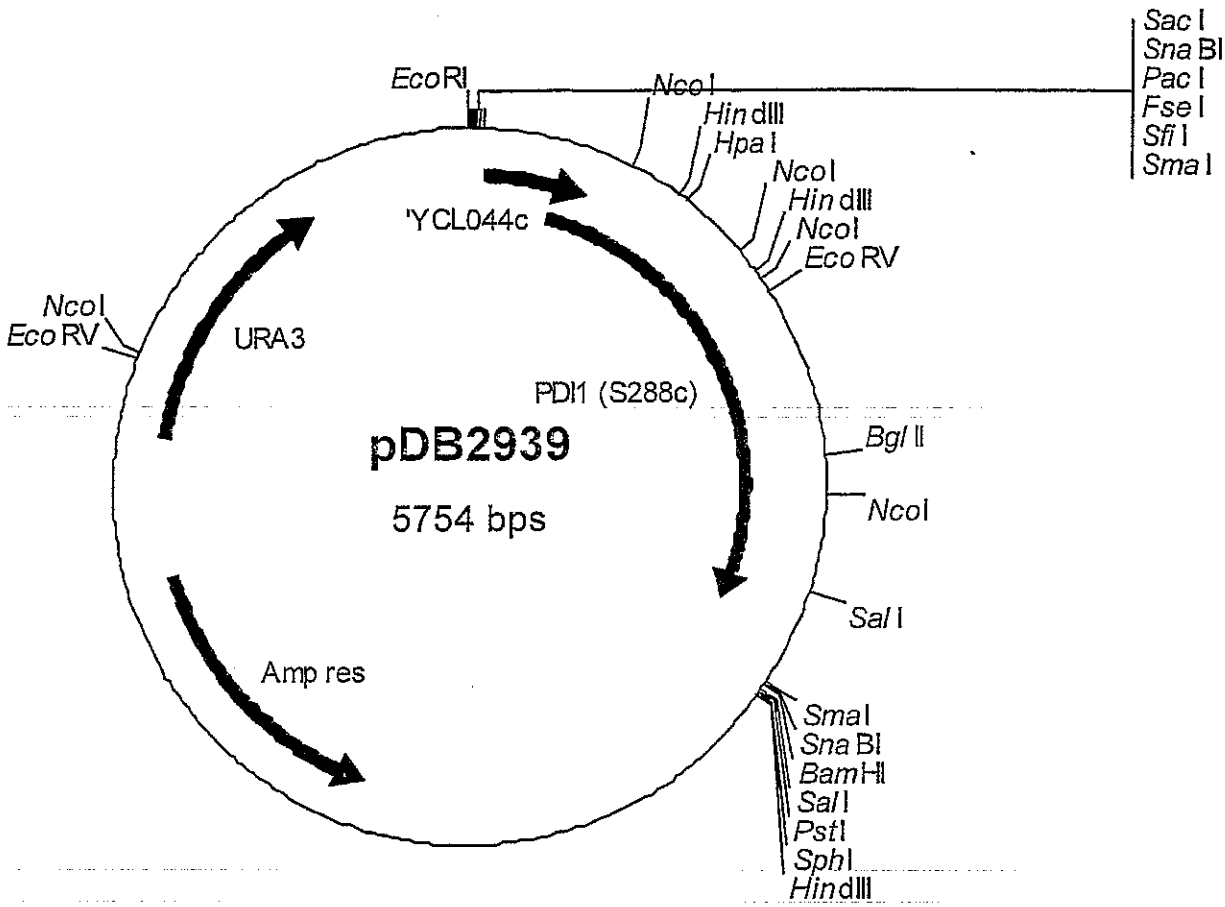

Sac I
Sna BI
Pac I
Fse I
Sfi I
Sma I
EcoRI
Nco I
Hin dIII
Hpa I
Nco I
Hin dIII
Nco I
Eco RV
'YCL044c
Nco I
Eco RV
URA3
PDI1 (S288c)
pDB2939
5754 bps
Bgl II
Nco I
Sal I
Amp res
Sma I
Sna BI
BamHI
Sal I
Pst I
Sph I
Hin dIII

Figure 43

PCR Primers DS248 and DS250 for amplification of *S. cerevisiae PDI1* genes

DS248

```
                                        SfiI
                                    --------+-----
                SacI          PacI
               -----+       ------+--
        EcoRI        SnaBI             FseI       SmaI
       -+-----       ---+---          -------+-    ---+--
5'-GTCAGAATTC GAGCTCTACG TATTAATTAA GGCCGGCCAG GCCCGGGCTA GTCTCTTTTT CCAATTTGCC ACCGTGTAGC ATTTTGTTGT-3'

                                                >>.................'YCL044c'.................>>
```

DS250

```
                SnaBI
               ---+--
        BamHI         SmaI
       -+-----       ---+---
5'-GTCAGGATCC TACGTACCCG GGTAAGGCGT TCGTGCAGTG TGACGAATAT AGCG-3'
```

Figure 44

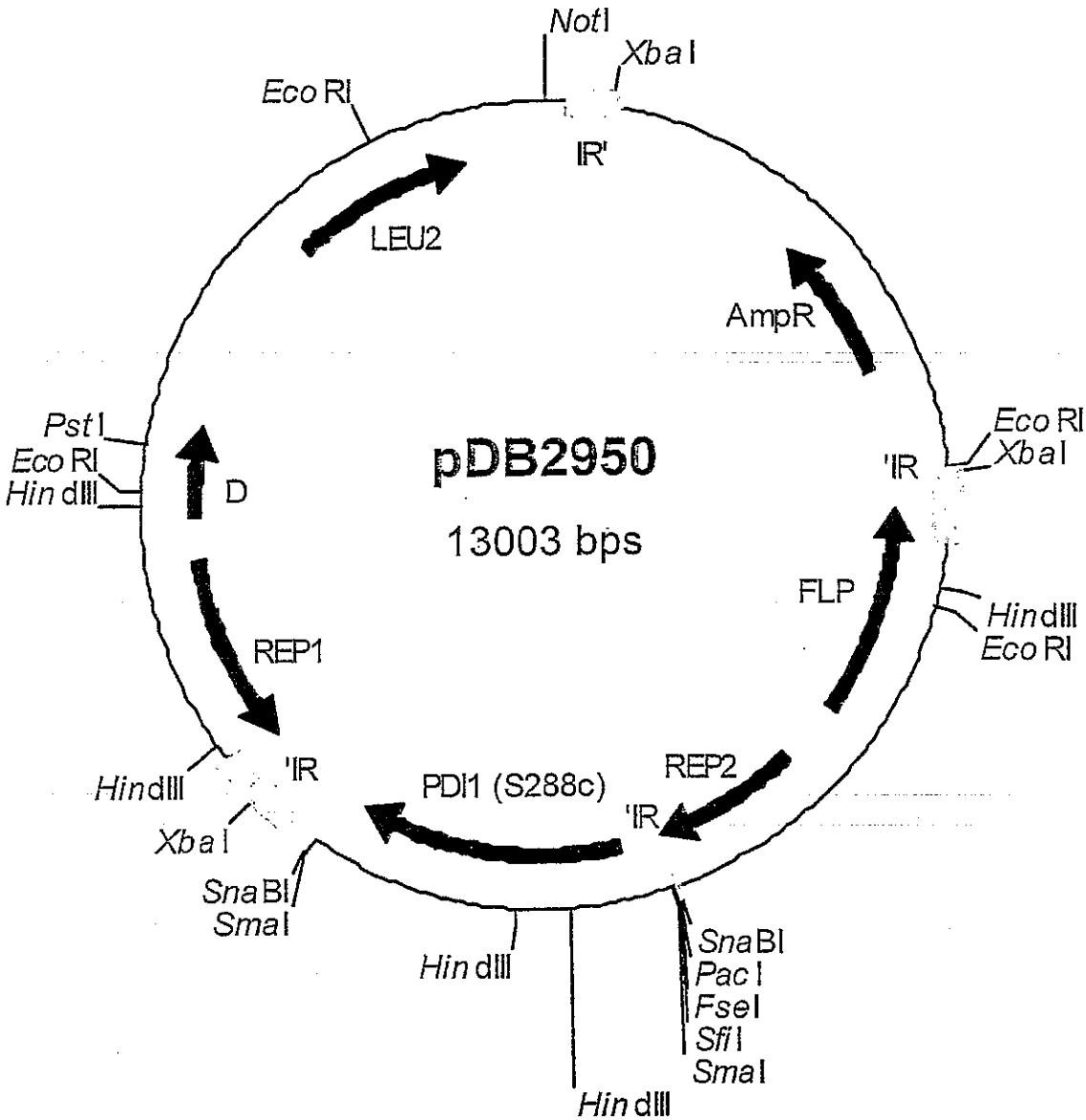

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IR'
LEU2
AmpR
EcoRI
XbaI
'IR
pDB2950
13003 bps
PstI
EcoRI
HindIII
D
FLP
HindIII
EcoRI
REP1
HindIII
'IR
PDI1 (S288c)
REP2
'IR
XbaI
SnaBI
SmaI
HindIII
SnaBI
PacI
FseI
SfiI
SmaI
HindIII

Figure 45

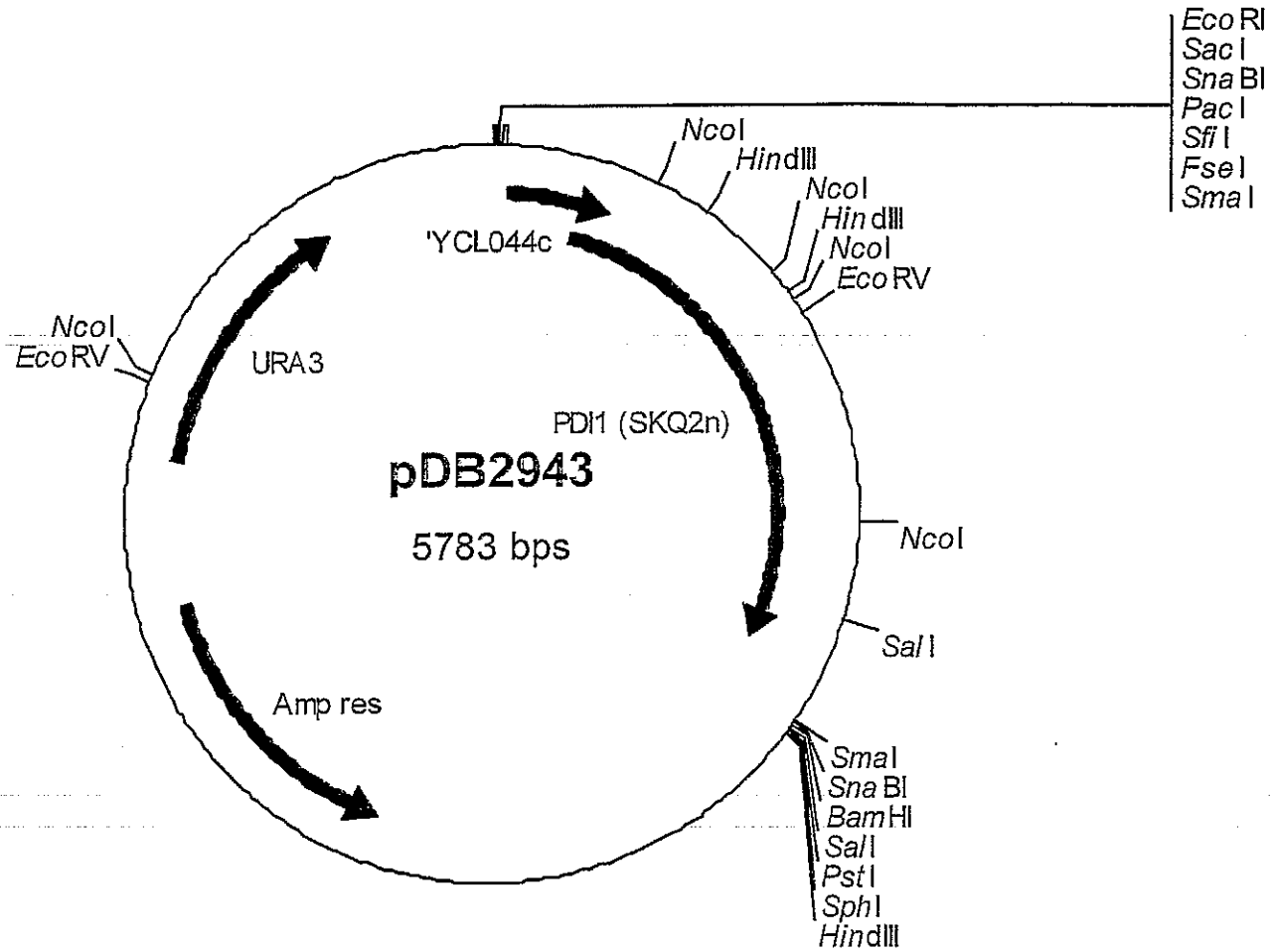

EcoRI
SacI
SnaBI
PacI
SfiI
FseI
SmaI
NcoI
HindIII
NcoI
HindIII
NcoI
EcoRV
'YCL044c
NcoI
EcoRV
URA3
PDI1 (SKQ2n)
pDB2943
5783 bps
NcoI
SalI
Amp res
SmaI
SnaBI
BamHI
SalI
PstI
SphI
HindIII

Figure 46

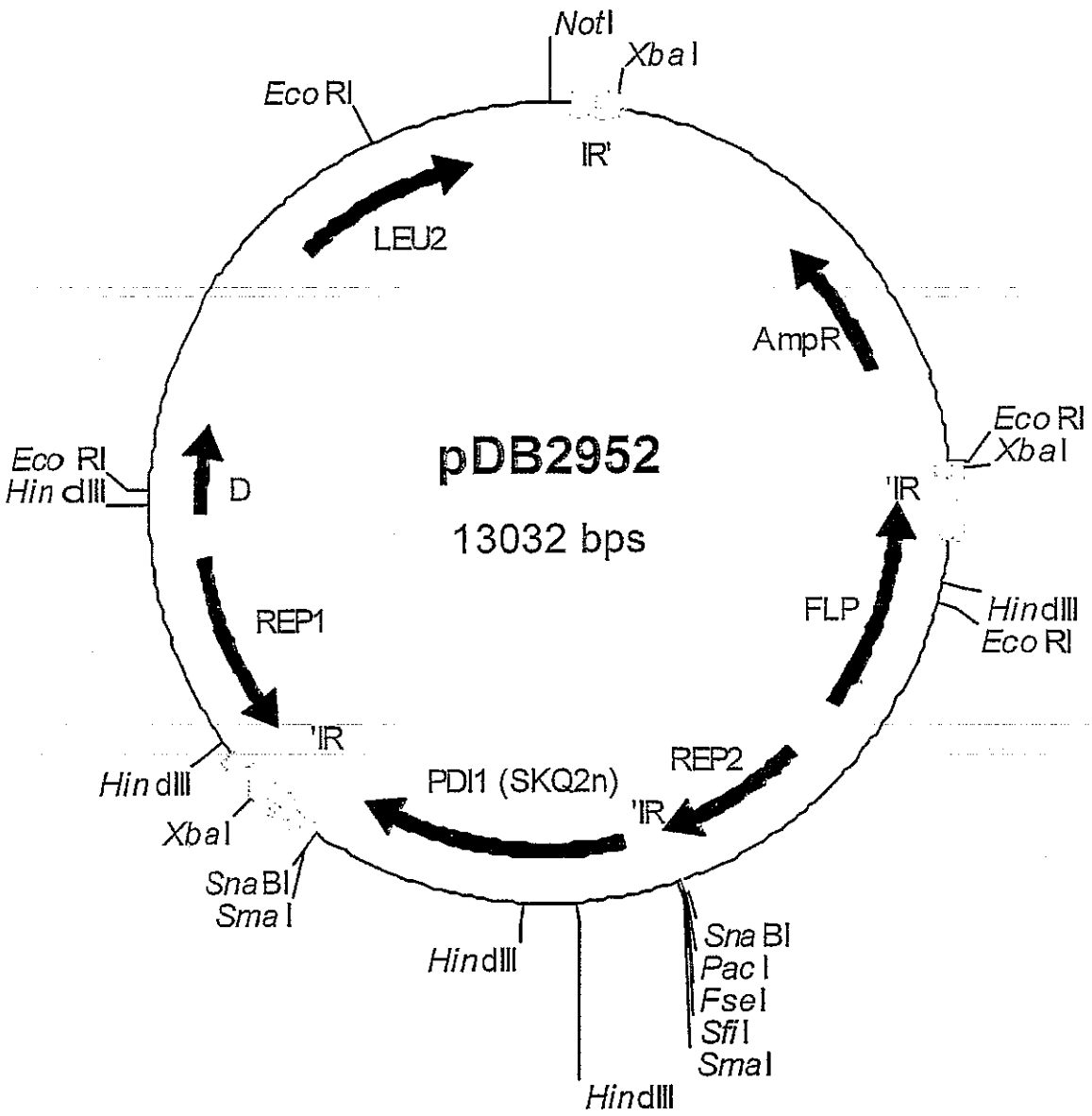
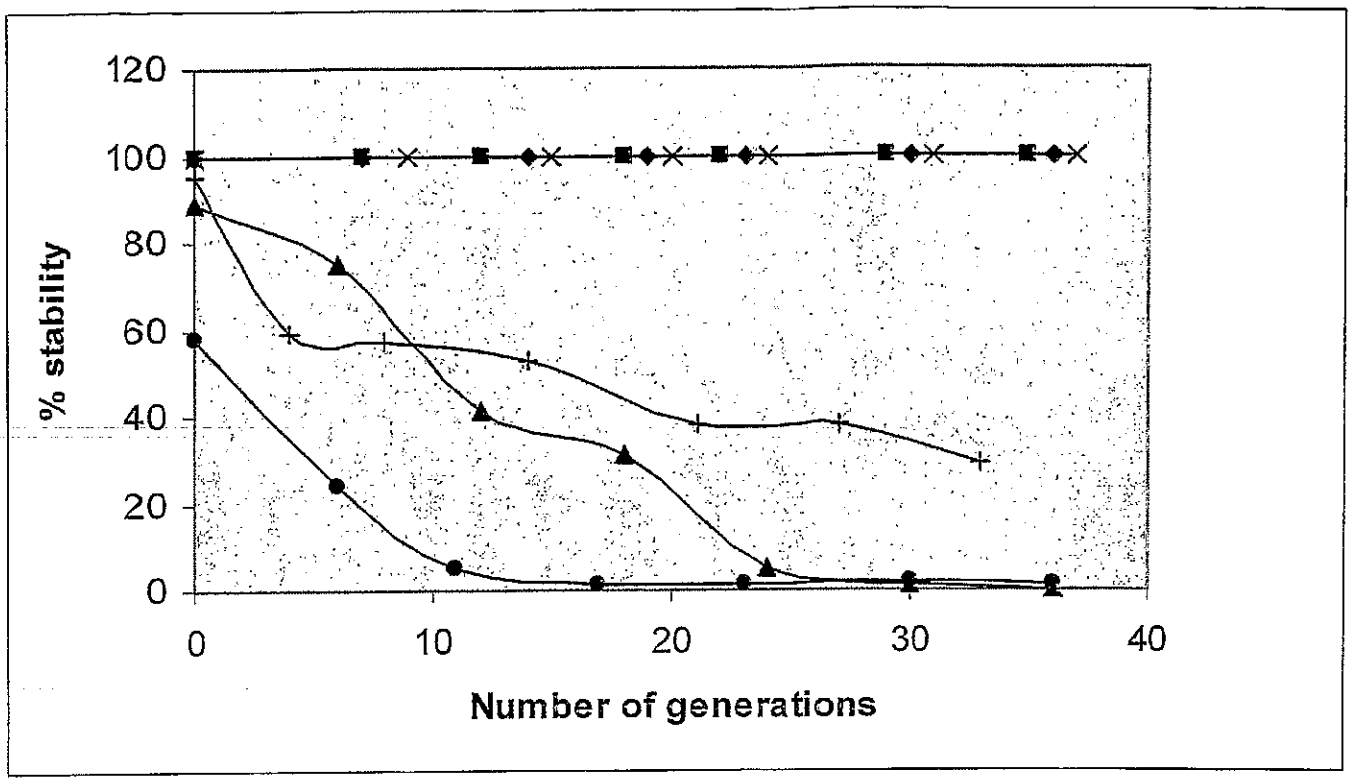

NotI
XbaI
EcoRI
IR'
LEU2
AmpR
EcoRI
XbaI
pDB2952
13032 bps
EcoRI
HindIII
D
'IR
FLP
HindIII
EcoRI
REP1
'IR
REP2
HindIII
PDI1 (SKQ2n)
'IR
XbaI
SnaBI
SmaI
HindIII
SnaBI
PacI
FseI
SfiI
SmaI
HindIII

Figure 47


120
100
80
60
40
20
0
% stability
0
10
20
30
40
Number of generations

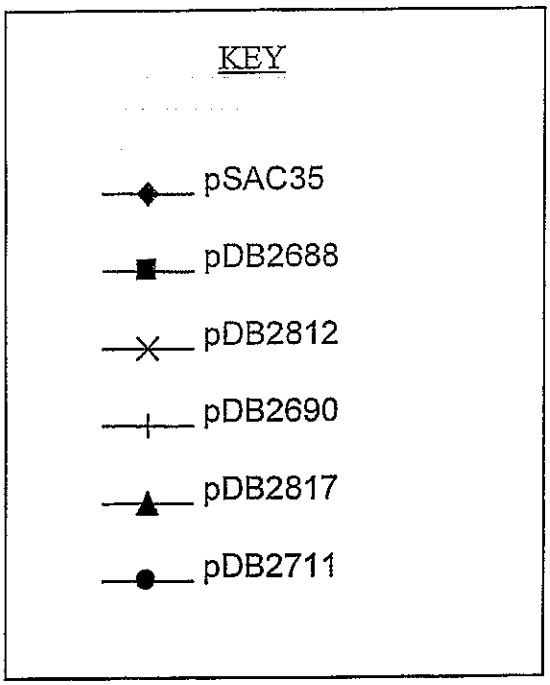

KEY
pSAC35
pDB2688
pDB2812
pDB2690
pDB2817
pDB2711

FIGURE 48

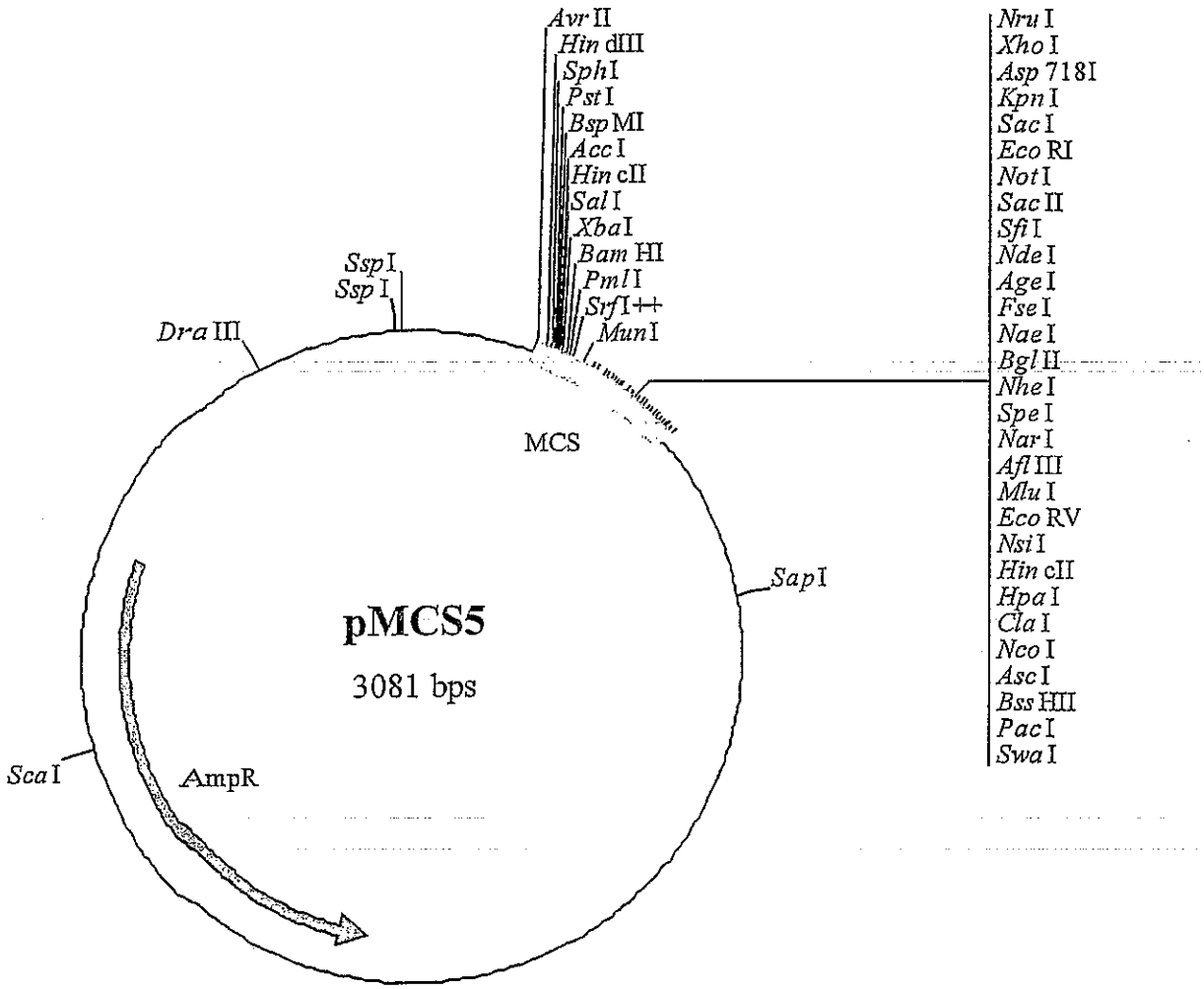

Avr II
Hin dIII
Sph I
Pst I
Bsp MI
Acc I
Hin cII
Sal I
Xba I
Bam HI
Pml I
Srf I++
Mun I
Nru I
Xho I
Asp 718I
Kpn I
Sac I
Eco RI
Not I
Sac II
Sfi I
Nde I
Age I
Fse I
Nae I
Bgl II
Nhe I
Spe I
Nar I
Afl III
Mlu I
Eco RV
Nsi I
Hin cII
Hpa I
Cla I
Nco I
Asc I
Bss HII
Pac I
Swa I
Ssp I
Ssp I
Dra III
MCS
Sap I
pMCS5
3081 bps
Sca I
AmpR

FIGURE 49

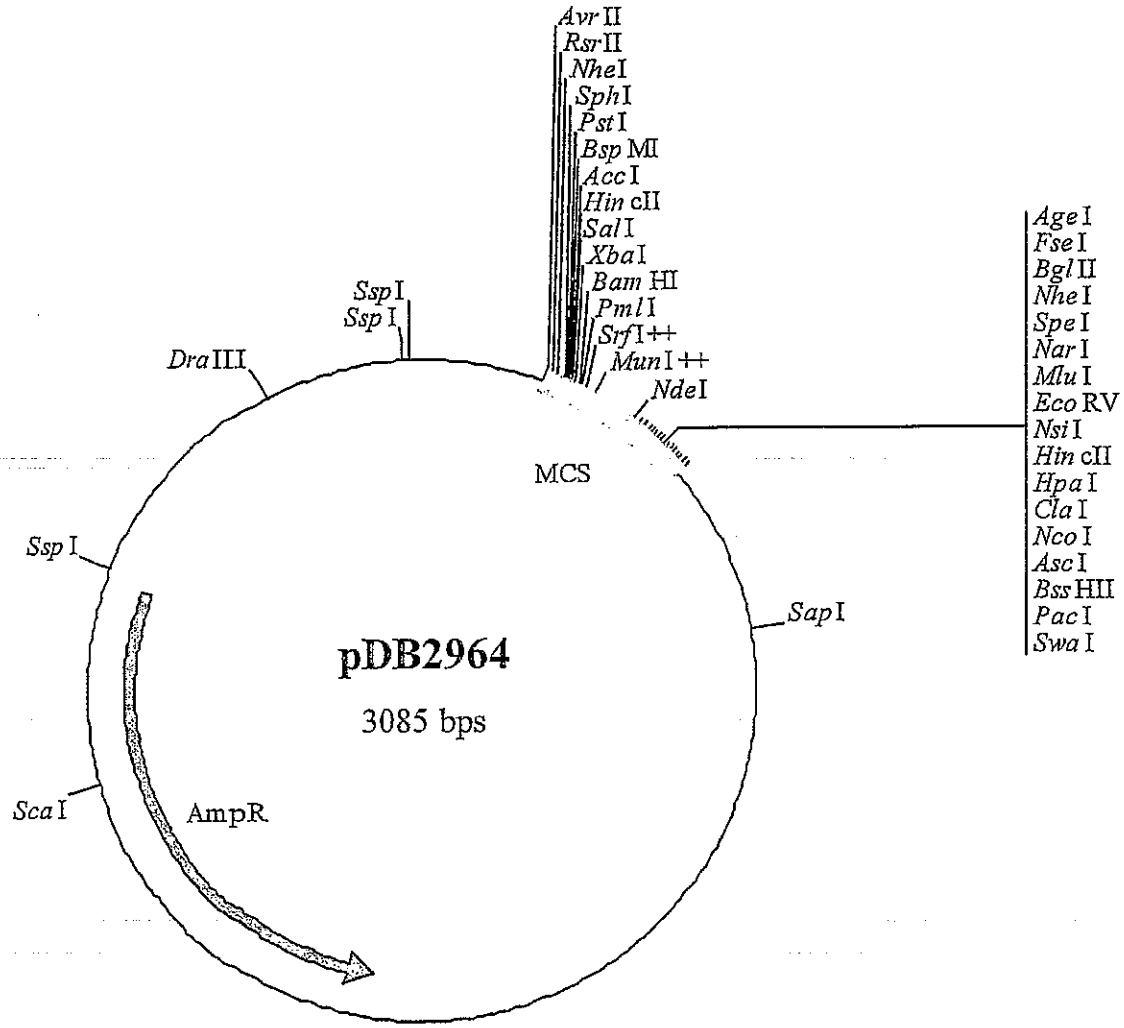

AvrII
RsrII
NheI
SphI
PstI
BspMI
AccI
HincII
SalI
XbaI
BamHI
PmlI
SrfI++
MunI++
NdeI
AgeI
FseI
BglII
NheI
SpeI
NarI
MluI
EcoRV
NsiI
HincII
HpaI
ClaI
NcoI
AscI
BssHII
PacI
SwaI
SspI
SspI
DraIII
SspI
SapI
MCS
pDB2964
3085 bps
ScaI
AmpR

FIGURE 50

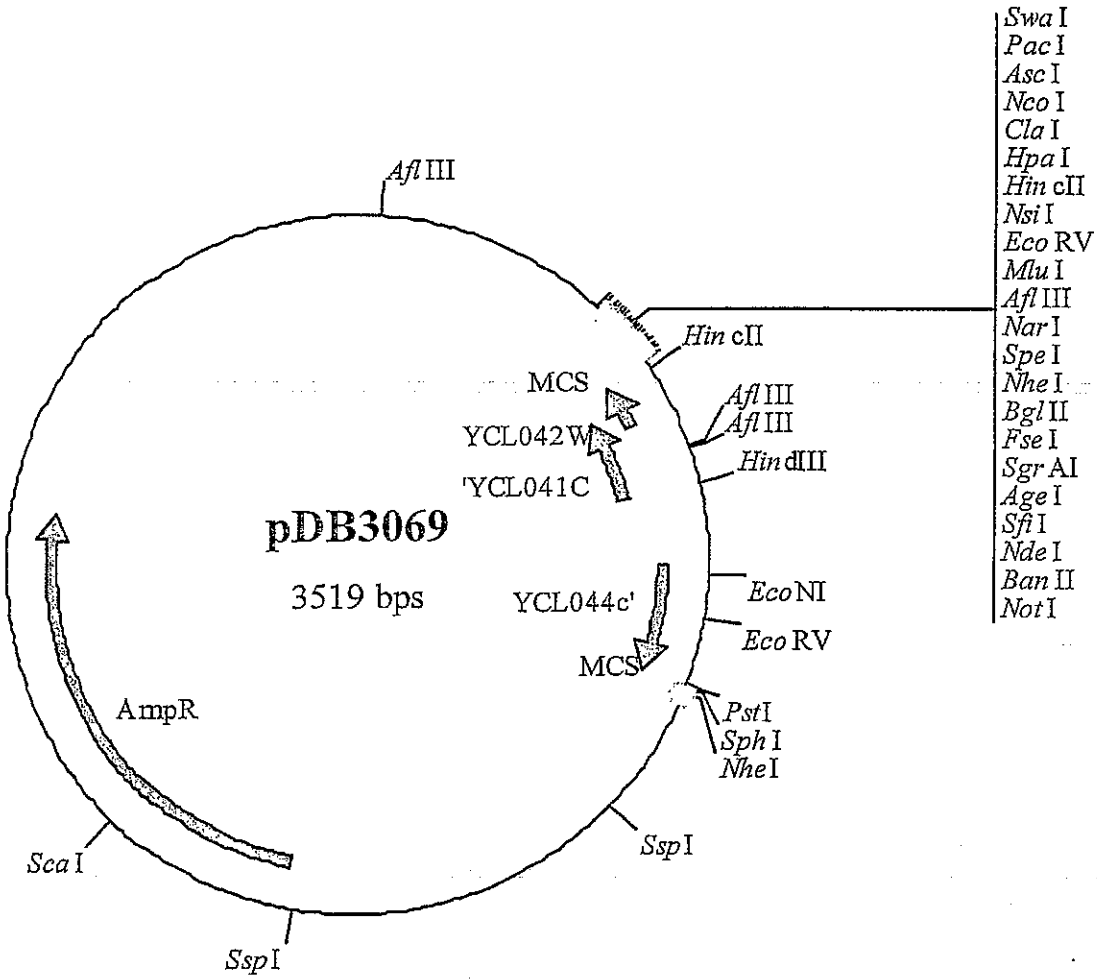

pDB3069
3519 bps
AfI III
Hin cII
MCS
YCL042W
'YCL041C
AfI III
AfI III
Hin dIII
YCL044c'
Eco NI
Eco RV
MCS
Pst I
Sph I
Nhe I
Ssp I
Sca I
Ssp I
AmpR
Swa I
Pac I
Asc I
Nco I
Cla I
Hpa I
Hin cII
Nsi I
Eco RV
Mlu I
AfI III
Nar I
Spe I
Nhe I
Bgl II
Fse I
Sgr AI
Age I
Sfi I
Nde I
Ban II
Not I

FIGURE 51

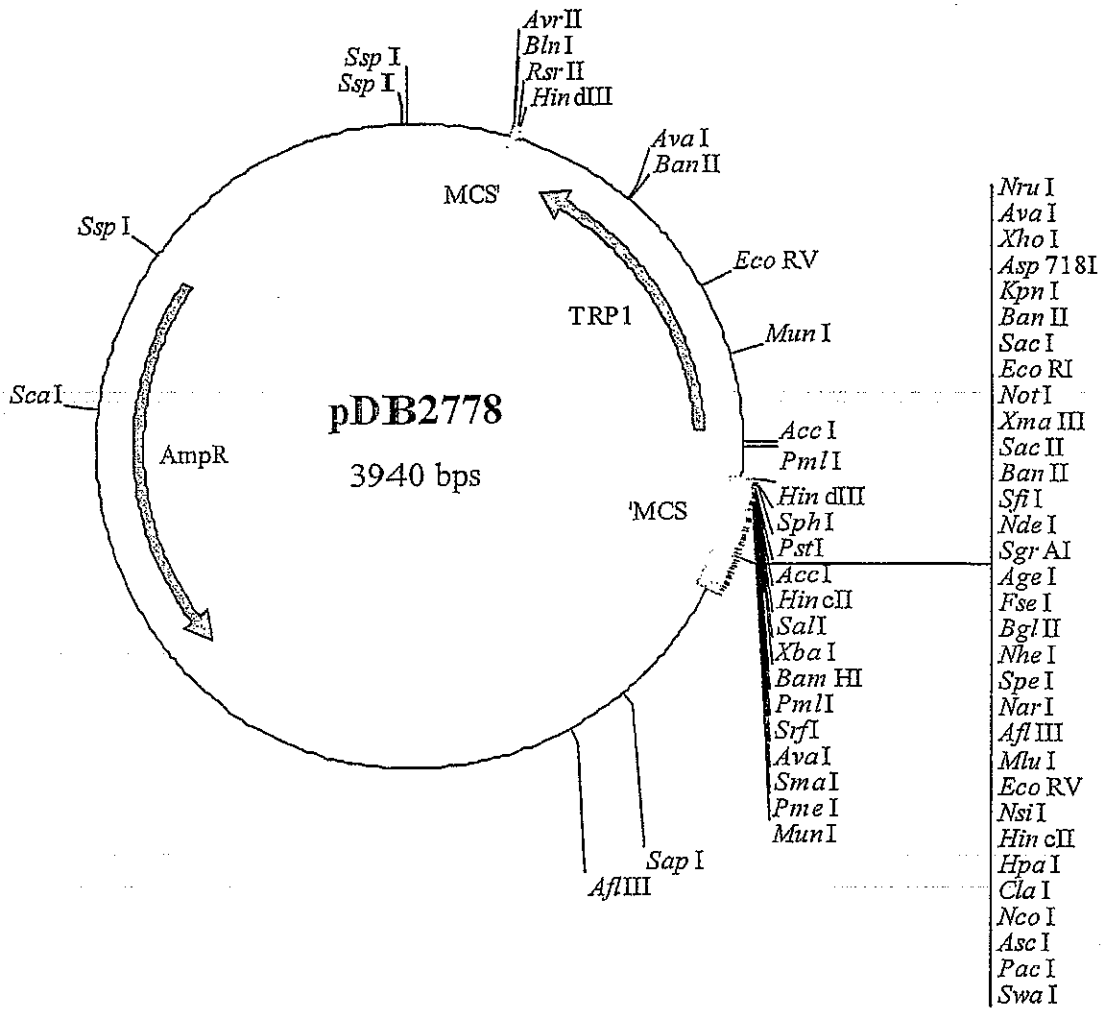

pDB2778
3940 bps
AmpR
TRP1
MCS'
'MCS
Ssp I
Ssp I
Avr II
Bln I
Rsr II
Hin dIII
Ava I
Ban II
Eco RV
Mun I
Sca I
Ssp I
Acc I
Pml I
Hin dIII
Sph I
Pst I
Acc I
Hin cII
Sal I
Xba I
Bam HI
Pml I
Sfr I
Ava I
Sma I
Pme I
Mun I
Sap I
Afl III
Nru I
Ava I
Xho I
Asp 718I
Kpn I
Ban II
Sac I
Eco RI
Not I
Xma III
Sac II
Ban II
Sfi I
Nde I
Sgr AI
Age I
Fse I
Bgl II
Nhe I
Spe I
Nar I
Afl III
Mlu I
Eco RV
Nsi I
Hin cII
Hpa I
Cla I
Nco I
Asc I
Pac I
Swa I

FIGURE 52

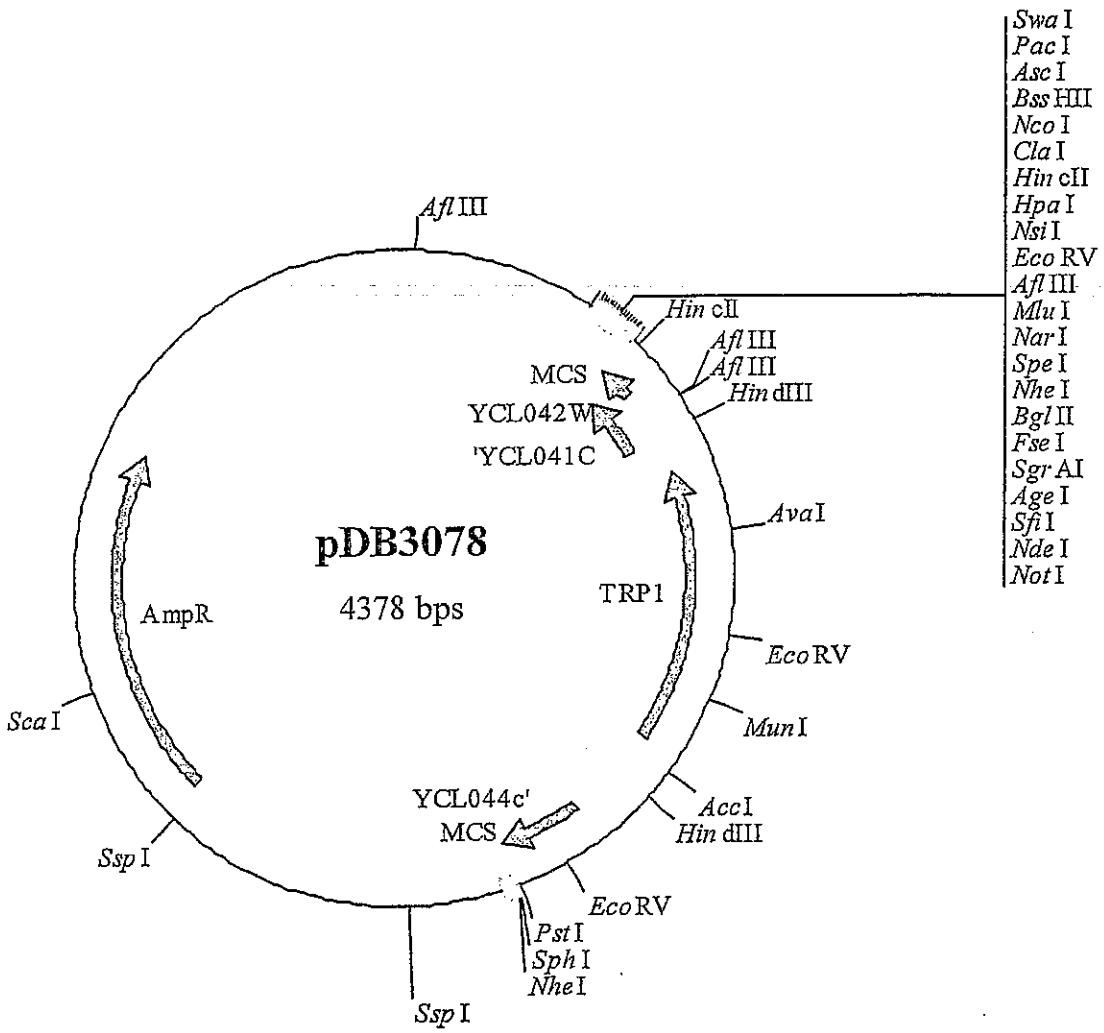

pDB3078
4378 bps
AmpR
TRP1
MCS
YCL042W
'YCL041C
YCL044c'
MCS
AflIII
HincII
AflIII
AflIII
HindIII
AvaI
EcoRV
MunI
AccI
HindIII
EcoRV
PstI
SphI
NheI
SspI
ScaI
SspI
SwaI
PacI
AscI
BssHII
NcoI
ClaI
HincII
HpaI
NsiI
EcoRV
AflIII
MluI
NarI
SpeI
NheI
BglII
FseI
SgrAI
AgeI
SfiI
NdeI
NotI

FIGURE 53

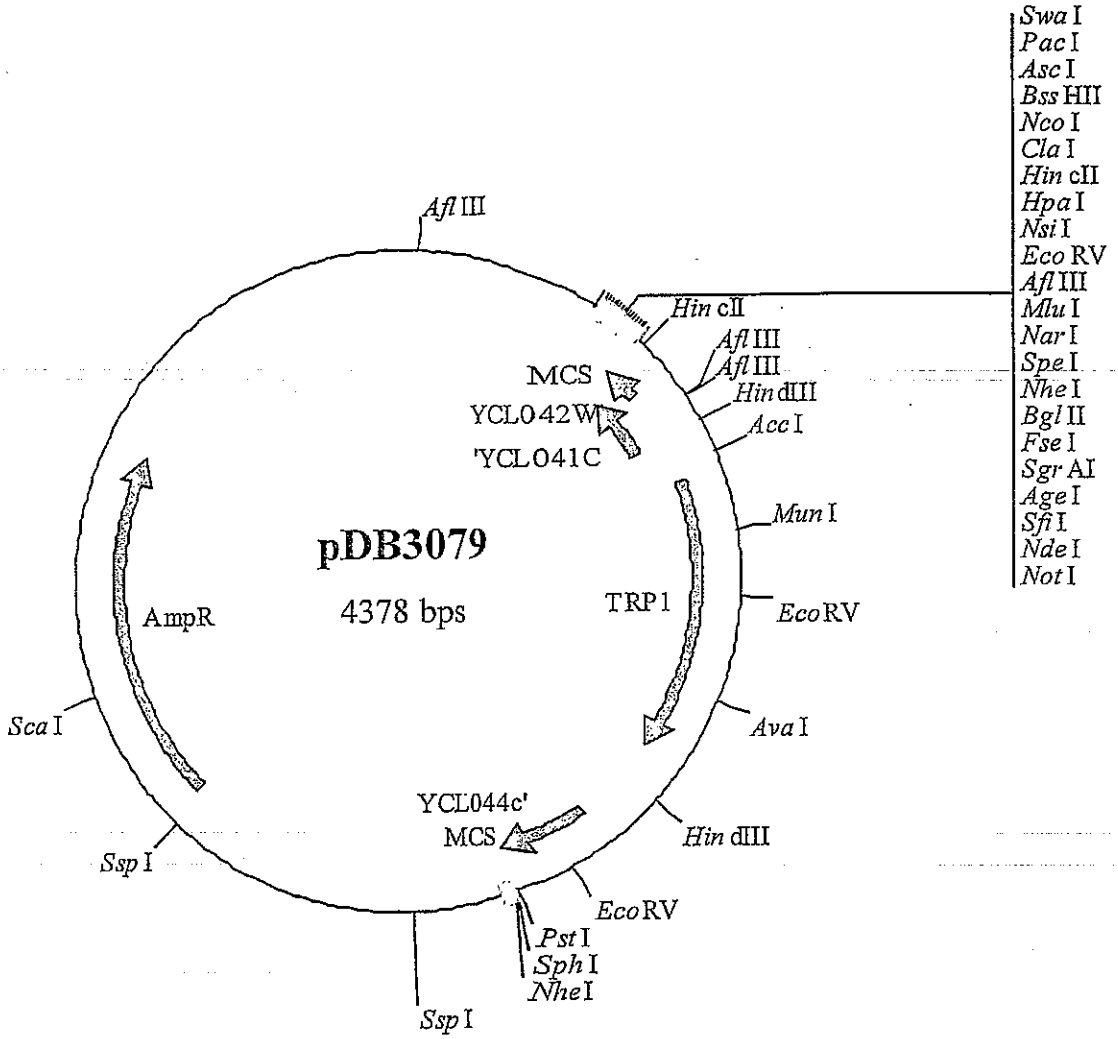

Swa I
Pac I
Asc I
Bss HII
Nco I
Cla I
Hin cII
Hpa I
Nsi I
Eco RV
Afl III
Mlu I
Nar I
Spe I
Nhe I
Bgl II
Fse I
Sgr AI
Age I
Sfi I
Nde I
Not I
Afl III
Hin cII
Afl III
Afl III
Hin dIII
Acc I
MCS
YCL042W
'YCL041C
Mun I
pDB3079
4378 bps
AmpR
TRP1
EcoRV
Sca I
Ava I
YCL044c'
MCS
Hin dIII
Ssp I
EcoRV
Pst I
Sph I
Nhe I
Ssp I

FIGURE 54

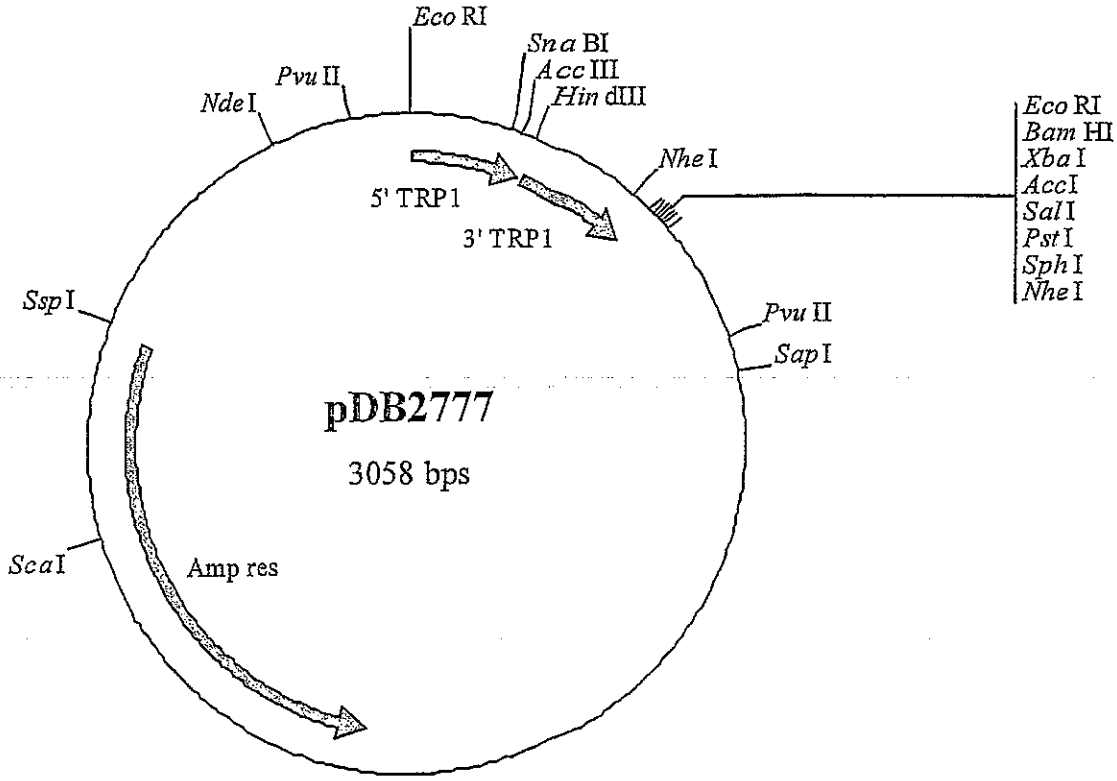

Eco RI
Sna BI
Acc III
Hin dIII
Pvu II
Nde I
Nhe I
5' TRP1
3' TRP1
Eco RI
Bam HI
Xba I
Acc I
Sal I
Pst I
Sph I
Nhe I
Ssp I
Pvu II
Sap I
pDB2777
3058 bps
Sca I
Amp res

FIGURE 55

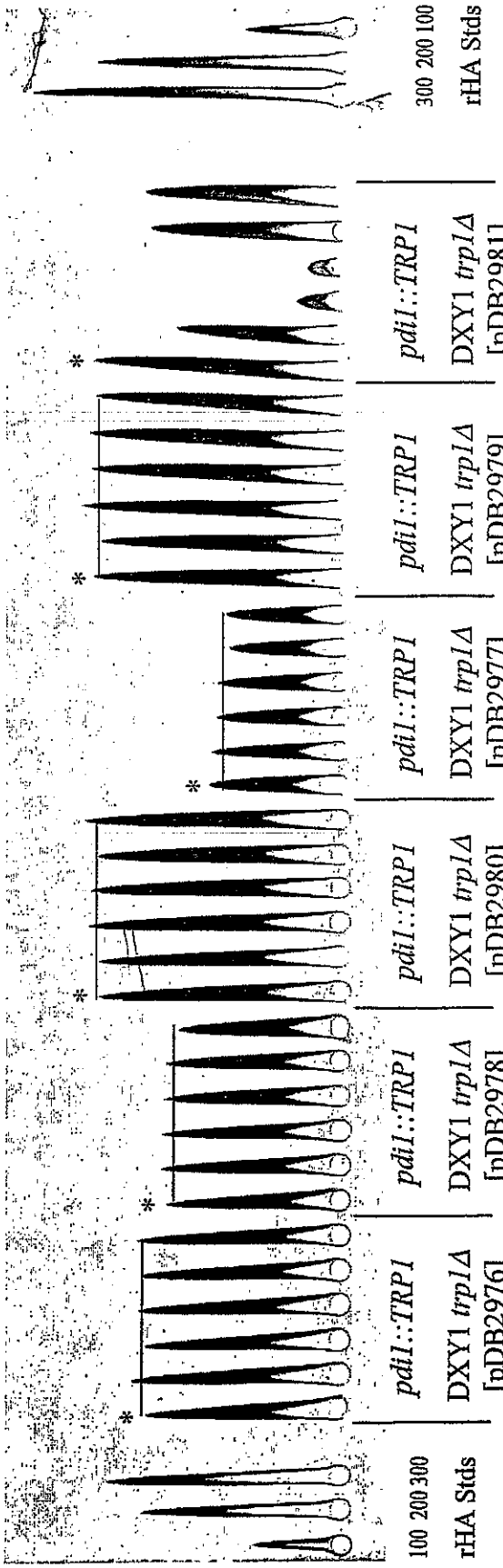

100 200 300
rHA Stds
pdi1::TRP1
DXY1 trp1Δ
[pDB2976]
pdi1::TRP1
DXY1 trp1Δ
[pDB2978]
pdi1::TRP1
DXY1 trp1Δ
[pDB2980]
pdi1::TRP1
DXY1 trp1Δ
[pDB2977]
pdi1::TRP1
DXY1 trp1Δ
[pDB2979]
pdi1::TRP1
DXY1 trp1Δ
[pDB2981]
300 200 100
rHA Stds

FIGURE 56

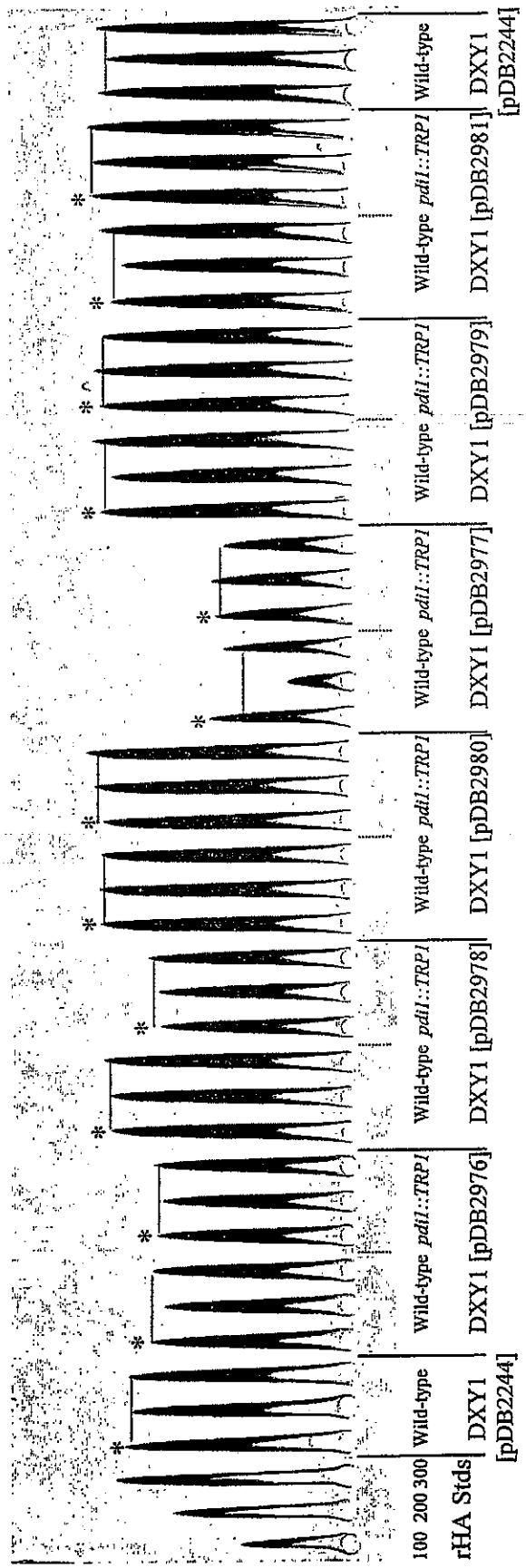

rHA Stds
100 200 300
DXY1 [pDB2244]
Wild-type
DXY1 [pDB2976]
Wild-type pdi1::TRP1
DXY1 [pDB2978]
Wild-type pdi1::TRP1
DXY1 [pDB2980]
Wild-type pdi1::TRP1
DXY1 [pDB2977]
Wild-type pdi1::TRP1
DXY1 [pDB2979]
Wild-type pdi1::TRP1
DXY1 [pDB2981]
Wild-type
DXY1 [pDB2244]

Figure 57

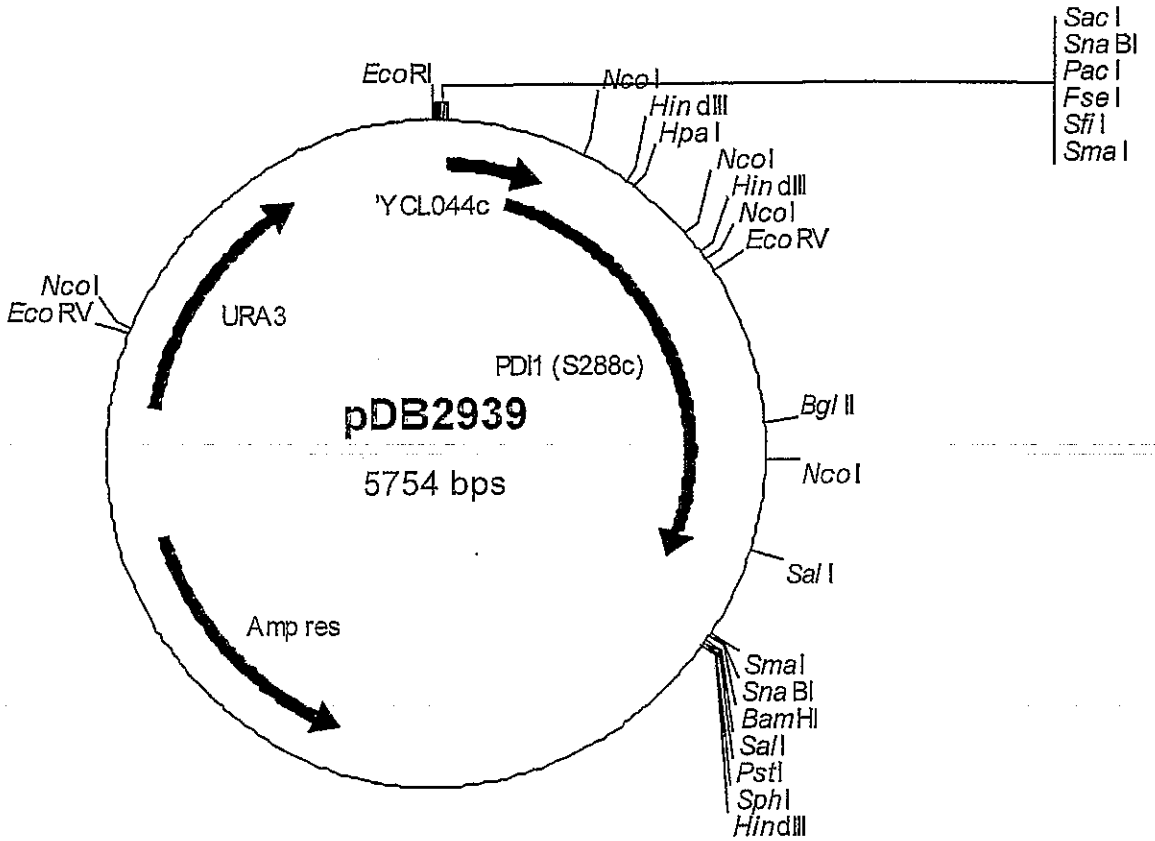

Sac I
Sna BI
Pac I
Fse I
Sfi I
Sma I
EcoRI
Nco I
Hin dIII
Hpa I
Nco I
Hin dIII
Nco I
Eco RV
'YCL044c
Nco I
Eco RV
URA3
PDI1 (S288c)
pDB2939
5754 bps
Bgl II
Nco I
Sal I
Amp res
Sma I
Sna BI
BamHI
Sal I
Pst I
Sph I
Hind III

Figure 58

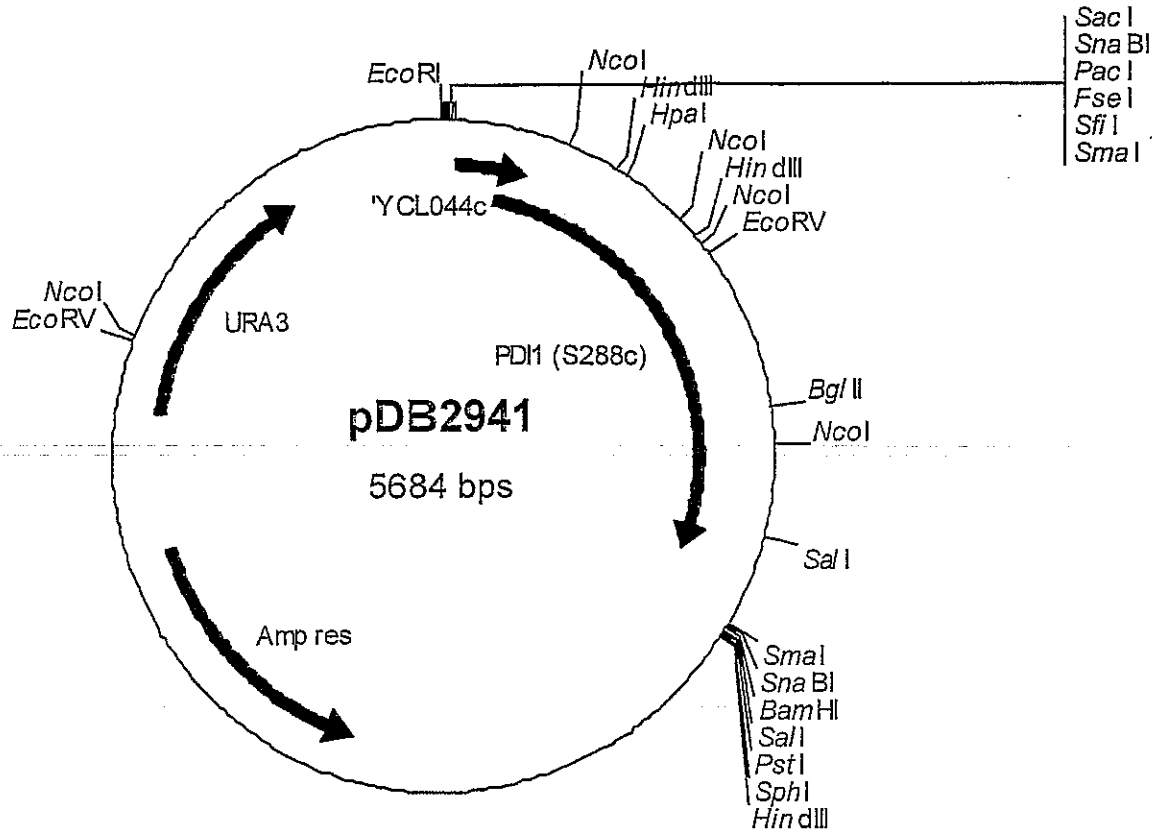

Sac I
Sna BI
Pac I
Fse I
Sfi I
Sma I
EcoRI
NcoI
HindIII
HpaI
NcoI
HindIII
NcoI
EcoRV
'YCL044c
NcoI
EcoRV
URA3
PDI1 (S288c)
pDB2941
5684 bps
BglII
NcoI
SalI
Amp res
SmaI
SnaBI
BamHI
SalI
PstI
SphI
HindIII

Figure 59

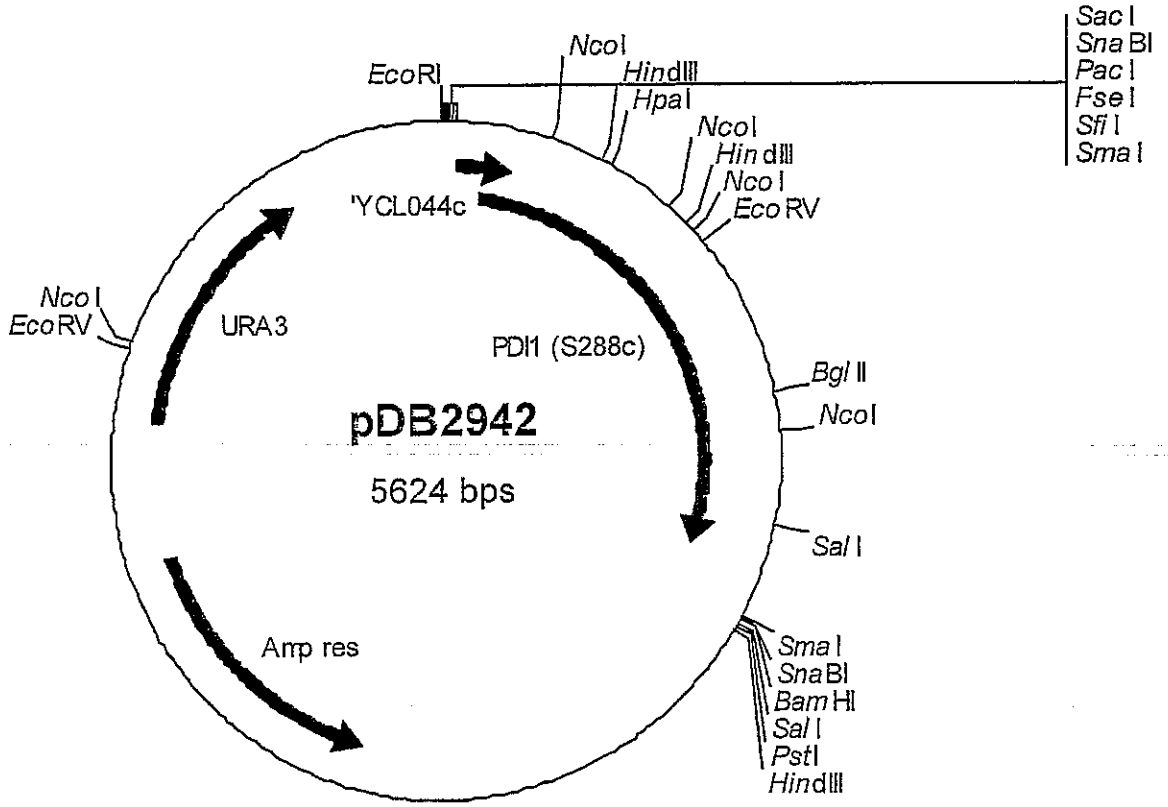

Sac I
Sna BI
Pac I
Fse I
Sfi I
Sma I
Nco I
EcoRI
Hind III
Hpa I
Nco I
Hin d III
Nco I
Eco RV
'YCL044c
Nco I
Eco RV
URA3
PDI1 (S288c)
pDB2942
5624 bps
Bgl II
Nco I
Sal I
Amp res
Sma I
Sna BI
Bam HI
Sal I
Pst I
Hind III

Figure 60

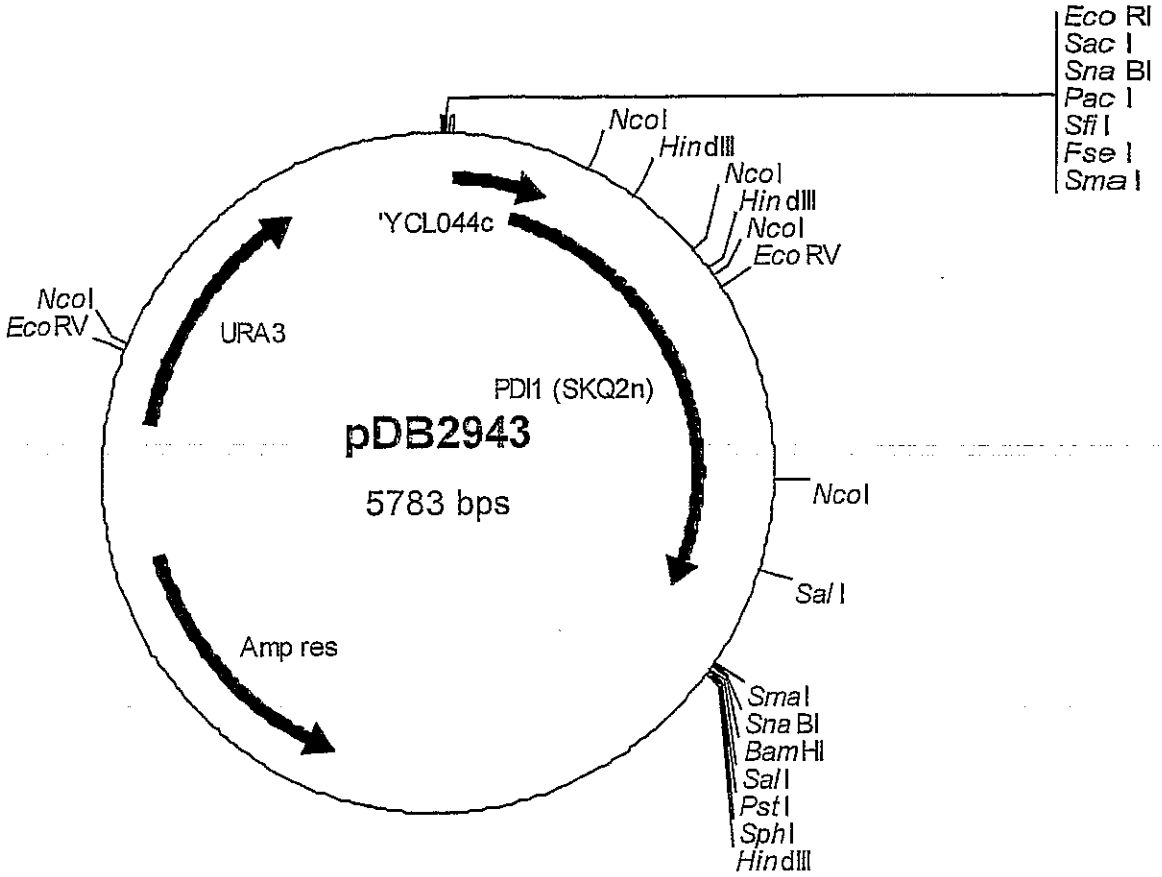

Eco RI
Sac I
Sna BI
Pac I
Sfi I
Fse I
Sma I
NcoI
HindIII
NcoI
HindIII
NcoI
EcoRV
'YCL044c
NcoI
EcoRV
URA3
PDI1 (SKQ2n)
pDB2943
5783 bps
NcoI
SalI
Amp res
SmaI
SnaBI
BamHI
SalI
PstI
SphI
HindIII

Figure 61

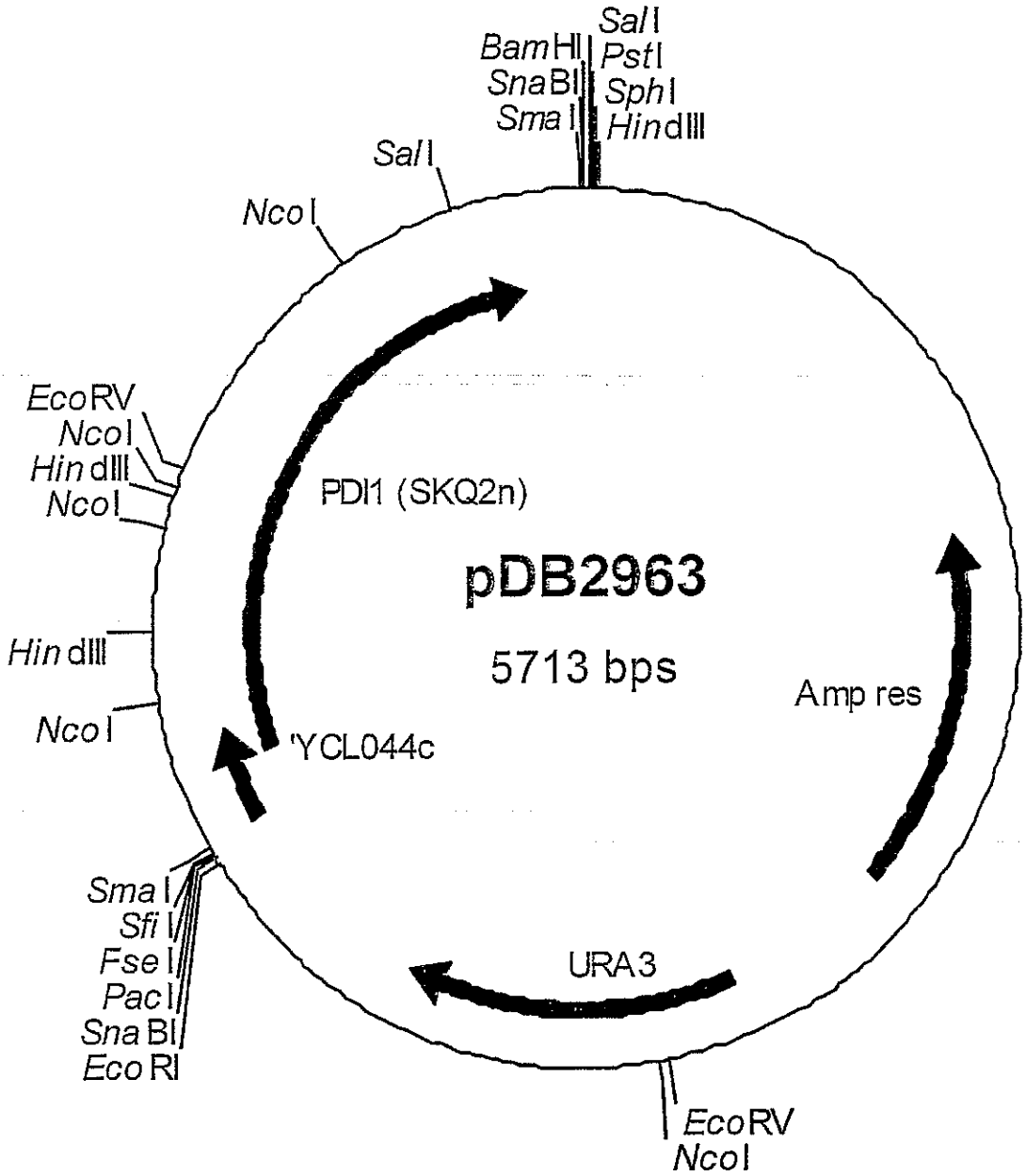

Sal I
Bam HI
Pst I
Sna BI
Sph I
Sma I
Hin dIII
Sal I
Nco I
EcoRV
Nco I
Hin dIII
Nco I
PDI1 (SKQ2n)
pDB2963
5713 bps
Amp res
Hin dIII
Nco I
'YCL044c
Sma I
Sfi I
Fse I
Pac I
Sna BI
Eco RI
URA3
EcoRV
Nco I

Figure 62

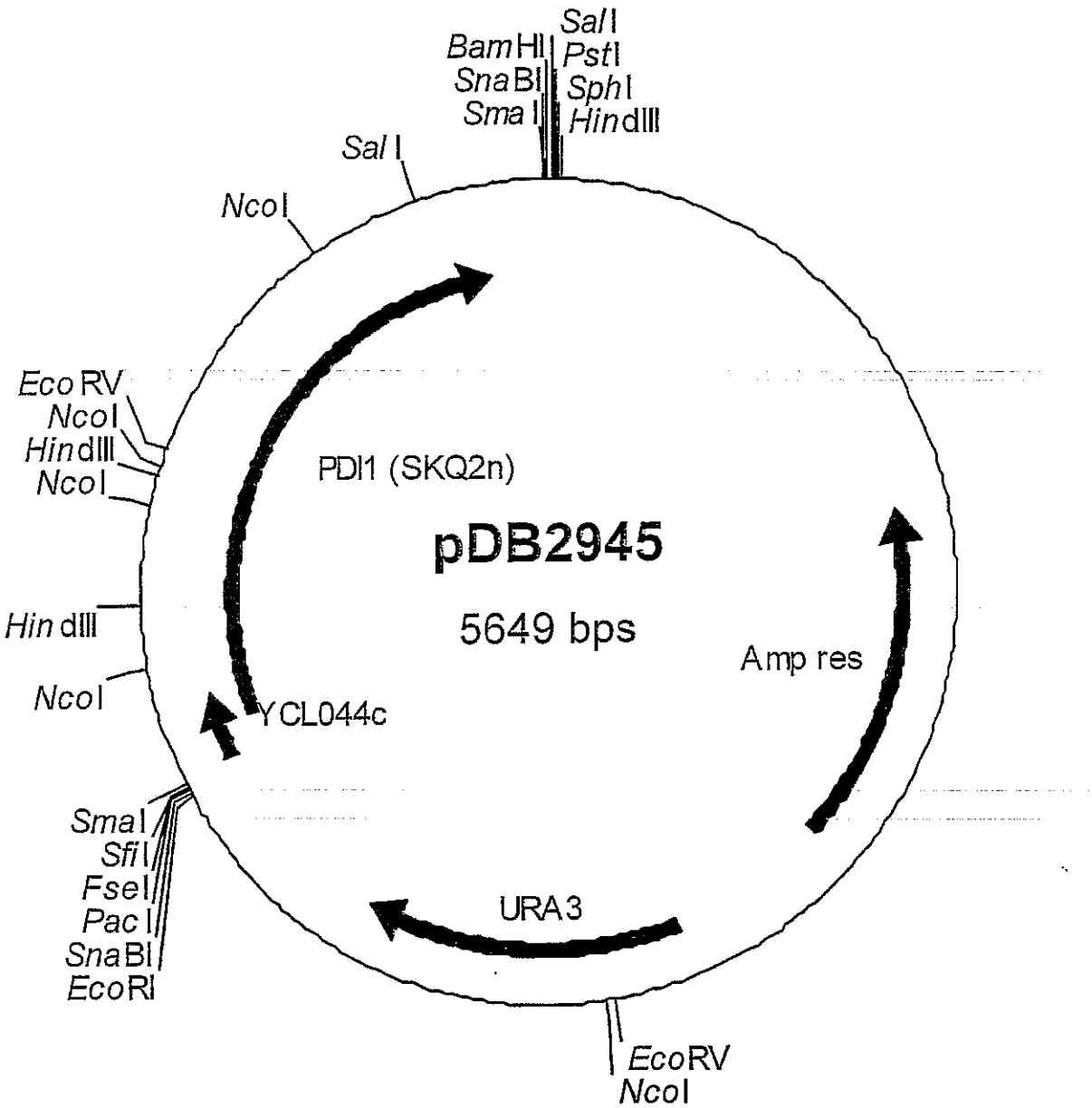

SalI
BamHI
PstI
SnaBI
SphI
SmaI
HindIII
SalI
NcoI
EcoRV
NcoI
HindIII
NcoI
PDI1 (SKQ2n)
pDB2945
5649 bps
Amp res
HindIII
NcoI
YCL044c
SmaI
SfiI
FseI
PacI
SnaBI
EcoRI
URA3
EcoRV
NcoI

Figure 63

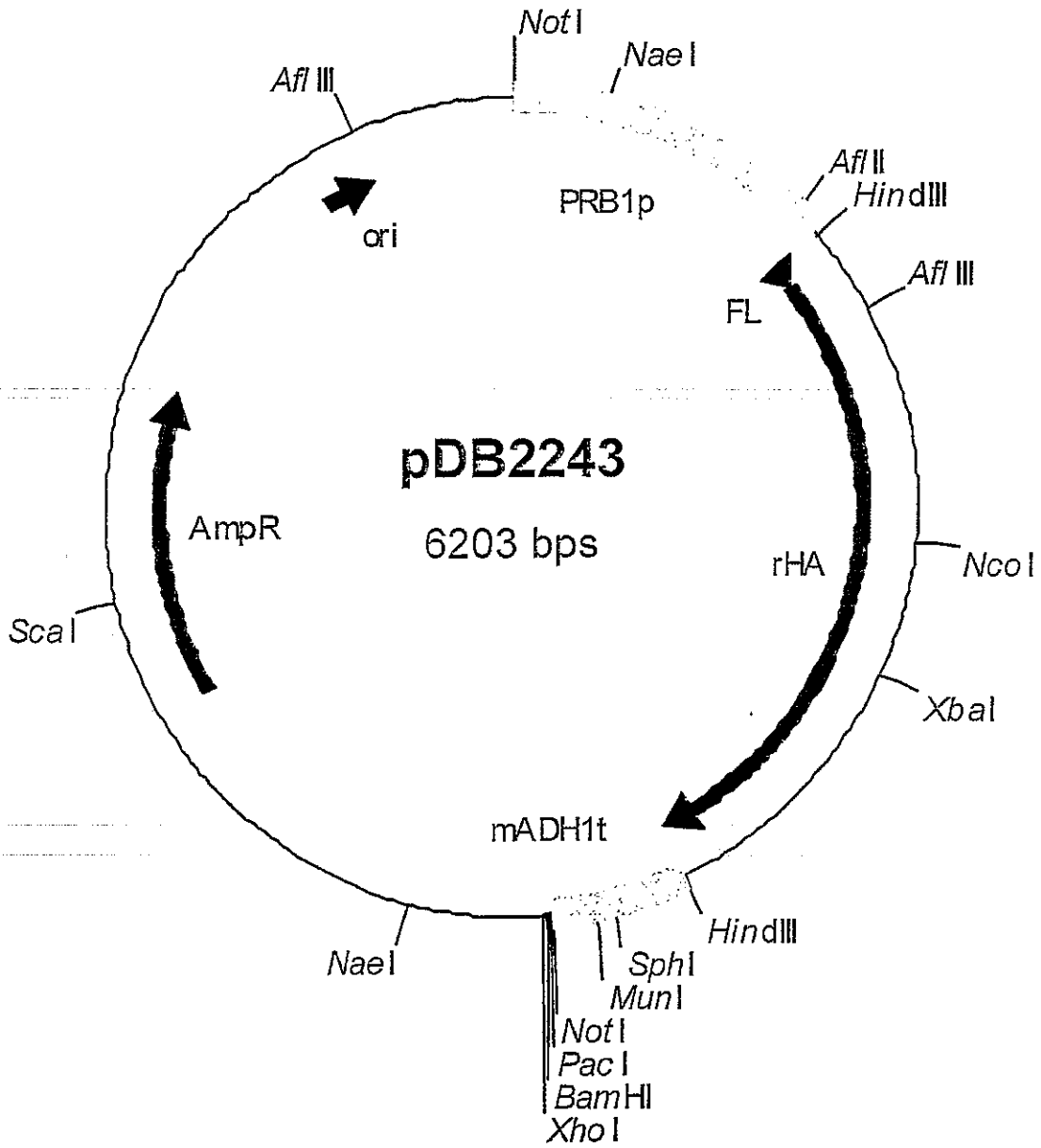

Not I
Nae I
Afl III
Afl II
Hin dIII
PRB1p
ori
Afl III
FL
pDB2243
6203 bps
AmpR
rHA
Nco I
Sca I
Xba I
mADH1t
Hin dIII
Nae I
Sph I
Mun I
Not I
Pac I
Bam HI
Xho I

Figure 64

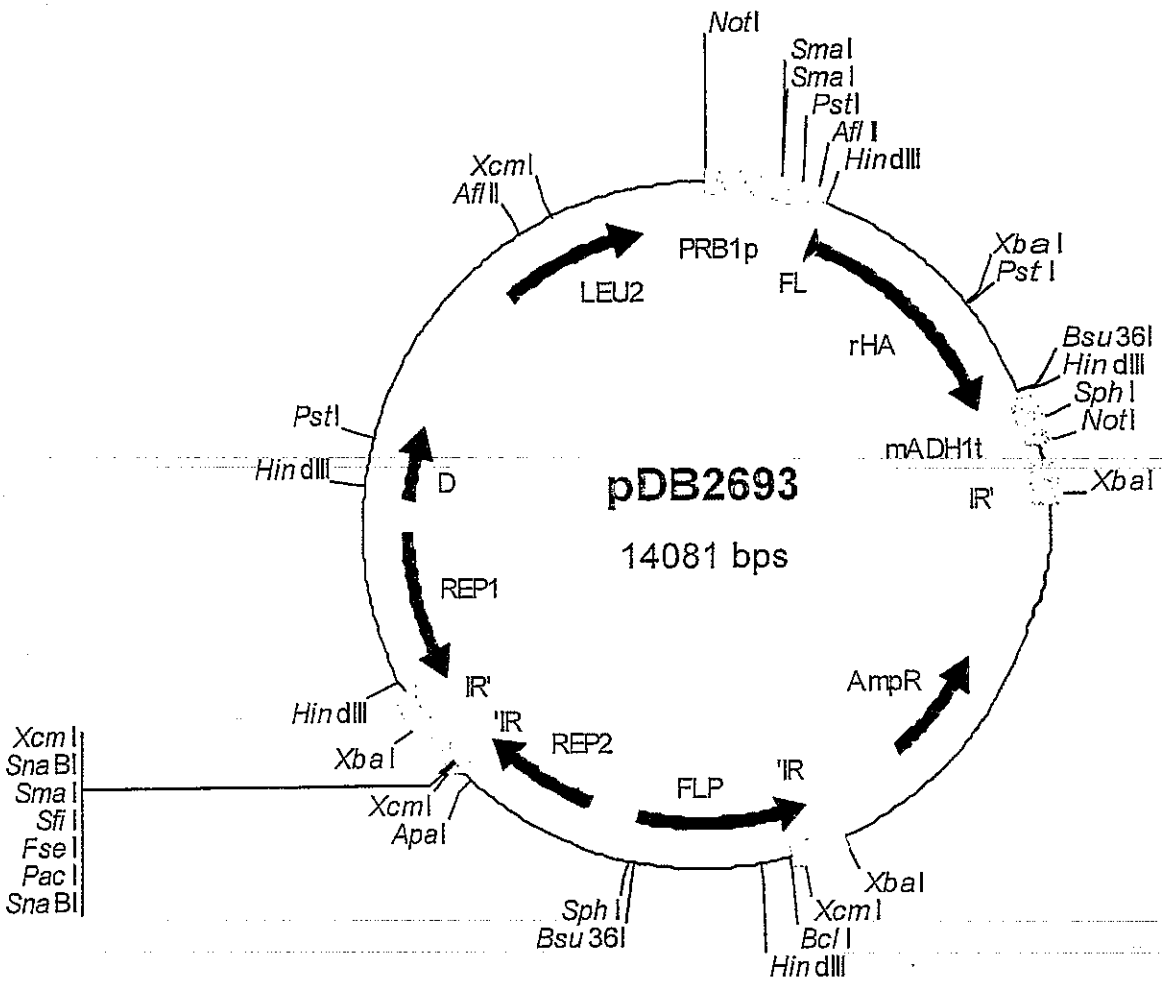

NotI
SmaI
SmaI
PstI
AflI
HindIII
XcmI
AflII
PRB1p
FL
XbaI
PstI
LEU2
rHA
Bsu36I
HindIII
SphI
NotI
PstI
HindIII
D
pDB2693
14081 bps
mADH1t
IR'
XbaI
REP1
IR'
AmpR
HindIII
'IR
REP2
XcmI
SnaBI
SmaI
SfiI
FseI
PacI
SnaBI
XbaI
FLP
'IR
XcmI
ApaI
XbaI
SphI
Bsu36I
XcmI
BclI
HindIII

FIGURE 65

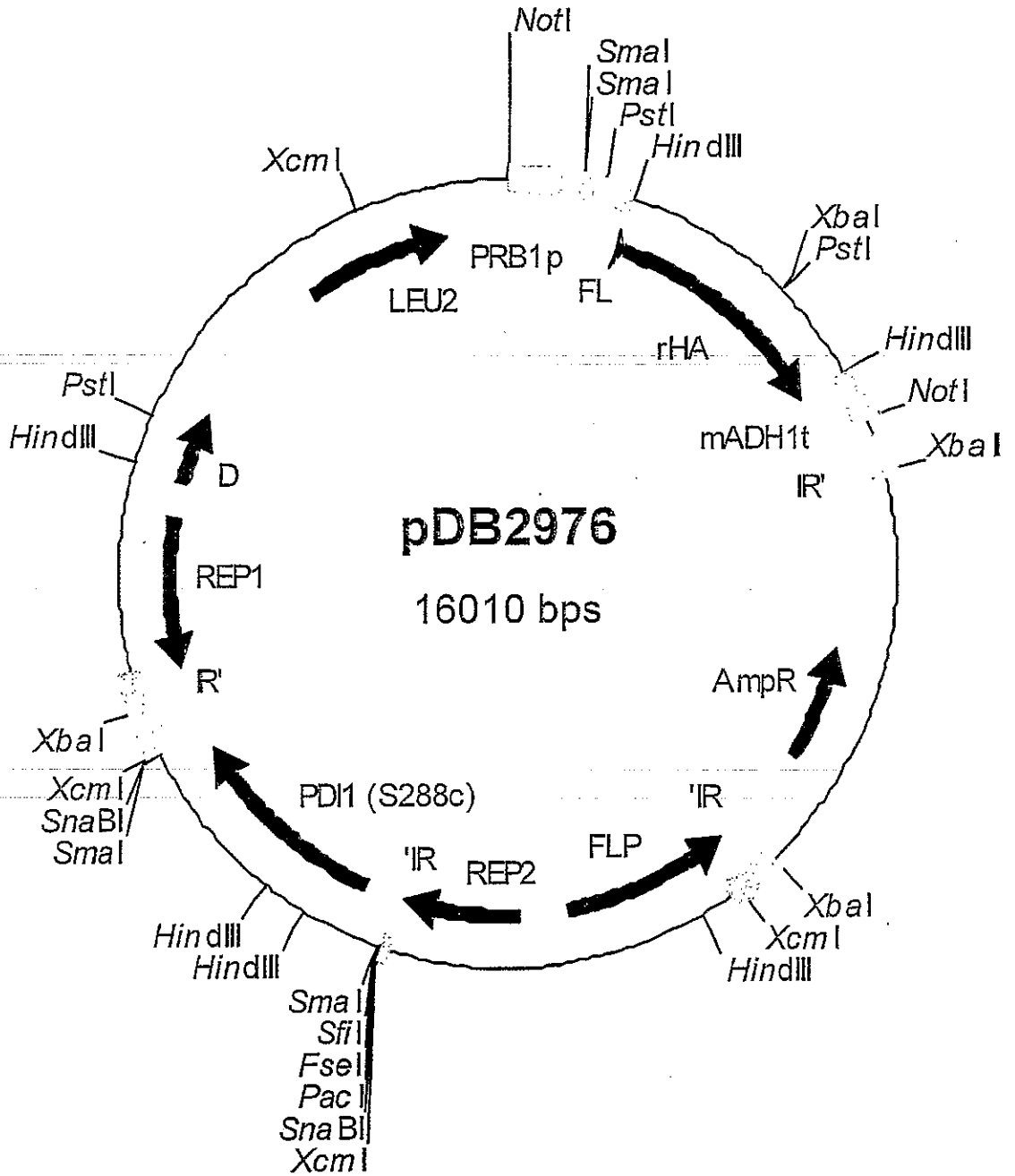

NotI
SmaI
SmaI
PstI
HindIII
XcmI
XbaI
PstI
PRB1p
LEU2
FL
rHA
HindIII
NotI
XbaI
mADH1t
IR'
PstI
HindIII
D
pDB2976
16010 bps
REP1
R'
AmpR
XbaI
XcmI
SnaBI
SmaI
PDI1 (S288c)
'IR
'IR
REP2
FLP
XbaI
XcmI
HindIII
HindIII
HindIII
SmaI
SfiI
FseI
PacI
SnaBI
XcmI

FIGURE 66

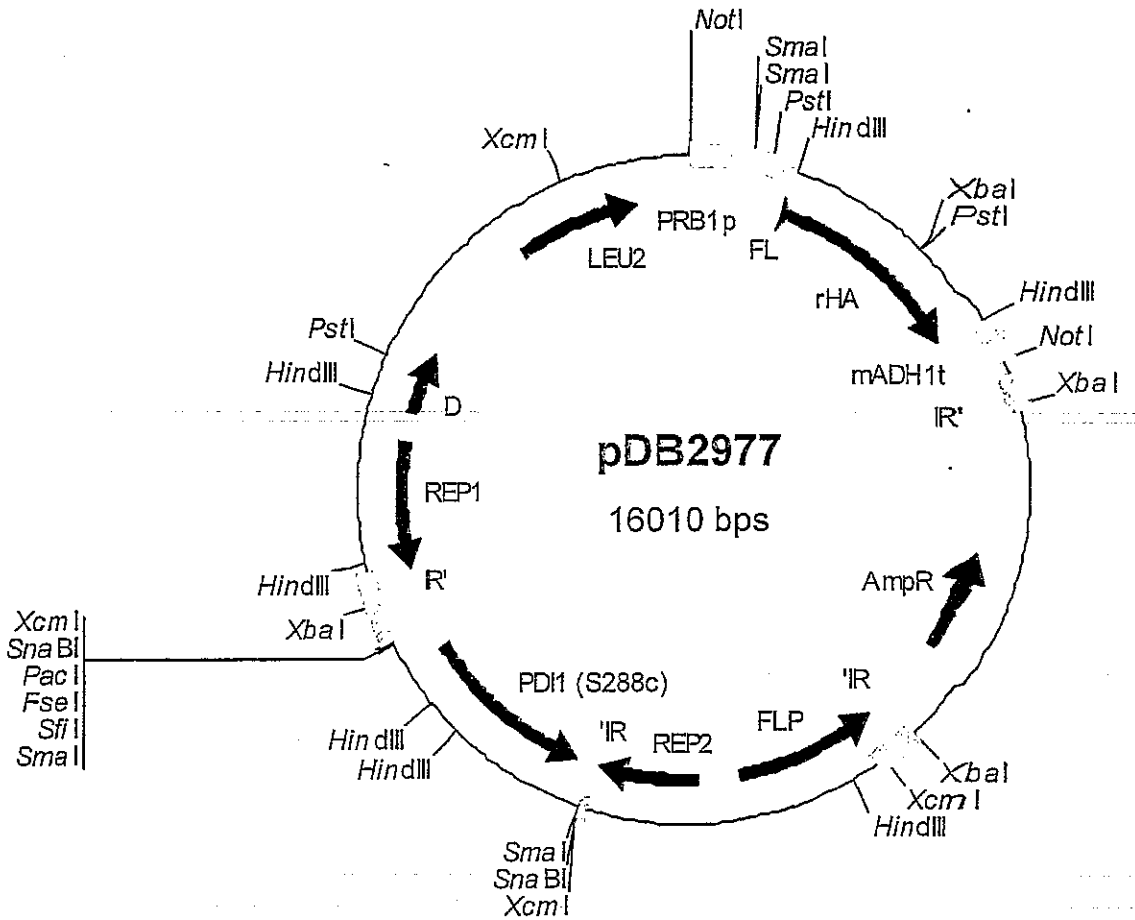

pDB2977
16010 bps
NotI
SmaI
SmaI
PstI
HindIII
XbaI
PstI
HindIII
NotI
XbaI
'IR
AmpR
'IR
FLP
'IR
REP2
PDI1 (S288c)
'IR
HindIII
XbaI
HindIII
PstI
HindIII
D
REP1
R'
XcmI
SnaBI
PacI
FseI
SfiI
SmaI
XcmI
LEU2
PRB1p
FL
rHA
mADH1t
IR'
HindIII
XbaI
XcmI
SmaI
SnaBI
XcmI

FIGURE 67

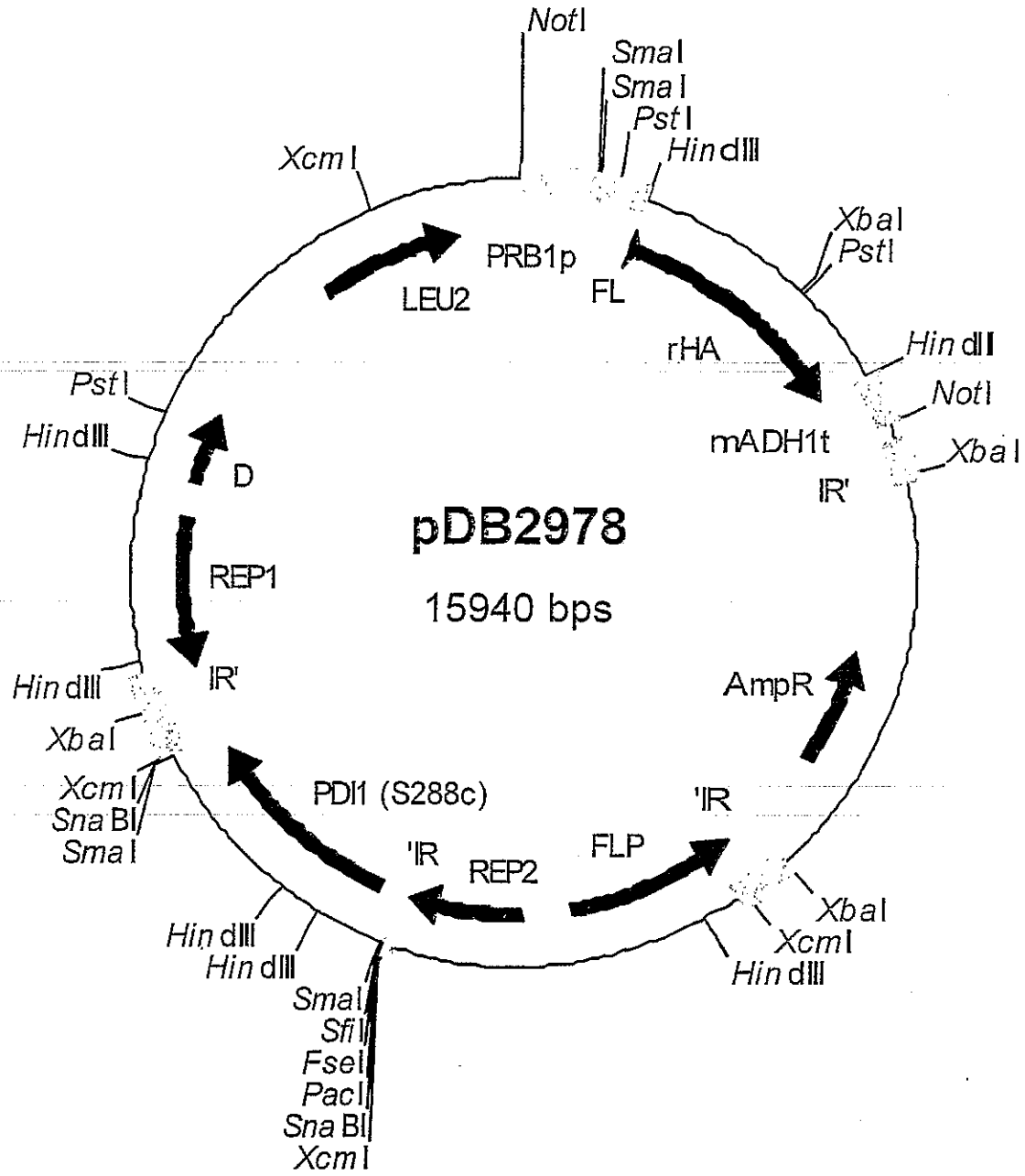

NotI
SmaI
SmaI
PstI
HindIII
XcmI
XbaI
PstI
PRB1p
LEU2
FL
rHA
HindIII
NotI
XbaI
mADH1t
IR'
PstI
HindIII
D
pDB2978
15940 bps
REP1
IR'
HindIII
XbaI
AmpR
XcmI
SnaBI
SmaI
PDI1 (S288c)
'IR
'IR
REP2
FLP
XbaI
XcmI
HindIII
HindIII
HindIII
SmaI
SfiI
FseI
PacI
SnaBI
XcmI

FIGURE 68

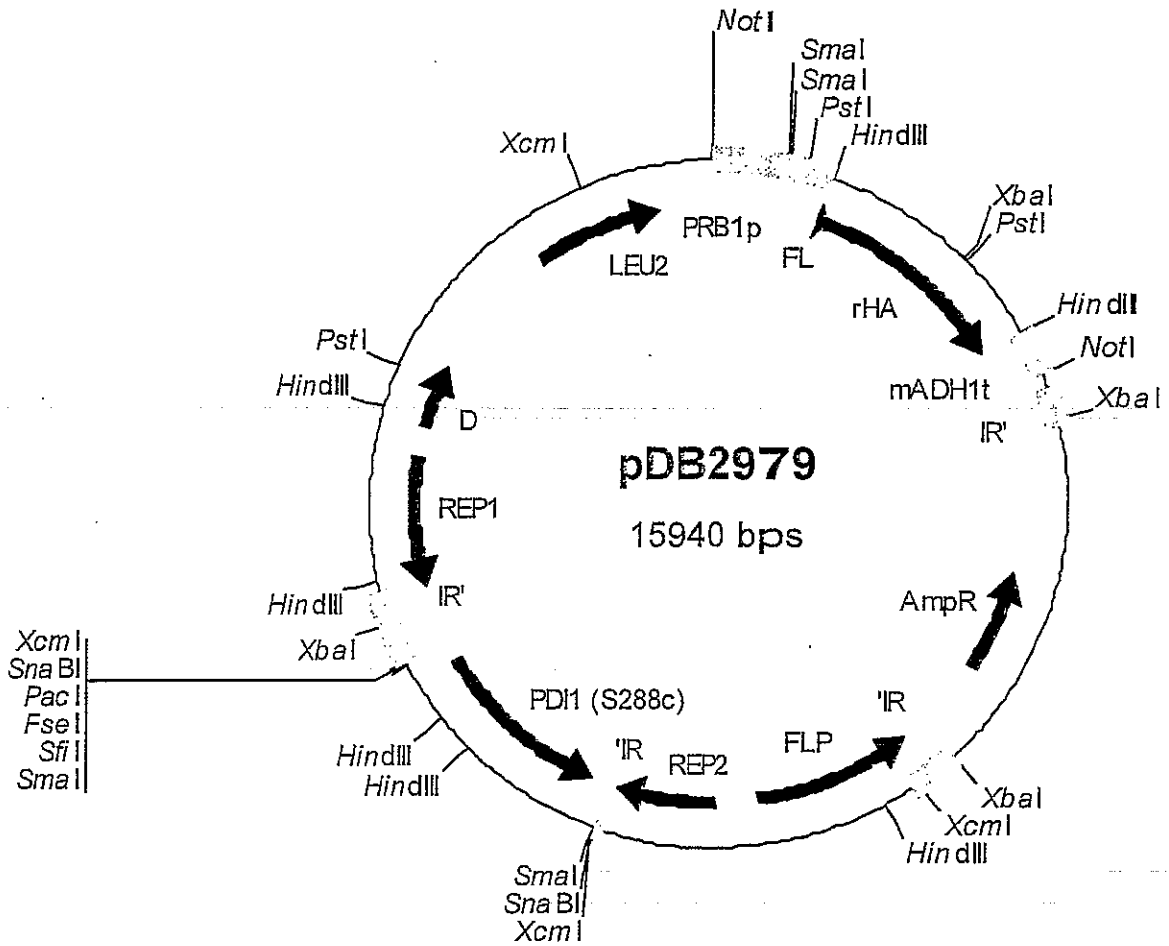

pDB2979
15940 bps
Not I
Sma I
Sma I
Pst I
Hin dIII
Xcm I
Xba I
Pst I
Hin dII
Not I
Xba I
PRB1p
LEU2
FL
rHA
mADH1t
IR'
Pst I
Hin dIII
D
REP1
IR'
Hin dIII
Xba I
Xcm I
Sna BI
Pac I
Fse I
Sfi I
Sma I
AmpR
PDI1 (S288c)
'IR
REP2
FLP
'IR
Hin dIII
Hin dIII
Xba I
Xcm I
Hin dIII
Sma I
Sna BI
Xcm I

FIGURE 69

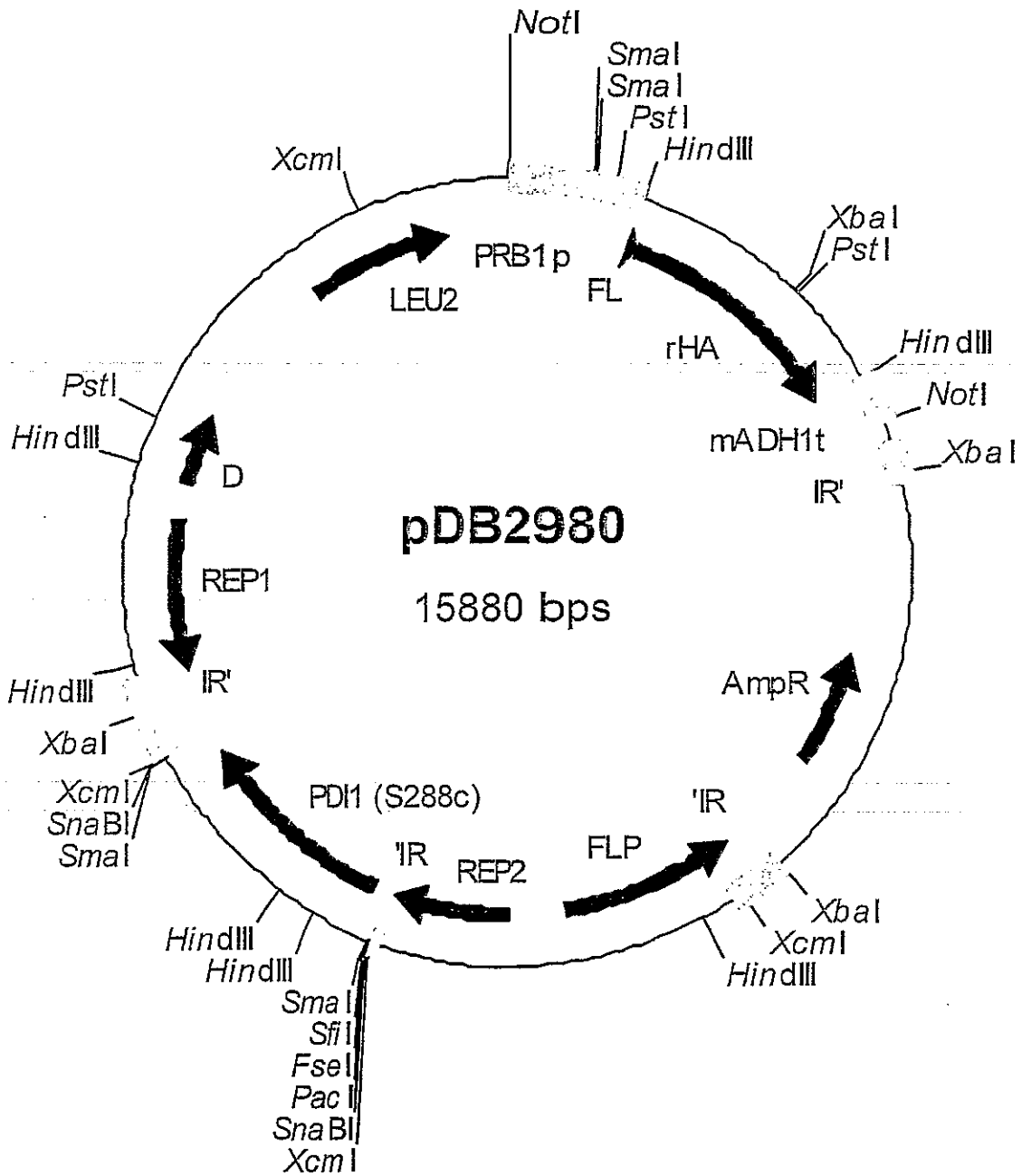

NotI
SmaI
SmaI
PstI
HindIII
XcmI
XbaI
PstI
PRB1p
LEU2
FL
rHA
HindIII
NotI
XbaI
mADH1t
IR'
PstI
HindIII
D
REP1
pDB2980
15880 bps
HindIII
IR'
AmpR
XbaI
XcmI
SnaBI
SmaI
PDI1 (S288c)
'IR
'IR
REP2
FLP
XbaI
XcmI
HindIII
HindIII
HindIII
SmaI
SfiI
FseI
PacI
SnaBI
XcmI

FIGURE 70

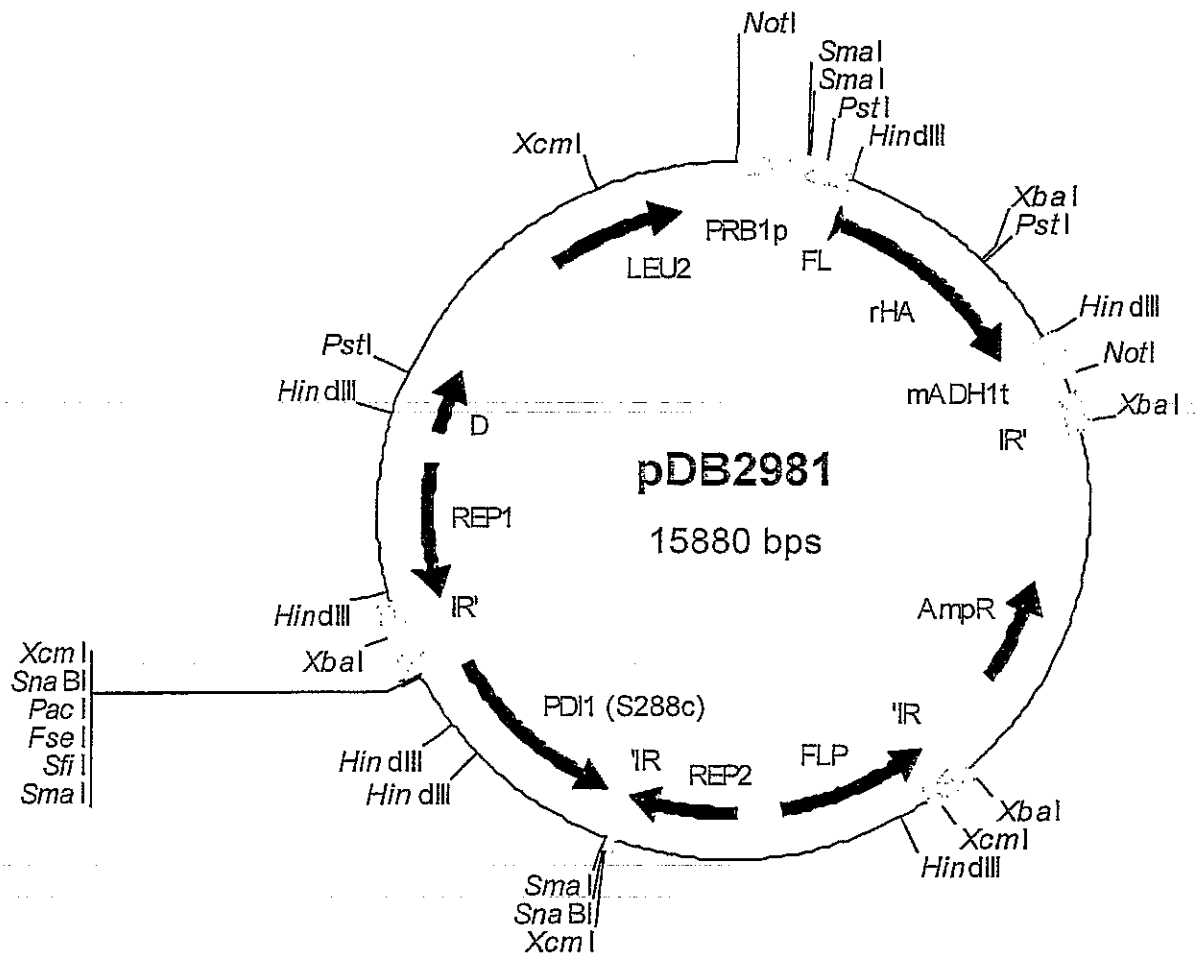

NotI
SmaI
SmaI
PstI
HindIII
XcmI
XbaI
PstI
PRB1p
LEU2
FL
rHA
HindIII
NotI
XbaI
mADH1t
IR'
PstI
HindIII
D
pDB2981
15880 bps
REP1
IR'
HindIII
XbaI
AmpR
XcmI
SnaBI
PacI
FseI
SfiI
SmaI
PDI1 (S288c)
'IR
'IR
REP2
FLP
HindIII
HindIII
XbaI
XcmI
HindIII
SmaI
SnaBI
XcmI

FIGURE 71

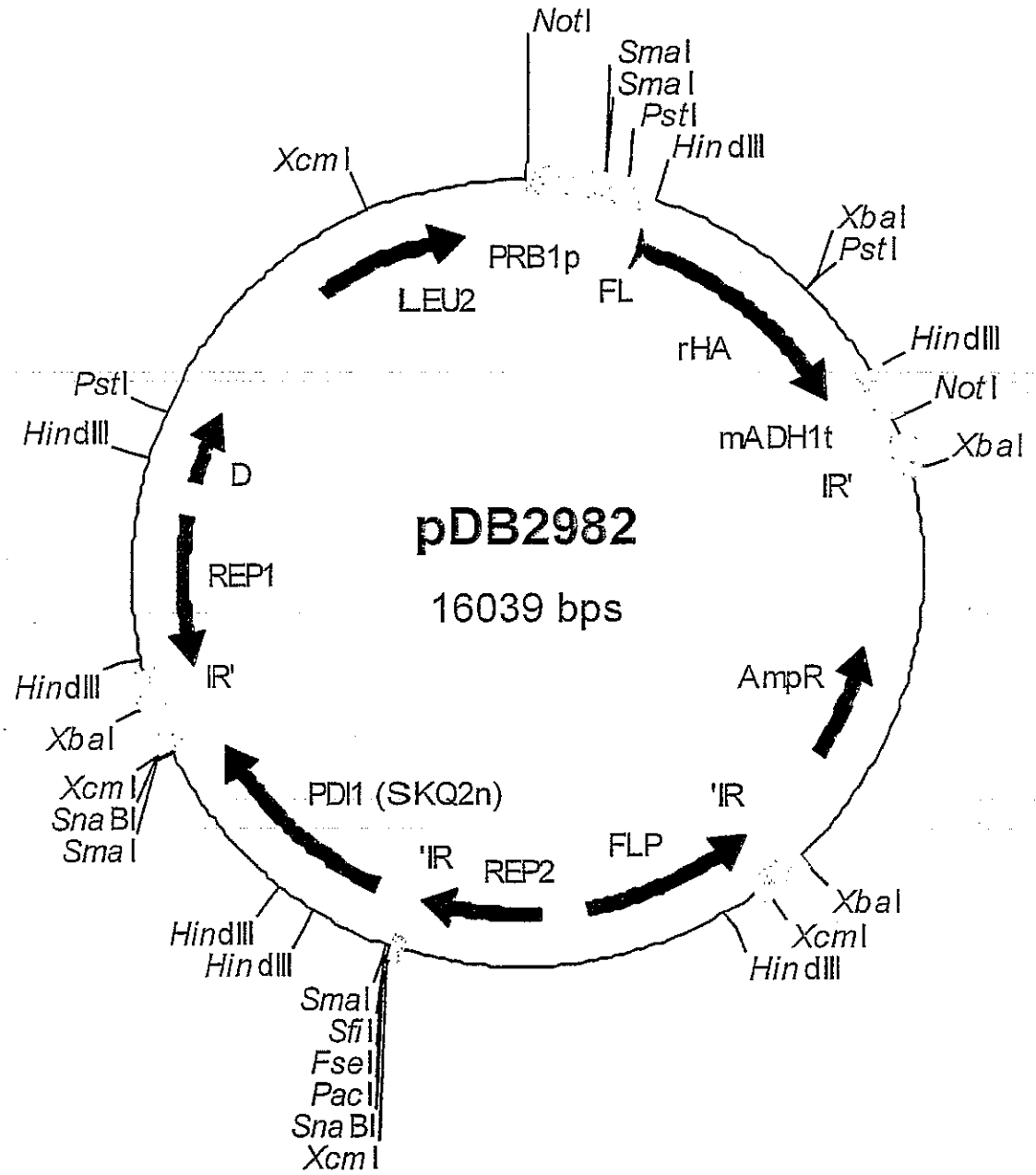

NotI
SmaI
SmaI
PstI
HindIII
XcmI
XbaI
PstI
PRB1p
LEU2
FL
rHA
HindIII
NotI
XbaI
mADH1t
IR'
PstI
HindIII
D
pDB2982
16039 bps
REP1
IR'
AmpR
HindIII
XbaI
XcmI
SnaBI
SmaI
PDI1 (SKQ2n)
'IR
'IR
REP2
FLP
XbaI
XcmI
HindIII
HindIII
HindIII
SmaI
SfiI
FseI
PacI
SnaBI
XcmI

FIGURE 72

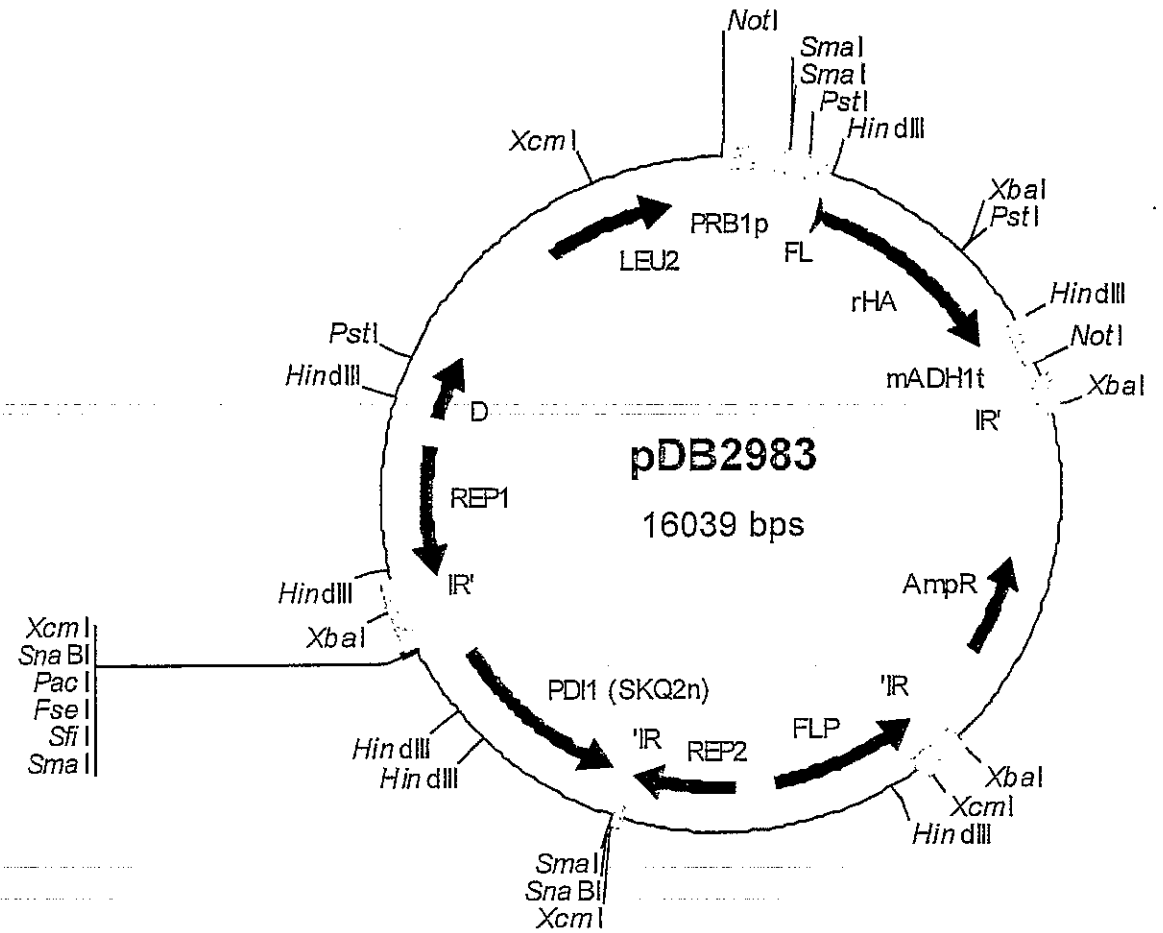

NotI
SmaI
SmaI
PstI
HindIII
XcmI
XbaI
PstI
PRB1p
LEU2
FL
rHA
HindIII
NotI
XbaI
mADH1t
IR'
PstI
HindIII
D
pDB2983
16039 bps
REP1
IR'
HindIII
XbaI
AmpR
XcmI
SnaBI
PacI
FseI
SfiI
SmaI
PDI1 (SKQ2n)
'IR
REP2
FLP
'IR
HindIII
HindIII
XbaI
XcmI
HindIII
SmaI
SnaBI
XcmI

FIGURE 73

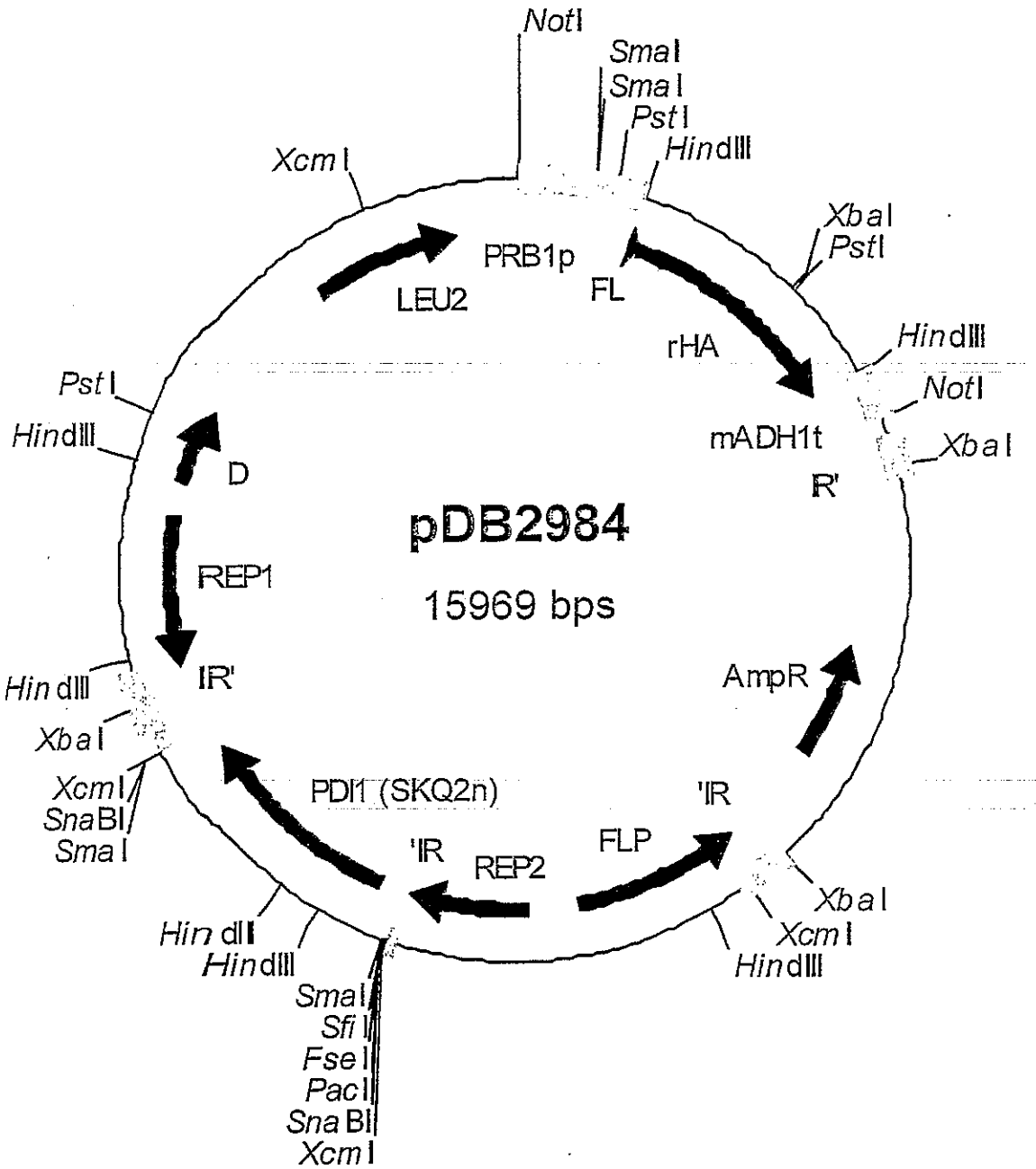

NotI
SmaI
SmaI
PstI
HindIII
XcmI
XbaI
PstI
PRB1p
LEU2
FL
rHA
HindIII
NotI
XbaI
mADH1t
R'
PstI
HindIII
D
REP1
IR'
HindIII
XbaI
XcmI
SnaBI
SmaI
pDB2984
15969 bps
AmpR
PDI1 (SKQ2n)
'IR
'IR
REP2
FLP
XbaI
XcmI
HindIII
HindIII
SmaI
SfiI
FseI
PacI
SnaBI
XcmI

FIGURE 74

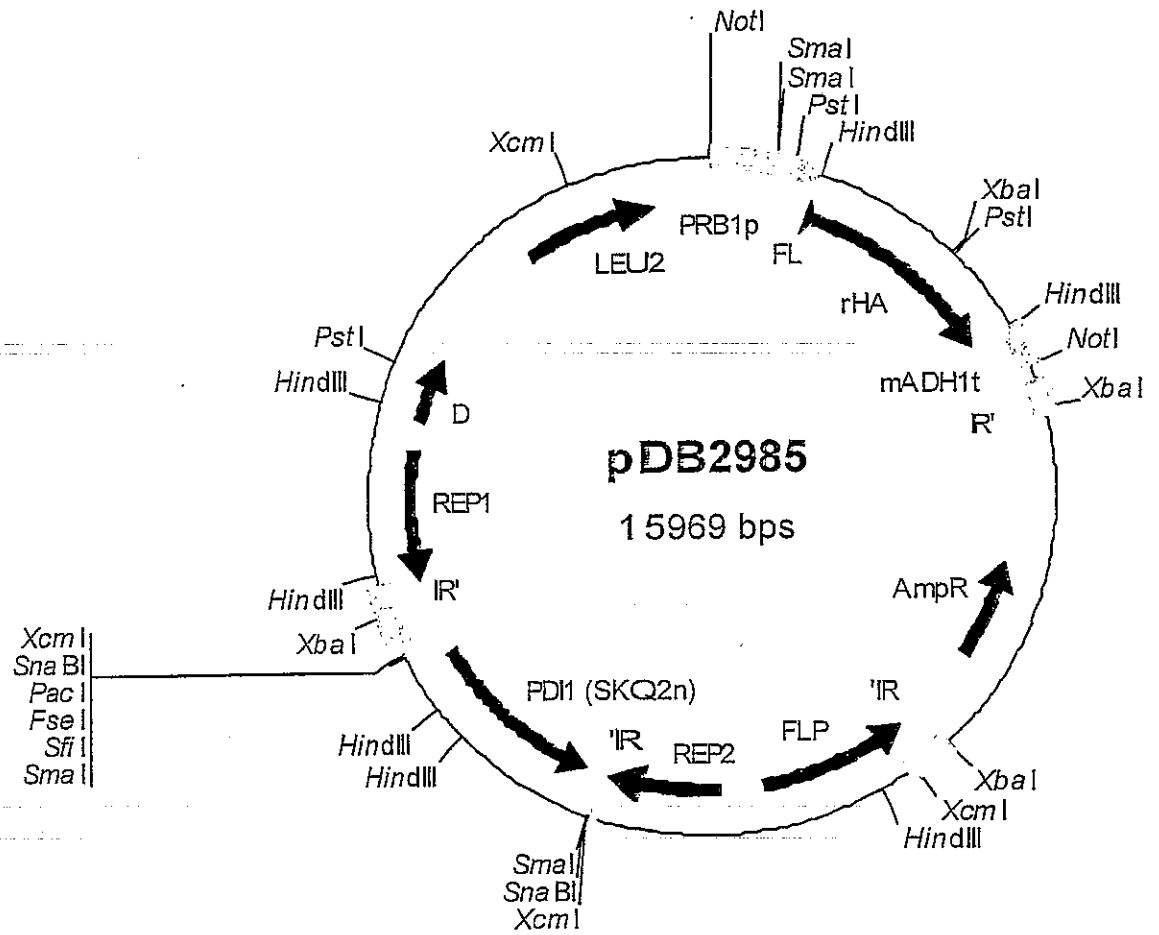

NotI
SmaI
SmaI
PstI
HindIII
XcmI
XbaI
PstI
PRB1p
LEU2
FL
rHA
HindIII
NotI
XbaI
mADH1t
R'
PstI
HindIII
D
pDB2985
15969 bps
REP1
IR'
HindIII
XbaI
AmpR
XcmI
SnaBI
PacI
FseI
SfiI
SmaI
PDI1 (SKQ2n)
'IR
'IR
REP2
FLP
HindIII
HindIII
XbaI
XcmI
HindIII
SmaI
SnaBI
XcmI

FIGURE 75

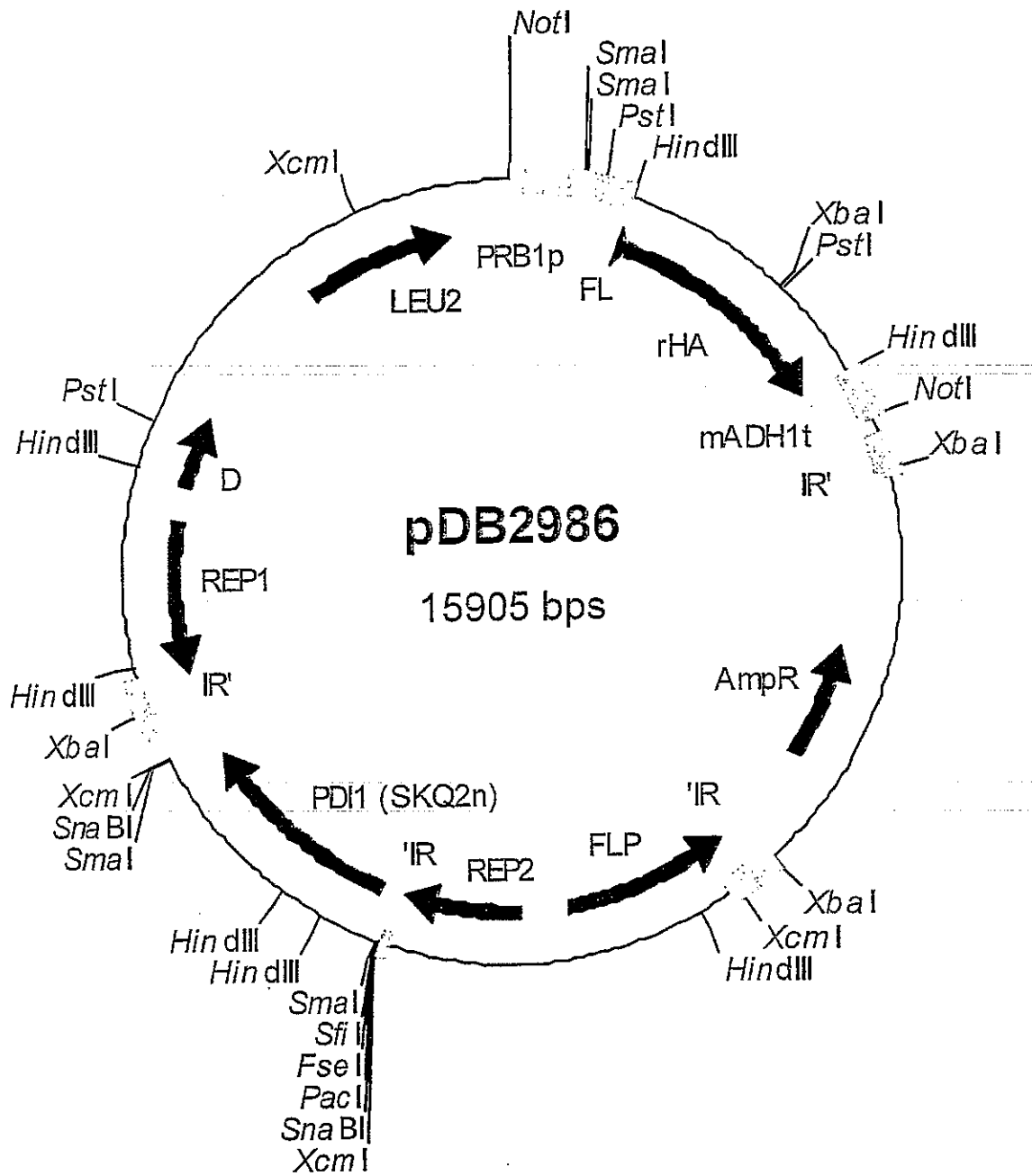
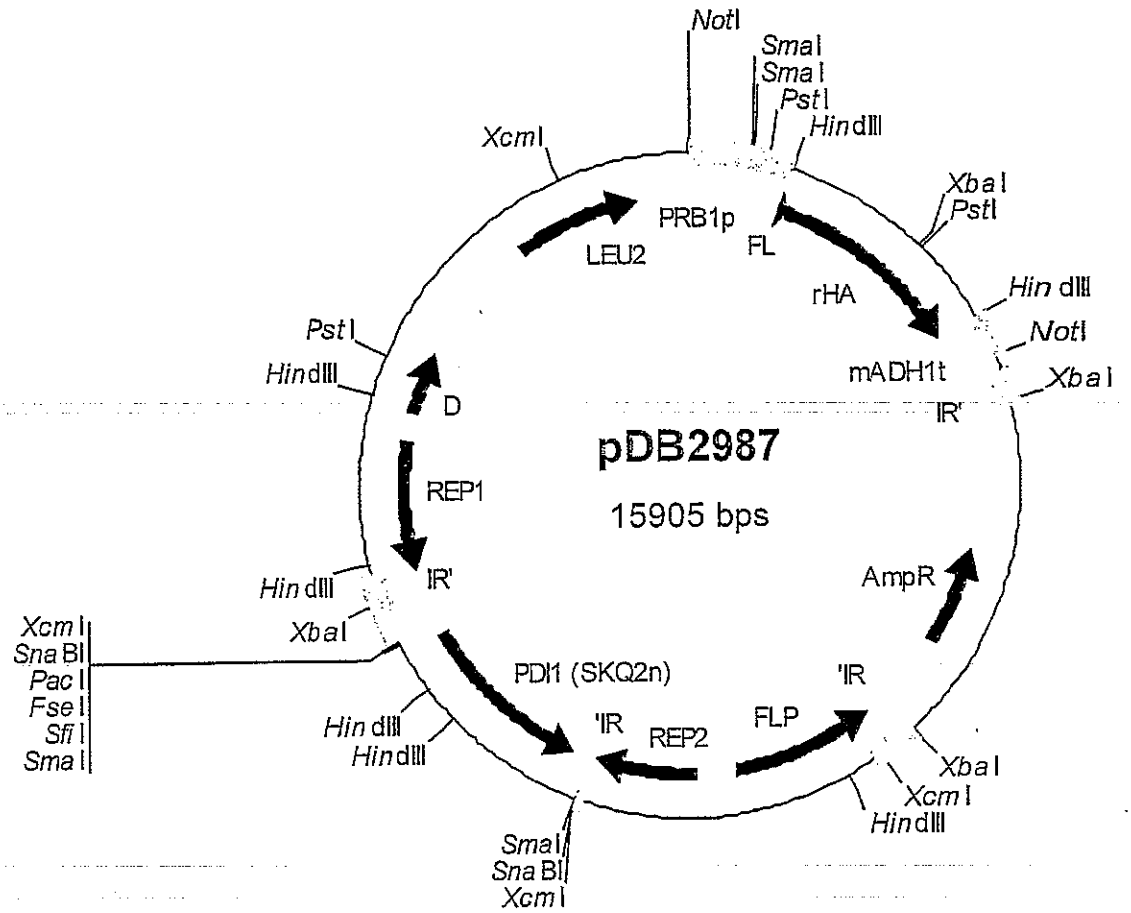

pDB2986
15905 bps
NotI
SmaI
SmaI
PstI
HindIII
XcmI
XbaI
PstI
PRB1p
LEU2
FL
rHA
HindIII
NotI
XbaI
mADH1t
IR'
PstI
HindIII
D
REP1
IR'
HindIII
XbaI
XcmI
SnaBI
SmaI
PDI1 (SKQ2n)
'IR
'IR
REP2
FLP
AmpR
XbaI
XcmI
HindIII
HindIII
HindIII
SmaI
SfiI
FseI
PacI
SnaBI
XcmI

FIGURE 76


NotI
SmaI
SmaI
PstI
HindIII
XcmI
XbaI
PstI
PRB1p
LEU2
FL
rHA
Hin dIII
NotI
XbaI
mADH1t
IR'
PstI
HindIII
D
pDB2987
15905 bps
REP1
IR'
Hin dIII
XbaI
XcmI
SnaBI
PacI
FseI
SfiI
SmaI
PDI1 (SKQ2n)
'IR
REP2
FLP
'IR
AmpR
Hin dIII
HindIII
XbaI
XcmI
HindIII
SmaI
SnaBI
XcmI