

RAT
pcDNA3-barr1(FLAG) Sequence

	10	20	30	40	50	
	1234567890	1234567890	1234567890	1234567890	1234567890	
	AAGCTTGGTAA	COGAGCTGGG	ATCCACTAGT	AACGGCCGCC	AGTGTGCTGG	50
	<i>HindIII</i>	<i>KpnI</i>				
	AATTCTGCAG	ATCGGACCAT	GGGCGACAAA	GGGACACGAG	TGTTCAAGAA	100
	<i>PstI</i>	<i>M</i>	<i>G D K</i>	<i>G T R V</i>	<i>F K K</i>	
	GGCAAGCCCC	AATGGAAAGC	TCACCGTCTA	CCTGGGAAAG	CGGGACTTTG	150
	A S P	N G K L	T V Y	L G K	R D F V	
	TGGACCACAT	TGACCTGGTG	GACCCCGTGG	ATGGCGTGGT	CCTGGTGGAT	200
	D H I	D L V	D P V D	G V V	L V D	
	CCTGAGTATC	TCAAAGAAAG	GCGAGTCTAC	GTGACACTGA	CCTGCGCCTT	250
	P E Y L	K E R	R V Y	V T L T	C A F	
	COGGTATGGC	CGGTAAGACC	TGGATGTCCT	GGGCTGACT	TTTCGCAAAG	300
	R Y G	R E D L	^{10A} D ₅₁ V L	G L T	F R K D	
	ACCTGTTTGT	GGCTAAGTIG	CAGTCCCTCC	CACCGGCCCC	TGAGGACAAG	350
	L F V	A N V	Q S F P	P A P	E D K	
	AAGOCACCTGA	CTCGGCTACA	AGAGCGACTC	ATCAAGAAGC	TGGGCGAGCA	400
	K P L T	R L Q	E R L	I K K L	G E H	¹⁰ WEM
	TGCTACCCCC	TTCACCTTTG	AGATCCCGCC	AAACCTTCCG	TGCTCAGTCA	450
	A Y P	F T F E	I P P	N L P	C S V T	
	CATTGCAACC	TGGGCTGAG	GACACAGGGA	AGGCTGCGG	TGTGGATTAT	500
	L Q P	G P E	D T G K	A C G	V D Y	
	GAAGTGAAG	CCTTCTGTGC	TGAGAACCCTG	GAGGAGAAGA	TCCACAAAAG	550
	E V K A	F C A	E N L	E E K I	H K R	
<i>EcoRI</i>	GAATTC	TGCTGAG	CGGCTAGTCA	TCCGGAAGGT	TCAATATGCC	600
	N S V	R L V I	R K V	Q Y A	P E R P	
	CTGGCCCTCA	GCCACGGCT	GAGACCACCA	GACAGTTCCT	CATGTCCGAC	650
	G P Q	P T A	E T T R	Q F L	M S D	
	AAGCCCTGTC	ACCTTGAGGC	ATCTCTGGAT	AAGGAGATCT	ATTATCATGG	700
	^{K^A} ^{P^A} ^{L^A} H	^{L^A} E A	S L D	K E I Y	Y H G	
	AGAACCACATC	AGCGTCAATG	TCCATGTCAC	CAACAACACC	AACAAGACTG	750
	E P I	S V N V	H V T	N N T	N K T V	
	TGAAGAAGAT	CAAGATCTCG	GTGCGCCAGT	ATGCAGACAT	CTGTCTCTTC	800
	K K I	K I S	V R Q Y	A D I	C L F	
	AACACAGCTC	AGTACAAGTG	CCGAGTGGCC	¹⁰² ATGAGGAAG	CTGATGATAC	850
	N T A Q	Y K C	P V A	M E E A	D D T	
	TGTGGCACCC	AGCTCAACAT	TCTGCAAGGT	CTACACACTG	ACTCCCTTCC	900
	V A P	S S T F	C K V	Y T L	T P F L	

Enzymes listed can be used to excise insert

I routinely use HindIII + XbaI



EcoRI
NciI
443

11/28/00

Both Barr1 FLAG and Barr2 FLAG are cloned in pcDNA3

pcDNA3-barr1(FLAG) Sequence

10	20	30	40	50	
1234567890	1234567890	1234567890	1234567890	1234567890	
TGGCAAACAA	CAGAGAGAAG	CGGGGGCTTG	COCTOGAOGG	GAAGCTCAAG	950
A N N	R E K	R G L A	L D G	K L K	Nar I
CATGAAGACA	CAAATCTGGC	TTCCAGCACT	CTGTTGOGGG	AAAGGCGCCAA	1000
H E D T	N L A	S S T	L L R E	G A N	
CCGTGAAATC	CTGGGTATCA	TTGTTTCTTA	CAAAGTCAAA	GTGAAGCTGG	1050
R E I	L G I I	V S Y	K V K	V K L V	
TGGTGTCCCG	GGGCGGCGCTG	TTGGGAGAAC	TTGCATCCAG	TGACGTGGCT	1100
V S R	G G L	L G D L	A S S	D V A	
GTGGAAGTGC	CCTTTACCTT	AATGCACCCC	AAGCCTAAAG	AGGAGCCCCC	1150
V E L P	F T L	M H P	K P K E	E P P	
ACATOGGGAA	GTTCAGAGA	GCGAGACTCC	AGTAGACACC	AATCTCATAG	1200
H R E	V P E S	E T P	V D T	N L I E	
AGCTTGACAC	CAATGATGAC	GACATTGTGT	TTGAGGACTT	TGCTOGTCAG	1250
L D T	N D D	D I V F	E D F	A R Q	
CGGCTGAAAG	GCATGAAGGA	TGACAAGGAC	GAAGAGGATG	ATGGCACCGG	1300
R L K G	M K D	D K D	E E D D	G T G	
CTCTOCACAC	CTCAACAACA	GAGACTACAA	AGAAGATGAC	GACAAGTAAG	1350
S P H	L N N R	D Y K	D D D	D K	
ATCCATCACA	CTGGCGGCGG	CTCGAGCATG	CATCTAGAGG	GCCC	1394
	Not I Xho I		Xba I Apa I		

CTCGAG

gTCgAc

C T C G A C
g A g C T g

pcDNA3-barr1(FLAG) Restriction Sites

Map Name	# Sites	"All Enzymes" Sites
AatII	0	
Acc65I	1	8
AccI	4	128, 227, 881, 1184
AciI	11	37, 141, 427, 488, 561, 921, 987, 1064, 1251, 1365, 1369
AflII	0	
AflIII	0	
AgeI	0	
AhaII	1	995
AluI	10	4, 16, 120, 390, 808, 841, 863, 945, 1047, 1203
AlwI	7	28, 15, 206, 193, 417, 534, 1345
AlwNI	1	1254
ApaI	1	1394
ApaLI	0	
ApoI	2	51, 552
AscI	0	
AseI	0	
AvaI	2	1058, 1372
AvaII	4	65, 153, 171, 189
AvrII	0	
BalI	1	829
BamHI	2	20, 198
BanI	4	8, 855, 994, 1294
BanII	3	18, 1148, 1394
EbsI	2	272, 962
EcoV	0	
BclI	0	
BfaI	3	27, 565, 1385
BglI	0	
BglII	2	686, 764
Bpu1102I	0	
BsaAI	1	231
BsaBI	1	763
BsaHI	1	995
BsaI	2	616, 1070
BsaJI	9	68, 132, 176, 460, 614, 830, 1011, 1058, 1059
BsiEI	2	37, 1369

pcDNA3-barr1(FLAG) Restriction Sites

Map Name	# Sites	"All Enzymes" Sites
BsiWI	7	181, 309, 596, 645, 938, 1154, 1155
BsmAI	5	616, 799, 1070, 1168, 1316
BsmI	0	
Bsp1286I	5	18, 445, 823, 1148, 1394
BspDI	0	
BspEI	1	572
BspHI	0	
BspMI	1	250
BspMII	1	572
BsrFI	2	333, 1297
BsrI	1	1366
BssHII	0	
Bst1107I	0	
BstBI	0	
BstEII	0	
BstNI	9	133, 166, 193, 271, 461, 529, 602, 901, 1012
BstUI	0	
BstXI	3	47, 1095, 1361
BstYI	7	20, 198, 422, 539, 686, 764, 1350
Bsu36I	2	341, 467
Cfr10I	2	333, 1297
ClaI	0	
DdeI	9	203, 341, 444, 467, 521, 593, 608, 620, 809
DpnI	9	22, 62, 200, 424, 541, 688, 760, 766, 1352
DpnII	9	20, 60, 198, 422, 539, 686, 758, 764, 1350
DraI	0	
DraIII	0	
DrdI	1	274
EaeI	4	34, 258, 827, 1366
EagI	2	34, 1366
Eam1105I	0	
EarI	2	802, 1277
Ecl136II	1	16
Eco47III	0	

pcDNA3-barr1(FLAG) Restriction Sites

Map Name	# Sites	"All Enzymes" Sites
EcoNI	0	
EcoO109I	1	1390
EcoRI	2	51, 552
EcoRV	0	
Esp3I	0	
EspI	0	
Fru4HI	6	37, 562, 1065, 1252, 1366, 1369
FokI	6	193, 286, 556, 1071, 1282, 1300
FspI	0	
HaeII	1	998
HaeIII	11	36, 260, 337, 465, 484, 600, 605, 829, 1067, 1368, 1392
HgaI	1	702
HgiAI	2	18, 445
HhaI	3	247, 776, 997
HincII	0	
HindIII	1	2
HinfI	5	224, 360, 377, 891, 1176
HinPI	3	245, 774, 995
HpaI	0	
HpaII	6	252, 261, 334, 573, 1059, 1298
HphI	3	114, 405, 720
KasI	1	994
KpnI	1	12
MaeI	3	27, 565, 1385
MaeII	3	230, 317, 1094
MaeIII	5	29, 231, 447, 726, 1090
MboI	9	20, 60, 198, 422, 539, 686, 758, 764, 1350
MboII	7	277, 549, 765, 768, 789, 967, 1294
MluI	0	
MnlI	14	335, 461, 524, 589, 617, 649, 659, 827, 943, 1133, 1226, 1277, 1321, 1380
MscI	1	829
MseI	1	1120
MspI	6	252, 261, 334, 573, 1059, 1298
MnI	0	
NaeI	0	
NarI	1	995

pcDNA3-barr1(FLAG) Restriction Sites

Map Name	# Sites	"All Enzymes" Sites
NciI	3	262, 1059, 1060
NcoI	2	68, 830
NdeI	0	
NgcMI	0	
NheI	0	
NlaIII	9	72, 403, 645, 700, 727, 834, 955, 1266, 1381
NlaIV	10	10, 22, 173, 200, 338, 857, 996, 1145, 1296, 1392
NotI	1	1366
NruI	0	
NsiI	1	1383
NspBII	1	1251
NspHI	2	403, 1381
PacI	0	
PaeR7I	1	1372
PflMI	0	
PleI	5	232, 354, 371, 885, 1170
PmeI	0	
PmlI	0	
PpuMI	0	
PstI	1	60
PvuI	0	
PvuII	0	
RsaI	2	10, 814
RsrII	0	
SacI	1	18
SacII	0	
SalI	0	
Sau3aI	9	20, 60, 198, 422, 539, 686, 758, 764, 1350
Sau96I	9	65, 153, 171, 189, 336, 463, 604, 1390, 1391
ScaI	0	
ScrFI	12	133, 166, 193, 262, 271, 461, 529, 602, 901, 1012, 1059, 1060
SecI	9	68, 132, 176, 460, 614, 830, 1011, 1058, 1059
SfaNI	3	679, 1093, 1390

pcDNA3-barr1(FLAG) Restriction Sites

Map Name	# Sites	"All Enzymes" Sites
SfcI	1	56
SfiI	0	
SmaI	1	1060
SnaBI	0	
SpeI	1	26
SphI	2	403, 1381
SplI	0	
SspI	0	
StuI	2	484, 600
StyI	2	68, 830
TaqI	2	935, 1373
TfiI	0	
Tth111I	0	
XbaI	1	1384
XcmI	0	
XhoI	1	1372
XraI	1	1058
XrnI	1	511