The λgt11 expression vector was constructed to allow rapid antibody screening of β-galactosidase fusion proteins encoded by DNA cloned into a unique EcoRI site within the 3´ end of the *lac*Z gene (1,2). Unfortunately, the EcoRI sites are often lost during cloning, making it difficult to excise the insert for subcloning or sequencing. Many researchers have opted to double-digest λgt11 with SacI and KpnI to liberate a DNA fragment containing inserted DNA at the EcoRI restriction site. The SacI–KpnI fragment can then be subcloned into pUC19 or another suitable vector for sequencing, mapping or further analysis.

NEB has sequenced the SacI–KpnI region of λgt11 (3). Previously, this sequence was deduced from the sequences of the various constructs used to create the phage. This sequence agrees with the *lacZ* gene sequence and the *lacZ*/λ fusion junction of λp*lac* 5 and λ*c*I857 (precursors of λgt11) (4-7). The sequence also contains two open reading frames (ORFs) (the first consists of two thirds of the membrane protein *lom* fused to a normally untranslated ORF and the second is the host specificity protein J) in the opposite orientation from the *lacZ* ORF, possibly accounting for the occasionally observed translation of proteins in that orientation (5). Several unique restriction targets in the SacI–KpnI sequence have been identified. These may be helpful for restriction mapping DNA cloned into the EcoRI cloning site. In addition, several enzymes have been identified which cleave the SacI–KpnI region twice, once on each side of the EcoRI cloning site. These may be useful for subcloning the region surrounding the EcoRI cloning site into other vectors should the EcoRI site contain multiple inserts or the SacI and KpnI sites be inconvenient (for example if the cloned DNA contains a SacI or KpnI site). The enzyme ApoI (Pu/AATTPy) may be useful for removing some fragments with mutated EcoRI sites.

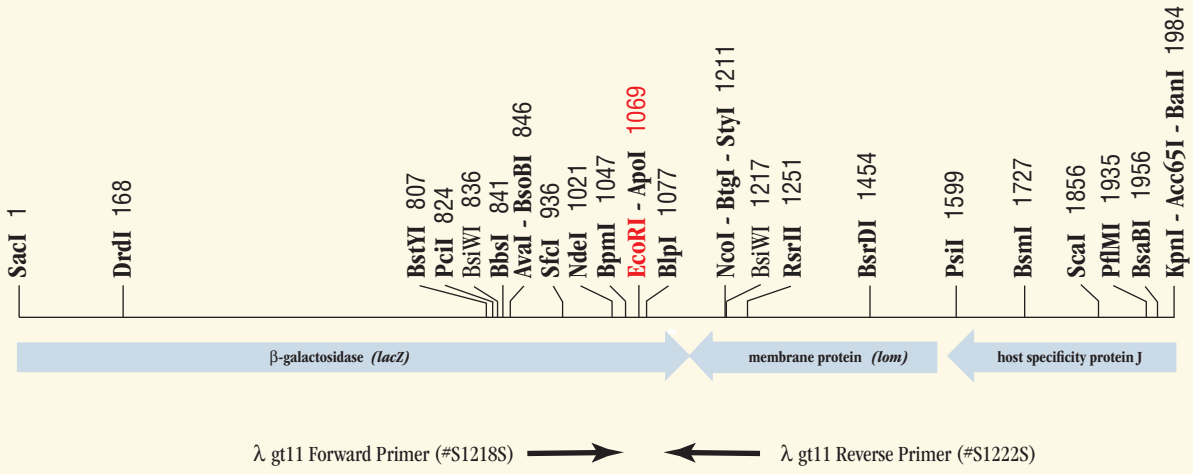
Positions of sequencing primers available from NEB (not drawn to scale) and positions of restriction endonucleases which cleave the SacI–KpnI region only once are presented in the figure below. The accompanying chart lists those restriction endonucleases which cleave the fragment either once or twice and also provides the recognition site coordinates. Those which cleave twice and whose sites flank the EcoRI site are followed by an asterisk (*). Of particular interest are the locations of the BsiWI sites which cleave only 233 and 148 base pairs to the left and right of the EcoRI position; the BsiWI fragment can then be cloned for further analysis.

Single Sites	Location
Acc65I	1984
ApoI	1069
AvaI	846
BanI	1984
BbsI*	841
BlpI	1077
BpmI	1047
BsaBI	1956
BsmI	1727
BsoBI	846
BsrDI	1454
BstYI	807
BtgI	1211
DrdI	168
EcoRI	1069
KpnI	1984
NcoI	1211
NdeI	1021
PciI	824
PflMI	1935
PsiI	1599
RsrII	1251
SacI	1
ScaI	1856
SfcI	936
StyI	1211
TspGWI(x)*	1378

(x) = enzyme not available from NEB

Two Sites	Locations	
AccI	828	1706
AgeI	1249	1428
AleI	350	896
AlwI	807	1758
ApaLI	410	525
AvaII	1185	1252
BaeI	1426	1803
BanII	1	1050
BciVI	1303	1432
BglI	332	1201
BsaHI	212	384
BsaXI*	1301	1522
BsiWI	836	1217
BsmFI*	252*	871
BsrBI*	566	730
BstXI	278	895
BstZ17I	828	1706
BtgZI*	967*	1692
BtsI	207	522
EaeI	1208	1256
HincII	941	1786
HinfI	1042	1421
MboII*	842*	988
MluI	147	572
MlyI*	1042*	1421
NlaVI	1049	1984
NspI	824	1782
Nt.BstNBI*	1042*	1421
PleI*	1042*	1421
PvuII	712	1075
TatI (x)	1176	1856

SacI - KpnI region of λgt11



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