

### MCS Restriction Sites in pLW01

Enzyme name	Site orientation Recognition sequence	Site position	Downstream fragment
BglII	a/gatct	638	2792
XbaI	t/ctaga	696	2734
<b>NcoI</b>	<b>c/catgg</b>	<b>735</b>	<b>2695</b>
<b>NheI</b>	<b>g/ctagc</b>	<b>740</b>	<b>2690</b>
<b>BamHI</b>	<b>g/gatcc</b>	<b>771</b>	<b>2659</b>
<b>EcoRI</b>	<b>g/aatcc</b>	<b>777</b>	<b>2653</b>
<b>SacI</b>	<b>gagct/c</b>	<b>783</b>	<b>2647</b>
<b>SalI</b>	<b>g/tcgac</b>	<b>790</b>	<b>2640</b>
<b>HindIII</b>	<b>a/agctt</b>	<b>796</b>	<b>2634</b>
<b>NotI</b>	<b>gc/ggccgc</b>	<b>802</b>	<b>2628</b>
<b>EagI</b>	<b>c/ggccg</b>	<b>803</b>	<b>2627</b>
<b>XhoI</b>	<b>c/tcgag</b>	<b>811</b>	<b>2619</b>

T7 promoter XbaI

gaaatt**taatac****gactcactat**agggagaccacaacggtttccct**ctaga**aataatTTTgTTTaaCTTTaa  
 cTTTaa**attatgctgagt**gatatccctctggtgTTgCCaaaggg**agatct**TTTaaTaaacaaattgaaatt

RBS NcoI BamHI SacI

**gaaggag**atata**ccatgg**ctagcatgactggtggacagcaaatgggTc**ggatcc**gaatt**cgagctc**cgTc  
 c**ttcctc**tatat**ggtacc**gatcgtactgaccacctgTcgtttacc**cagcctaggc**TTa**agctcgagcag**  
EcoRI SalI

HindIII XhoI 6xHis Stop

gacaagcttgcggccgcact**cgagc**accaccaccaccaccact**gagat**ccggctgctaacaaagcccga  
ctg**ttcgaac**gccggcgt**gagctc**gtggtggtggtggtggt**gact**ctaggccgacgattgTTTcgggctt  
NotI/EagI

T7 terminator

aggaagctgagttggctgctgccaccgctgagcaataact**tagcataacc**cttggggcctctaaacgggt  
 tccttcgactcaaccgacgacggtggcgactcgttattgat**cgattgggga**acc**ccggagattgcca**

**cttgaggggTTTTTg**  
**gaactccccaaaaaac**

Forward sequencing primer: ttaatac**gactcactat**agg

Reverse sequencing primer: ctagttattgctcagcggTg