

Plasmid	End Structure	% Ligated BglII Site	% Ligated XhoI Site
pTW423-0	CAATTGTAGAGACTATCCACAAGGACAATATT GTTAACATCTCTGATAGGTGTTCTGTTATAA	87	77
pTW423-31	CAATTGTAGAGACTATCCACAAGGACAATATT GTTAACATCTCTGATAGGTGTTCTGTTATAA	54	83
pTW423-32	CAATTGTAGAGACTATCCACAAGGACAATATT GTTAACATCTCTGATAGGTGTTCTGTTATAA	62	85
pTW423-34	CAATTGTAGAGACTATCCACAAGGACAATATT GTTAACATCTCTGATAGGTGTTCTGTTATAA	88	88
pTW423-AT	CAATTGTAGAAACTATCCACAAGGACAATATT GTTAACATCTCTGATAGGTGTTCTGTTATAA	80	86
pTW423-GC	CAATTGTAGAGACGATCCACAAGGACAATATT GTTAACATCTCTGCTAGGTGTTCTGTTATAA	66	84
pTW423-36	CAATTGTAGAGACTATCCACAAGGACAATATT GTTAACATCTCTGATAGGTGTTCTGTTATAA	97	90
pTW423-38	CAATTGTAGAGACTATCCACAAGGACAATATT GTTAACATCTCTGATAGGTGTTCTGTTATAA	85	89
pTW423-310	CAATTGTAGAGACTATCCACAAGGACAATATT GTTAACATCTCTGATAGGTGTTCTGTTATAA	82	88
pTW423-313	CAATTGTAGAGACTATCCACAAGGACAATATT GTTAACATCTCTGATAGGTGTTCTGTTATAA	80	68
pTW423-313/4	CAATTGTAGAGACTATCCACAAGGACAATATT GTTAACAA GGTGTTCTGTTATAA	83	91
pTW423-313/9	CAATTGTAGAGACTATCCAC CAATATT GTTAACAA GGTGTTCTGTTATAA	79	90
pTW423-317	CAATTGTAGAGACTATCCACAAGGACAATATT GTTAACATCTCTGATAGGTGTTCTGTTATAA	89	74
pTW423-322	CAATTGTAGAGACTATCCACAAGGA CAATATT GTTAACATCTCTGATAGGTGTTCTGTTATAA	85	75
pTW423-51	CAATTGTAGAGACTATCCACAAGGACAATATT GTTAACATCTCTGATAGGTGTTCTGTTATAA	99	81
pTW423-52	CAATTGTAGAGACTATCCACAAGGACAATATT GTTAACATCTCTGATAGGTGTTCTGTTATAA	93	63
pTW423-54	CAATTGTAGAGACTATCCACAAGGACAATATT GTTAACATCTCTGATAGGTGTTCTGTTATAA	93	88
pTW423-56	CAATTGTAGAGACTATCCACAAGGACAATATT GTTAACATCTCTGATA GGTGTTCTGTTATAA	79	88
pTW423-58	CAATTGTAGAGACTATCCACAAGGACAATATT GTTAACATCTCTGATAGGTGTTCTGTTATAA	84	84
pTW423-510	CAATTGTAGAGACTATCCACAAGGACAATATT GTTAACATCTCTGATAGGTGTTCTGTTATAA	79	88
pTW423-513	CAATTGTAGAGACTATCCACAAGGACAATATT GTTAACATCTCTGATAGGTGTTCTGTTATAA	63	79
pTW423-513F	$\begin{array}{c} \text{A}_G \text{A} \\ \text{CAATTGTAGAGACTATCCACAAGGACAATATT} \\ \text{GTTAACATCTCTGATA} \text{GGTGTGTTCTGTTATAA} \\ \text{G}_{\text{TG}} \\ \updownarrow \\ \text{A}_G \text{A} \\ \text{CAATTGTAGAGACTATCCACAAGGACAATATT} \\ \text{GTTAACATCTCTGATAGGTGTTCTGTTATAA} \\ \text{GG} \text{T}^G \end{array}$	99	81
pTW423-517	CAATTGTAGAGACTATCCACAAGGACAATATT GTTAACATCTCTGATAGGTGTTCTGTTATAA	66	73
pTW423-522	CAATTGTAGAGACTATCCACAAGGACAATATT GTTAACATCTCTGATAGGTGTTCTGTTATAA	61	80

Supplemental Figure 1. Structure and ligation efficiency of oligonucleotide-modified plasmids. Primer extension was used to measure the ligation efficiency of each oligonucleotide-modified plasmid.

