Troubleshooting Guide for Cloning

Problem	Cause	Solution
Few or no transformants	Incomplete Cleavage	● Confirm cleavage of the vector on an agarose gel (BP160). If cleavage isn't complete, repeat digestion or gel purify linear vector. ● Redesign primers (suggest at least 6 n.t. upstream of the recognition site)
	Incompatible Ends	● Confirm the presence of the recognition sites in the vector, inserts and primers.
	Missing Phosphate Group	● Vector and insert should have a 5'-phosphate for ligation. If no phosphate is present, use T4 Polynucleotide Kinase to phosphorylate vector and insert (BP8098).
	Inefficient Ligation	● Vary the molar ratio of vector to insert (1:1 to 1:15 vector:insert). ● Repeat the ligation reaction with fresh buffer. ● Heat inactivation of T4 DNA Ligase (BP8099) may help increase transformation efficiency. ● Total DNA concentration should be less than 10ng/µl to limit concatamer formation. ● Purify the DNA (NaCl, EDTA, and other contaminants may effect ligation efficiency). ● Confirm ends of the vector are not damaged: Perform a control ligation of a single cut, dephorylated vector that's incubated with T4 Polynucleotide Kinase.
	Insert toxicity to cell	● Use T4 DNA Polymerase (BP8105) or Klenow (BP8106) to blunt vector and/or insert.
	Ends not blunted correctly	● Try using a different competent cell line for transformation (*Transmax* * competent cells, BP4100)
	DNA damaged during purification	● Analyze agarose gels using longwave UV (360nM) to minimize UV exposure
Transformants contain no insert	Vector Self Ligation	● Dephosphorylate the vector DNA using Alkaline Phosphatase (BP8097)
	Incomplete cleavage of vector	● Check for self-ligation of vector. If incomplete cleavage, repeat digestion and re-purify linear vector.
Wrong inserts/constructs in transformants	Used wrong amplicon	● Gel purify the PCR product
	Non-specific amplicons	● Gel purify the PCR product
	Sequence error in amplicon	● Amplify the PCR fragment using a high-fidelity Taq Polymerase
	Incorrect primer use	● Confirm the primer sequence
	Insert toxicity to cell	● Try using a different competent cell line for transformation (*Transmax* * competent cells, BP4100)
No plasmid in transformants	Low antibiotics level	● Increase the amount of antibiotic in the agar (BP1423)
	Used satellite colonies	● Pick larger, well established colonies for amplification in LB broth (BP1426)