

Table 1

| | Number of Colonies | Background Reduction (%) |
|---------------------------|--------------------|--------------------------|
| <u>5' Overhang</u> | | |
| EcoR I-cut | | |
| pUC 19 vector | | |
| Control | 583 | |
| 1 μ l SuperSAP | 5 | 99% |
| <u>3' Overhang</u> | | |
| Pst I-cut | | |
| pUC 19 vector | | |
| Control | 1987 | |
| 1 μ l SuperSAP | 0 | 100% |
| <u>Blunt End</u> | | |
| Hinc II-cut | | |
| pUC 19 vector | | |
| Control | 490 | |
| 1 μ l SuperSAP | 2 | 99.6% |

Rapid SuperSAP™ dephosphorylation for 5 min prevents recirculation of vector. pUC19 vector was digested as indicated, purified using a spin column and re-suspended in 10mM Tris-HCl, pH 8.5. 5 μ g was treated with 1 μ l of SuperSAP and incubated for 5 min at 37°C followed by heat-inactivation at 65°C for 15 min. 50 ng was self-ligated directly using the USB Ligate-IT Rapid Ligation Kit (PN 78400), 2.5 ng was transformed into *E. coli* DH5- α and 0.5 ng was plated on selective medium.