

Cloning Integration Sites From Dicty Genomes Following REMI Mutagenesis

1. Prepare genomic DNA from REMI strains (CsCl purification is essential).
2. Following Southern Blot analysis of genomic DNA from the REMI strain (using the mutagenic plasmid as a probe), select several restriction enzymes that give fragment sizes that are about 1-8 Kb larger than the mutagenic plasmid (up to 12Kb total) and produce cohesive ends.
3. Digest 10 μ g genomic DNA with the chosen restriction enzyme: 30 μ l final volume, 3 μ l (30U) enzyme, incubate overnight.
4. Add 70 μ l TE, extract once with phenol and once with chloroform. Back extract with 100 μ l TE, the final volume of the pooled aqueous phases will be 200 μ l. Add 20 μ l 3M NaOAc pH5.2 and 440 μ l Ethanol, mix and spin at top speed in eppi test tubes. Wash the pellet once with 70% ethanol, resuspend in 20 μ l DDW. Allow the DNA to dissolve for several hours.
5. combine 5 μ l DDW, 10 μ l DNA, 4 μ l ligase buffer X5 (BRL) and 1 μ l ligase (BRL). Incubate at 37°C for 2h.
6. Use 3 μ l for electroporation of highly competent E. coli SURE cells, plate each transformation reaction on one LB+carb (amp) plate and grow at 37°C overnight. If necessary (i.e. for strains that contain additional plasmids), perform filter lifting and colony hybridization with a probe for the BSR gene.