

Protocol for making markerless insertions into the *M. maripaludis* chromosome

1. Clone your gene into the *AscI* site of pBIPrt.
2. Transform your construct into the appropriate *M.maripaludis* strain using standard transformation protocol.
3. Take 0.2 ml of your 4 hour or o/n out-growth and, instead of plating, inoculate into McCas tube with Neo and grow 2 days.
4. Inoculate 0.05 mls into another McCas + Neo tube and grow o/n.
5. Take o/n growth and inoculate 0.05ml into McCas only tube and grow o/n.
6. Take o/n growth and inoculate 0.05 ml into McCas + 0.25 ug/ml 8-azahypoxanthine and 0.25 ug/ml 6-azauracil and grow o/n.
7. Now streak out o/n culture onto a plate and grow 2-3 days. This should be your strain.

\* NOTE: IT IS IMPERATIVE YOU USE CASAMINO ACIDS INSTEAD OF YEAST EXTRACT.