# CHEF GEL WITH EMBEDDED YEAST DNA

1) Inoculate a single colony into 50 ml YPD. Grow in shaker to an $OD_{600}$ of > 1.0 at $30^{o}$ C.

2) Turn on $50^{o}$ C water bath. When the desired OD is reached, centrifuge the cells at 5,000 x g, 10 minutes at $4^{o}$ C. Decant the supernatant and resuspend the pellet in 10 ml cold 50 mM EDTA pH 8.0.

3) Determine the cell concentration by direct cell counts. 1:100 dilution usually works. You want 6 x $10^{8}$ cells/ml of agarose plugs (3 x $10^{8}$ cells per 0.5 ml plugs).

4) Microwave the 2% CleanCut agarose solution to melt, equilibrate to $50^{o}$ C.

5) Remove appropriate number of cells for amount of plugs desired. Resuspend the cells in 500 µl (250 µl) of cell suspension buffer and bring suspension to $50^{o}$ C. (Numbers in parentheses refers to the amount for 0.5 µl of plugs).

6) Just prior to mixing cells with agarose add 50 µl (25 µl) of 20 mg/ml Lyticase and proceed immediately to next step.

7) Combine warmed cell suspension with 500 µl (250 µl) of 2% CleanCut agarose and mix gently but thoroughly. Transfer the mixture to plug molds. Allow the agarose to solidify- 15 minutes at $4^{o}$ C.

8) In a Falcon tube add 5 ml (2.5 ml) of lyticase buffer per strain/plugs. Push the solidified plugs, using the provided tool, into the Falcon tube with lyticase buffer. Incubate the plugs 1 hour at $37^{o}$ C without agitation.

9) Remove the lyticase buffer and rinse the plugs with $ddH_2O$. Remove $ddH_2O$ completely and add 5 ml (2.5 ml) of Proteinase K reaction buffer. Incubate the plugs overnight at $50^{o}$ C without agitation.

10) Wash the plugs 4x in 30-50 ml of wash buffer, 1 hour per wash. Wash at room temperature with gentle agitation. Wash with 1 mM PMSF during the second or third wash if the plugs are to be used in subsequent enzymatic reactions.

11) Store the plugs at 4o C. The plugs are stable for 3 months to 1 year. For long term storage, store in wash buffer.

Cell Suspension Buffer
10 mM Tris, pH 7.2
20 mM NaCl
50 mM EDTA

Proteinase K Reaction Buffer
100 mM EDTA, pH 8.0
0.2% Sodium deoxycholate
1% Sodium lauryl sarcosine
1 mg/ml Proteinase K

Lyticase Buffer
10 mM Tris, pH 7.2
50 mM EDTA
1 mg/ml lyticase

1X Wash Buffer
20 mM Tris, pH 8.0
50 mM EDTA