Yeast Genomic Library

Concept:

Genomic DNA – Sau3AI partial digestion
Vector DNA – BamHI full digestion partial

Ligate and transform above products

Vector Information:

- use centromeric plasmid to avoid cross-supressing clones
- selectable marker (amp/ura)
- unique BamHI site preferably in the middle of the mcs

Preparing Vector:

1) digest 3-4ug of library vector with BamHI for 2-4hrs in a total volume of 20ul. (note, the digestion can go overnight if needed to achieve complete digestion – check by gel purification)
2) Dilute sample to 45ul with sterile, molecular biology grade water and add 5ul of 10X CIP Buffer and 0.5-1ul CIP
3) Incubate at 37°C for 20min.
4) Stop the reaction by Phenol/Chloroform extraction
   - add equal volume of PCI to each tube
   - vortex and spin, max speed for 5min at room temp
   - transfer aqueous phase to new, sterile 1.5 ml tube
   - add equal volume chloroform to tube
   - vortex and spin, max speed for 5 min at room temp
   - transfer aqueous phase equally to 4 separate tubes
   - add 0.1 volume of sodium acetate and 2.5 volumes of 100% ETOH to each tube
   - mix by inverting the tube a few times and precipitate the DNA by keeping the tubes on dry ice (or in -80C freezer) until frozen solid. Alternatively, ppt overnight at -20C)
   - thaw tubes on ice
   - centrifuge at max speed for 20 minutes at 4C
   - discard supernatant and wash pellet 1x with 70% ETOH
   - spin at max speed for 5 min at room temperature and carefully remove all supernatant (by hand, not vacuum)
   - air dry pellets at room temp for ~30 min
   - resuspend each pellet in 50ul of sterile, molecular biology grade water

Preparing Fragmented Yeast Genomic DNA

1) Grow 250mls yeast in YPD O/N to saturation
2) Spin and resuspend in SE buffer (0.9M sorbitol, 0.1M EDTA pH 8.0)
3) Add 10ul BME
4) Add 2.5ml of 2mg/ml Zymolase 100K in SE buffer (ICN)
5) Incubate 45min at 37C until spheroplasts are formed
6) Spin 5 min, 5000 rpm
7) Resuspend in 20mls TE
8) Add 4.5ml TSE solution and mix (1.2ml of 2M Tris pH 7.6, 1.2ml 10% SDS, 3ml 0.5M EDTA pH 8.0)
9) Incubate 30min at 65C
10) Add 4mls of 5M potassium acetate
11) Put on ice for at least 60min
12) Spin 20min 10,000rpm
13) ppt supernatant with 2x vol. ETOH
14) Spin 5min 8,000rpm
15) Rinse 70% ETOH
16) Resuspend O/N in 12.5ml TE

Day 2:

1) Spin 5min 8,000rpm
2) Transfer supernatant and add 65ul 10mg/ml RNase A
3) Incubate 30min at 37C
4) Add 13ml 2-propanol, mix

5) Spin F/S and rinse pellet with 70% ETOH
6) Air dry
7) Resuspend O/N in TE at ~1mg/ml

Day 3: partial digestion of genomic DNA with Sau3AI
Reaction mix:
- 100ug DNA
- 120ul 10X Sau3AI buffer
- 30ul of (X) units/ml of Sau3AI restriction enzyme where (X) is the appropriate diluted amount of enzyme
- add water to a final volume of 1.2 mls
- digest genomic DNA with appropriate dilution for 1hr at 37C (NOTE: do not bother with small fragments unless you want a control. Those fragments will not be large enough to carry a gene but are a good size to test for vector/insert ligation and electroporation)

Values for (X) (as determined by gel electrophoresis following 1hr digestion of genomic DNA):
Large fragments: 1 unit enzyme/250ul water
Medium fragments: 1 unit enzyme/100ul water
Small fragments: 1 unit enzyme/50ul water

Electroelution of DNA fragments:
- following digestion, split reaction into two tubes and extract DNA by phenol/chloroform as described above (step 4 in "preparing vector")
- resuspend DNA in 50ul of water and mix with 5ul of orange loading dye
- run each sample on a fresh small 1% agarose gel (load a ladder in the far outside lanes)
- excise the bands of interest, large and medium fragments
- load the gel pieces into dialysis tubing and clamp at each end
- place the dialysis tubing into an agarose gel chamber and run at 100V for 1hr to run the DNA pieces out of the gel but into the dialysis tubing
- remove the liquid from the dialysis tubing and ETOH ppt as described above (at the end of step 4 in "preparing the vector")
- air dry pellets and resuspend in 50ul of sterile water

Ligation of digested genomic DNA to the Digested Vector DNA:
Library Ligation:
Reaction mix:
12ul genomic DNA fragments, size specific (300-500ng/ul)
10ul cut vector (100ng/ul)
2.5ul 10x T4 ligase buffer
1ul T4 ligase
sterile water to 25ul
incubate at 16C overnight (at least 16hrs)

Electroporate into electrocompetent e-coli (using Jones lab electroporator) and plate – 1 ligation reaction onto 15 plates