# Vectorette PCR of Yeast DNA

**Carl Friddle**

1) Cut 1-3 µg of clean DNA overnight with 8-10U of blunt cutting enzyme in 20µl

Most problems come from dirty, uncut DNA. Phenol/glass bead/RNase prepared DNA works well

RsaI, AluI and DraI provide good results.

2) Heat inactivate enzyme

3) Add:

- 3µl 10x NEBuffer used in digest
- 1µl annealed anchor bubble
- 1µl (400U) ligase
- 0.5µl of 5mM ATP (50µM ATP final)
- 25.5µl Water

4) Incubate at 16 C for 9-24 hours.

5) Use 5µl in 100µl PCR. Perkin Elmer Ampliwax is recommended for hot start.

- 5 µl of ligation
- 2.5 µl of 20µM specific primer [M13(-47) for mTn3 library]
- 2.5 µl of 20µM 224 primer
- 8 µl of 2.5 mM dNTPs
- 10 µl of Taq PCR buffer
- 71µl Water
- 1µl Taq DNA polymerase (5U)
- Transfer to Perkin Elmer 9600 Thermal Cycler
    - Denature 92C, 2 minutes
    - 35 Cycles [92C, 20sec; 67C, 30sec; 72C, 45-180sec (>1 min/1 kb)]
    - 72C, 90sec

6) Gel purify 80 µl of PCR product in 1-3% SeaKem GTG, extract with Qiaex (Qiagen), elute with 12 µl of ddWater

7) Sequence 7 µl with Sequenase kit from Amersham.

Use 1 µl of 200-600µM specific primer [M13(-47) for mTn3]. Undiluted 10 OD synthesis from Genset works well.

Use high specific activity S-35 (>1000 Ci/mmol, Amersham AG1000)

Boil 10' and fast cool in ice water.

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**Anchor Bubble primers**

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3'   GAGAGGGAAGAGAGCAGGCAAGGAATGGAAGCTGTCTGTCGCAGGAGAGGAAG 5'
     ||||||||||||   ||    || | | | ||| |  |   ||||||||||||
5' GACTCTCCCTTCTCGAATCGTAACCGTTCGTACGAGAATCGCTGTCCTCTCCTTC  3'


PRIMER 224   5' CGAATCGTAACCGTTCGTACGAGAATCGCT 3'
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To anneal bubble primers, heat a 2-4µM (in ddWater) to 65 C for 5 minutes, then add $MgCl_2$ to 1-2 mM and allow to cool to room temperature.

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**References:**


- Riley et al, Nucleic Acids Research 18: 2887-2890, 1990
- Foote et al, Science 258: 60-66, 1992
- Burns et al, Genes Dev. 8(9): 1087-1105, 1994
- Vollrath, Large DNA Course, Cold Spring Harbor Laboratory, 1995
- Transposon Library Web Site


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