Effect of template DNA concentration on PCR

1. Prepare 10 ng/µl, 1 ng/µl, 0.1 ng/µl and 0.01 ng/µl dilutions from your 2 DNA samples

2. Label 9 PCR tubes as follows: 10 O, 10 F, 1 O, 1F, 0.1 O, 0.1 F, 0.01 O. 0.01 F, 0.

3..Add 18 µl of PCR master mix to each tube

4.Add 2 µl of DNA to designated tubes, or 2 µl water to the “0” tubes

5.Mix, then run 3’ @ 94˚ C, then 30 cycles of 15" @ 94°, 50” – 1”/cycle @ 50°, 15" @ 72°

6.Add 4 µl EZ vision-2 loading dye to each tube, then load 10 µl on 2% agarose gel (melt 0.6 g agarose in 30 mls bionic buffer). Load 10 µl Hyperladder V in outside lane

7. Run 5’ @ 80 volts, then increase to 150 volts until dye is 2/3 way down gel.

Take picture of gel.

Corn primers =, R-ZMSODCC. ZMSODCC - L

Corn master mix

• 40 µl 5 x PCR buffer

• 2 µl 100 pmol/µl R-ZMSODCC (1 pmol/µl final)

• 2. µl 100 pmol/µl ZMSODCC - L (1 pmol/µl final)

• 4 µl 10 mM dNTP (200 µM final)

• 1 µl one*Taq* DNA polymerase

•131 µl water

Tobacco primers = R- Tobacco NcoI, Tobacco NcoI-L

Tobacco Master mix

• 40 µl 5 x PCR buffer

• 2 µl 100 pmol/µl R- Tobacco NcoI, (1 pmol/µl final)

• 2 µl 100 pmol/µl Tobacco NcoI-L (1 pmol/µl final)

• 4 µl 10 mM dNTP (200 µM final)

• 1 µl one*Taq* DNA polymerase

• 131 µl water