STEPS OF CLONING

Exercise 6: to extract generic DNA from the Columbia wild-type strain of Arabidopsis thaliana

Before extraction

After extraction

Exercise 6: to confirm the amplification of the target region
- to clone the amplified DNA into a TEV vector
- to create recombinant plasmids
- to transform recombinant plasmids into E. coli cells
- to transform the平板s

Confirmation of amplification

Before cloning

After cloning

Exercise 5: to amplify a small fragment from the 770 gene in order to clone it into a cloning vector

Before amplification

After amplification

Before transformation

After transformation

Propagation

Confirmation of transformation and propagation
Objectives:

a) to confirm that the cloned DNA fragment, digested last week, is indeed the 1.3 kb DNA from Arabidopsis TTG gene

b) to detect genetic similarity between the cloned fragment and a probe

To achieve objective (a): electrophorese the digested DNA fragments

To achieve objective (b): denature the digested DNA fragments on the gel and do the Southern blotting
b) What is Southern blotting?

Southern blotting is a process to transfer DNA fragments from an agarose gel to a solid support such as a nylon membrane, for the purpose of DNA-probe hybridization and detection of genetic similarity between the probe and the DNA of interest.

The transfer is needed because the hybridization and part of the detection process take place at 42° and 65°C, respectively; agarose gel melts at these temperatures.

How does Southern blotting work?

4. Cut the top left corner of the gel; photograph the gel along with a ruler (the 0 mark of the ruler aligned with the well of the gel).

5. Wash the gel in 0.25N HCl for 20 min. DNA is nicked by brief depurination.

6. Rinse the gel in distilled water: not essential!

7. Wash the gel in denaturation solution for 20 min. Note that after this wash the DNA denatures on the gel as illustrated below:

**Procedure**

1. You have tubes W-E, B-E, W-H, and B-H.

2. Add 5 µL of gel loading dye to each tube, mix well, and centrifuge briefly. The total volume should now be 25 µL (20 µL digested reaction + 5 µL loading dye).

3. Load the gel as shown on right, and electrophorese for 30-35 min at 90 volts.
8. Rinse the gel in distilled water; not essential.

9. Wash the gel in neutralization solution for 20 min.

10. Wash the gel in 1xSSC buffer for 5 min.

11. Set up the Southern as illustrated below:

12. Allow the transfer to take place overnight (usually 18 hours).

13. Take down the set up, mark the trace of the gel's wells on the membrane, and immerse the membrane in 1xSSC buffer to remove pieces of agarose sticking to it.

14. While still wet, transfer the membrane to a UV cross-linker and cross-link it. After cross-linking the DNA will stay on the membrane permanently.

15. Air-dry the membrane and store in a refrigerator until the next laboratory session.

Evaluation of Your Performance

DNA bands visible on gel...5