

IPCR Cloning for pSKI015 Activation-tagging Lines

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1. Genomic DNA isolation. We use Nucleon Phytopure Plant DNA Extraction Kit (Amersham Pharmacia). Two to three weeks old Arabidopsis rosette leaves are very good starting material for this purpose.
2. Purify genomic DNA with a commercially available purification kit. We use Genomic DNA purification Kit from Q-BIO gene. This step is very important for getting high quality genomic DNA.
3. Pick up about 100 ng purified genomic DNA and digest with KpnI or XhoI in a 100ul volume at 37 C overnight.
4. After digestion, the restriction enzyme was heat-inactivated at 70C for 5min, and the reaction was purified with a CONCERT Nucleic Acid purification kit. Elute the digested DNA in 80 ul dd H₂O.
5. Set up self-ligation reaction with T4 DNA ligase in a 100ul volume, 16C overnight.

Digested/purified genomic DNA from step 4 ~80 ul

T4 DNA ligase buffer	10 ul
T4 DNA ligase (from Takara)	1ul
<u>ddH₂O</u>	<u>9 ul</u>

100 ul total volume

6. Purify the ligation reaction with CONCERT Nucleic Acid purification kit. And elute the sample to a volume of about 60ul. Use 4ul of the purified self-ligated solution as template for inverse PCR.

5ul 10x PCR buffer
4ul dNTP mix (2.5 mM each)
0.5 ul T3 primer (20uM)
0.5 ul AtRB primer (20 uM)
1.0 ExTaq (from Takara)
4.0 ul self-ligated solution
<u>35.0 ul dd H₂O</u>

50 ul total volume

Annealing temperature is 63C , and we use 2 min extension for the PCR.

T3 Primer: AATTAACCCTCACTAAAGGGAAC

AtRB primer: GATATCTAGATCCGAAACTATCAGTG

7. If you get single band PCR product that means the PCR reaction is very specific and now you are ready to send it for sequencing.

(Any further question, please send email to Huachun Wang at hw00d@mizzou.edu)