SCHNABLE LAB
Plant Genomics

2035C Roy J. Carver Co-Laboratory
Plant Sciences Institute
Iowa State University
Ames, Iowa 50011-3650
515 294-7209
FAX 515 294-5256

DLA for amplifying *Mu* flanking sequence

Digestion-ligation-amplification (DLA), a novel adaptor-mediated PCR-based method that uses a single-stranded oligo as the adaptor, was developed to overcome difficulties of amplifying unknown sequences flanking known DNA sequences in large genomes (LIU *et al.* 2009). If you have questions or comments about this protocol please contact Dr. Schnable at schnable@iastate.edu. The citation for this protocol is Liu et al., 2009 (see below).

1. Digest genomic DNA from maize plants that carry a *Mu*-induced mutant with *Nsp* I (R*CATG^Y)

1,000 ng gDNA
4 µl 10x NEB#2 Buffer
0.4 µl BSA (100mg/ml)
1µl *Nsp* I (NEB, Cat#:R0602L, 10,000U/ml) = 10U
ddH2O to 40 µl

Incubate at 37°C for 1.5hr

2. Ligation using adaptor

Prepare 50uM NspI-5B Adaptor Mix (100ul):

100uM NspI-5B*(oligo)	50ul
10X NEB#2 buffer	5ul
ddH2O	45ul

* see below for primer sequences

After 1.5hrs incubation, add to genomic DNA digestion reaction:

5.0 µl NspI-5B Adaptor Mix (50uM)
3.0 µl ddH2O
1.0 µl 10X Ligase Buffer
1.0 µl T4 ligase (NEB, Cat# M0202L)

Total volume per reaction is now 50 µl. Incubate for 3 hrs at 16°C in the thermocycler (Program "Dig-Lig", see below).

3. Purification – Follow the protocol provided for PCR purification with the Qiaquick PCR purification kit (QIAGEN, Cat# 28106 or Cat# 28104).

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4. (Optional) Blocking using ddNTP

Purified Dig-Lig product	50-200ng
Klenow 10X buffer	5
2mM **ddNTP**	2
Klenow 5U/ul	0.8
ddH2O	Up to 50ul

(See below for the program: Klenow30)

Note: This is an optional step. You can try to use the protocol without this step first. If too much background amplification occurs, repeat with the addition of this step.

5. Purification – Follow the protocol provided for PCR purification with the Qiaquick PCR purification kit (QIAGEN, Cat# 28106 or Cat# 28104).

CAUTION: It's important to fully remove ddNTP. It is therefore suggested to conduct the PE wash step twice.

6. 1st PCR (PCR program: HL60-30, see below.)

GeneAmp 10X PCR Buffer II	2ul
2mM dNTP	2ul
MgCl2 (25mM)	1.2ul
MuTIR primer (5uM)*	1.2ul
NspI-5 primer (5uM)*	1.2ul
Purified DNA (from step 3)	50~100ng
AmpliTaq Gold (5U/ul)**	0.2ul
Water	To 20ul

* see below for primer sequences
** AmpliTaq Gold® DNA Polymerase (Applied Biosystems, Cat#: N808-0241)

7. Nested PCR (PCR program: HL60-35, see below)

GeneAmp 10X PCR Buffer II	3ul
2mM dNTP	4ul
MgCl2 (25mM)	1.8ul
Mu53s* (5uM)	1.5ul
NspI-P* (5uM)	1.5ul
10X diluted 1st PCR product (from step 4)	2ul

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AmpliTaq Gold (5U/ul)	0.2ul
Water	To 30ul

* see below for primer sequences

8. Oligo sequences:

1) MuTIR 32 mer
   5' AGAGAAGCCAACGCCAWCGCCTCYATTTCGTC
2) Mu53s 19 mer
   5' GCCTCYATTTCGTCGAATC
3) NspI-5B 22 mer
   5' CAGAACGTCACAGCATGTCATG
4) NspI-5 20 mer
   5' GAACGTCACAGCATGTCATG
5) NspI-P 19 mer
   5' AACGTCACAGCATGTCATG

9. PCR programs

1) Dig-Lig
   1.5h @ 37°C
   4°C
   3h @ 16°C
   4°C forever
2) Klenow30
   0.5h @ 30°C
   75°C 10min
   4°C forever
3) HL60-30
   94°C 10min
   30 cycles { 94°C 30s; 60°C 45s; 72°C 2.5min }
   72°C 10min
   4°C forever

4) HL60-35
   94°C 10min
   35 cycles { 94°C 30s; 60°C 45s }

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72°C 2.5min
72°C 10min
4°C forever

References

LIU, S., C. R. DIETRICH and P. S. SCHNABLE, 2009 DLA-Based Strategies for Cloning Insertion Mutants: Cloning the *gl4* Locus of Maize using *Mu* Transposon Tagged Alleles. Genetics **in press.**