1

Dec 01, 2021

Genotyping Arabidopsis T-DNA lines V.1

Steven J Burgess[1]

[1]University of Illinois at Urbana-Champaign

1

dx.doi.org/10.17504/protocols.io.b2huqb6w

Burgess Lab UIUC

Steven Burgess
University of Illinois at Urbana-Champaign

Citation: Steven J Burgess Genotyping Arabidopsis T-DNA lines https://dx.doi.org/10.17504/protocols.io.b2huqb6w

This protocol is used for genotyping Arabidopsis seedlings to test for the presence of a transfer DNA (T-DNA) insertion. By using two primer sets it is possible to determine whether a seedling is homozygous, heterozygous or azygous for an insertion in the predicted genomic location.

To identify lines with T-DNA insertions in a gene of interest, you need the Arabidopsis Genome Identifier (AGI) number corresponding to the genomic locus (e.g. RCS1A = AT1G67090), then visit the Salk Institute T-DNA Express site to find all the mapped insertions at your locus of interest.

Genotyping primers have been pre-designed for each T-DNA line, these can be retrieved from the Salk Institute T-DNA primer site, and ordered at any supplier of DNA oligonucleotides before starting the protocol.

In the US T-DNA lines can be purchased from the Arabidopsis Biological Resource Center (ABRC) and in the UK and EU from the European Arabidopsis Stock Center (NASC).

Recommended reading

- http://signal.salk.edu/tdnaprimers.2.html

Setting up the PCR reaction

Genotyping is performed with the Phire Direct PCR Mix, this includes the polymerase, nucleotides and salts necessary for amplification. We use the “dilution protocol” which involves taking a small leaf disk and homogenizing it in dilution buffer using a gel tip (see manufacturer’s instructions for more details.)

DOI

dx.doi.org/10.17504/protocols.io.b2huqb6w

Steven J Burgess 2021. Genotyping Arabidopsis T-DNA lines. **protocols.io** https://dx.doi.org/10.17504/protocols.io.b2huqb6w

protocol

DOI: 10.1126/science.1086391

Arabidopsis, Genotyping

protocol ,

Dec 01, 2021

Dec 01, 2021

55572

- Phire Direct Master Mix (Thermo Fisher Scientific; F170S)
- Genotyping primers (LBb1.3 ATT TTG CCG ATT TCG GAA C)
- Swiss Line Core Sampling Tool 0.5 mm, 0.50 mm I.D., 0.80 mm O.D. (Fisher Scientific; NC1310089) /(Electron Microscopy Sciences; 69039-05)
- 100bp ladder (New England Biolabs; N0467S)
- 10x Tris-Acetate-EDTA (TAE) Buffer (see Sigma website for recipe)
- 10,000x SYBR™ Safe DNA stain (Thermo Fisher Scientific; S33102)
- 6x Gel Loading Dye (New England Biolabs; B7024S)

:

Prepare primer working solution

1 Re-suspend lyophilized primers in dH_2O to a stock concentration of 100 mM. Note: primer sequences can be obtained from SALK T-DNA express if you have the T-DNA accession number (http://signal.salk.edu/tdnaprimers.2.html)

2 Create a 10 mM working solution of the primer by diluting 10 µL of the stock into 90 µL of water.

Preparing the PCR template

3 Using a 0.5 mm sampling device take a single leaf punch and add to 20 µL of dilution buffer.

4 Mash the sample with the end of a pipette tip

Citation: Steven J Burgess Genotyping Arabidopsis T-DNA lines https://dx.doi.org/10.17504/protocols.io.b2huqb6w

5 Incubate sample at room temperature for 5 mins

6 Spin down sample for 1 min max speed in a mini centrifuge

Setting up the PCR reaction

7 Add the following components to a PCR tube. Set up one reaction with the diluted template from the putative T-DNA insertion line and one with WT arabidopsis as a negative control. The reaction will result in amplification if the T-DNA insert is present.

A	B
Component	**Amount**
2x Phire Master Mix	10 µL
Diluted template	1 µL
LBb1.3 primer [10 mM]	1 µL
RP [10 mM]	1 µL
dH2O	7 µL

Note: LBb1.3 (ATTTTGCCGATTTCGGAAC) is the recommended sequencing primer for SALK lines. If you have SAIL, Wisc_Lox or GABI-KAT lines you will need to use a different border primer. See the SALK website for details http://signal.salk.edu/tdnaprimers.2.html

8 In a second PCR tube set up the following reaction. Set up one reaction for the putative T-DNA insertion line and one for WT arabidopsis as a positive control. This reaction tests for the absence of a TDNA insertion. If a T-DNA insertion is present there will be no amplification product as the distance between the L and R primers. The WT control should give a product, and heterozygous lines will also give a product as there is one WT allele.

A	B
Component	**Amount**
2x Phire Master Mix	10 µL
Diluted template	0.5 µL
LP [10 mM]	1 µL
RP [10 mM]	1 µL
dH2O	7.5 µL

Citation: Steven J Burgess Genotyping Arabidopsis T-DNA lines https://dx.doi.org/10.17504/protocols.io.b2huqb6w

9 Run the following PCR program

A	B	C	D
Step	**Temperature**	**Time**	**Cycles**
Initial denaturation	98	5 min	1
Denaturation	98	5s	40
Annealing	*	5s	
Extension	72	20s	
Final Extension	72	1 min	1

*indicates annealing temperature to be adjusted to primer

10 Analyze the resulting products by gel electrophoresis

Citation: Steven J Burgess Genotyping Arabidopsis T-DNA lines https://dx.doi.org/10.17504/protocols.io.b2huqb6w