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Generation of *Arabidopsis* transposon lines

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Abstract

Positive and negative selection for unlinked transposition has been used to generate a large collection of *Arabidopsis* transposon lines.

Significance and context

Insertional mutagens such as T-DNAs (from *Agrobacterium tumefaciens*) and transposons are powerful tools for both 'forward' and 'reverse' genetic screens in *Arabidopsis*. Whereas the forward genetic approach starts with genes identified by mutation, reverse genetics involves screening for mutations in a given DNA sequence. Large collections of T-DNA insertion lines exist, but these often harbor complex and/or multiple insertions. Transposons offer the advantage that excision can cause reversion of the mutant phenotype or create new mutant alleles. Transposons, however, are unstable in the presence of their transposase and tend to move to linked sites, rendering genome-wide coverage difficult. To select for transposition events further from the insertion site and for loss of the transposase after transposon reinsertion, an elegant technique based on positive and negative selection has been designed by Rob Martienssen's group. The study by Tissier *et al*. presents a different version of positive/ negative selection that can be used for large-scale mutagenesis. A large library of lines with single, stable inserts was tested in reverse genetic screens, and 1,200 insertion sites were sequenced.

Key results

The single T-DNA cassette used for transposon mutagenesis contains the following: a defective *dSpm* (suppressor-mutator) transposon element; a *GUS* reporter gene to monitor excision; two terminators in opposite orientations to block host gene expression after *dSpm* reinsertion; the transposase gene fragment; a *BAR* herbicide-resistance gene within the *dSpm* element to select for T-DNA insertion and transposon reinsertion; and a proherbicide-sensitivity counterselectable marker to select for loss of the T-DNA. Progeny of the primary transformants resistant to both positive and negative selection have undergone transposition and lost the original T-DNA by segregation or recombination. The frequencies of germinal excision (10^{-2}) and of unlinked transposition ($2.5\text{-}10 \times 10^{-4}$) were lower than expected. A total of 48,000 single-insert lines were collected in 960 pools of 50; 1,200 insertion sites were then amplified by PCR and sequenced. BLAST searches showed that the sequences appear to be randomly distributed throughout the genome and that 70% fall within coding regions. Reverse genetic screens

were carried out on 29,000 lines by PCR and inverse display of insertions, and 25 insertions were found for 41 genes searched. Overall, the collection most probably represents about 38,000 independent insertions.

Links

The insertion sequences have been deposited in the Sequenced Insertion Sites database (SINS). The database can be browsed in annotated form or subjected to keyword search. The set of sequences can also be downloaded for BLAST searches.

Reporter's comments

The experimental design ensures that single, independent, stable transposon inserts are introduced throughout the *Arabidopsis* genome. Although the design is not novel, the constructs described in this paper can readily be used for large-scale mutagenesis. Saturating the *Arabidopsis* genome with mutations, however, will probably require a combination of different mutagens. One disadvantage of single, as opposed to multiple, insert lines is that a larger number of lines need to be screened in order to score a gene of interest in both forward and reverse genetic screens. The advantage, however, is that the interpretation of knockout phenotypes is unambiguous. The performance of this library in reverse genetic screens is promising, with a higher percentage of insertions in coding sequences than is observed with T-DNA libraries. One of the most promising aspects of this study is the establishment of a database of 1,200 insertion site sequences, the first of its kind. Larger bodies of similar data would enable reverse genetics to be carried out *in silico* eliminating the need for tedious molecular screens. Generating such databases is labor intensive, however, and should be a priority of the *Arabidopsis* community as a whole.

Table of links

Plant Cell

Sequenced Insertion Sites database

References

1. Tissier AF, Marillonet S, Klimyuk V, Patel K, Torres MA, Murphy G, Jones J: Multiple independent defective suppressor-mutator transposon insertions in *Arabidopsis*: a tool for functional genomics. Plant Cell. 1999, 11: 1841-1852. 1040-4651