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Successful PCR-based reverse genetic screens using an *En-1*-mutagenised *Arabidopsis thaliana* population generated via single-seed descent

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Abstract The development of an Arabidopsis population via single-seed descent is described which includes 3,000 lines that carry approximately 15,000 independent insertions of the autonomous maize element *En-1*. A PCR strategy is outlined which allows the recovery of *En-1*-insertion mutants among this population in any random gene sequence of *Arabidopsis thaliana*. The method employs PCR reactions on pooled DNA. Positive amplification using a target-specific primer and an *En-1*-specific primer on row, column and single-tray pools identifies the putative insertion mutant. In a control experiment two insertion mutants of the *PIN* gene were successfully identified. In addition, a new independent insertion in the *PIN* gene was detected which was transmitted to the next generation and showed cosegregation with the *pin* phenotype. These examples demonstrate that the inheritance of inserts of the autonomous element *En-1* is stable enough to make a proper genetic analysis feasible in a genomic background with multiple *En-1* inserts.

Key words Arabidopsis · *En-1* · Reverse genetics · *PIN* · Transposable element

Introduction

Arabidopsis thaliana has become a model system in plant science because of its small size, its short life cycle and the ease with which transgenic plants can be obtained (Meyerowitz 1987). However, while world-wide mapping and DNA-sequencing efforts will soon decipher the entire genome of this plant, the function of many of the gene sequences being defined in this genome project remains unclear. Such information might come from the analysis of mutants. Mutant phenotypes can be studied in plants in which the function of the gene is abolished. Hence, a technique "reverse genetic screening" has been developed which allows the identification of insertion mutations in any gene whose sequence is available. This method has been successfully employed in Drosophila and Caenorhabditis populations saturated with transposable element insertions (Ballinger and Benzer 1989; Kaiser and Goodwin 1990; Rushford et al. 1993; Zwaal et al. 1993). In petunia and maize transposable element-mutagenised populations, knock-out mutants have also been successfully identified using this strategy (Koes et al. 1995; Mena et al. 1996). As a first step, the method exploits DNA insertions to create random mutations throughout the genome. In the second step, polymerase chain reaction (PCR) screens identify the particular insertion mutant. A primer, specific for the gene of interest, is used in combination with primers for the end of the transposable element. Amplification follows only if there is an insert actually within the gene of interest or within a short distance of it. The efficiency of these screens can be increased if pools of mutagenised plants are screened rather than individual plants. Subsequently, individual plants have to be tested with PCR to identify the putative insertion mutant. The method allows the recovery of mutant alleles for any gene of interest in a relatively short time.

For reverse genetic technology to be established in Arabidopsis, the development of a population saturated with insertions is highly desirable. In Arabidopsis, however, an endogenous transposable element with high activity is not available for insertion mutagenesis.

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Therefore, the integration of T-DNAs via *Agrobacterium tumefaciens* transformation has been employed as an alternative system for insertion mutagenesis (Feldmann 1991; Koncz et al. 1992; Azpiroz-Leehan and Feldmann 1997). Using reverse genetic techniques McKinney et al. (1995) identified two null mutants in two different actin genes, ACT2 and ACT4, in a T-DNA population of plants described in Forsthoefel et al. (1992). Moreover, an insertion was detected in 25% of the genes of interest tested (Krysan et al. 1996) in a collection of 9,100 independent Arabidopsis T-DNA transformants described in Feldmann (1991) and Forsthoefel et al. (1992).

The maize transposable elements *Ac*/*Ds* and *En*/*Spm* have been introduced into Arabidopsis in order to generate insertions (Kunze et al. 1997). The advantage in the use of transposable elements in 'reverse genetic screens' is their ability to transpose. Excision of the transposable element from the gene of interest often restores gene function, which leads to a wild-type phenotype. The isolation of wild-type revertants can be used as direct proof for the identity of the gene. Furthermore, the tendency of *En*/*Spm* to transpose to linked sites can be used for targeted insertion in the ORF or in specific domains of the gene whose function is studied, once an insertion is identified in the proximity of the gene. Local transposition has been shown for *En*/*Spm* in maize, tobacco and Arabidopsis (Peterson 1970; Novic and Peterson 1981; Cardon et al. 1993a, b; Aarts et al. 1995). To establish the reverse genetic technology in Arabidopsis, we have generated a population of 3,000 lines, which carry approximately 15,000 independent insertions of the autonomous maize transposable element *En-1*. Primers specific for *En-1* were designed and PCR protocols developed which enable the identification of single *En-1*-insertion mutants.

Materials and methods

Plant material

After the introduction of *En-1* into the ecotype Columbia (Cardon et al. 1993a), five descendants of the primary transformant were selected which carried 1–3 transposed copies of *En-1* but no T-DNA (designated G11, G24, G37, G48 and G69). One hundred progeny derived from each of the five plants were propagated by single-seed descent (SSD) (Wisman et al. 1998). After 6 generations, 24 lines were identified that carried 6–15 independent *En-1* insertions. Of these 24 lines, 6 were derived from G11, 2 from G24, 6 from G37, 6 from G48 and 4 from G69. The *En-1*-mutagenised population consisted of 100 progeny from each of the 24 lines, 400 progeny of the original SSD lines in generation 6 and 200 F_6 descendants derived from crosses between G11, G24, G37, G48 and G69. These 3,000 lines were propagated for 5 additional SSD generations. In generation 4 Southern hybridisations were performed to estimate the average number of independent *En-1* insertions. In generation 5, leaf material and seeds were sampled from all lines.

Pooling strategy

To reduce the PCR screening effort and provide extra controls for the identification of insertion mutants, we used a three-dimension multiplex pooling strategy to arrange the plant material for DNA preparation. Approximately 3,000 plants were grown in 85 trays of 35 plants each (Fig. 2B). Each of the four different pools of DNA consisted of one of four single leaves sampled from opposite parts of the rosette of each plant. One leaf went into the pool representing the single trays (1–85), two other leaves were sampled according to rows (1–7) and columns (A–E) over sub-populations of 18 trays while the fourth pool was comprised from single leaves from each of the plants growing in three consecutive trays. Due to the number of trays, one 4-tray pool was sampled. Thus, a single tray pool represented 35 plants; a 3-tray pool, 105 plants; a row pool, 90 plants; and a column pool, 126 plants. The pooling strategy allows not only the identification of each plant by its tray, column and row number but also avoids the identification of *En-1* inserts that are not recovered in the progeny. The loss of prior identified insertions in subsequent generations might occur due to the somatic activity of the autonomous element. When somatic sectors carrying a transposed *En-1* element do not contribute to the reproductive tissue, the inserts will not be transmitted to the next generation. However, because a positive screen included the detection of the insert in four leaves taken from different parts of the rosette, the chance of hitting a small somatic insertion was greatly reduced, and the chance of recovering the insert in the progeny was large.

Genomic DNA isolation

The pooled leaf material was first ground in liquid nitrogen to ensure homogenisation and thorough mixing and then used for DNA isolation. DNA was prepared following the procedure of Rodger and Bendich (1988) with some modifications. A 7.5-ml aliquot of extraction buffer (2% CTAB, 100 mM TRIS (pH 8), 20 m*M* EDTA (pH 8), 1.4 *M* NaCl and 1% polyvinylpyrrolidone Mr (40,000) was added to 0.5 g of frozen leaf powder and then incubated at 65°C for 20 min. The solution was extracted with an equal volume of chloroform and precipitated with 0.7-volume iso-propanol. The DNA pellet was resuspended in 1 ml TE (10 m*M* TRIS/1 m*M* EDTA, pH 8) and treated with 3 µl RNAse (10 mg/ml) for 40 min at 37°C. The solution was then extracted with an equal volume of chloroform/iso-amylalcohol (24 : 1) and again precipitated with 0.7-volume of iso-propanol. The pellet was dissolved in 400 µl TE (10 m*M* TRIS/1 m*M* EDTA, pH 8). For PCR screens the DNA solutions were further diluted to a final concentration of 25 ng/µl.

PCR reactions

The annealing position of the primers is depicted in Fig. 3. The 5′ outward *En-1*-specific primers were En205 (5′-AGA AGC ACG ACG GCT GTA GAA TAG GA-3′) and En91 (5′-TGC AGC AAA ACC CAC ACT TTT ACT TC-3′). The 3′ outward *En-1*-specific primers were En8130 (5′-GAG CGT CGG TCC CCA CAC TTC TAT AC-3′) and En7706 (5′-GCT CCA ATG ACC CAC CAA CAG AAT G-3′). The primers annealing to the *PIN* gene were Pin166 (5′- CCT CGC TTA CGG CTC TGT CAA ATG-3′) and Pin609 (5′ -CCT GCT GTG TCT GGA AAC TGC TCG-3′). The numbers of the primers indicate the 5′ annealing position of the primer. The EMBL Genbank accession number of *En-1* is M25427. Primers were obtained from MWG, Germany. PCR was carried out in 50-µl reaction volume containing 50 ng DNA, 20 pmol of gene-specific primer, 20 pmol of *En-1* specific primer, 50-µ*M* dNTP, 1.25 U *Taq* polymerase (Boehringer Mannheim), 1 × PCR reaction buffer (Boehringer Mannheim). The following PCR conditions were used: an initial step at 85°C for 2 min; then 40 cycles of 94.0°C for 40 s, 65°C

for 1 min, 72°C for 2 min. An elongation step was carried out for 5 min at 72°C. The MJ Research model TETRAD thermal cycler was used.

Southern blotting and hybridisation

The PCR products were separated on a 1.5% agarose gel. As the amplification products cannot always be recognised directly on agarose gels, the gels were blotted onto nylon membranes under alkaline transfer conditions (0.4 *N* NaOH for 2 h). The blots were hybridised with a [^{32}P]-radioactive-labelled probe specific for the gene of interest. Pre-hybridisation was carried out for 2 h at 65°–68°C in a solution containing 3% SDS, 7 × mol SSPE, 200 mg/l PVP, 200 mg/l Ficoll. Hybridisation (1% SDS, 3.5 × SSPE, 200 mg/l PVP, 200 mg/l Ficoll, 5 mg/l herringsperm DNA) was performed for 16 h at 65–68°C using as a probe 25–100 ng of the cDNA of the gene of interest labelled by random priming (108–109 dpm/mg). The filters were washed at a moderate stringency of 2 × SSPE, 0.1% SDS for 1–2 h and 10 min in 0.2 × SSPE, 0.1% SDS, at 65°–68°C. Autoradiography was performed at − 80°C for 4–48 h using Kodak XAR film and an intensifying screen.

Cloning and sequencing of amplified products

PCR products were purified with Qiaquick columns from Qiagen to remove free nucleotides and excess PCR primers. They were then cloned into pGEM T vector (Promega). Cloned inserts were sequenced at the ADIS sequencing unit of the MPIZ with T7 and universal primers using an Applied Biosystems 377 DNA sequencer.

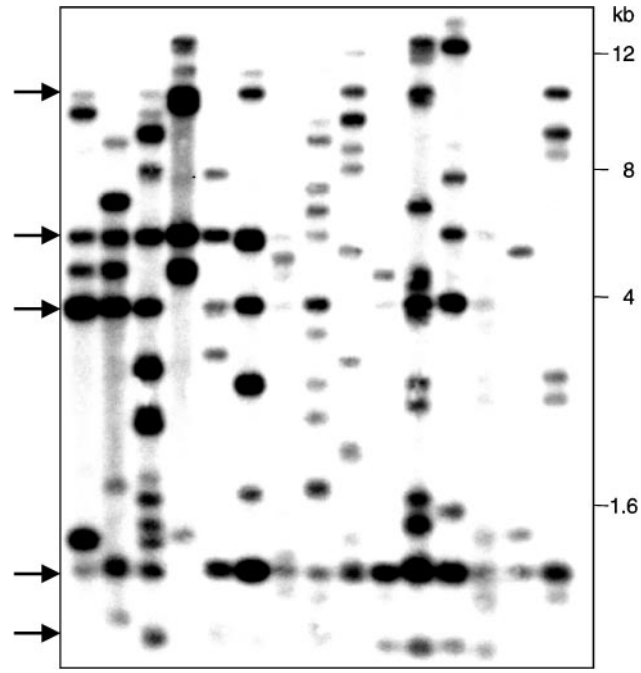

Fig. 1 Southern blot showing the number of independent *En-1* inserts in 15 individual SSD lines of generation 4 which were derived from a single plant carrying 8 copies of *En-1*. DNA isolated from individual SSD lines was digested with *Eco*RV, separated on agarose gels, blotted and hybridised with a probe representing the 3′ end of *En-1*. Each hybridising band represents a single insertion. The *arrows* indicate the *En-1* inserts which were present in the parent. Weak hybridising signals may represent hemizygous inserts or somatic insertion events

Results and discussion

The population

The activity of *En-1* in Arabidopsis can be used to achieve saturation of the genome with insertions. *En-1* transposes via a cut and paste mechanism in which excision from the donor site is regularly followed by reinsertion at other positions in the genome. The continuously changing position of *En-1* makes the development of multiple-copy lines feasible. As a first step, lines that carried 6–15 independent *En-1*-insertions were identified and progeny consisting of 100 plants per line were grown. To generate independent insertions, we chose the single-seed descent method for propagation. In single-seed descent (SSD) one seed is taken from each plant to give rise to the next generation. In the first SSD generation, plants from 1 family exhibited insertion patterns which were quite similar to those of the parental plants. After a few SSD generations, however, the insertion positions changed greatly due to regular excision and re-integration of the element. As shown in Fig. 1, many new bands appeared in the descendants after 4 generations of SSD, whereas three out of eight parental bands were not found again. In addition, 150 descendants derived from 10 parents were tested on Southern blots for their insertion pattern. It was estimated that after 4 generations of SSD each plant carried on average 5 new, and thus independent, insertions, i.e. the entire population of 3,000 plants (see materials and methods) carried 15,000 independent insertions. Each line represented by a single plant was given an identification number according to its row and column position in the tray (Fig. 2). Leaf tissue of the single plants of SSD generation 5 was harvested for DNA preparations. These DNAs served as templates for the PCR screens.

PCR screens

To identify inserts in genes, we used a primer complementary to the gene in combination with primers complementary to the ends of *En-1* in PCR reactions. The screens were performed in two rounds to reduce the number of PCR reactions. In the first round of screening, DNAs from approximately 3,000 plants that were grown and harvested in twenty-seven 3-tray pools and one 4-tray pool were tested with four different PCR primer combinations (En205, En8130 and two gene-specific primers, Fig. 2A). *En-1*-specific primers were chosen that gave little background in the PCR reactions. The optimal DNA concentration in relation to

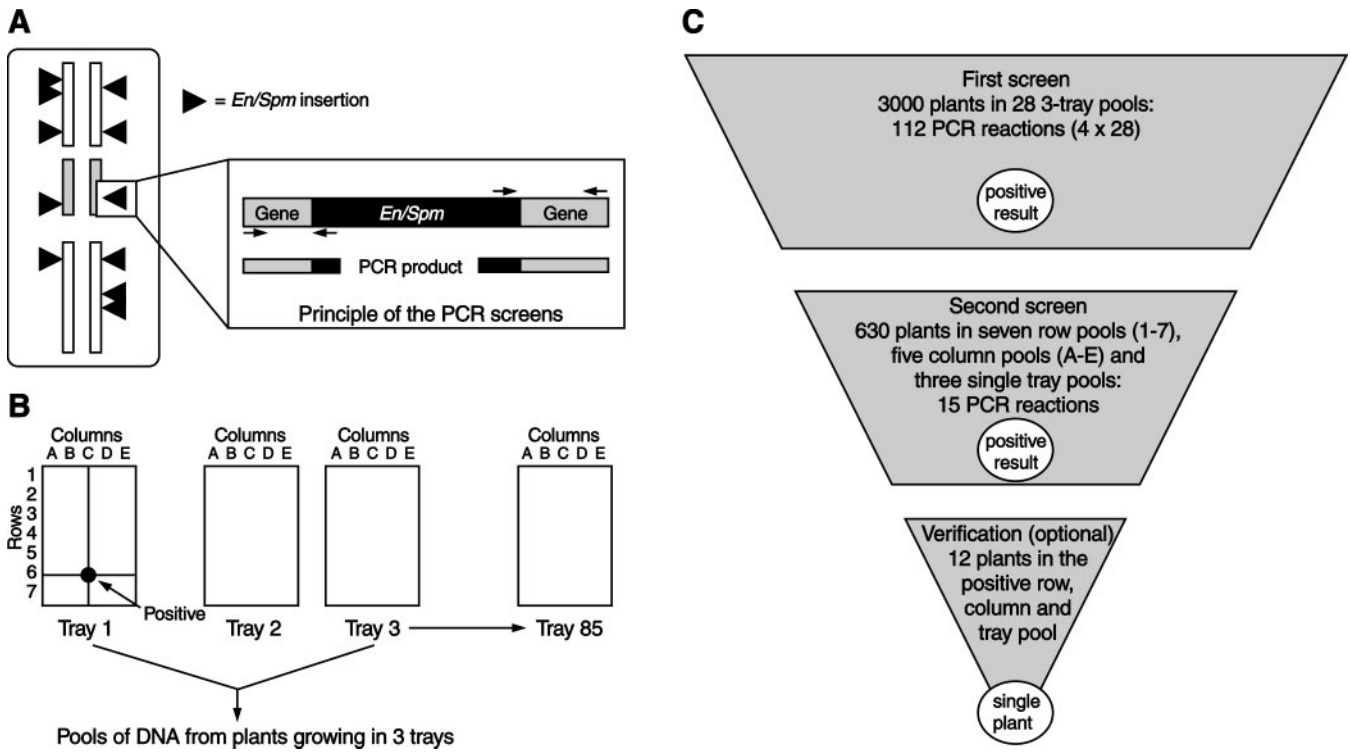

Fig. 2A–C Principle of the reverse genetic PCR screens on *En-1*-insertion Arabidopsis lines. **A** The genetic structure of a typical *En-1* insertion plant and the principle of the PCR screens using combinations of gene and *En-1*-specific primers to amplify insertion-specific sequences. **B** The pooling strategy used for collecting the leaves that yielded the DNAs used in the PCR screens described. Leafs were pooled on the basis of rows, columns, single trays and combinations of 3 trays. **C** The different rounds of PCR screens on the entire population necessary to identify a single plant that contains an *En-1* insertion in the gene of interest. • (**2B**) indicates a plant carrying an insertion in the gene of interest that is positively identified at position C6 in tray 1

primer concentration and reaction volume was also established. These parameters were shown to influence the amount of PCR product in relation to background. A total of 112 PCR reactions were used to screen the DNA pools. PCR products were separated on agarose gels, blotted and hybridised to a gene-specific DNA fragment. Three-tray pools that resulted in an amplification product led to a second round screens of 15 PCR reactions on DNAs from 630 plants arranged in the 3 single-tray pools (I–III), in seven-row pools (1–7) and five-column pools (A–E) with the successful primer combination. The detection of an amplification product of the same size in 1 tray, row and column pool identified the position in the tray and thus the putative mutant. In some cases the result was not that clear. For example, hybridisation signals were detected in more than the expected number of pools. Other experiments resulted in amplification products in two out of three pools. In these cases the second round was repeated by using two alternative primers specific for *En-1* resulting in a size shift of the amplification products due to their different positions on the *En-1* sequence. PCR reactions performed with primer En91 should give rise to a 100-bp shorter product than those with primer En205, and primer En7706, a 430-bp longer product than with primer En8130 (Figs. 3 and 5). Following this strategy unspecific background bands were distinguished from gene-specific products.

After a positive rescreen the potential insertion mutant was identified, and selfed seeds of the identified plant were planted to confirm the insert in individual progeny by amplifying the expected PCR fragment. The segregation ratio of plants carrying the expected PCR product determined if the insertion mutant was heterozygous or homozygous for the insertion. Alternatively, Southern blot analysis was employed using a restriction enzyme that yielded a restriction fragment length polymorphism (RFLP) between the wild- type and the insertion allele. Plants homozygous for the insert could be scored for a phenotype.

Screening for insertions in the *PIN* gene

To test the feasibility of the pooling strategy and PCR protocols, we planted a mutant heterozygous for a *pin* allele with an *En-1* insert at position 238 of the *PIN* gene (Gälweiler and Palme, personal communication)

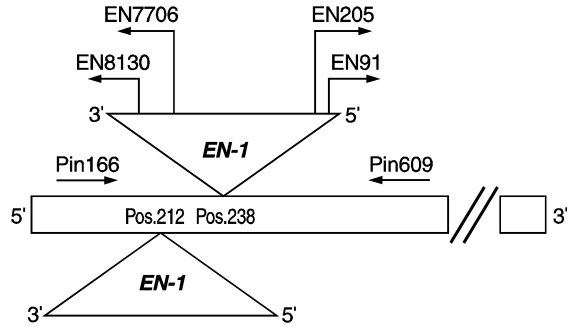

Fig. 3 The position of the *En-1*- and *PIN*-specific primers used in the control experiment

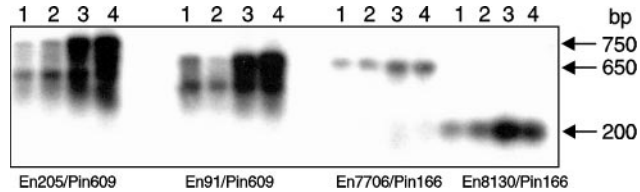

Fig. 5 Southern hybridisations of nested PCR reactions to confirm the identity of the *pin* insertion mutant. Shown is the expected PCR product length polymorphism using the primer combinations En8130/Pin166, En7706/Pin166, En91/Pin609 and En205/Pin609. *Lane 1* three-tray pool 25–27, *lane 2* tray pool 25, *lane 3* row pool 2, *lane 4* column pool E

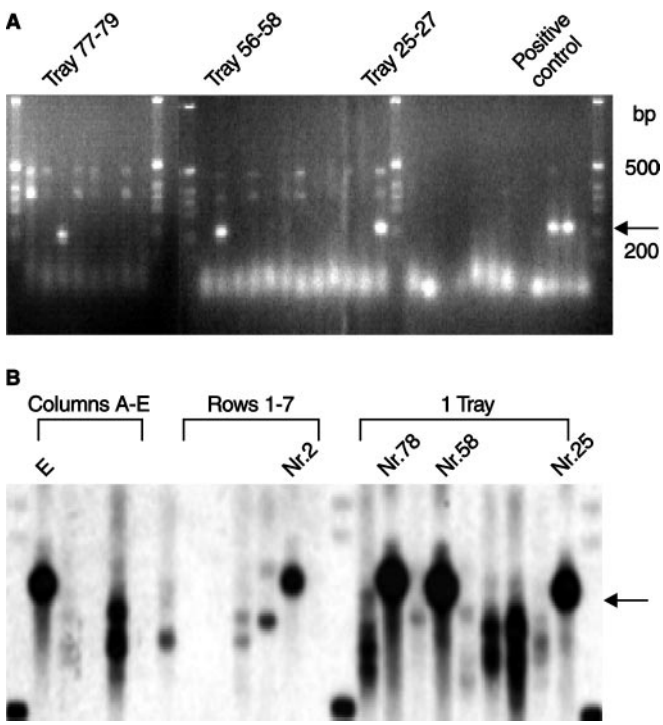

Fig. 4A, B Identification of plants carrying an *En-1* insertion in the *PIN* gene via reverse genetics. **A** Agarose gel of PCR products amplified with the primer Pin166 and En8130 using DNA of the three-tray pools as template. **B** Southern analysis of PCR products with DNAs isolated from row, column and single-tray pools. The blot was hybridised with a radiolabelled fragment of the *PIN* cDNA. The *arrow* indicates the PCR products amplified with primers Pin166 and En8130

at two random positions in the population. DNA of the 3-tray pools was tested using primer En8130 in combination with the *PIN*-specific primers. After separation of the PCR products on agarose gels the expected 215-bp product was detected in 3-tray pools 25–27 and 56–58 (Fig. 4A). In addition a third positive plant was identified in trays 77–79. Subsequently, the row, column and the single-tray pools were screened with PCR and the positions of the mutants were identified in all three dimensions. Hybridisation to a *PIN*-specific cDNA probe confirmed that the PCR products were specific for the *PIN* gene. Figure 4B shows that the position of the first control plant was tray 25, row 2, column E. The second control plant could be found in tray 58, row 1, column E. The third, newly detected putative *pin* mutant was found in tray 78, row 4 and column A. This position represented DNA of plant no.13,413. Additional PCR products arose during the screening procedure, most likely due to non-specific priming of either *En-1* or the *PIN*-gene specific primers to the Arabidopsis genome (Fig. 4A). In some cases even hybridisation signals of these additional products were observed (Fig. 4B). The identity of these additional PCR products was tested by cloning and sequencing. In the three cases tested, the sequence did not reveal any homology with the gene of interest, demonstrating that these products were not specific.

To confirm the identity of the *pin*-insertion mutants, we tested the positive pools again using the same primer combination, En8130/Pin166, as well as primer combinations En7706/Pin166, En205/Pin609 and En91/Pin609. The use of En7706 should give rise to a product of 650 bp, whereas the other two primer combinations which amplify the 5′ border region of the *En-1* insertion should amplify products of approximately 750 and 650 bp, respectively (Fig. 3). The new primer combinations amplified products with the expected size (Fig. 5), indicating that primer En7706, and also the 5′ outward primers of *En-1*, En205 and En91, were functional. In addition, bands of different size arose when using primer Pin609. These products gave also rise to hybridisation signals in the Southern analysis (Fig. 5). The sequence of the shorter fragment showed a deletion in sequence that is probably the result from the formation of an internal DNA loop that is not amplified by the *Taq* polymerase.

To verify whether plant no. 13,413 indeed carried an *En-1* insertion in the *PIN* gene, we tested 51 progeny plants for the presence of the amplification product specific for the *En-1* insertion. The progeny segregated in 36 normal and 15 plants showing the phenotype of a homozygous *pin* mutant described in Goto et al. (1991). As expected, the *pin* mutant plants carried the *En-1*-insertion allele as well as 26 of the wild-type

plants. Progeny testing of the latter confirmed that all of these plants were heterozygous for the insertion allele. The remaining 10 wild-type plants carried no *En-1* insertion in the *PIN* gene and were homozygous wild-type plants. The sequence of the PCR fragment from the newly identified *pin* mutant demonstrated that the insertion was at position 212, which is 26 bp upstream from the original *En-1* insertion of the control mutant. A similar genetic analysis of progenies of the 2 heterozygous *pin* control plants demonstrated that the inheritance of the insertion is stable and that it segregates with the *pin* phenotype. Despite the somatic activity of the autonomous elements the insertion allele segregates in a Mendelian fashion. Our genetic analysis was also not hindered by the additional *En-1* inserts in the genome of the multiple-copy lines. It can be concluded that the use of the reverse genetic approach allowed the detection of a new *pin* mutant among 3,000 plants carrying multiple inserts of *En-1*. Hence, it is expected that the pooling strategy and the PCR protocols will be effective tools for the identification of insertions in any Arabidopsis sequence in this population.

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References

Aarts GM, Corzaan P, Stiekema WJ, Pereira A (1995) A two-element Enhancer-Inhibitor transposable element system in *Arabidopsis thaliana*. Mol Gen Genet 247:555–564

Azpiroz-Leehan R, Feldmann KA (1997) T-DNA insertion mutagenesis in Arabidopsis: going back and forth. Trends Genet 13:152–156

Ballinger DG, Benzer S (1989) Targeted gene mutations in Drosophila. Proc Natl Acad Sci USA 86:9402–9406

Cardon GH, Frey M, Gierl A (1993a) Definition and characterisation of an artificial *En/Spm*-based transposon tagging system in transgenic tobacco. Plant Mol Biol 23:157–178

Cardon GH, Frey M, Saedler H, Gierl A (1993b) Mobility of the maize transposable element *En/Spm* in *Arabidopsis thaliana*. Plant J 3:773–784

Feldmann KA (1991) T-DNA insertion mutagenesis in *Arabidopsis*: mutational spectrum. Plant J 1:71–82

Forsthoefel NR, Wu Y, Schulz B, Bennett MJ, Feldmann KA (1992) T-DNA insertion mutagenesis in *Arabidopsis*: prospects and perspectives. Aust J Plant Physiol 19:353–366

Goto M, Katoh N, Kranz AR (1991) Morphogenesis of floral Arabidopsis: predominant carpel formation of the *pin*-formed mutant. Jpn J Genet 66:551–557

Kaiser K, Goodwin SF (1990) "Site-selected" transposable element mutagenesis in Drosophila. Proc Natl Acad Sci USA 87:1686–1690

Koes R, Souer E, van Houwelingen A, Mur L, Spelt C, Quattrochio F, Wing J, Oppedijk B, Ahmed S, Maes T, Gerats T, Hoogeveen P, Meesters M, Kloos D, Mol JNM (1995) Targeted gene inactivation in petunia by PCR-based selection of transposable element insertion mutants. Proc Natl Acad Sci USA 92:8149–8153

Koncz C, Nemeth K, Redei, GP, Schell J (1992) T-DNA insertional mutagenesis in *Arabidopsis*. Plant Mol Biol 20:963–976

Krysan JK, Young JC, Tax F, Sussman MR (1996) Identification of transferred DNA insertions within Arabidopsis genes involved in signal transduction and ion transport. Proc Natl Acad Sci USA 93:8145–8150

Kunze R, Saedler H, Lönnig WE (1997) Plant transposable elements. Adv Bot Res 27:331–470

McKinney EC, Ali N, Traut A, Feldmann KA, Belostotsky DA, McDowell JA, Meagher RB (1995) Sequence-based identification of T-DNA mutations in Arabidopsis: mutations in actin genes *act2-1* and *act4-1*. Plant J 8:613–622

Mena M, Ambrose BA, Meeley RB, Briggs SP, Yanofsky MF, Schmidt RJ (1996) Diversification of C-function activity in maize flower development. Science 274:1537–1540

Meyerowitz EM (1987) *Arabidopsis thaliana*. Annu Rev Genet 21:93–111

Novick EM, Peterson PA (1981) Transposition of the *Enhancer* controlling element system of maize. Mol Gen Genet 183:440–448

Peterson PA (1970) The *En* mutable system in maize. III. Transposition associated with mutational events. Theor Appl Genet 40:367–377

Rodger P, Bendich D (1988) A quick DNA minipreparation for PCR analysis of plant tissue. Plant Mol Biol Manual A6:1–10

Rushforth AM, Saari B, Anderson P (1993) Site-selected insertion of the transposable element *Tc1* into a *Caenorhabditis elegans* myosin light chain gene. Mol Cell Biol 13:902–910

Wisman E, Cardon GH, Fransz P, Saedler H (1998) The behaviour of the autonomous maize transposable element *En/Spm* in *Arabidopsis thaliana* allows efficient mutagenesis. Plant Mol Biol (in press)

Zwaal RR, Broeks A, van Meurs J, Groenin JTM, Plasterk RHA (1993) Target-selected gene inactivation in *Caenorhabditis elegans* by using a frozen transposon insertion mutant bank. Proc Natl Acad Sci USA 90:7431–7435