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TECHNICAL UPDATE

Unexpectedly rapid *IS*1 transposition into an Arabidopsis chromatin remodeling gene

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Abstract Common cloning is often associated with instability of certain classes of DNA. Here we report on *IS*1 transposition as possible source of such instability. During the cloning of *Arabidopsis thaliana* gene into commercially available vector maintained in widely used *Escherichia coli* host the insertion of complete *IS*1 element into the intron of cloned gene was found. The transposition of the *IS*1 element was remarkably rapid and is likely to be sequence-specific. The use of *E. coli* strains that lower the copy number of vector or avoiding the presence of the problematic sequence is a solution to the inadvertent transposition of *IS*1. The transposition of *IS*1 is rare but it can occur and might confound functional studies of a plant gene.

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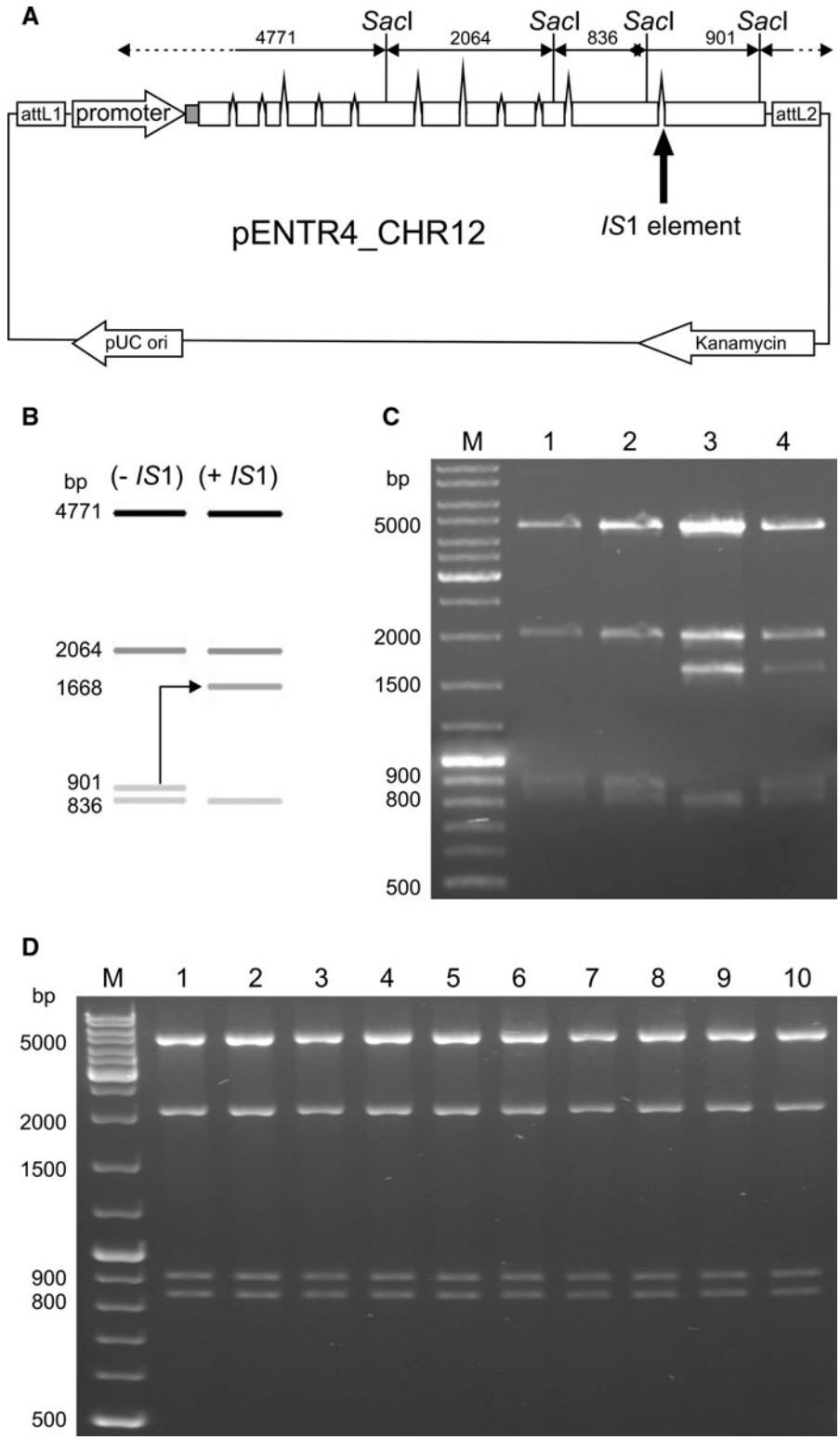
During investigations into the role of the *Arabidopsis thaliana AtCHR12* gene in plant stress responses (Mlynarova et al. 2007), many frustrating failures were unexpectedly encountered. Upon a more detailed analysis, the reason was due to a remarkably rapid transposition of the *IS*1 element from the widely used *Escherichia coli* host strain into this particular plant gene. A genomic copy of *AtCHR12* including its promoter (8804 bp) was isolated from Arabidopsis genomic DNA by PCR and cloned into a Gateway plasmid (Invitrogen). Following standard *E. coli* DH5α transformation, clones with the expected restriction pattern were generated (Fig. 1a). One clone (pENTR4_CHR12) was selected and sequenced, confirming the presence of the genomic sequence. When re-isolated from a new overnight culture, restriction analysis revealed the presence of new DNA. By sequencing and BLAST against the *E. coli* genome, this new DNA was found to be the complete *IS*1 element that had become inserted into the last intron of the *AtCHR12* gene (Fig. 1a). The *IS*1 insert was flanked by a 9-bp direct repeat (GGTAATCTC) derived from the acceptor sequence. No other rearrangements of the acceptor sequence were found. The *IS*1 element is the smallest bacterial insertion sequence known (768 bp; (Mahillon and Chandler


A
SacI
SacI
SacI
SacI
4771
2064
836
901
attL1
promoter
attL2
pENTR4_CHR12
IS1 element
pUC ori
Kanamycin
B
bp
(- IS1)
(+ IS1)
4771
2064
1668
901
836
C
M
1
2
3
4
bp
5000
2000
1500
900
800
500
D
M
1
2
3
4
5
6
7
8
9
10
bp
5000
2000
1500
900
800
500

◀ **Fig. 1** **a** Schematic map of pENTR4_CHR12 with indicated *Sac*I restriction sites and the size of expected restriction fragments (bp). **b** *Sac*I restriction pattern of pENTR4_CHR12 with and without *IS*1 insertion. The arrow indicates the shift in fragment size after transposition. **c** Gel electrophoresis of *Sac*I digestion of 4 plasmid isolates after re-transformation of clean plasmid to *E. coli*. *Lanes 1* and *2* are plasmid isolates without *IS*1, *lane 3* with *IS*1 element and *lane 4* carries both sequence variants. **d** *Sac*I digestion of new plasmid isolations shows that none of the clones analysed contains *IS*1. Plasmid preparations were obtained after re-transformation of the isolated plasmid to CopyCutter^{TM} EPI400 *E. coli* cells. Ten randomly picked clones were propagated in 3 rounds of uninduced overnight (16 h) culture before new pDNA isolation. The *E. coli* cells were obtained from Epicentre (www.epibio.com). M, GeneRuler^{TM} DNA Ladder mix (*Thermo Scientific*)

1998)). It is present in many *E. coli* genomes and can cause spontaneous insertion mutations in both plasmid and chromosomal DNA (Prather et al. 2006). This unfortunate integration was in hindsight occurring in several cloning attempts of the same gene at about the same insertion site (last intron/last exon boundary). When deliberately retested, pENTR4_CHR12 showed a hitherto unreported fast rate of *IS*1 transposition, irrespective of the method used to introduce the clean plasmid in *E. coli* cells (Fig. 1c). After one overnight (16 h) culture that comprises an estimated 40 generations, half of the clones tested contained at least 50 % of plasmid DNA with an *IS*1 insertion (Fig. 1c). This rate of transposition is well above the frequency published elsewhere of less than 0.1 % in the same LB medium or up to 10 % in another, more poor medium (Prather et al. 2006). Nucleotide composition of the flanking sequences (30 bases) of the *IS*1 integration sites analyzed does not show these to be particularly AT-rich compared to the rest of the gene sequence. More integration sites should be carefully analyzed in order to establish any precise sequence preference of *IS*1 insertion for the *AtCHR12* sequence. The unexpected *IS*1 transposition here reported can confound functional studies of a plant gene. Also *IS*10 was reported to transpose (Kovarik et al. 2001), but not as fast as here observed. From a biological containment perspective, it is remarkable that a widely used *E. coli* host contains—and is allowed to contain—such an active *IS* element. Upon the use of *E. coli* cell line that lowers the copy number of vectors (CopyCutter from Epicentre), no *IS*1 transposition was observed (Fig. 1d). Its use therefore is a convenient solution to the inadvertent transposition of *IS*1. Both the tomato and the potato genome, both genomes of our research interest, was sequenced with conventional (Sanger) and Next Generation Sequencing (NGS) technologies by a combination of cloning-based (notably bacterial artificial chromosomes) and whole genome shotgun approaches without cloning steps. No occurrence of *IS*1 was found by BLAST in the tomato or potato genome assembly or in any genome assembly of plant genomes present in Phytozome (www.phytozome.org), except for *Zea mays*, *Vitis vinifera* and *Brassica rapa*. Known *E. coli* sequences are apparently efficiently filtered away in most genome assembly procedures. In some individual plant sequences present at NCBI, however, from for example tomato, presence of *IS*1 elements can be detected by BLAST.

References

Kovarik A, Matzke MA, Matzke AJ, Koulakova B (2001) Transposition of IS10 from the host *Escherichia coli* genome to a plasmid may lead to cloning artefacts. Mol Genet Genomics 266:216–222

Mahillon J, Chandler M (1998) Insertion sequences. Microbiol Mol Biol Rev 62:725–774

Mlynarova L, Nap JP, Bisseling T (2007) The SWI/SNF chromatin-remodeling gene AtCHR12 mediates temporary growth arrest in Arabidopsis thaliana upon perceiving environmental stress. Plant J 51:874–885

Prather KL, Edmonds MC, Herod JW (2006) Identification and characterization of IS1 transposition in plasmid amplification mutants of E. coli clones producing DNA vaccines. Appl Microbiol Biotechnol 73:815–826