# GENETICS 

Supporting Information<br>http://www.genetics.org/cgi/content/full/genetics.110.117606/DC1

# Miniature Inverted-Repeat Transposable Elements of Stowaway Are Active in Potato 

Masaki Momose, Yutaka Abe and Yoshihiro Ozeki

Copyright © 2010 by the Genetics Society of America DOI: 10.1534/genetics.110.117606


Figure Sl.-Approximate positions of primers used in this study. Arrow heads show primers and their directions used for the analysis of (A) F3'5'H (pseudo) genes as described in supplementary methods, (B) the insertion locus of $d T$ stu1-2 and (C) MITE display. Coding regions are marked by shaded boxes and the Stowaway MITE is indicated by a black bar.


Figure S2.-MITE display using primers designed from sequences of $d T s t u 1$ and $d T s t u 1-2$. Mse+T was used as a selective primer. The left panels show peaks from 0 base to 120 bases, the middle from 120 to 360 bases and the right from 360 to 600 bases. Differences of 'JKR' and 'JKP' as compared with ' 72218 ' are indicated by arrows. An arrowhead shows a new peak of 315 bases in size.


Figure S3.-Tuber appearance of one of the plants regenerated from protoplasts of '72218'. Arrows indicate purple variegation (which appear as a dark line in the picture) present against the red background.

## File S1

## Supporting Methods

Isolation and sequences determination of the genomic DNA for $F 3^{\prime} 5^{\prime} H$ genes: For the isolation of $F 3^{\prime} 5^{\prime} H$ pseudo-gene, $f 3^{\prime} 5^{\prime} h 2$, inverse PCR was carried out with primers No. 1 and 7 for the 1 st, and No. 2 and 8 for the 2nd amplification using as a template self-ligated genomic DNA from 'JKP' which had been digested by MboI. Primers No. 7 and 8 were based on the highly conserved region among P 450 or $F 3^{\prime} 5^{\prime} H$ genes. The product was cloned and its sequence enabled the design of primers No. 9, 10 and 11, which were used in inverse PCR (with primers No. 2 and 9 for the 1 st and No. 10 and 11 for the 2nd amplification) on self-ligated $X b a I$-digested genomic DNA of ' JKP '. The sequence of the resulting 5 kb product provided information to design primers No. 12 and 13 that were used in PGR reactions on genomic DNA from 'JKP' and '72218' to compare their $f 3^{\prime} 5^{\prime} h 2$ alleles (primers No. 5 and 12 for the 1 st and No. 5 and 13 for the 2 nd amplification). The third copy of $F 3^{\prime} 5^{\prime} H$ pseudo-gene, $f 3^{\prime} 5^{\prime} h 3$, was isolated after a series of inverse PCR reactions that yielded sequence to design primers that were used in subsequent rounds. Inverse PGRs were done on HincII-digested genomic DNA followed by self-ligation with primers No. 14 and 15 and then self-ligated EcoRI-digested DNA with a primer set of No. 16 and 17 for the 1st followed by a set of No. 17 and 18 for the 2nd amplification. The penultimate round of inverse PCR was performed on HindIII-digested and self-ligated DNA with primers No. 18 and 19 for the 1st, and No. 20 and 21 for the 2nd amplifications. Finally, primers could be designed (No. 22 and 23 for the 1 st and No. 18 and 24 for the 2 nd amplification) to amplify $f 3^{\prime} 5^{\prime} h 3$ from both 'JKP' and '72218' genomic DNAs as templates (see Figure Sl). The extension time in all PCRs was 5 min.

## TABLE S1

Primer sequences used in this study

| Primer | Sequence |
| :---: | :---: |
| 1 | 5'-AACATTTTTGTCAATAAAKCATCAAA-3' |
| 2 | 5'-CCTTGTAAATCCATCCAAGCTA-3' |
| 3 | 5'-CGGAATTCAAGGTTTATATTATATCTTCGATTTT-3' |
| 4 | 5'-GGCATTACGTATTAGTGAGTTG-3' |
| 5 | 5'-CCTTCTACTTCATTCTCACTCT-3' |
| 6 | 5'-AGCAAATATGTTGCACTATAAATG-3' |
| 7 | 5'-CCTGATTTTCTTGATKTTRTTATGG-3' |
| 8 | 5'-GGGATAATTCTGAAGGAGAAAG-3' |
| 9 | 5'-TATTCCAAGTTGTTGACACCCA-3' |
| 10 | 5'-ACTGAAGTAGCCATCCAAAGAC-3' |
| 11 | 5'-TCAACGAACACTCTCTTACTTAA-3' |
| 12 | 5'-GCTCACTACACAATGCACATG-3' |
| 13 | 5'-TCATGAAATGCATCGACAATTTAT-3' |
| 14 | 5'-GCTAATCCAAAAGATTCCTCCA-3' |
| 15 | 5'-TGCAAAAAGCTGTCCCTCTTG-3' |
| 16 | 5'-TTACGTTACGGTCTTCAACAG-3' |
| 17 | 5'-AGAGGAGGATAACAAACTTGTAT-3' |
| 18 | 5'-AACAGATACGTTGCACTATAACT-3' |
| 19 | 5'-CCTAGTCCCCATTTCACTACA-3' |
| 20 | 5'-GAACATGAGTTTACGTGAACCG-3' |
| 21 | 5'-AGGCATCCTTCGAAATCCACA-3' |
| 22 | 5'-TTGTAAAGTGCACCCATCATCT-3' |
| 23 | 5'-CATTGGTCCTAACGATGGACA-3' |
| 24 | 5'-ATTCAGATCCTCCCGATGAATT-3' |
| 25 | 5'-ATTCATTTTGGACCACAAGTTTTA-3' |
| 26 | 5'-TGTTTTTTGCAGTTATCTTATTTCA-3' |
| 27 | 5'-CAAGGGGAGACATTTAGG-3' |
| 28 | 5'-AGACATTTCATAGGCAAATTGTTA-3' |
| 29 | 5'-AGCTGAAATATGAGATTGAAATTAG-3' |
| 30 | 5'-ATTTTGCTATATCCACAATGACTT-3' |
| 31 | 5'-CATTCTTTTTGGGACTGACTA-3' |
| 32 | 5'-ATAAAWTGGGACRGAGGGAGTA-3' |
| Mse+0 | 5'-GACGATGAGTCCTGAGTAA-3' |
| Mse+N | 5'-GACGATGAGTCCTGAGTAAN-3' |

