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A transient assay for recombination demonstrates that *Arabidopsis SNM1* and *XRCC3* enhance non-homologous recombination

R.A. Johnson[1,2], R.P. Hellens[1] and D.R. Love[2,3]

[1]The New Zealand Institute for Plant & Food Research Limited, Sandringham, Auckland, New Zealand
[2]School of Biological Sciences, University of Auckland, Auckland, New Zealand
[3]LabPlus, Diagnostic Genetics, Auckland Hospital, Auckland, New Zealand

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Corresponding author: R.A. Johnson
E-mail: rjoh064@aucklanduni.ac.nz

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ABSTRACT. Replacement of endogenous genes by homologous recombination is rare in plants; the majority of genetic modifications are the result of transforming DNA molecules undergoing random genomic insertion by way of non-homologous recombination. Factors that affect chromatin remodeling and DNA repair are thought to have the potential to enhance the frequency of homologous recombination in plants. Conventional tools to study the frequencies of genetic recombination often rely on stable transformation-based approaches, with these systems being rarely capable of high-throughput or combinatorial analysis. We developed a series of vectors that use chemiluminescent (*LUC* and *REN*) reporter genes to assay the relative frequency of homologous and non-homologous recombination in plants. These transient assay vectors were used to screen 14 candidate

genes for their effects on recombination frequencies in *Nicotiana benthamiana* plants. Over-expression of *Arabidopsis* genes with sequence similarity to *SNM1* from yeast and *XRCC3* from humans enhanced the frequency of non-homologous recombination when assayed using two different donor vectors. Transient *N. benthamiana* leaf systems were also used in an alternative assay for preliminary measurements of homologous recombination frequencies, which were found to be enhanced by over-expression of *RAD52*, *MIM* and *RAD51* from yeast, as well as *CHR24* from *Arabidopsis*. The findings for the assays described here are in line with previous studies that analyzed recombination frequencies using stable transformation. The assays we report have revealed functions in non-homologous recombination for the *Arabidopsis SNM1* and *XRCC3* genes, so the suppression of these genes' expression offers a potential means to enhance the gene targeting frequency in plants. Furthermore, our findings also indicate that plant gene targeting frequencies could be enhanced by over-expression of *RAD52*, *MIM*, *CHR24*, and *RAD51* genes.

Key words: Extra-chromosomal recombination; *Agro*-infiltration; Non-homologous recombination; Homologous recombination; Plant genetic modification; Transient Dual-Luciferase® assays