

Additional File 4. Values used to calculate the frequency of loss-of-function mutation by EMS treatment

Spontaneous reversion frequency of C/G to T/A transition	$10^{-7} \sim 10^{-8}$ per base pair per plant <sup>a, b</sup>
Specificity of C/G to T/A transition in EMS mutagenesis	$\sim 100\%$ <sup>c</sup>
Fold increase of C/G to T/A transition by EMS treatment	26 ~ 370 fold <sup>b</sup>
Mutation rate for C or G base	$3.7 \times 10^{-5} \sim 2.6 \times 10^{-6}$ per base pair per plant
GC content in coding sequence	44.1% <sup>d</sup>
Average gene size	1900 bp <sup>d</sup>
Expected mutation rate in EMS mutagenesis	$3.1 \times 10^{-2} \sim 2.2 \times 10^{-3}$ per gene
% ratio of non-silent (missense and nonsense) mutation in EMS mutagenesis	53.6% <sup>c</sup>
Expected frequency of non-silent mutation in EMS mutagenesis	$1.7 \times 10^{-2} \sim 1.2 \times 10^{-3}$ per gene

<sup>a</sup> **Kovalchuk, I., Kovalchuk, O., and Hohn, B.** (2000). Genome-wide variation of the somatic mutation frequency in transgenic plants. *Embo J* **19**, 4431-4438.

<sup>b</sup> **Van der Auwera, G., Baute, J., Bauwens, M., Peck, I., Piette, D., Pycke, M., Asselman, P., and Depicker, A.** (2008). Development and Application of Novel Constructs to Score C:G-to-T:A Transitions and Homologous Recombination in Arabidopsis. *Plant Physiol* **146**, 22-31

<sup>c</sup> **Greene, E.A., Codomo, C.A., Taylor, N.E., Henikoff, J.G., Till, B.J., Reynolds, S.H., Enns, L.C., Burtner, C., Johnson, J.E., Odden, A.R., Comai, L., and Henikoff, S.** (2003). Spectrum of chemically induced mutations from a large-scale reverse-genetic screen in Arabidopsis. *Genetics* **164**, 731-740.

<sup>d</sup> **The Arabidopsis Genome Initiative** (2000). Analysis of the genome sequence of the flowering plant Arabidopsis thaliana. *Nature* **408**, 796-815.