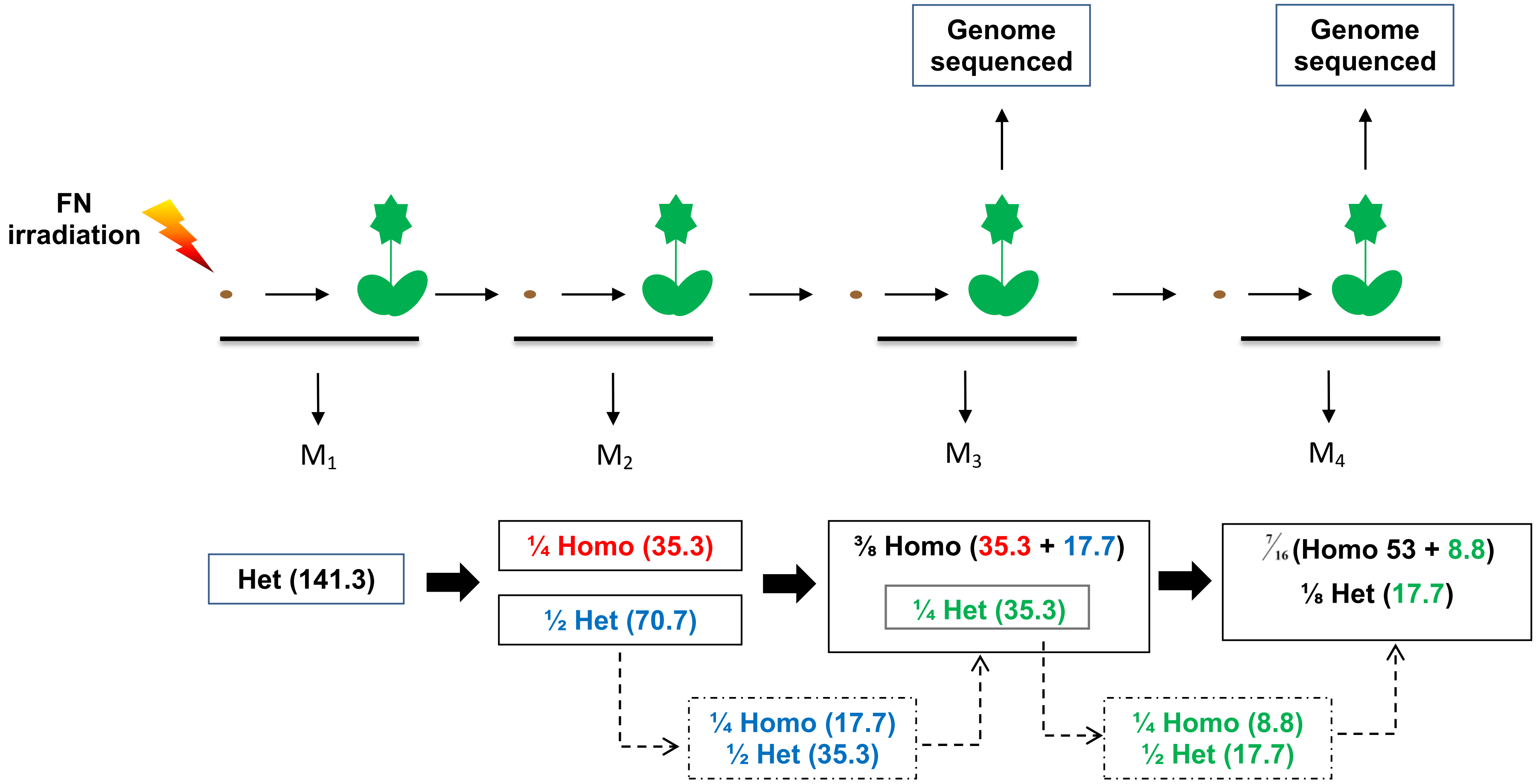

Supplemental Figure 1. Estimation of numbers of mutations in E99 and E125 M_4 plants. To assist in the determination of the spontaneous mutation rate calculation in our FN-exposed mutant lines we genome sequenced the progeny of two individual M_4 plants (progeny of E99 and

E125 genome-sequenced M_3 lines) and compared the number of predicted homozygous M_4 mutations to the number of observed M_4 mutations identified in E99 and E125 DNA sequencing datasets. Mendelian segregation laws were used to estimate the number of variants, both homozygous (Homo) and heterozygous (Het) in the preceding generations of E99 and E125 M_3 mutant lines genomes sequenced. As shown, all ‘fixed’ homozygous mutations in the M_2 (in red text) would have passed onto the subsequent M_3 generations. In addition, a quarter of the heterozygous mutations (70.7; blue text) in the M_2 lines would have segregated in a Mendelian 1:2:1 (homozygous mutation:heterozygous mutation:homozygous non-mutant) ratio, becoming homozygous (17.7; blue text) in the following generation. A total of 53 homozygous variants along with a further 35.3 segregating heterozygous variants (green text) are predicted in the M_3 generations. In the M_4 generation, a further 8.8 homozygous variants (green text) are predicted following segregation (in a Mendelian 1:2:1 ratio) of the M_3 heterozygous variants in addition to 17.7 heterozygous mutations (green text). In the original FN exposed M_1 lines a total of 141.3 (black text) heterozygous mutations are predicted to have been FN-induced and a quarter of these should become homozygous in the M_2 generation (35.3; red text), half becoming heterozygous (70.7; blue text) and a quarter would be the original DNA sequence. The numbers of predicted and observed mutations in E99 and E125 lines in the M_3 and M_4 generations are summarized in Supplemental Table 4.