Supplemental Figure 1. Estimation of numbers of mutations in E99 and E125 M₄ 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 M₄ plants (progeny of E99 and
E125 genome-sequenced M₃ lines) and compared the number of predicted homozygous M₄ mutations to the number of observed M₄ 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 M₃ mutant lines genomes sequenced. As shown, all ‘fixed’ homozygous mutations in the M₂ (in red text) would have passed onto the subsequent M₃ generations. In addition, a quarter of the heterozygous mutations (70.7; blue text) in the M₂ 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 M₃ generations. In the M₄ generation, a further 8.8 homozygous variants (green text) are predicted following segregation (in a Mendelian 1:2:1 ratio) of the M₃ heterozygous variants in addition to 17.7 heterozygous mutations (green text). In the original FN exposed M₁ 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 M₂ 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 M₃ and M₄ generations are summarized in Supplemental Table 4.