|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  | **gRNA-ABA/1/364** | | | **gRNA-ABA/1/364+ TREX2** | | | **gRNA-ABA/2/323** | | | **gRNA-ABA/2/323+ TREX2** | | |
|  |  |  | genome | | | genome | | | genome | | | genome | | |
|  |  |  | A | B | R | A | B | R | A | B | R | A | B | R |
| **Mutation type frequency [%]** | 1 bp | del | 0.37 | 0.25 | 0.59 | 0.09 | 0.04 | 0.00 | 36.23 | 39.06 | 41.78 | 0.89 | 0.15 | 0.04 |
| ins | 0.03 | 0.04 | 0.07 | 0.00 | 0.02 | 0.00 | 19.29 | 26.31 | 21.60 | 0.00 | 0.00 | 0.00 |
| sub | 3.01 | 0.17 | 1.23 | 0.01 | 0.15 | 0.00 | 2.67 | 1.23 | 0.38 | 0.09 | 0.30 | 0.00 |
| 2-10 bp | del | 28.92 | 30.46 | 28.07 | 14.04 | 12.00 | 11.24 | 32.65 | 28.70 | 35.48 | 14.42 | 15.19 | 12.31 |
| ins | 0.00 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 | 0.23 | 0.00 | 0.00 | 0.00 | 0.00 |
| sub | 0.08 | 0.17 | 0.62 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.05 | 0.00 | 0.00 |
| 11-99 bp | del | 66.08 | 66.30 | 68.06 | 83.88 | 85.01 | 86.37 | 2.82 | 2.46 | 0.66 | 82.60 | 83.64 | 84.09 |
| ins | 0.55 | 0.66 | 0.13 | 0.02 | 0.06 | 0.00 | 6.34 | 2.01 | 0.09 | 0.00 | 0.00 | 0.00 |
| sub | 0.06 | 0.92 | 0.00 | 0.01 | 1.80 | 0.15 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| >100 bp | del | 0.28 | 0.04 | 0.57 | 1.39 | 0.89 | 2.03 | 0.00 | 0.00 | 0.00 | 1.60 | 0.41 | 3.49 |
| ins | 0.07 | 0.00 | 0.04 | 0.01 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| sub | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Complex modifications | | 0.54 | 0.99 | 0.62 | 0.43 | 0.04 | 0.19 | 0.00 | 0.00 | 0.00 | 0.34 | 0.32 | 0.07 |

**Table S2**. Mutation frequencies in Triticale protoplasts acquired by deep-sequencing of short reads. Type and size of mutation generated by individual constructs was quantified as a percentage of the total construct efficiency.