Addendum to Petit-Houdenot et al. "A clone resource of *Magnaporthe oryzae* effectors": internal BsaI restriction enzyme sites

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We recently described a clone resource of 195 effectors of the blast pathogen *Magnaporthe oryzae* and related species (Petit-Houdenot et al. 2020. Molecular Plant-Microbe Interactions, https://doi.org/10.1094/MPMI-03-20-0052-A). These clones are freely available as Golden Gate compatible entry plasmids as an open source clone library to be used in a variety of functional studies. This addendum is to raise awareness of the presence of internal BsaI restriction enzyme sites in ten of the sequences.

Brief explanation of the observation. We noticed that ten out of the 195 effector sequences published in this dataset contain internal BsaI-restriction enzyme sites (Supplementary File 1), the enzyme used for Golden Gate cloning reactions to assemble level 1 expression constructs. These sites were likely introduced during manual codon-optimization. All sites are in reverse orientation (consensus sequence: XXXXnGAGACC; X=overhang, n=variable nucleotide).

Problem caused by sites. It is possible that these sites interfere with individual Golden Gate cloning reactions due to internal cleavage of the effector sequence. This might lead to assembly of fragmented effector sequences into level 1 expression plasmids.

If our level 0 plasmids are used to generate new level 0 plasmids with "non-standard" overhangs (other overhangs than AATG / GCTT) by PCR, custom overhangs should be compared to the internal overhangs generated by these sites.

Suggested solution. We have not noticed any problems during cloning using the plasmids in question. In our experience the effectors reassembled in the correct order in level 1 pGADT7 yeast two-hybrid plasmids. However, we suggest control digestions followed by agarose gel electrophoresis and/or Sanger sequencing of the final plasmids to confirm correct plasmid assemblies. If these plasmids are used in PCR to generate custom Golden Gate cloning fragments, overhangs should be compared to exclude misassembly or internal sites can be removed during the PCR.

Supplementary File 1. Ten effector sequences with internal BsaI restriction enzyme sites. Fasta sequences of effector candidates with internal BsaI restriction enzyme sites. BsaI-recognition sites are marked in red. Cleavage sites (overhangs created by BsaI-cleavage) are marked in yellow.