Supplementary belongs to:

Biochemical characterization of the functional roles of residues in the active site of the β-galactosidase from Bacillus circulans ATCC 31382

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Table S1. The primer pairs used for site-directed mutagenesis of BgaD-D.

| Primer name  | DNA sequence (5' to 3')                                                                 |
|--------------|----------------------------------------------------------------------------------------|
| Arg185-F     | CACCCAGCCGAGCAGCNGKCTTGCATTCCGGGAGCG                                                 |
| Arg185-R     | CGCTCCCAATACCCAMNNNGCTCTCGGCTGGGTG                                                   |
| Asp481-F     | GATCGGCGAGNKNKAAAACCC                                                                  |
| Asp481-R     | GGTTTTMNNCTCTCGGCGATC                                                                 |
| Asp481Lys-F  | GATCGGCGAGAAGAAACC                                                                    |
| Asp481Lys-R  | GGTTTTCTTCTCGCGGATC                                                                  |
| Asp481Arg-F  | GATCGGCGAGGGAACCC                                                                    |
| Asp481Arg-R  | GGTTTTTCCGCTCGGCGATC                                                                  |
| Asp481Asn-F  | GATCGGCGAGAACAACCC                                                                  |
| Asp481Asn-R  | GGTTTTGTTCTCGGCGATC                                                                  |
| Asp481Gln-F  | GATCGGCGAGGGAACCC                                                                  |
| Asp481Gln-R  | GGTTTTCTGCTCGGCGATC                                                                  |
| Asp481Leu-F  | GATCGGCGAGGGAACCC                                                                  |
| Asp481Leu-R  | GGTTTTCACTCGGCGATC                                                                  |
| Asp481Trp-F  | GATCGGCGAGGGAACCC                                                                  |
| Asp481Trp-R  | GGTTTTCACTCGGCGATC                                                                  |
| Asp481Gly-F  | GATCGGCGAGGGAACCC                                                                  |
| Asp481Gly-R  | GGTTTTCCCTCGGCGATC                                                                  |
| Lys487-F     | CGCGGAGACNNKGTAAATGTTCAC                                                              |
| Lys487-R     | GTGTAAACATTTCACNNNGTTTCGCC                                                          |
| Tyr511-F     | GGACTGAACNKAGCGAGAACACTATGAGGCC                                                      |
| Tyr511-R     | GCCATCATAGTTGCTCGCTMNNGTTCAGTCC                                                         |
| Tyr511Phe-F  | GGACTGAACATAGCGAGGAGAACAACCTATGAGGCC                                                   |
| Tyr511Phe-R  | GCCATCATAGTTGCTCGCTMNNGTTCAGTCC                                                         |
| Tyr511Trp-F  | GGACTGAACATGCGAGGAGAACAACCTATGAGGCC                                                   |
| Tyr511Trp-R  | GCCATCATAGTTGCTCGCTMNNGTTCAGTCC                                                         |
| Trp570-F     | GTCGGCNNKGGAGCAGAAGCTGCGAGAAG                                                       |
| Trp570-R     | CTTCGCGATCTGCGMNNNGCCGAC                                                             |
| Trp570Tyr-F  | GTCGGCTACCGAGCAGAAGCTGCGAGAAG                                                       |
| Trp570Tyr-R  | GCAGTTTCGGCTCGTACGCGAGCAGCAGG                                                       |
| Trp570Phe-F  | GTCGGCTTGGAGCAGAAGCTGCGAGAAG                                                       |
| Trp570Phe-R  | GCAGTTTCGCTCGAAAGGAGCAGCAGG                                                       |
| Trp570Ala-F  | GTCGGCCTGCGGAGCAGAAGCTGCGAGAAG                                                       |
| Trp570Ala-R  | GCAGTTTCGTCAGCGGCGAGCAGCAGG                                                       |
| Trp570Val-F  | GTCGGCCGTGGAGCAGAAGCTGCGAGAAG                                                       |
| Trp570Val-R  | GCAGTTTCGTCAGCGGCGAGCAGCAGG                                                       |
| Trp570Leu-F  | GTCGGCCCTTGGAGCAGAAGCTGCGAGAAG                                                       |
| Trp570Leu-R  | GCAGTTTCGTCAGCGGCGAGCAGCAGG                                                       |
| Trp570Cys-F  | GTCGGCTCGGAGCAGAAGCTGCGAGAAG                                                       |
| Trp570Cys-R  | GCAGTTTCGTCGCGAGCAGCAGCAGG                                                       |
| Trp593-F     | ACCTGAGCATATTGCAGGGCAATTTCNTKACCGGGCTTTGATTATTAGG                                    |
| Trp593-R     | CCAAATATAATCAAGGCGAGTCMNNGATAATAATTCCTCGCAAATGCTGAGT                                  |
| Glu601-F     | CCGGCCCTTGGATTATATGCGCGNKNCCGACGCCATATTATAATTC                                           |
|              |                                                                                       |
|              |                                                                                       |
| Codon            | Sequence                                      |
|------------------|-----------------------------------------------|
| Glu601-R         | GGAATTATAATATGGCCTCGGACGACCGATATAATCAAAGCCGG  |
| Glu601Asp-F      | CCGGCTTTGATTATATTGCGACCGATATATATTATAATCCGG    |
| Glu601Asp-R      | GGAATTATAATATGGCCTCGGACGACCGATATAATCAAAGCCGG  |
| Phe616-F         | GCAAAAAGCTCTATATNNKGGTGCTTGGATACCGG           |
| Phe616-R         | CCGTATCCACAGCACCNNATAGGACGCGTTTTTCG           |
| Phe616Trp-F      | GCAAAAAGCTCTATATTTGCGTGCTTGGATACCGG           |
| Phe616Trp-R      | CCGTATCCACAGCACCNNATAGGACGCGTTTTTCG           |
| Phe616His-F      | GCTCTATACAGTGCTTGGATAC                        |
| Phe616His-R      | GTATCCACAGCACCAGATAGGAC                       |
| Phe616Ser-F      | GCTCTATACAGTGCTTGGATAC                        |
| Phe616Ser-R      | GTATCCACAGCACCAGATAGGAC                       |
| Phe616Thr-F      | GCTCTATACAGTGCTTGGATAC                        |
| Phe616Thr –R     | GTATCCACAGCACCAGATAGGAC                       |
| Phe616Asn-F      | GCTCTATAGTGCTTGGATAC                         |
| Phe616Asn-R      | GTATCCACAGCACCAGATAGGAC                       |
| Phe616Cys-F      | GCTCTATACAGTGCTTGGATAC                        |
| Phe616Cys-R      | GTATCCACAGCACCAGATAGGAC                       |
| Phe616Pro-F      | GCTCTATACAGTGCTTGGATAC                        |
| Phe616Pro-R      | GTATCCACAGCACCAGATAGGAC                       |
| Phe616Ala-F      | GCTCTATAGTGCTTGGATAC                         |
| Phe616Ala-R      | GTATCCACAGCACCAGATAGGAC                       |
| Phe616Ile-F      | GCTCTATACAGTGCTTGGATAC                        |
| Phe616Ile-R      | GTATCCACAGCACCAGATAGGAC                       |
| Phe616Met-F      | GCTCTATAGTGCTTGGATAC                         |
| Phe616Met-R      | GTATCCACAGCACCAGATAGGAC                       |
| Phe616Tyr-F      | GCTCTATACAGTGCTTGGATAC                        |
| Phe616Tyr-R      | GTATCCACAGCACCAGATAGGAC                       |

The “N” base in the primers stands for a “A or T or C or G” base, and “K” stands for a “G or T” base. “NNK” codons are commonly used in screens for codon substitutions to reduce the presence of some codon-rich amino acids, thereby reducing their over representation in a particular library. Additionally, using NNK codons removes 2 out of 3 possible stop codons, which also limits the number of sequences in a library that produce unwanted truncated gene products. 1
**Figure S1.** SDS-PAGE gel analysis of the wild-type BgaD-D and the mutant enzymes (loaded with 5 µL of 0.1 mg/mL protein solutions). M, Marker proteins; 1, WT; 2, Trp570Gly; 3, Trp570Thr; 4, Trp570Arg; 5, Trp570Glu; 6, Trp570Tyr; 7, Trp570Phe; 8, Trp570Ala; 9, Trp570Val; 10, Trp570Cys; 11, Trp570Leu; 12, Trp593Tyr; 13, Trp593Phe; 14, Phe616Val; 15, Phe616Glu; 16, Phe616Gly; 17, Phe616Lys; 18, Phe616Gln; 19, Phe616Arg; 20, Phe616Asp; 21, Phe616Leu; 22, Phe616Trp; 23, Phe616His; 24, Phe616Ser; 25, Phe616Thr; 26, Phe616Asn; 27, Phe616Cys; 28, Phe616Pro; 29, Phe616Ala; 30, Phe616Ile; 31, Phe616Met; 32, Phe616Tyr; 33, Asp481Glu; 34, Asp481His; 35, Asp481Ser; 36, Asp481Asn; 37, Asp481Gln; 38, Lys487Met; 39, Lys487Phe; 40, Lys487Leu; 41, Lys487Gln; 42, Lys487Ser; 43, Lys487Gly; 44, Lys487Asn; 45, Lys487Cys.
Reference:

1. Tang, L., Gao, H., Zhu, X., Wang, X., Zhou, M., and Jiang, R. (2012) Construction of “small-intelligent” focused mutagenesis libraries using well-designed combinatorial degenerate primers. *Biotechniques*, 52 (3), 149–158.