

Supplementary Materials:

Rational Design of Recombinant Papain-Like Cysteine Protease: Optimal Domain Structure and Expression Conditions for Wheat-Derived Enzyme Triticain- α

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Table S1. Oligonucleotides used for cloning of Triticain- α expression vectors.

Construct	Oligonucleotide (Forward/Reverse)*
pET26-Triticain- α -GM	5'-TATACATATGTCGATCGTGTCTCGTACGG/ 5'-TATTAAGCTTTTAGCCCGTCTTCGTCCGG
pET28-6HIS-Triticain- α -GM	5'-TATACATATGTCGATCGTGTCTCGTACGG/ 5'-TATTAAGCTTTTAGCCCGTCTTCGTCCGG
pET28-Triticain- α -GM-6HIS	5'-TATACCATGGCGGACATGTCGATCGTGTCTCG/ 5'-TATTCGAGGCCCGTCTTCGTCCGGTAG
pET26-pelB-Triticain- α -GM-6HIS	5'-TATACCATGGCGGACATGTCGATCGTGTCTCG/ 5'-TATTCGAGGCCCGTCTTCGTCCGGTAG
pQE80-6HIS-Triticain- α -CatD	5'-TGGATCCCTGCCGGAGACCGTCCG/ 5'-TATTAAGCTTTTAGCCCGTCTTCGTCCGG
pET28-6HIS-Triticain- α -CatD	5'-TATCATATGCTGCCGGAGACCGTCCG/ 5'-TATTAAGCTTTTAGCCCGTCTTCGTCCGG
pET26-Triticain- α -CatD	5'-TATCATATGCTGCCGGAGACCGTCCG/ 5'-TATTAAGCTTTTAGCCCGTCTTCGTCCGG
pQE80-6HIS-Triticain- α -ProD	5'-ATGGATCCATCGTGTCTGACGGGGAG/ 5'-TATTAAGCTTTACTCCTCGTTGTCGTCG
pET30-Triticain- α -CatD-6HIS	5'-TATCATATGCTGCCGGAGACCGTCCG/ 5'-TTCGAGGCCCGTCTTCGTCCGGT
pGEX-GST-Triticain- α -CatD	5'-ATGGATCCCTGCCGGAGACCGTCCGAC/ 5'-TTCGAGTTAGCCCGTCTTCGTCCGG
pET15-6HIS-Triticain- α -GM	5'-TATACATATGTCGATCGTGTCTCGTACGG/ 5'-TTCGAGTTAGCCCGTCTTCGTCCGG
pET15-Triticain- α -CatD -6HIS	5'-ATACCATGGCGCTGCCGGAGACCGTCCG/ 5'-ATTTCGAGTCACTGGTGGTGGTGGTGGCCCGTCTTCGTCCGGT
pET15-Triticain- α -GM-6HIS	5'-TATACCATGGCGGACATGTCGATCGTGTCTCG/ 5'-ATTTCGAGTCACTGGTGGTGGTGGTGGTGGCCCGTCTTCGTCCGGT
pPIC9-Triticain- α -GM	5'-TGAATTCATCCATCGTGTCTCGTACGGG/ 5'-ATTCGGGCCGCTTAGCCCGTCTTCGTCCGG
pPIC9K-Triticain- α -G	5'-TGAATTCATCCATCGTGTCTCGTACGGG/ 5'-ATTCGGGCCGCTTAGCCCGTCTTCGTCCGG
	<i>Mutagenic Megaprimer:</i>
pET15-6HIS-extP-Triticain- α -CatD	5'-GCCGGAGACCGTCCGATTGGAGGAAGAAGG 5'-TTACATATGAATTATGAAGAAGTTATAAAAAAATATAGAGG/ 5'-ATTCATATGGAAATTTTCTTCTCCTCTATATTTTTTATAA
pET15-6HIS-extP-Triticain- α -CatD ^{C154A}	5'-TTACATATGAATTATGAAGAAGTTATAAAAAAATATAGAGG/ 5'-ATTCATATGGAAATTTTCTTCTCCTCTATATTTTTTATAA
pET15[6HIS-Triticain- α -CatD+6HIS-Triticain- α -ProD]	5'-ATAAGATCTAAATCATAAAAAAATTTATTGTC/ 5'-ATAAGATCTATTCTCACCAATAAAAAAACG

* Restriction sites are underlined