082218 Mayer lab DnaK-J domain chimeric plasmid

Original pBAD Forward primer read for pMPM1416 sample #2:

NNNNNNNNNNGNNNNNNNNNNNNNCNGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCATACCCGTTTTTTTGGATGGAGTGAAAGGAGGAATTCACCATGGCTAAGCAAGATTATTACGAGATTTTAGGCGTTTCCAAAACAGCGGAAGAGCGTGAAATCAGAAAGGCCTACAAACGCCTGGCCATGAAATACCACCCGGACCGTAACCAGGGTGACAAAGAGGCCGAGGCGAAATTTAAAGAGATCAAGGAAGCTTATGAAGTTCTGACCGACTCGCAAAAACGTGCGGCATACGATCAGTATGGTCATGCTGCGTTTGAGCAAGGTGGCATGGGCGGCGGCGGTTTTGGCGGCGGCGCAGACTTCAGCGATATTTTTGGTGACGTTTTCGGCGATATTTTTGGCGGCGGATCTTCTCATATGGGTAAAATAATTGGTATCGACCTGGGTACTACCAACTCTTGTGTAGCGATTATGGATGGCACCACTCCTCGCGTGCTGGAGAACGCCGAAGGCGATCGCACCACGCCTTCTATCATTGCCTATACCCAGGATGGTTGTACACTAGTTGGTCAGCCGGCTAAACGTCAGGCAGTGACGAACCCGCAAAACACTCTGTTTGCGATTAAACGCCTGATTGGTCGCCGCTTCCAGGACGAAGAAGTACAGCGTGATGTTTCCATCATGCCGTTCAAAATTATTGCTGCTGATAACGGCGACGCATGGGTCGAAGTTAAAGGCCAGAAAATGGCACCGCCGCAGATTTCTGCTGAAGTGCTGAAAAAAATGAAGAAAACCGCTGAAGATTACCTGGGTGAACCGGTAACTGAAGCTGTTATCACCGTACCGGCATACTTTAACGATGCTCAGCGTCAGGCAACCAAAGACGCAGGCCGTATCGCTGGTCTGGAAGTAAAACGTATCATCAACGAACCGACCGCAGCTGCGCTGGCTTACGGTCTGGACAAAGGCACTGGCAACCGTACTATCGCGGGTTNATGACCTGGGTGGTGGCGCCTTCNNATATTTCTATTATCGAAATCGACGAAGTTGANNGCGAAAAANNTTCGAAGTNCTGGCAACCAACGGTGANNNCCACCTGGGGGGGTGAANACTTCGACNGCCGNCTGATNANTANCNNNNNANNNNNNNNNANNNTNNNNNNNGACNGNNCNANNATCNCNNGCANNGCNNNCNNNNNANNNNNNNAAAANNNNAAATCNANNNNNNNCNNNANNNNNNCNNNNNNNNNNANNNTNNNNNNNNNNNNNNNNNNNGNNNNNNANNNNNCNNNNNN

DnaK-JD F primer read for pMPM1416 sample #2:

NNNNNNNNNNNNNNGTNTNNNCATGCCGTTCNAAATTATTGCTGCTGATAACGGCGACGCATGGGTCGAAGTTAAAGGCCAGAAAATGGCACCGCCGCAGATTTCTGCTGAAGTGCTGAAAAAAATGAAGAAAACCGCTGAAGATTACCTGGGTGAACCGGTAACTGAAGCTGTTATCACCGTACCGGCATACTTTAACGATGCTCAGCGTCAGGCAACCAAAGACGCAGGCCGTATCGCTGGTCTGGAAGTAAAACGTATCATCAACGAACCGACCGCAGCTGCGCTGGCTTACGGTCTGGACAAAGGCACTGGCAACCGTACTATCGCGGTTTATGACCTGGGTGGTGGCGCCTTCGATATTTCTATTATCGAAATCGACGAAGTTGACGGCGAAAAAACCTTCGAAGTTCTGGCAACCAACGGTGATACCCACCTGGGGGGTGAAGACTTCGACAGCCGTCTGATCAACTATCTGGTTGAAGAATTCAAGAAAGATCAGGGCATTGACCTGCGCAACGATCCGCTGGCAATGCAGCGCCTGAAAGAAGCGGCAGAAAAAGCGAAAATCGAACTGTCTTCCGCTCAGCAGACCGACGTTAACCTGCCATACATCACTGCAGACGCGACCGGTCCGAAACACATGAACATCAAAGTGACTCGTGCGAAACTGGAAAGCCTGGTTGAAGATCTGGTAAACCGTTCCATTGAGCCGCTGAAAGTTGCACTGCAGGACGCTGGCCTGTCCGTATCTGATATCGACGACGTTATCCTCGTTGGTGGTCAGACTCGTATGCCAATGGTTCANAAGAAAGTTGCTGAGTTCTTTGGTAAAGAGCCGCGTAAAGACGTTAACCCGGACGAAGCTGTAGCAATCGGTGCTGCTGTTCNGGGTGGTGTTCTGACTGGTGACGTAAAAGACGTACTGCTGCTGGACGTTACCCCGCTGTCTCTGGGTATCGAAACCATGGGCGGTGTGATGACNACGCTGATCGCGAAAAANACCACTATCCCGACCAAGCACAGCCAGGTGTTCTCTACCGCTGANANANCAGTCTGNNNAACCATCCATGTGCTGCNGGNGAACGTAACGTGNGNTGATAACAATCNNCTGGNNCAGTTCNNNNANNTGGNATCACCCNGCNCNGNNNNGNNNGCCNCANATNNNANTANNNNANNTCNNTGCNNANNNNNCNNCNNNTTTNCNNNAAANANNAAAANCNNNNGNNAANNANNNNN

DnaK-JD R primer read for pMPM1416 sample #2:

NGNNNTGGNNNNCCNNNNCCNNNNNNNNNCNGNNNNANNCNAAGNCGATCGNNNNCNNCTTCTATCNTNCNATNCCCNGANNTGTACNNTANNNTCAGCNGNTAAACGTCAGNCAGTGACNNNCCGCAAANCNNTCTGTTTGCGATTAAACNNNGNNGGTCGNCGCTTCCAGGACGAAGAAGTACAGCGTGATGTTTCCATCATGCCGTTCAAAATTATTGCTGCTGATAACGGCGACGCATGGGTCGAAGTTAAAGGCCAGAAAATGGCACCGCCGCAGATTTCTGCTGAAGTGCTGAAAAAAATGAAGAAAACCGCTGAAGATTACCTGGGTGAACCGGTAACTGAAGCTGTTATCACCGTACCGGCATACTTTAACGATGNTCAGCGTCAGGCAACCAAAGACGCAGGCCGTATCGCTGGTCTGGAAGTAAAACGTATCATCAACGAACCGACCGCAGCTGCGCTGGCTTACGGTCTGGACAAAGGCACTGGCAACCGTACTATCGCGGTTTATGACCTGGGTGGTGGCGCCTTCGATATTTCTATTATCGAAATCGACGAAGTTGACGGCGAAAAAACCTTCGAAGTTCTGGCAACCAACGGTGATACCCACCTGGGGGGTGAAGACTTCGACAGCCGTCTGATCAACTATCTGGTTGAAGAATTCAAGAAAGATCAGGGCATTGACCTGCGCAACGATCCGCTGGCAATGCAGCGCCTGAAAGAAGCGGCAGAAAAAGCGAAAATCGAACTGTCTTCCGCTCAGCAGACCGACGTTAACCTGCCATACATCACTGCAGACGCGACCGGTCCGAAACACATGAACATCAAAGTGACTCGTGCGAAACTGGAAAGCCTGGTTGAAGATCTGGTAAACCGTTCCATTGAGCCGCTGAAAGTTGCACTGCAGGACGCTGGCCTGTCCGTATCTGATATCGACGACGTTATCCTCGTTGGTGGTCAGACTCGTATGCCAATGGTTCAGAAGAAAGTTGCTGAGTTCTTTGGTAAAGAGCCGCGTAAAGACGTTAACCCGGACGAAGCTGTAGCAATCGGTGCTGCTGTTCAGGGTGGTGTTCTGACTGGTGACGTAAAAGACGTACTGCTGCTGGACGTTACCCCGCTGTCTCTGGGTATCGAAACCATGGGCGGTGTGATGACGACGCTGATCGCGAAAAACACCNNNNNNNNNNNNNNN

Original pBAD Reverse primer read for pMPM1416 sample #2:

NNNNNNANNNNNTGNNNNNTNNNNNNNNAANNNNNNNNGNAAAANCNTNGNNNNNNNNNNNNNNNNNNNNNNCNNNNNNGGGNNNAAGNNTNGNNNNNCNTNTNNTNNNTNTNNNNTGAAGANNNNNAAAGATNCNNNNNNTTGACNTGNGCANCGATNCNNNNNNAATNCNNNNNCTGAANNNNCNGNNNNAAAAAGCGAAAATCGAANNNTNTTTCCGCTCAGCAGNCNGACGTNNNCNTNCCNNACATCNCTGCAGNCGCGACCNGNNCGNAACACATGAACATCAAAGTGANTCGTGCGAAACTGGAAAGCCTGGNTGAAGATNTTGTAAACNGTTCCNTGAGCCGNNGAAAGNTGCACTGCAGGACGCTGGCCTGTCCGTATCTGATATCGACGACGTTATCNTCGTTGGTGNTCAGACTCGTATGNCAATGGTTCAGAAGAAAGTTGNTGAGTTCTTTGGTAAAGAGCCGCGTAAAGACGTTAACCCGGACGAAGCTGTAGCAATCGGTGCTGCTGTTCAGGGTGGTGTTCTGACTGGTGACGTAAAAGACGTACTGCTGCTGGACGTTACCCCGCTGTCTCTGGGTATCGAAACCATGGGCGGTGTGATGACGACGCTGATCGCGAAAAACACCACTATCCCGACCAAGCACAGCCAGGTGTTCTCTACCGCTGAAGACAACCAGTCTGCGGTAACCATCCATGTGCTGCAGGGTGAACGTAAACGTGCGGCTGATAACAAATCTCTGGGTCAGTTCAACCTAGATGGTATCAACCCGGCACCGCGCGGCATGCCGCAGATCGAAGTTACCTTCGATATCGATGCTGACGGTATCCTGCACGTTTCCGCGAAAGATAAAAACAGCGGTAAAGAGCAGAAGATCACCATCAAGGCTTCTTCTGGTCTGAACGAAGATGAAATCCAGAAAATGGTACGCGACGCAGAAGCTAACGCCGAAGCTGACCGTAAGTGTGAGGAGCTGGTACAGACTCGCAACCAGGGCGACCATCTGCTGCACAGCACCCGTAAGCAGGTTGAAGAAGCAGGCGACAAACTGCCGGCTGACGACAAAACTGCTATCGAGTCTGCGCTGACTGCACTGGAAACTGCTCTGAAAGGTGAAGACAAAGCCGCTATCGAAGCGAAAATGCAGGAACTGGCACAGGTTTCCCAGAAACTGATGGAAATCGCCCAGCAGCAACATCACCATCATCACCATTAATAAGCTTATCGATACCGTCGACCTCGAGGGGGGGCAGCTGGCTGNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN

Upon translation of the cds (see plasmid map), aa1-105 align with DnaJ sequence and aa109-714 align with DnaK sequence, except for the following point mutations to DnaK, as described by Kityk et al:

1. E47C of DnaK (C155 of DnaK-JD)
2. T199A of DnaK (A307 of DnaK-JD)
3. F529C of DnaK (C637 of DnaK-JD)