**A:**

**pMV261:** **4488 bp**

**Ref:** Stover *et al*., 1991 Nature 351:456



5’gctagccaacaaagcgacgttgtgtctcaaaatctctgatgttacattgcacaagataaaaatatatcatcatgaacaataaaactgtctgcttacataaacagtaatacaaggggtgttatgagccatattcaacgggaaacgtcttgctcgaggccgcgattaaattccaacatggatgctgatttatatgggtataaatgggctcgcgataatgtcgggcaatcaggtgcgacaatctatcgcttgtatgggaagccccatgcgccagagttgtttctgaaacatggcaaaggtagcgttgccaatgatgttacagatgagatggtcagactaaactggctgacggaatttatgcctcttccgaccatcaagcattttatccgtactcctgatgatgcatggttactcaccactgcgatccccgggaaaacagcattccaggtattagaagaatatcctgattcaggtgaaaatattgttgatgcgctggcagtgttcctgcgccggttgcattcgattcctgtttgtaattgtccttttaacagcgatcgcgtatttcgtctcgctcaggcgcaatcacgaatgaataacggtttggttgatgcgagtgattttgatgacgagcgtaatggctggcctgttgaacaagtctggaaagaaatgcataatcttttgccattctcaccggattcagtcgtcactcatggtgatttctcacttgataaccttatttttgacgaggggaaattaataggttgtattgatgttggacgagtcggaatcgcagaccgataccaggatcttgccatcctatggaactgcctcggtgagttttctccttcattacagaaacggctttttcaaaaatatggtattgataatcctgatatgaataaattgcagtttcatttgatgctcgatgagtttttctaatcagaattggttaattggttgtaacactggcagagcattacgctgacttgacgggacggcggctttgttgaataaatcgaacttttgctgagttgaaggatcagatcacgcatcttcccgacaacgcagaccgttccgtggcaaagcaaaagttcaaaatcaccaactggtccacctacaacaaagctctcatcaaccgtggctccctcactttctggctggatgatggggcgattcaggcctggtatgagtcagcaacaccttcttcacgaggcagacctcactagttccactgagcgtcagaccccgtagaaaagatcaaaggatcttcttgagatcctttttttctgcgcgtaatctgctgcttgcaaacaaaaaaaccaccgctaccagcggtggtttgtttgccggatcaagagctaccaactctttttccgaaggtaactggcttcagcagagcgcagataccaaatactgtccttctagtgtagccgtagttaggccaccacttcaagaactctgtagcaccgcctacatacctcgctctgctaatcctgttaccagtggctgctgccagtggcgataagtcgtgtcttaccgggttggactcaagacgatagttaccggataaggcgcagcggtcgggctgaacggggggttcgtgcacacagcccagcttggagcgaacgacctacaccgaactgagatacctacagcgtgagcattgagaaagcgccacgcttcccgaagggagaaaggcggacaggtatccggtaagcggcagggtcggaacaggagagcgcacgagggagcttccagggggaaacgcctggtatctttatagtcctgtcgggtttcgccacctctgacttgagcgtcgatttttgtgatgctcgtcaggggggcggagcctatggaaaaacgccagcaacgcggcctttttacggttcctggccttttgctggccttttgctcacatgttctttcctgcgttatcccctgattctgtggataaccgtattaccgcctttgagtgagctgataccgctcgccgcagccgaacgaccgagcgcaacgcgtgagcccaccagctccgtaagttcgggtgctgtgtggctcgtacccgcgcattcaggcggcagggggtctaacgggtctaaggcggcgtgtacggccgccacagcggctcttagcggcccggaaacgtcctcgaaacgacgcatgtgttcctcctggttggtacaggtggttgggggtgctcggctgtcgctggtgtttcatcatcagggctcgacgggagagcgggggagtgtgcagttgtggggtggcccctcagcgaaatatctgacttggagctcgtgtcggaccatacaccggtgattaatcgtggtttattatcaagcgtgagccacgtcgccgacgaatttgagcagctctggctgccgtactggtccctggcaagcgacgatctgctcgaggggatctaccgccaaagccgcgcgtcggccctaggccgccggtacatcgaggcgaacccaacagcgctggcaaacctgctggtcgtggacgtagaccatccagacgcagcgctccgagcgctcagcgcccgggggtcccatccgctgcccaacgcgatcgtgggcaatcgcgccaacggccacgcacacgcagtgtgggcactcaacgcccctgttccacgcaccgaatacgcgcggcgtaagccgctcgcatacatggcggcgtgcgccgaaggccttcggcgcgccgtcgatggcgaccgcagttactcaggcctcatgaccaaaaaccccggccacatcgcctgggaaacggaatggctccactcagatctctacacactcagccacatcgaggccgagctcggcgcgaacatgccaccgccgcgctggcgtcagcagaccacgtacaaagcggctccgacgccgctagggcggaattgcgcactgttcgattccgtcaggttgtgggcctatcttcccgccctcatgcggatctacctgccgacccggaacgtggacggactcggccgcgcgatctatgccgagtgccacgcgcgaaacgccgaatttccgtgcaacgacgtgtgtcccggaccgctaccggacagcgaggtccgcgccatcgccaacagcatttggcgttggatcacaaccaagtcgcgcatttgggcggacgggatcgtggtctacgaggccacactcagtgcgcgccatgcggccatctcgcggaagggcgcagcagcgcgcacggcggcgagcacagttgcgcggcgcgcaaagtccgcgtcagccatggaggcattgctatgagcgacggctacagcgacggctacagcgacggctacaactggcagccgactgtccgcaaaaagcggcgcgtgaccgccgccgaaggcgctcgaatcaccggactatccgaacgccacgtcgtccggctcgtggcgcaggaacgcagcgagtggttcgccgagcaggctgcacgccgcgaacgcatccgcgcctatcacgacgacgagggccactcttggccgcaaacggccaaacatttcgggctgcatctggacaccgttaagcgactcggctatcgggcgaggaaagagcgtgcggcagaacaggaagcggctcaaaaggcccacaacgaagccgacaatccaccgctgttctaacgcaattggggagcgggtgtcgcgggggttccgtggggggttccgttgcaacgggtcggacaggtaaaagtcctggtagacgctagttttctggtttgggccatgcctgtctcgttgcgtgtttcgttgcgtccgttttgaataccagccagacgagacggggttctacgaatcttggtcgataccaagccatttccgctgaatatcgtggagctcaccgccagaatcggtggttgtggtgatgtacgtggcgaactccgttgtagtgcttgtggtggcatccgtggcgcggccgcggtaccagatctttaaatctagaggtgaccacaacgacgcgcccgctttgatcggggacgtctgcggccgaccatttacgggtcttgttgtcgttggcggtcatgggccgaacatactcacccggatcggagggccgaggacaaggtcgaacgaggggcatgacccggtgcggggcttcttgcactcggcataggcgagtgctaagaataacgttggcactcgcgaccggtgagtcgtaggtcgggacggtgaggccaggcccgtcgtcgcagcgagtggcagcgaggacaacttgagccgtccgtcgcgggcactgcgcccggccagcgtaagtagcggggttgccgtcacccggtgacccccggtttcatccccgatccggaggaatcacttcgcaatggccaagacaattgcggatccagctgcagaattcgaagcttatcgatgtcgacgtagttaactagcgtacgatcgactgccaggcatcaaataaaacgaaaggctcagtcgaaagactgggcctttcgttttatctgttgtttgtccggccatcatggccgcggtgatca3’

ttcgaa: *Bst*B1; gttaac: *Hpa*I

*hsp60* promoter: gaccacaa…………….ggccaagac

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**B:**

***cfp-29* (MSMEG\_5830) - 798 bp**

atgaacaacctctatcgcgacctcgccccgatcaccgaatccgcttgggccgagatcgaactggaggcgacccgcacgttcaagcgtcacatcgccggacgccgggtggtcgacgtcagcgggcccaacggtccgacgaccgcgagcgtcagcacgggtcatctgctcgacgtgagcccgcccggcgacggcgtcatcgcgcatcttcgcgatgccaaaccgctcgtgcgcctgcgggtgccgttcacggtggcgcgcagggacatcgacgacgtcgagcgcggctcgcaggactccgactgggatccggtcaaggacgccgccaagaagctcgcgttcgtcgaggaccgcgcgatcttcgagggctatgccgccgcgtcgatcgagggcatccgcagttccagctccaaccccgcgctcgcactgcccgacgacgcccgcgagatccccgacgtgatcgcccaggccctctccgagctgcgtctggccggtgtcgacgggccctactcggtgctgctctcggccgagacctacaccaaggtcagcgagaccaccgcacacggatatccgatccgcgagcacatcaaccgcctcgtcgacggtgagatcatctgggcgcccgcgatcgacggtgcgttcgtgttgtcaacgcgcggcggtgatttcgacctgcagctcggcaccgacgtgtccatcggctacctgtcccatgacgccgaggtggtccacctctacatggaggagaccatgacgttcctgtgctacaccgctgaggcctctgtcgcgctgaccccctga

**Note:** TGA: Stop codon. Do not include the stop codon when designing reverse primer for fusion of *cfp-29* with either *mCherry*, *esxA* or *esxA::FLAG*-tag amplification. When NOT using any fusions, the stop codon MUST be included in the reverse primer.

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**C:**

**mCherry: red fluorescent protein (711 bp)**

5’ATGGTGAGCAAGGGCGAGGAGGATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCATGTACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGTACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAACGTCAACATCAAGTTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAACGCGCCGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGTAG3’

**Note:** TAG: Stop codon.

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**D:**

***esxA* (Rv 3875)**

5’ATGACAGAGCAGCAGTGGAATTTCGCGGGTATCGAGGCCGCGGCAAGCGCAATCCAGGGAAATGTCACGTCCATTCATTCCCTCCTTGACGAGGGGAAGCAGTCCCTGACCAAGCTCGCAGCGGCCTGGGGCGGTAGCGGTTCGGAGGCGTACCAGGGTGTCCAGCAAAAATGGGACGCCACGGCTACCGAGCTGAACAACGCGCTGCAGAACCTGGCGCGGACGATCAGCGAAGCCGGTCAGGCAATGGCTTCGACCGAAGGCAACGTCACTGGGATGTTCGCATAG3’

**Note:** TAG: Stop codon. Do not include the stop codon when designing reverse primer for *esxA::3X FLAG*-tag amplification. When NOT using the FLAG-tag, the stop codon MUST be included in the reverse primer.

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