

07/06/2016

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| Additive: DMSO | | 0 | % | | | | |
|----------------|--------------------|------|---|-------------------------|---------------------|-------|-----|
| 50 uL | 1X_65ul 1.5ul Mg+ | 1ul | x | Master Mix (no primers) | | 7 x | |
| Amount | Compound | | | Amount | Compound | | |
| 35.5 uL | ddH ₂ O | 0.71 | | 249 uL | ddH ₂ O | 248.5 | |
| 0 uL | DMSO 100% | 0 | | 0 uL | DMSO 100% | 0 | |
| 5 uL | 10x PCR buffer | 0.1 | | 35 uL | 10x PCR buffer | 35 | |
| 4 uL | dNTPs (2.5mM each) | 0.08 | | 28 uL | dNTPs (2.5mM each) | 28 | |
| 3 uL | MgCl ₂ | 0.06 | | 21 uL | MgCl ₂ | 21 | |
| 0.5 uL | Pf, 10µM | 0.01 | | 3.5 uL | Pf, 5µM | 332.5 | 3.5 |
| 0.5 uL | Pr, 10 µM | 0.01 | | 3.5 uL | Pr, 5 µM | | 3.5 |
| 1 uL | DNA template | 0.02 | | 7 uL | DNA template | 339.5 | |
| 0.5 uL | Polymerase | 0.01 | | 3.5 uL | Polymerase | | |
| 50 uL | Total | 1 | | 350 uL | Total mm per25 ul: | 48.5 | 50 |

original protocol with 5x enhancer

Notes:

Genomic DNA BL2 tested in 16S PCR reaction, 2 types with and with no DMSO

94 C - 5 min

94 C, 45 s: 48 C, 1 min: 72 C, 2 min: 33x

72 C, 5 min

4 C, inf

primers done from stock: 9f 400, 1492r 200: by dilution to 50

| | ul | stock: uM | h2o ul | resulting uM | ul resulting |
|-------|----|-----------|--------|--------------|--------------|
| 9f | 20 | | 400 | 140 | 50 |
| | | | | | 160 |
| 1492r | 40 | | 200 | 120 | 50 |
| | | | | | 160 |

| Prepare Mmix Taq | | 0 | % | | | | |
|------------------|--------------------|------|---|-------------------------|---------------------|-----|------|
| 1000 uL | 1X_65ul 1.5ul Mg+ | 1ul | x | Master Mix (no primers) | | 1 x | |
| Amount | Compound | | | Amount | Compound | | |
| 690 uL | ddH ₂ O | 0.69 | | 690 uL | ddH ₂ O | 690 | |
| 0 uL | DMSO 100% | 0 | | 0 uL | DMSO 100% | 0 | |
| 100 uL | 10x PCR buffer | 0.1 | | 100 uL | 10x PCR buffer | 100 | |
| 80 uL | dNTPs (2.5mM each) | 0.08 | | 80 uL | dNTPs (2.5mM each) | 80 | |
| 60 uL | MgCl ₂ | 0.06 | | 60 uL | MgCl ₂ | 60 | |
| 20 uL | Pf, 5µM | 0.02 | | 20 uL | Pf, 5µM | 930 | |
| 20 uL | Pr, 5 µM | 0.02 | | 20 uL | Pr, 5 µM | | |
| 20 uL | DNA template | 0.02 | | 20 uL | DNA template | | |
| 10 uL | Polymerase | 0.01 | | 10 uL | Polymerase | | |
| 1000 uL | Total | 1 | | 1000 uL | Total mm per25 ul: | 930 | 1000 |