

| 01/03/2016 | | | | 01/03/2016 12:12 | | | |
|----------------|--------------------|--------|----------|-------------------------|-------|---------|---|
| Additive: DMSO | | 5 | % | | | | |
| 0 uL | 1X_65ul 1.5ul Mg+ | 1 | x | Master Mix (no primers) | | 0 | x |
| Amount | Compound | Amount | Compound | | | | |
| 0 uL | ddH ₂ O | 0.64 | 0 uL | ddH ₂ O | 0 | | |
| 0 uL | DMSO 100% | 0.05 | 0 uL | DMSO 100% | 0 | | |
| 0 uL | 10x PCR buffer | 0.1 | 0 uL | 10x PCR buffer | 0 | | |
| 0 uL | dNTPs (2.5mM each) | 0.08 | 0 uL | dNTPs (2.5mM each) | 0 | | |
| 0 uL | MgCl ₂ | 0.06 | 0 uL | MgCl ₂ | 0 | | |
| 0 uL | Pf, 5µM | 0.02 | 0 uL | Pf, 5µM | 0 | | |
| 0 uL | Pr, 5 µM | 0.02 | 0 uL | Pr, 5 µM | 0 | | |
| 0 uL | DNA template | 0.02 | 0 uL | DNA template | 0 | | |
| 0 uL | Polymerase | 0.01 | 0 uL | Polymerase | 0 | | |
| 0 uL | Total | 1 | 0 uL | Total mm per25 ul: | ##### | #DIV/0! | |

! Previous PCR at 87.5 C didn't work

Doing Temperature Ramp from 1) 67.3 ; 2) 68

| | | | | | |
|--------|----|---|--------|--------------|--------------|
| Notes: | | Sample #1 DNA only + Sample 20 control + BL2 | | | |
| | | 94 C - 5 min | | | |
| | | 94 C, 45 s: anneal C, 1 min: 69 C, 1 min: 31x | | | |
| | | 72 C, 10 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 |

| Additive: DMSO | | | | 0 | % | | | | |
|----------------|----|--------------------|--|------|--------|-------------------------|---------------------|-----|---------|
| 30 uL | | 1X_65ul 1.5ul Mg+ | | 1ul | x | Master Mix (no primers) | | 6 x | |
| Amount | | Compound | | | Amount | | Compound | | |
| 20.7 | uL | ddH ₂ O | | 0.69 | 124 | uL | ddH ₂ O | | 124.2 |
| 0 | uL | DMSO 100% | | 0 | 0 | uL | DMSO 100% | | 0 |
| 3 | uL | 10x PCR buffer | | 0.1 | 18 | uL | 10x PCR buffer | | 18 |
| 2.4 | uL | dNTPs (2.5mM each) | | 0.08 | 14.4 | uL | dNTPs (2.5mM each) | | 14.4 |
| 1.8 | uL | MgCl ₂ | | 0.06 | 10.8 | uL | MgCl ₂ | | 10.8 |
| 0.6 | uL | Pf, 5μM | | 0.02 | 3.6 | uL | Pf, 5μM | | 167.4 |
| 0.6 | uL | Pr, 5 μM | | 0.02 | 3.6 | uL | Pr, 5 μM | | |
| 0.6 | uL | DNA template | | 0.02 | 3.6 | uL | DNA template | | |
| 0.3 | uL | Polymerase | | 0.01 | 1.8 | uL | Polymerase | | |
| 30 | uL | Total | | 1 | 180 | uL | Total mm per25 ul: | | 27.9 30 |