| *reaction components (µL)* | | MIX 1 |
|---|---|---|
| *number of rxns:* | 1 | 23 |
| PCR water | 13.65 | 313.95 |
| 10x buffer | 2 | 46 |
| 25mM MgCl2 | 0.8 | 18.4 |
| dNTPs (8mM) | 1.25 | 28.75 |
| primer 1 (10 µM) | 0.6 | 13.8 |
| primer 2 (10 µM) | 0.6 | 13.8 |
| HotStar Taq | 0.1 | 2.3 |
| DNA template | 1 | |
| Total Volume | 20 | |
| *forward primer:* | COI_Leray_F | |
| *reverse primer:* | COI_Leray_R | |

| | |
|---|---|
| Thermocycler: | Tetraid left |
| Block: | 2 |
| Dilution: | 1:10 |
| Sites: | Hood Canal |
| Month: | May'17 |

| | *temp* | *time* | | |
|---|---|---|---|---|
| initialization | 95 | 15 min | | |
| number of cycles: | | 16 then 25 | | |
| denature | 95 | 15 | | |
| annealing | 62-46 | 30 | | |
| extension | 72 | 1 min | 16x | 25x |
| final ext. | 72 | 10min | | |
| program name: | | | | |
| HSLeray | | | | |

Notes

Continue with Amplicon production. Do all 06 samples, 1:10 dilutions, K1 1:100 as positive control. This will become the .1 replicates. Add a test for the re-extractions of 09 samples (1:10). PCR regime starts with a TouchDown from 62 to 46 in 15cycles, then 25 more cycles at 46

*SAMPLES*

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1 | SA06_1:10_A | 9 | LL06_1:10_C | 17 | NTC | 25 | |
| 2 | SA06_1:10_B | 10 | PO06_1:10_A | 18 | TR09_1:10_C | 26 | |
| 3 | SA06_1:10_C | 11 | PO06_1:10_B | 19 | LL09_1:10_C | 27 | |
| 4 | TR06_1:10_A | 12 | PO06_1:10_C | 20 | TW09_1:10_A | 28 | |
| 5 | TR06_1:10_B | 13 | TW06_1:10_A | 21 | TW09_1:10_B | 29 | |
| 6 | TR06_1:10_C | 14 | TW06_1:10_B | 22 | TW09_1:10_C | 30 | |
| 7 | LL06_1:10_A | 15 | TW06_1:10_C | 23 | | 31 | |
| 8 | LL06_1:10_B | 16 | K1_1:100 | 24 | | 32 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 49 | | 57 | | 65 | | 73 | |
| 50 | | 58 | | 66 | | 74 | |
| 51 | | 59 | | 67 | | 75 | |
| 52 | | 60 | | 68 | | 76 | |
| 53 | | 61 | | 69 | | 77 | |
| 54 | | 62 | | 70 | | 78 | |
| 55 | | 63 | | 71 | | 79 | |
| 56 | | 64 | | 72 | | 80 | |