Worksheet 2018: Summer 2017 Amphibian samples to test for Ranavirus using Kimble et al. primers (qPCR) and SYBR Green SsoAdvance Universal mix; standards -6, -7, -8, -9; Pespeni machine

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | S | S | S | S | NTC | NEG | T2-1-13 | CC2-1-10 | Y2-1-10 | O2-1-4 | BB2-1-16 | B2-1-5 |
| B | S | S | S | S | NTC | NEG | T2-1-13 | CC2-1-10 | Y2-1-10 | O2-1-4 | BB2-1-16 | B2-1-5 |
| C | N2-1-10 | X2-1-1 | N2-1-25 | H2-1-9 | J2-1-13 | I1-1-14 | J2-1-8 | X2-1-2 | M2-1-2 | S2-1-4 | Y2-1-30 | Y2-1-25 |
| D | N2-1-10 | X2-1-1 | N2-1-25 | H2-1-9 | J2-1-13 | I1-1-14 | J2-1-8 | X2-1-2 | M2-1-2 | S2-1-4 | Y2-1-30 | Y2-1-25 |
| E | N2-1-15 | C2-1-10 | AA2-1-9 | S2-1-10 | K2-1-10 | F2-1-2 | K2-1-16 | AA2-1-10 | U2-1-27 | S2-1-8 | CC2-1-12 | T2-1-10 |
| F | N2-1-15 | C2-1-10 | AA2-1-9 | S2-1-10 | K2-1-10 | F2-1-2 | K2-1-16 | AA2-1-10 | U2-1-27 | S2-1-8 | CC2-1-12 | T2-1-10 |
| G | C2-1-9 | S2-1-14 | E2-1-29 | S2-1-19 | CC2-1-8 | N2-1-17 | O2-1-5 | Y2-1-22 | F2-1-11 | BB2-1-7 | D2-1-5 | W2-1-26 |
| H | C2-1-9 | S2-1-14 | E2-1-29 | S2-1-19 | CC2-1-8 | N2-1-17 | O2-1-5 | Y2-1-22 | F2-1-11 | BB2-1-7 | D2-1-5 | W2-1-26 |

MCP – Sybr green

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Stock** | **Final konc** | **20 µl** | **10 µl** |  | **101** |
| **H2O** |  |  |  | 2.5 |  | **252.5** |
| **SSOAdvanced Universal l SYBR Green mix** | 2x | 1x |  | 5 |  | **505** |
| **Primer 1** | 10 μM | 0.2 μM |  | 0.25 |  | **25.25** |
| **Primer 2** | 10 μM | 0.2 μM |  | 0.25 |  | **25.25** |
| **Template DNA** |  |  |  | 2 |  | **2** |
|  |  |  |  |  |  |  |

Remember to Spin? Completed \_\_\_\_\_\_\_\_