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 | target |
| A | S | S | S | S | NTC | NEG | H2-1-12 | E2-1-3 | W2-1-6 | X2-1-11 | R2-1-2 | P2-1-15 | MCP |
| B | S | S | S | S | NTC | NEG | H2-1-12 | E2-1-3 | W2-1-6 | X2-1-11 | R2-1-2 | P2-1-15 |  |
| C | O2-1-3 | Q2-1-24 | E2-1-27 | K2-1-4 | I2-1-27 | E2-1-4 | J2-1-20 | S2-1-27 | AA2-1-1 | B2-1-3 | J2-1-12 | X2-1-26 |  |
| D | O2-1-3 | Q2-1-24 | E2-1-27 | K2-1-4 | I2-1-27 | E2-1-4 | J2-1-20 | S2-1-27 | AA2-1-1 | B2-1-3 | J2-1-12 | X2-1-26 |  |
| E | I2-1-1 | V2-1-12 | Y2-1-20 | Y2-1-27 | H2-1-10 | BB2-1-1 | E2-1-1 | S2-1-28 | CC2-1-15 | I2-1-28 | Q2-1-15 | AA2-1-2 |  |
| F | I2-1-1 | V2-1-12 | Y2-1-20 | Y2-1-27 | H2-1-10 | BB2-1-1 | E2-1-1 | S2-1-28 | CC2-1-15 | I2-1-28 | Q2-1-15 | AA2-1-2 |  |
| G | S2-1-18 | H2-1-7 | I2-1-4 | Q2-1-20 | CC2-1-14 | B2-1-9 | U2-1-28 | U2-1-26 | T2-1-4 | P2-1-1 | N2-1-3 | V2-1-17 |  |
| H | S2-1-18 | H2-1-7 | I2-1-4 | Q2-1-20 | CC2-1-14 | B2-1-9 | U2-1-28 | U2-1-26 | T2-1-4 | P2-1-1 | N2-1-3 | V2-1-17 |  |

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 \_\_\_\_\_\_\_\_