Worksheet 2018: Summer 2016 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 | I7-8 | G4-16 | F3-6 | L2-10 | K6-38 | C7-33 | MCP |
| B | S | S | S | S | NTC | NEG | I7-8 | G4-16 | F3-6 | L2-10 | K6-38 | C7-33 |  |
| C | K6-23 | O4-2 | J6-7 | K6-39 | A2-1 | O6-3 | L7-3 | F2-9 | P4-6 | D6-2 | I4-24 | C4-29 |  |
| D | K6-23 | O4-2 | J6-7 | K6-39 | A2-1 | O6-3 | L7-3 | F2-9 | P4-6 | D6-2 | I4-24 | C4-29 |  |
| E | Q5-17 | Q4-30 | L2-4 | J5-1 | I5-24 | K3-1 | C2-15 | F7-23 | M5-13 | H7-6 | J4-3 | K7-1 |  |
| F | Q5-17 | Q4-30 | L2-4 | J5-1 | I5-24 | K3-1 | C2-15 | F7-23 | M5-13 | H7-6 | J4-3 | K7-1 |  |
| G | E7-8 | N6-5 | Q3-23 | O7-2 | I1-2 | F4-3 | K6-5 | C7-31 | J1-5 | B7-16 | K7-16 | M4-16 |  |
| H | E7-8 | N6-5 | Q3-23 | O7-2 | I1-2 | F4-3 | K6-5 | C7-31 | J1-5 | B7-16 | K7-16 | M4-16 |  |

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