**Duplex Chytridiomycosis Detection**

***B. dendrobatidis & B. salamandrivorians*  qPCR positive Control Plasmid**

**100µL/tube**

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| **Dilution Tube** | **Quantity** |
| BdBs #1 | 1.3 x 10e6 Molecules/µL |
| BdBs #2 | 1.3 x 10e5 Molecules/µL |
| BdBs #3 | 1.3 x 10e4 Molecules/µL |
| BdBs #4 | 1.3 x 10e3 Molecules/µL |
| BdBs #5 | 1.3 x 10e2 Molecules/µL |
| BdBs #6 | 1.3 x 10e1 Molecules/µL |
| BdBs #7 | 1.3 Molecules/µL |

Important notes:

* Dilutions should be stored at -20°C between uses and are stable through multiple freeze-thaw cycles.
* Dilutions must be thoroughly vortexed and spun down (2x vortex ≥30 seconds & spin) before each use for the most accurate and log-linear standard curve.
* BdBs #7, the lowest concentration dilution (1.3 molecules/µL) will occasionally fail due to stochastic sampling error. (With 2µL BdBs #7 added to a qPCR reaction [2.6 molecules, on average], the reaction should fail approximately 7% of the time).
* The BdBs control plasmid contains target sequence for *B. dendrobatidis* and *B. salamandrivorans* (ITS-1 rRNA gene of *B. dendrobatidis* and 5.8s rRNA gene of *B. salamandrivorans*).
* Multiplex assays are complex with multiple primers, probes, and DNA targets. If one of the targets (Bd or Bs) is present in high concentration in a sample the ability to amplify a low copy number of the other target in the reaction will be reduced. During the development of this assay we found that using Qiagen’s *QuantiFast Pathogen PCR +IC Kit®* (catalogue #211352), which also includes an internal positive control, resulted in a more robust multiplex reaction than when using a generic Taq and master mix.

References:

Blooi, M. J. Clin. Microbiol. Dec 2013 vol. 51 no. 12, 4173-4177

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