**REFERENCE DYES:** ROX dye (ROX): provided with the Stargene Brilliant PCR reagents (standard), Red-Spacer 9-Spaced 9 (S9): synthesized using Red-Spacer 9 (S9) synthesis using ROX CPG & a PEG spacer amine; the spacer is to increase stability & stability.

**Purpose:** to test various reference dyes (B2) in real-time PCR.

**Ruthenium Complex (R6G, R1, R2, and R3): various Ruthenium Complexes, which exists at 460 nm and luminesce at ~620 nm. This large Stokes’ shift makes them very attractive for the ABI 7700, because it has one excitation source of 488 nm.

**Conclusions:**

- The best reference dye was ROX for this study followed closely by CR18. This is based on Ct spread, signal reliability for many replicates of several concentrations, linearity of exponential portion of amplification curve.

- Buffer A performed very well, but exhibited some ROX drop.

- The CR18 performed better than the R9. This which could be attributed to the longer spacer on the CR18, which may prevent interaction with the PCR and improve stability. ROX performed very well—similar to Buffer A. A new compound consisting of ROX and two spacers may work as well as CR18.

- Blue 665, with emission at ~665 nm, is still very interesting and warrants further study.

- The difference in performance between the FRET oligos, F118 and Buffer A, was unexpected and indicates that the dye in Buffer A is more stable for this particular assay. This matrix further comparisons, because this may be template or probe and primer dependent

**Acknowledgements:**

We would like to thank Renee Horner for her assistance with the 7700 machine. We would also like to thank Julia Vold for her assistance with running the 7700. For the use of the Rotor-Gene, we would like to thank Photos Research Products, and especially Todd Dopp for his assistance with the software.