A single-step bioluminescent endpoint assay for Nucleic Acid Amplification

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The generation of DNA in Nucleic Acid Amplification Technologies (NAATs) is necessarily accompanied by the production of pyrophosphate. Since a sensitive bioluminescent assay for pyrophosphate is available (the ELIDA method), in principle, the outcome of any particular NAAT can be assayed using an ELIDA. However a complication in performing such an endpoint ELIDA is the presence of dATP in the NAAT that can act as a substrate for the firefly luciferase used in the bioluminescent assay. This has two deleterious effects: firstly, it produces a high background level of light from samples regardless of whether pyrophosphate is present or not, secondly, the rate of decay of light emission from the ELIDA becomes increasingly high as the amount of pyrophosphate in the sample increases. These factors seriously affect the sensitivity and reproducibility of such endpoint assays. Previously, attempts to use an endpoint ELIDA to follow, in particular, PCR, have had to be in the form of (at least) two-step assays to obtain reliable results, essentially because of the high levels of dATP in PCR. Clearly, a one-step mix-and-measure assay would be preferable.

We demonstrate here, a single-step, endpoint ELIDA for PCR that is sensitive and reliable. This was possible by substituting d-alpha-S-ATP for dATP in the PCR reaction with appropriate optimisation of PCR for use with d-alpha-S-ATP. The result of this substitution is twofold: firstly it greatly reduces the background bioluminescence, since d-alpha-S-ATP is not a substrate for firefly luciferase, secondly, it stabilises the light emission from assays, even at high pyrophosphate concentrations. These improvements greatly increase the utility of endpoint, ELIDA-based assays for PCR and other NAATs.