Bicistronic Design for Precise and Reliable Gene Expression

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Despite having progressed extensively in the field of synthetic biology in terms of DNA synthesis, analysis and transplanting, we still cannot reliably, quantitatively measure expression of new genetic constructs. We engineered a biobrick compatible expression cassette to control transcription and translation initiation which can be reused in new genetic contexts. Previous research has shown that the Bicistronic design have much lesser variations in expression with varying genes of interest as compared to the regular monocistronic design.(Mutalik, Endy, Guimaraes, Cambray, Lam, Juul, Tran & Paull, 2013) The Bicistronic design(BCD) consists of two Shine-Dalgarno sequences in its translation element which when combined with indiscriminate gene of interests are known to reliably express within twofold of the relative target expression window. The expression levels can be controlled with the sequence of the Shine Dalgarno and promoter sequences. The four original BCDs driving Red fluorescent protein were chosen from V.Mutalik's and D.Endy's designs and they have very low, low, medium and high expressions. The fluorescence expression was measured using flow cytometry. The parts were made biobrick compatible using the RFC25 assembly standard. Results from the biobrick BCDs were similar as the original BCDs, which implies that scars from the restriction sites did not affect the expression levels. These parts will be submitted to partsregistry and made available to the public to be reused by other research groups.