E. Coli MoClo assembly & part library: Applications in traditional biological research
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CIDAR MoClo Assembly Format

E. coli Standard Parts and Vectors

CIDAR MoClo Assembly Format

α-aldolase

Example Application – Equal expression of variants

Our collaborator is attempting to express a wildtype aldolase protein (with N-terminal His tag) at the same level as an untagged mutant CDS and isolate heterotetramers. The N-terminal His tag has influenced expression, dramatically reducing levels of HRA.

Solution: using MoClo and a previous data to predict equal co-expression conditions, we made 12 plasmids with a pairwise combinations of similar high expression transcriptional units for each protein sequence.

Data reported as Molecules of Equivalent fluorescein (MEFL) units, a normalization which provides a measure of molecules rather than arbitrary units.

Rational design and predictable behavior.
(a) Comparison of 28 pairs of plasmids in which each pair differed only in the choice of coding sequence, either GFP or RFP, in a transcriptional unit with a given promoters and RBS part. (b) Single TU expression compared to the same TU when expressed in the same plasmid with another TU. Expression of a single TU is consistent when assembled into a larger device.

Preliminary results – Western Blots

Left: anti-His antibody identifies levels of His-tagged wildtype aldolase (HRA) in BL21 cells expressing both proteins. This preliminary data showed that the new dual expression clones all had an increase in HRA expression compared to previous clones (HRA control). Right: anti-aldolase antibodies recognize both HRA and R42A (mutant, no his-tag) versions of the aldolase protein and shows approximately equal expression of both versions (doublet bands) especially in the case of the 6-5 clone. Data provided by our collaborator: Dr. Dean Tolan and his student, Quinn Ho at Boston University.

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The part library is available from Addgene (#1000000059) and all sequence information is available online with links at www.cidarlab.org/moclo.