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Non-Confidential Description - PSU No. 2516
"pST44 Bacterial Polycistronic Expression System"

Keywords:

Bacterial expression, protein complexes, polycistronic, reconstitution

Links:

Inventor website

Related Publication

Inventors:

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Background

Many cellular processes (gene regulation, cell-cycle control, and metabolism, among others) require enzymatic or regulatory multi-component protein complexes. Isolating such complexes for biochemical and biophysical studies has proven challenging, especially for those present in scarce quantities. One current method involves over-expressing and purifying recombinant versions of each component, then reconstituting the complex *in vitro*. This provides micro- to milligram quantities: a low yield for such a tedious and complex process. An attractive alternative to *in vitro* reconstitution is *in vivo* reconstitution by co-expression, because individual components of a complex which might not fold properly when expressed individually may fold together into a complex when co-expressed in a cellular environment. Thus a new method of *in vivo* reconstitution could yield greater quantities of protein complexes, while simplifying the production process.

Invention Description

We have developed a second generation *Escherichia coli* polycistronic expression system with improved modularity over our original system. This pST44 expression system simplifies the construction of polycistronic plasmids, particularly of variant plasmids expressing deletion or point mutations in any subunit. To facilitate purification of the expressed complex, we have prepared a suite of 72 plasmids which allows individual subunits to be tagged at the N- or C-terminus with six permanent or cleavable peptide affinity tags. We have demonstrated these new features in a detailed deletion analysis of a three-protein yeast Piccolo NuA4 histone acetyltransferase complex, and in the affinity purification of a human Piccolo NuA4 complex. We have also utilized the modular design to show that the order of expression of the three subunits along the polycistronic plasmid does not affect the reconstitution of the yeast Piccolo complex in *E. coli*.

Advantages / Applications

- Simplifies the process of isolating protein complexes
- Yields a greater amount of protein complexes than previous solutions
- Modular design allows researchers greater control over order of expression

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