Submitted parts

TM- HAD:

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TM- GFP:

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GBS- GFP:

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<u>EC- HAD:</u>

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EC- GFP:

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<u>Gln-H</u>

The Gln HPQ high-affinity glutamine transport system is a member of the ATP-Binding Cassette (ABC) Superfamily of transporters [Wu95]. Based on sequence similarity, GlnH is the periplasmic glutamine-binding protein, GlnQ is the ATP-binding component, and GlnP is the membrane component of the ABC transporter. Mutation of glnP results in the impaired ability to transport glutamine as well as the inability to utilized glutamine as a sole source of carbon [Masters81, Nohno86]. Expression of the cloned glnHPQ genes on a plasmid vector restored the glnH, glnP and glnQ mutants' abilities to transport glutamine and utilize glutamine as a sole carbon source [Nohno86].

<u>HAD</u>

YniC is a sugar phosphatase belonging to the superfamily of haloacid dehalogenase (HAD)-like hydrolases. Its preferred substrate is 2-deoxyglucose-6-phosphate [Kuznetsova06]. The phosphatase activity of YniC was first discovered in a high-throughput screen of purified proteins [Kuznetsova05].

Phosphatase activity of YniC is dependent on the presence of a divalent cation such as Mg^{2+}, which appears to affect substrate binding [Kuznetsova06].

Mutagenesis of the predicted catalytic Asp residues in YniC results in loss of phosphatase activity. A yniC deletion mutant is more sensitive to the presence of 2-deoxyglucose in the growth medium than wild type, while a strain overexpressing yniC tolerates higher concentrations of 2-deoxyglucose [Kuznetsova06]. 2-deoxyglucose is taken up by E.coli and is phosphorylated to 2-deoxyglucose-6P, a toxic analog of glucose-6P.

GFP

The green fluorescent protein (GFP) is a protein composed of 238 amino acid residues (26.9 kDa) that exhibits bright green fluorescence when exposed to light in the blue to ultraviolet range. Although many other marine organisms have similar green fluorescent proteins, GFP traditionally refers to the protein first isolated from the jellyfish Aequorea victoria. The GFP from A. victoria has a major excitation peak at a wavelength of 395 nm and a minor one at 475 nm. Its emission peak is at 509 nm, which is in the lower green portion of the visible spectrum. The fluorescence quantum yield (QY) of GFP is 0.79. The GFP from the sea pansy (Renilla reniformis) has a single major excitation peak at 498 nm.

In cell and molecular biology, the GFP gene is frequently used as a reporter of expression.[4] In modified forms it has been used to make biosensors, and many animals have been created that express GFP as a proof-of-concept that a gene can be expressed throughout a given organism. The GFP gene can be introduced into organisms and maintained in their genome through breeding, injection with a viral vector, or cell transformation. To date, the GFP gene has been introduced and expressed in many Bacteria, Yeast and other Fungi, fish (such as zebrafish), plant, fly, and mammalian cells, including human. Martin Chalfie, Osamu Shimomura, and Roger Y. Tsien were awarded the 2008 Nobel Prize in Chemistry on 10 October 2008 for their discovery and development of the green fluorescent protein.

PETtr- plasmid expression

Contains a strong T7 promoter. The source of the T7 promoter is bacteriophage. This promoter is under the control of T7 operon lac, and so when we want to activate the reproduction we will add IPTG to the system, when we want to halt the operon we will substract lactose and enrich it with a different sugar as a glucose example of 1% which we used. Below is the plasmid map:

- MCS – Recognized areas of the restricted enzymes, we cloned the Gln-H gene with Nco1, Xho1.
- Amp R+
- HIS – used as a “glue” for the kolona and helps clean the proteins.

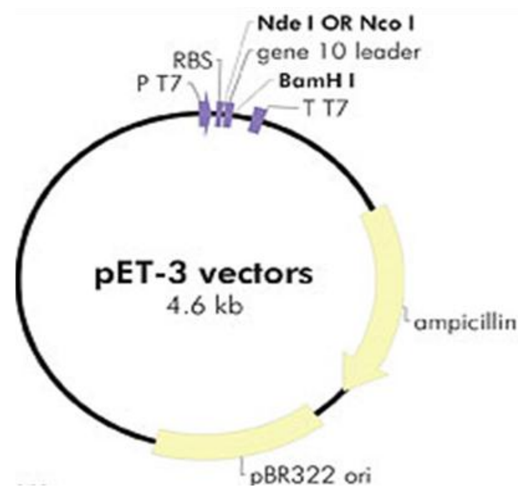

Fig 1: pET-3 vector

T7 promoter

The pET expression system is one of the most widely used systems for the cloning and in vivo expression of recombinant proteins in E.coli. This is due to the high selectivity of the pET system's bacteriophage T7 RNA polymerase for its cognate promoter sequences, the high level of activity of the polymerase and the high translation efficiency mediated by the T7 gene 10 translation initiation signals. In the

pET system, the protein coding sequence of interest is cloned downstream of the T7 promoter and gene 10 leader sequences, and then transformed into E.coli strains.

Protein expression is achieved either by IPTG induction of a chromosomally integrated cassette in which the T7 RNA polymerase is expressed from the lacUV5 promoter, or by infection with the polymerase-expressing bacteriophage lambda CE6. Due to the specificity of the T7 promoter, basal expression of cloned target genes is extremely low in strains lacking a source of T7 RNA polymerase. Upon induction the highly active polymerase essentially out-competes transcription by the host RNA polymerase. This phenomenon, together with high-efficiency translation, achieves expression levels in which the target protein may constitute the majority of the cellular protein—after only a few hours.