Protein Expression from a Chromosomally-Inserted Transposon

Darin J. Haskins, EPICENTRE Biotechnologies

Introduction

The expression of proteins in microorganisms for research or commercial applications is accomplished almost exclusively using plasmid-based expression systems. An example is the bacteriophage T7 expression system pioneered by Dr. William Studier,[1] which is used by many researchers to obtain a high-level expression of cloned proteins in *E. coli*. This system utilizes a plasmid in which the gene of interest is cloned downstream of the phage T7 gene 10 promoter and a host strain that is capable of expressing T7 RNA polymerase upon induction.

Although plasmid-based expression systems can be very useful, they are not without problems. Plasmids can place a large metabolic burden on host cells. Therefore, antibiotics may be required in the growth medium in order to avoid loss of the plasmid during cultivation.[2] Furthermore, employment of a strong plasmid selection method can kill cells that lose the plasmid, leading to an accumulation of non-viable and lysed cell matter, which complicates purification.[3] Overexpression of the cloned gene or other genes located on the plasmid can also lead to lower plasmid segregation stability.[2]

The standard practice of using antibiotic selection for plasmid maintenance also limits which plasmids and antibiotic resistance genes can be used for production of pharmaceutical grade proteins. This is because regulatory standards do not allow use of certain antibiotics because of the potential for patient allergic reactions to low levels of residual antibiotics.

An expression transposon, stably integrated into the chromosome of a microorganism, can alleviate many problems related to protein expression. The transposon can be inserted into the host chromosome using an EZ-Tn5™ Transposome™ complex (EPICENTRE Biotechnologies). In this method, the transposase is pre-bound *in vitro* to the 19-base mosaic ends (i.e., transposase recognition sequences) of an "artificial" transposon in the absence of Mg^{++}, and then electroporated into the microbe. Once inside the cell, this EZ-Tn5 Transposome is activated by Mg^{++} in the host's cellular environment, where the Transposome efficiently and randomly inserts its transposon DNA into the genomic DNA of the host cell. The transposon is called "artificial" because it lacks a gene that encodes the transposase. Therefore, the transposon is completely stable and cannot excise or reinsert itself into a new position on the chromosome.[4] Antibiotics or other selective pressures are not required to maintain the transposon insertion, and if desired, the antibiotic resistance marker used to select for the initial insertion can be removed by standard genetic engineering methods.

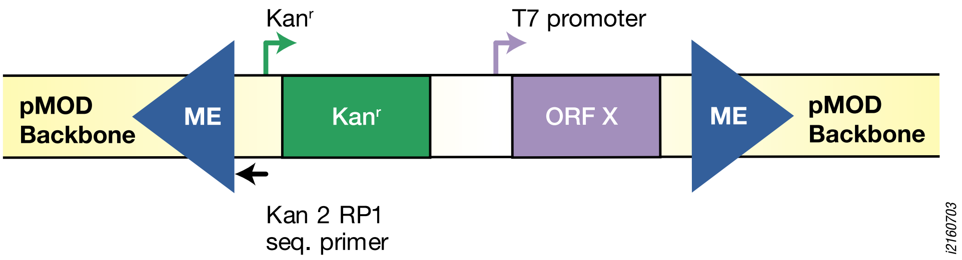

FIG 1. T7 expression cassettes were engineered into the EZ-Tn5™ pMOD™-6<KAN-2/MCS> Transposon Construction Vector in the configuration shown above.

Any DNA can be used to make an EZ-Tn5 transposon simply by adding transposase recognition sequences to both ends. Here, we report construction and use of EZ-Tn5 Transposomes for expression of two different proteins from the *E. coli* chromosome.

Methods and Results

Protein over-expression

T7 expression cassettes were engineered into the new EZ-Tn5™ pMOD™-6<KAN-2/MCS> Transposon Construction Vector in the configuration seen in FIG 1. Briefly, transcription of the gene to be expressed is driven by a T7 promoter located upstream of the open reading frame. The kanamycin resistance gene is included in the construct to provide a marker for the initial insertion event of the Transposome DNA into the *E. coli* chromosome. It is not necessary to utilize this marker in subsequent culturing, and it can be removed post-insertion if desired.

The Transposome complex was created by cutting the protein expression cassette from the pMOD vector using the *Pvu* II restriction sites outside of the mosaic ends. Alternatively, transposon DNA can be generated by standard PCR as described in EPICENTRE's product literature (www.EpiBio.com). The transposon DNA was then incubated with EZ-Tn5 transposase in the absence of Mg^{++}, allowing the stable Transposome complex to form.

The Transposome was then electroporated into the widely-used protein expression strain BL21(DE3). As described above, intracellular Mg^{++} then activates the transposase, allowing DNA insertion to occur. Chromosomal insertions were selected for by plating on kanamycin-containing LB solid medium, and confirmed by screening for the lack of a circular plasmid, potentially due to uncut pMOD vector background. The presence of each insertion on the chromosome can be confirmed by purifying total cellular DNA using the MasterPure™ DNA Purification Kit (EPICENTRE) and performing sequencing reactions using primers that anneal to the ends of the inserted EZ-Tn5 transposon.

Two different proteins were expressed in this manner to demonstrate the effectiveness of this approach. Three random chromosomal insertion clones were selected for each protein and assayed for the level of protein production as compared with the same proteins cloned into a pET T7 expression plasmid (Novagen). Protein yields were measured by lysing equivalent amounts of total cell mass and running them on an SDS-PAGE gel. The band intensities of the induced protein of interest were visually compared.

As seen in FIG 2, the expression of Protein X when using a chromosomally inserted EZ-Tn5 transposon was only very slightly lower than the expression achieved when using a pET plasmid. As for Protein Y, expression levels were very similar in *E. coli* cells, whether the protein was expressed using an EZ-Tn5 transposon or from a pET plasmid (FIG 3).

Conclusion

Chromosomal protein expression using transposon-mediated insertion is a viable alternative for high-level expression of proteins in *E. coli*. EZ-Tn5 transposon-based expression resulted in production of similar amounts

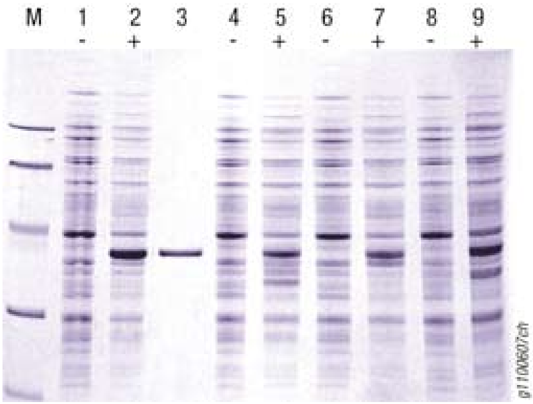

FIG 2. Expression of protein X from a pET plasmid or a chromosomally-inserted EZ-Tn5™ transposon. Equivalent amounts of un-induced(-) and induced(+) total cell lysates based on OD_{600} values were separated on a 10% SDS-PAGE. **Lanes 1-2**, pET expressed protein X; **Lane 3**, 250ng purified protein X standard; **Lanes 4-9**, chromosomal EZ-Tn5 transposon protein X expression clones.

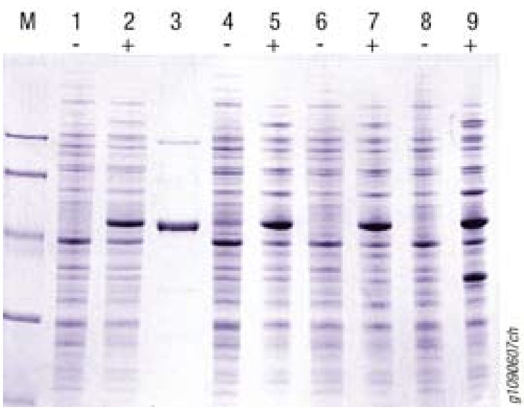

FIG 3: Expression of protein Y from a pET plasmid or a chromosomally-inserted EZ-Tn5™ transposon. Equivalent amount of un-induced(-) and induced(+) total cell lysates based on OD_{600} values were separated on a 10% SDS-PAGE. **Lanes 1-2**, pET expressed protein Y; **Lane 3**, 250ng purified protein Y standard; **Lanes 4-9**, chromosomal EZ-Tn5 transposon protein Y expression clones.

of two different proteins as a commonly used plasmid expression system. EZ-Tn5™ Transposomes provide researchers with an easy-to-use, well-characterized tool for creating these chromosomal expression strains.

Stable chromosomal insertion of heterologous genes in EZ-Tn5 transposons enable expression of high levels of proteins without use of antibiotics, which is desirable in all production situations, but especially when operating under pharmaceutical GMP conditions. The ability to stably maintain chromosomal expression strains without antibiotic selection additionally facilitates validation and verification of GMP production strains. Chromosomal expression of transposon-inserted genes also helps eliminate other problematic issues associated with plasmid based expression systems such as plasmid loss, and accumulation of excessive cellular debris from non-productive cells. Thus, chromosomal protein expression using the EZ-Tn5 system can increase recombinant protein yields, lower the costs associated with culture media, and simplify the protein purification process.

In addition to its uses for manufacturing proteins, the EZ-Tn5 Transposome expression technology can be used to genetically engineer bacterial strains to express non-native proteins for specific tasks, such as bio-remediation or for conversion of biomass into useful materials, and for production of biofuels.

EPICENTRE's EZ-Tn5 Transposome expression technology is a powerful tool for researchers and bioengineers who wish to "customize" microbes or express proteins at high level for downstream applications.

References

1. Studier, F. W. and Moffatt, B. A. (1986) *J. Mol. Biol.* **189**(1), 113.
2. Friehs, K. (2004) *Adv. Biochem. Eng. Biotechnol.* **86**, 47.
3. Jahic, M. *et al.,* (2003) *Microb. Cell Fact.* **2**(1), 6.
4. Goryshin, I. W. *et al.,* (2000) *Nat. Biotechnol.* **18**(1), 97.