Video Article

Inducible T7 RNA Polymerase-mediated Multigene Expression System, pMGX

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Abstract

Co-expression of multiple proteins is increasingly essential for synthetic biology, studying protein-protein complexes, and characterizing and harnessing biosynthetic pathways. In this manuscript, the use of a highly effective system for the construction of multigene synthetic operons under the control of an inducible T7 RNA polymerase is described. This system allows many genes to be expressed simultaneously from one plasmid. Here, a set of four related vectors, pMGX-A, pMGX-hisA, pMGX-K, and pMGX-hisK, with either the ampicillin or kanamycin resistance selectable marker (A and K) and either possessing or lacking an N-terminal hexahistidine tag (his) are disclosed. Detailed protocols for the construction of synthetic operons using this vector system are provided along with the corresponding data, showing that a pMGX-based system containing five genes can be readily constructed and used to produce all five encoded proteins in *Escherichia coli*. This system and protocol enables researchers to routinely express complex multi-component modules and pathways in *E. coli*.

Video Link

The video component of this article can be found at https://www.jove.com/video/55187/

Introduction

Co-expression of multiple proteins is increasingly essential, particularly in synthetic biology applications, where multiple functional modules must be expressed[1]; in studying protein-protein complexes, where expression and function often require co-expression[2,3]; and in characterizing and harnessing biosynthetic pathways, where each gene in the pathway must be expressed[4,5,6,7,8]. A number of systems have been developed for co-expression, particularly in the host organism *Escherichia coli*, the work horse for laboratory recombinant protein expression[9]. For example, multiple plasmids with differing selectable markers can be used to express individual proteins using a wealth of different expression vectors[10,11]. Single plasmid systems for multiple protein expression have used either multiple promoters to control the expression of each gene[10,12]; synthetic operons, where multiple genes are encoded on a single transcript[2,13]; or, in some cases, a single gene encoding a polypeptide that is ultimately proteolytically processed, yielding the desired proteins of interest[14].

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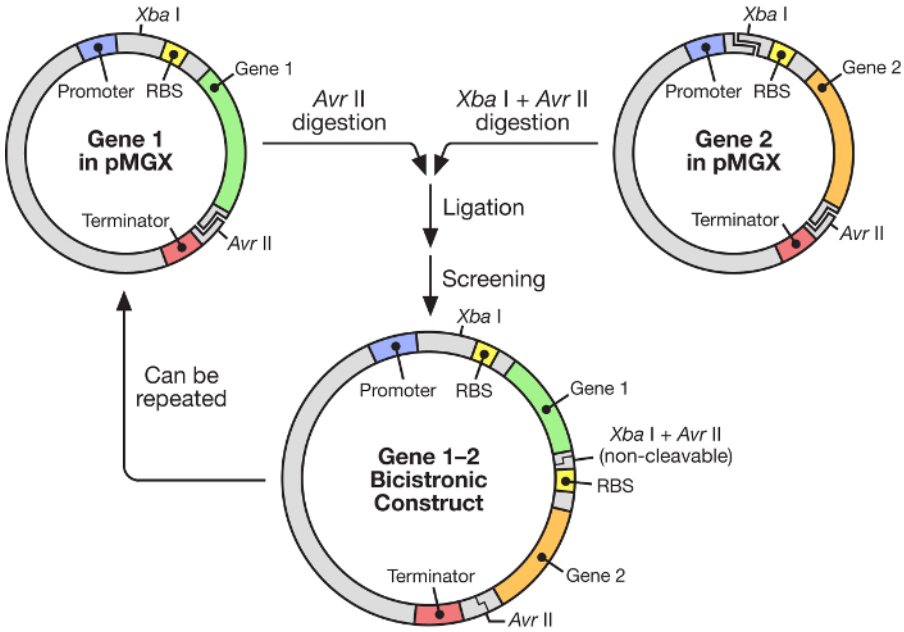

Figure 1: pMGX workflow showing the construction of a polycistronic vector. The pMGX system provides a flexible, easy-to-use strategy for the construction of synthetic operons under the control of an inducible T7 promoter. Please click here to view a larger version of this figure.

In this manuscript, the use of a highly effective system for the construction of multigene synthetic operons under the control of an inducible T7 RNA polymerase (**Figure 1**) is described. This system allows many genes to be expressed simultaneously from one plasmid. It is based on a plasmid system, originally called pKH22, that has been used successfully for a number of different applications[6,7,8]. Here, this plasmid set is expanded to include four related vectors: pMGX-A, an expression vector lacking any C- or N-terminal tags and with the ampicillin resistance marker; pMGX-hisA, an expression vector encoding an N-terminal hexahistidine tag and with the ampicillin resistance marker; pMGX-K, an expression vector lacking any C- or N-terminal tags and with the kanamycin resistance marker; and pMGX-hisK, an expression vector encoding an N-terminal hexahistidine tag and with the kanamycin resistance marker. In this study, the method for generating a polycistronic vector containing five genes using the pMGX system, specifically pMGX-A, is demonstrated along with the successful production of each individual protein in *Escherichia coli*.

Protocol

1. Obtaining Genes of Interest

1. **Design synthetic genes.**
    1. Optimize a gene sequence for *E. coli* expression.
    2. Remove any problematic restriction sites from the sequence (AvrII, NdeI, EcoRI, and XbaI).
    3. Incorporate restriction sites for cloning; a 5′-NdeI site and a 3′-EcoRI site are recommended. Other sites can be used, if necessary; refer to the multicloning region of the selected plasmid (**Figure S1-S4**). If desired, include a 5′ or 3′ encoding tag for Western blot detection.
    4. Commercially order the designed gene.
    5. Either blunt clone the gene into a blunt vector using a blunt cloning kit, as per the manufacturer's instructions; design primers to amplify the gene (then proceed to Step 1.2.2); or add additional 5′ and 3′ ends for direct cloning into a pMGX plasmid and proceed to Step 2. Here, the representative results are from blunt cloning the genes of interest.

2. **Amplify the desired gene (from a designed and optimized synthetic gene or from template DNA containing the desired gene)[15].**
    1. Design primers with restriction sites for cloning; a 5′-NdeI site and a 3′-EcoRI site are recommended. Other sites can be used, if necessary; refer to the multicloning region of the selected plasmid (**Figure S1-S4**). If desired, include either a 5′ or 3′ encoding tag for Western blot detection.
    2. PCR amplify the desired gene[15].
    3. Analyze the PCR by agarose gel electrophoresis[16].
    4. If there is unspecific amplification, clean up the amplified gene by gel extraction. If not, use an enzyme clean-up kit.
    5. Quantify the DNA using a spectrophotometer by checking the absorbance at 260 nm; blank with elution buffer[17].

2. Cloning Genes of Interest into a Multigene Expression System Vector, pMGX[18]

1. **Restriction digest the obtained gene of interest and the desired vector, pMGX, with NdeI and EcoRI.**
   NOTE: If a large amount of recircularized plasmids are obtained, allow the NdeI reaction to proceed for 1 h before adding EcoRI.
    1. Use a 40 µL digest reaction containing 0.5-1.5 µg of DNA and 1 µL of each enzyme with 4 µL of the appropriate 10x buffer.

2. For an NdeI and EcoRI digest, use EcoRI buffer and first add NdeI endonuclease. Digest for 1 h at 37 °C. Then add EcoRI endonuclease, and allow the digest to proceed for an additional hour. NdeI is sensitive to cleavages close to the end of DNA, so initial digestion by EcoRI can prevent effective digestion by NdeI.

2. Electrophorese the restriction digest on agarose gel (for a 1 kb gene, use a 0.7% agarose gel at 110 V for 55 min; to select the percent of agarose gel for differing gene sizes, refer to reference[16].
3. Excise insert and vector bands using a clean scalpel or razor blade and place the excised gel segment into a 1.5 mL tube.
4. Extract DNA from the agarose gel using a gel extraction kit according to the manufacturer's protocol.
5. **Ligate the gene of interest into the pMGX vector using a 3:1 insert-to-vector ratio; set up a negative ligation of digested pMGX without the insert.**
    1. Set up a 20 µL ligation reaction containing 1 µL of T4 DNA ligase, 0.15-0.5 µg of vector DNA (~5 µL), and an appropriate amount of insert based on a 3:1 insert-to-vector ratio and the size of the gene being inserted; the pMGX backbones are between 5,312 and 5,504 bp in size. Include a negative control reaction that contains everything but the gene being inserted. Ensure that the amount of vector DNA is equivalent in the negative control ligation and the vector plus insert ligation reactions.

6. **Transform ligation reactions into XL1-Blue chemically competent *E. coli* cells and ligated plasmids pMGX-*yfg1* (containing gene of interest 1), pMGX-*yfg2* (containing gene of interest 2) … pMGX-*yfgn* (containing gene of interest n). Use aseptic technique (inside a biosafety cabinet or under a flame).**
    1. Thaw 100 µL aliquots of chemically competent XL1-Blue *E. coli* on ice for 5 min and then add 5 µL of the ligation reactions. Incubate for 30 min on ice.
    2. Heat-shock the cells for 45 s in a water bath held at 42 °C and then add 200 µL of cold LB. Incubate them on ice for 2 min.
    3. Shake the cells at 37 °C, 220 rpm for 1 h and then spread plate 100 µL onto an LB-agar plate containing an appropriate selectable marker (either ampicillin or kanamycin).

7. **Screen the clones for positive transformants.**
    1. Compare the colony counts on the negative control and ligation plates. A colony count ratio greater than 1:2 is desired. If there are a large number of colonies on the negative control plate, go back to Step 2.1 and review the note.
    2. Select 4-8 individual colonies (depending on the negative control:ligation ratio) from each ligation reaction of the different genes inserted for screening (1 n) and inoculate a 4 mL LB and the appropriate antibiotic overnight culture/individual colony. Grow cultures overnight with shaking at 37 °C and 220 rpm.
    3. Isolate plasmid DNA using a plasmid DNA isolation kit according to the manufacturer's protocol.
    4. Set up a 20 µL NdeI + EcoRI digest reaction containing 150-500 ng of DNA and 1 µL of each enzyme with 2 µL of the appropriate 10x buffer. In this case, add NdeI and EcoRI at the same time that the gene of interest will be in between the restriction sites. A negative control with the empty pMGX vector is recommended. Digest for 2 h at 37 °C in a water bath.

3. Inserting Gene 2 into the pMGX Vector Containing Gene 1, pMGX-*yfg1*

1. **Restriction digest pMGX-*yfg1* with AvrII and treat with calf intestinal phosphatase (CIP).**
    1. Use a 40-µL digest reaction containing 0.5-1.5 µg of vector DNA (~5-10 µL of isolated DNA) and 1 µL of AvrII with 4 µL of the appropriate 10x buffer. Digest for 1.5 h at 37 °C and then add 1.5 µL of CIP. Leave it at 37 °C for an additional 30 min.

2. **Restriction digest pMGX-*yfg2* with AvrII and XbaI to liberate the gene of interest.**
    1. Use a 40 µL digest reaction containing 0.5-1.5 µg of DNA and 1 µL of each enzyme with 4 µL of the appropriate10x buffer. Digest for 2 h at 37 °C.

3. Electrophorese restriction digests on an appropriate percent (0.7%) agarose gel and excise the insert and vector bands using a clean scalpel/ razor blade (refer to Steps 2.2-2.3).
4. Extract the DNA using a gel extraction kit according to the manufacturer's protocol and quantify the DNA[17].
5. Ligate gene 2 into pMGX-*yfg1* using a 3:1 insert-to-vector ratio. Set up a negative ligation of digested pMGX-*yfg1* without the additional insert. Set up as above in Step 2.5.
6. Transform 5 µL of the ligation reactions into XL1-Blue chemically competent *E. coli* cells, ligated plasmids pMGX-*yfg1,2* (containing gene of interest 1 and 2), and negative pMGX-*yfg1* control, as seen in Step 2.6.
7. Compare the colony count on the negative control and ligation plates. A colony count ratio greater than 1:2 is desired. If there are a large number of colonies on the negative control plate, go back to Step 3.1 and review the CIP treatment.
8. Select 4-8 individual colonies (depending on the negative control:ligation ratio) from the ligation reaction and inoculate 4 mL of LB plus the appropriate antibiotic per individual colony and grow overnight at 37 °C and 220 rpm.
9. Isolate plasmid DNA using a plasmid DNA isolation kit according to the manufacturer's protocol and quantify the DNA[17].
10. **Screen the effective insertion of the second gene by performing a restriction digest of pMGX-*yfg1,2* with EcoRI.**
    1. Use a 20 µL digest reaction containing 150-500 ng of DNA and 1 µL of EcoRI enzyme with 2 µL of EcoRI 10x buffer. Digest for 2 h at 37 °C.

11. Electrophorese the restriction digest on an appropriate percent agarose gel; look for a band that corresponds to the size of gene 2 (see Step 2.2). A gene may insert into the vector in the undesired, reversed orientation.

4. Adding a Third Gene into the pMGX Vector Containing Genes 1 and 2, pMGX-*yfg1,2*

1. Restriction digest pMGX-*yfg1,2* with AvrII and treat with CIP, as seen in Step 3.1.
2. Restriction digest pMGX-*yfg3* with AvrII and XbaI, as seen in Step 3.2.

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3. Electrophorese the restriction digests on an appropriate percent agarose gel and excise insert and vector bands using a clean scalpel/razor blade; refer to Steps 2.2-2.3.
4. Extract the DNA from the agarose gel using a gel extraction kit and quantify the DNA[17].
5. Ligate gene 3 into pMGX-*yfg1,2* using a 3:1 insert-to-vector ratio and set up a negative ligation of digested pMGX-*yfg1,2* without an additional insert, as seen in Step 3.5.
6. Transform 5 µL of the ligation reactions into XL1-Blue chemically competent *E. coli* cells, ligated plasmid pMGX-*yfg1,2,3* (containing genes of interest 1, 2, and 3), and negative pMGX-*yfg1,2* control, as seen in Step 2.6.
7. Compare the colony count on the negative control and ligation plates. If there are a large number of colonies on the negative control plate, go back to Step 4.1 and review the CIP treatment.
8. Select 4-8 individual colonies (depending on the negative control:ligation ratio) from the ligation reaction and inoculate 4 mL of LB plus the appropriate antibiotic per individual colony; grow overnight at 37 °C and 220 rpm.
9. Isolate the plasmid DNA using a plasmid DNA isolation kit according to the manufacturer's protocol.
10. Screen the effective insertion of the third gene by performing a restriction digest of pMGX-*yfg1,2,3* with EcoRI, as seen in Step 3.10.
11. Electrophorese the restriction digest on an appropriate percent agarose gel; look for bands that correspond to the sizes of gene 2 and gene 3 (see Step 2.2). Note: gene 3 may insert into the vector in the undesired, reversed orientation. If genes 2 and 3 are the same size, another appropriate restriction digest site must be selected for screening.
    NOTE: Repeat as needed for each new gene.

5. Producing Proteins of Interest Using a Multigene Expression System and Assessing Production by Western Blotting

1. **Transform the positive clone containing all genes of interest into chemically competent, protein-production *E. coli*, such as BL21-(λDE3).**
    1. Thaw 100 µL aliquots of chemically competent BL21-(λDE3) *E. coli* on ice for 5 min and then add 1 µL of the positive cloned plasmid DNA; incubate for 30 min on ice.
    2. Heat shock the cells for 45 s in a water bath held at 42 °C and then add 200 µL of cold LB. Incubate on ice for 2 min.
    3. Shake the cells at 37 °C and 220 rpm for 1 h and then spread plate 100 µL onto an LB-agar plate containing the appropriate selectable marker (either ampicillin or kanamycin).

2. **Express the protein by isopropyl-β-D-1-thiogalactopyranoside (IPTG) induction.**
    1. Select an isolated colony from the B21-(λDE3) transformation plate and inoculate 4 mL of LB plus the appropriate antibiotic; grow overnight, shaking at 37 °C and 220 rpm.
    2. Inoculate a 100 mL LB plus the appropriate antibiotic culture using 1 mL of overnight culture.
    3. Grow at 37 °C with shaking at 220 rpm to an OD_{600} of 0.6.
    4. Induce the culture with 100 µM IPTG and grow for 15 h at 25 °C and 220 rpm.
    5. Remove 1 mL of the culture and centrifuge it at 13,000 x g for 1 min; discard the supernatant.

3. Lyse the cells using lysis solution as per the manufacturer's instructions and preform a Western blot of the soluble cell lysate to determine if all proteins were successfully produced[19].

Representative Results

In this study, the goal was to co-express five proteins from a single plasmid. The five-codon optimized synthetic gene fragments encoding either N- or C-terminal hexahistidine tags were purchased commercially. The synthetic genes were amplified by PCR and individually cloned into a PCR-blunt vector and sequenced. To generate the polycistronic plasmid, the five genes of interest were first cloned into a suitable pMGX plasmid, pMGX-A. **Figure 2** shows PCRBlunt-*yfg1* and PCRBlunt-*yfg2* in addition to pMGX-A digested with NdeI + EcoRI (see Step 2). To clone the first two genes into pMGX-A, the plasmid was digested with NdeI + EcoRI (**Figure 2**). Upon cloning the genes of interest into pMGX-A, the newly constructed plasmids, designated as pMGX-*yfg1* and pMGX-*yfg2*, were digested with AvrII + XbaI to confirm that successful clones were obtained, as shown in the 1.1-kb and 1.3-kb bands obtained in **Figure 3**.

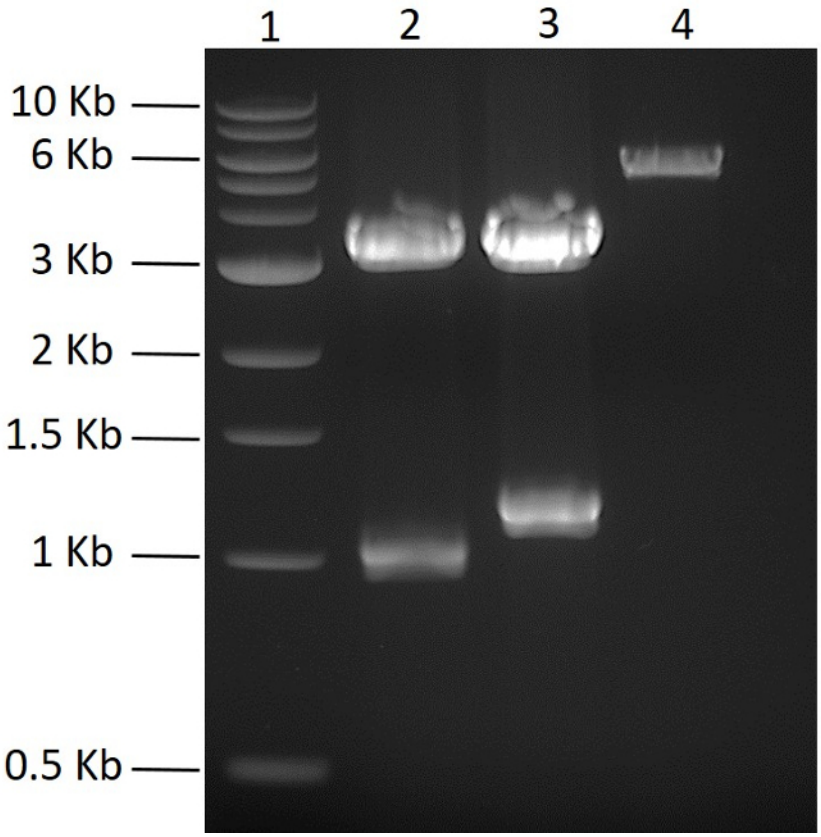

Figure 2: Cloning of pMGX plasmids. The expected results of PCR-blunt plasmids containing *yfg1* and *yfg2* after digestion with NdeI + EcoRI are shown on a 0.7% agarose gel (110 V, 55 min). Lane 1, 1 kb DNA ladder; lane 2, PCRBlunt-*yfg1*; lane 3, PCRBlunt-*yfg2*; lane 4, pMGX-A control. Please click here to view a larger version of this figure.

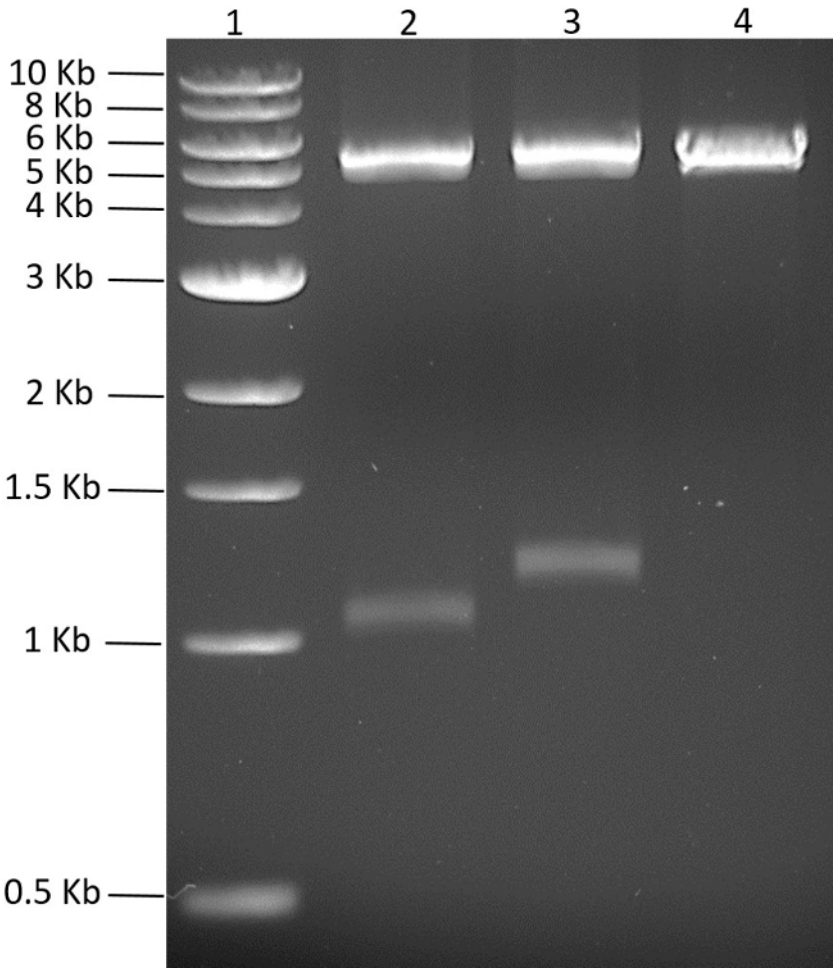

Figure 3: Screening of pMGX-*yfg1* and pMGX-*yfg2*. The 0.7% agarose gel (110 V, 55 min) shows the two bands that are generated by both pMGX-*yfg1* and pMGX-*yfg2* after digestion with AvrII + XbaI. Lane 1, 1-kb DNA ladder; lane 2, pMGX-*yfg1*; lane 3, pMGX-*yfg2*; lane 4, pMGX-A control. Please click here to view a larger version of this figure.

With both genes in the appropriate pMGX vector, the polycistronic plasmid was constructed. To generate the plasmid pMGX-*yfg1,2*, pMGX-*yfg1* was digested with AvrII, as shown in **Figure 4**. pMGX-*yfg2* was digested with AvrII + XbaI, and the 1.3-kb gene containing the fragment was ligated into the AvrII site of linearized pMGX-*yfg1*, generating pMGX-*yfg1,2*. Digestion of pMGX-*yfg1,2* with EcoRI confirmed the successful cloning of the desired plasmid and is shown in **Figure 5**.

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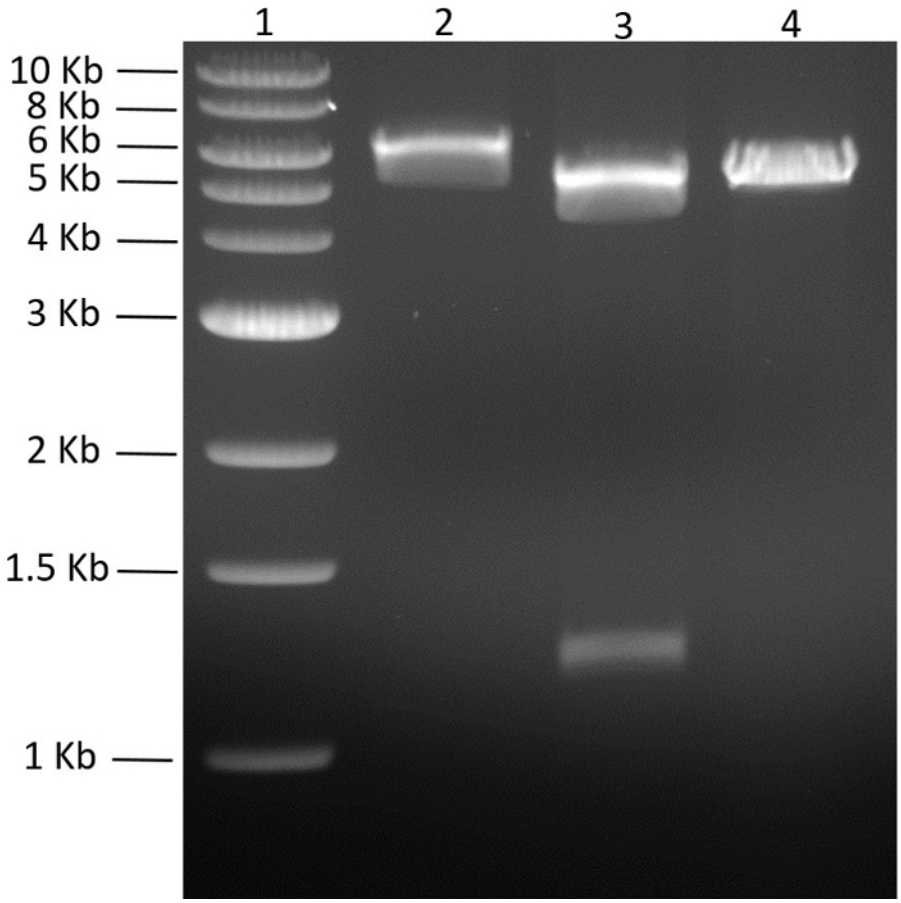

Figure 4: Generating the bicistronic plasmid pMGX-*yfg1,2*. The 0.7% agarose gel electrophoresis results (110 V, 55 min) of pMGX-*yfg1* digested with AvrII and pMGX-*yfg2* digested with AvrII + XbaI. Lane 1, 1-kb DNA ladder; lane 2, pMGX-*yfg1* digested with AvrII; lane 3, pMGX-*yfg2* digested with AvrII + XbaI; lane 4, pMGX-A control. Please click here to view a larger version of this figure.

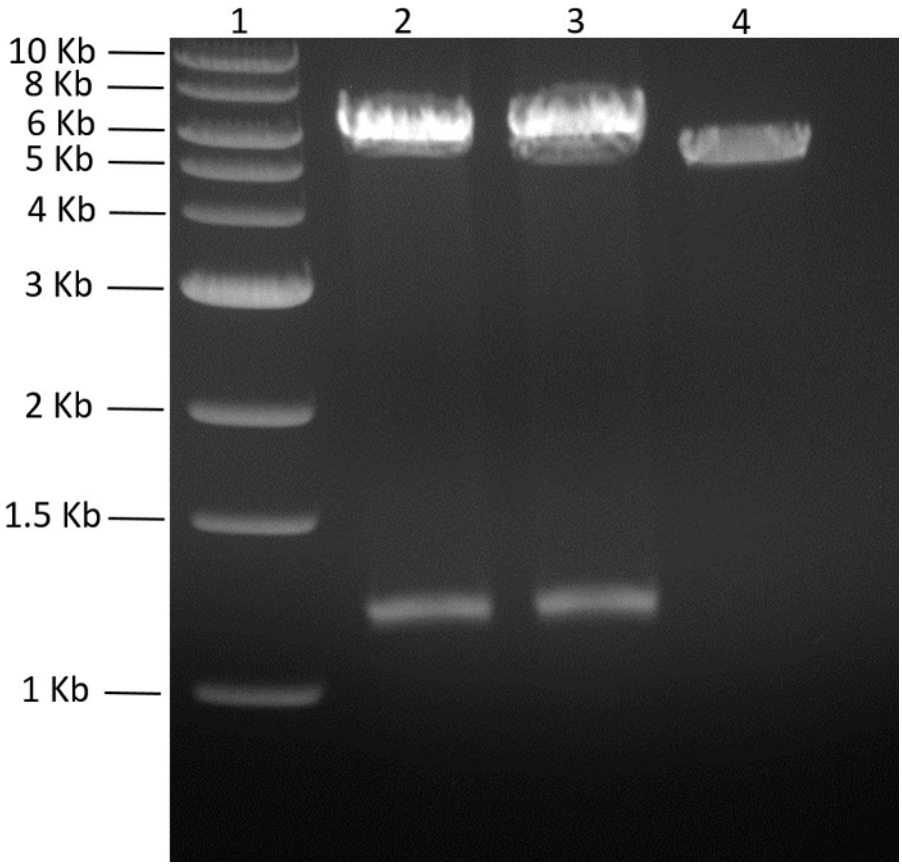

Figure 5: Screening of pMGX-*yfg1,2* clones. The resulting 0.7% agarose gel (110 V, 55 min) of pMGX-*yfg1,2* clones digested with EcoRI is shown. Successful clones will generate two bands, one for the inserted gene *yfg2* and the other corresponding to the backbone + *yfg1* (*pMGX-yfg1*). Lane 1, 1 kb DNA ladder; lane 2, pMGX-*yfg1,2* digested with EcoRI, clone 1; lane 3, pMGX-*yfg1,2* digested with EcoRI, clone 2; lane 4, pMGX-A control. Please click here to view a larger version of this figure.

When generating a polycistronic plasmid containing two or more genes, one may encounter an undesirable reversed insertion of the gene cloned into the AvrII site. **Figure 6** is a gel highlighting the difference between the results of a digested plasmid with a gene inserted in the correct orientation as opposed to a gene that is inserted in the reversed orientation. If the gene is ligated and cloned in the undesired orientation, the EcoRI site at the end of the gene will be inserted right next to the EcoRI site of the previous gene. This would yield a fragment size that is virtually undetectable by DNA gel electrophoresis (less than 50 bp). Additionally, the last inserted gene would appear in the backbone, which is readily identifiable due to the backbone size being larger than expected. The difference in the size of the backbone from the expected size will indicate the size of the last inserted gene.

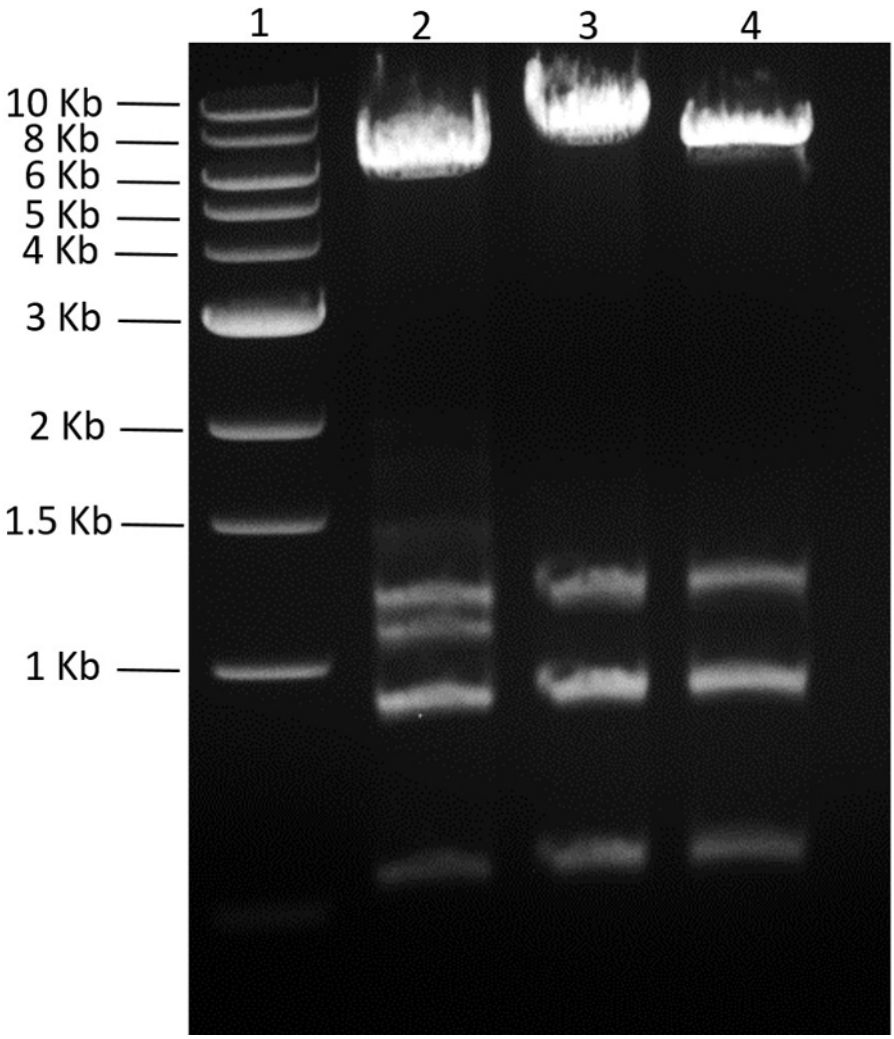

Figure 6: Screening of pMGX-*yfg1-5* clones. A 1.3% agarose gel (110 V, 65 min) depicting the results of pMGX-*yfg1-5* digested with EcoRI is shown. Successful clones (lane 2) will generate a band corresponding to the last inserted gene (*yfg5* in this case, 1.1 kb). Lane 1, 1-kb DNA ladder; lane 2, positive clone of pMGX-*yfg1-5,* digested with EcoRI; lane 3, negative clone of pMGX-*yfg1-5,* digested with EcoRI; lane 4, pMGX-*yfg1-4* control. Please click here to view a larger version of this figure.

To complete the five-gene polycistronic expression vector, the remaining three genes were cloned one after another into pMGX-*yfg1,2* to generate pMGX-*yfg1-5* (see Step 5). This plasmid was then transformed into BL21-(λDE3), and expression of the proteins was induced in a mid-log phase culture by the addition of IPTG. **Figure 7** is a Western blot of cell lysates, confirming that all five proteins from a single plasmid containing multiple genes in a single operon can be expressed. The expression of pMGX-*yfg1* (containing 1 gene), pMGX-*yfg1,2* (containing 2 genes), and pMGX-*yfg1-5* (containing 5 genes) are shown.

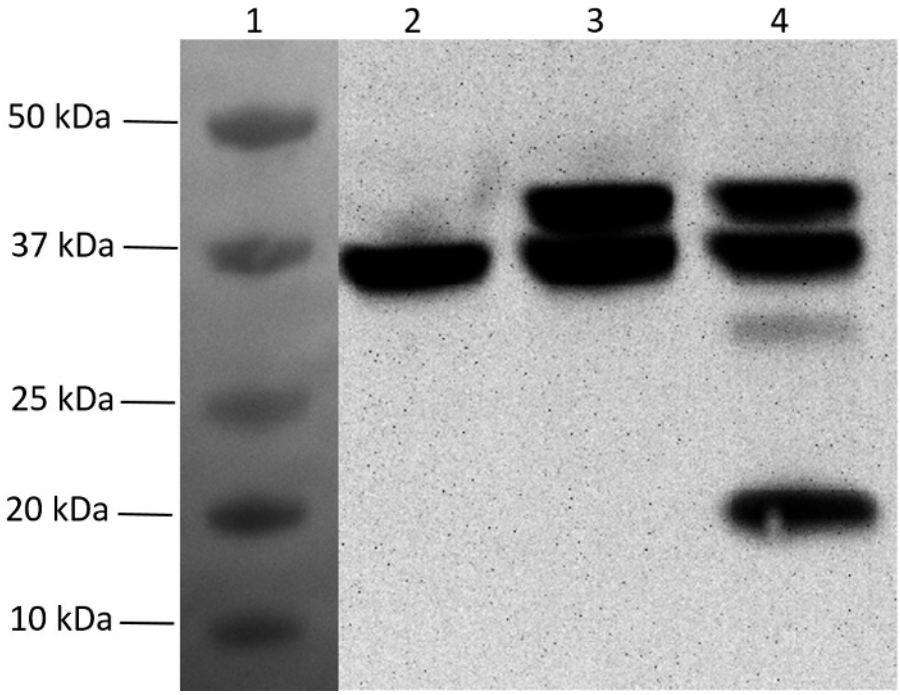

Figure 7: Western blot analysis of multi-gene expression using the pMGX system. Incorporation of either N- or C-terminal hexahistidine tags enabled the Western blot detection of protein expression. Samples were separated on pre-cast 4-20% gradient strain-free acrylamide gels (200 V, 35 min, 10 cm x 8.5 cm), and then a transfer (40 V, 90 min) onto a nitrocellulose membrane (0.45 µm, 10 cm x 7 cm) was performed. HRP-conjugated anti-His monoclonal antibody, which does not require a secondary antibody, was used. Blocking, transfer, and antibody dilution buffers were prepared as recommended by the antibody manufacturer. Detection was performed with Western Chemiluminescent HRP substrate following the manufacturer's instructions. The resulting membrane was visualized using an imager without a filter. Lane 1, dual-color standard transferred onto the membrane (not chemiluminescent); lane 2, His6pMGX-*yfg1*; lane 3, His6pMGX-*yfg1,2*; lane 4, His6pMGX-*yfg1-5.* Note: Protein produced by *yfg5* is the same size as the protein produced by *yfg1* (both are 36 kDa) and cannot be separated on the gel. Please click here to view a larger version of this figure.

Supplemental Figures: Please click here to download this file.

Discussion

Co-expression of multiple genes is increasingly essential, particularly in characterizing and reconstituting complex, multigene metabolic pathways[3,4,5]. The pMGX system makes multigene co-expression in *E. coli* routine[6,7,8] and accessible to diverse researchers. In this study, five proteins of interest were shown to be simultaneously produced from a single plasmid system using pMGX-A. Many co-expression systems currently available only allow for the insertion of two genes, generating bicistronic plasmids such as pET Duet vectors[12]. If one requires the addition of more genes, multiple bicistronic vectors with different selectable markers are required. In contrast to other multigene expression systems, the pMGX system allows for the facile cloning of multiple genes into one plasmid, with the ability to reuse restriction sites without the need for different donor plasmids or for removing multiple restriction enzyme sites from the genes[13,14]. Critical steps in this protocol include gene design, prevention of background recircularization of singly digested pMGX vectors, and screens of the polycistronic vectors for the correct orientation of the inserted gene.

In planning the cloning strategy to construct a polycistronic system, it is essential to ensure that the genes of interest do not contain restriction sites required for the downstream cloning. In particular, these include XbaI and AvrII, which are required for the insertion of the second and subsequent genes into the pMGX vector containing the first gene of interest (Steps 3 and 4). In addition, it is essential to eliminate restriction sites that will be used for the initial cloning of the genes of interest into pMGX (Step 2). Typically, these include NdeI or NheI at the 5′ end of the gene and BamHI or EcoRI at the 3′ end of the gene, although other restriction sites can also be used for this step, if necessary. Elimination of extra, unwanted restriction sites can be easily performed at the gene synthesis stage or, in the case of genes cloned from other sources, by synonymous nucleotide substitutions via site-directed mutagenesis[20].

The addition of subsequent genes of interest into pMGX containing the first gene of interest requires linearization of pMGX-*yfg1* with AvrII (Step 3.1; note that it can also be linearized with XbaI). This linearized vector will rapidly religate during the insertion of the second gene of interest (Step 3.5), leading to an extremely high number of background clones. To avoid this, it is essential to treat the linearized vector with a phosphatase to dephosphorylate the 5′ ends of the linearized vector. Typically, CIP is used, although others are available. Optimization of both the units of phosphatase and the incubation time may be necessary to optimize the ligations of subsequent genes of interest into the linearized vector.

Lastly, when generating a polycistronic system, the gene of interest is initially excised by digestion with XbaI and AvrII, generating complementary cohesive ends at the 5′ and 3′ ends of the gene (Steps 3.2 and 4.2). This insert can be ligated into the linearized vector in the forward or reverse direction. As all the cohesive ends are identical, no directionality is enforced through complementary annealing of the cohesive ends. It is thus necessary to screen the resultant clones for the correct insertion direction of the gene of interest. This can be easily

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done by screening clones with one of the restriction sites used to clone the original gene of interest into pMGX (Step 2). Typically, EcoRI is used, as the restriction site is at the 3′ end of the gene, and it is thus appropriate to screen for directionality, as shown in **Figure 6**. In most cases, 50% of the clones containing the gene of interest have the gene in the correct orientation and 50% have the gene in the opposite orientation.

Infrequently (~10% of ligations), all the clones from a particular ligation may have the gene of interest inserted in only one direction. This outcome appears to be sequence-dependent, since it is highly reproducible in these specific instances. If this occurs, and the gene of interest is continuously inserting in the reverse orientation, the desired vector can usually be accessed by switching the direction of cloning. For example, instead of cloning *yfg2* into the AvrII site of pMGX-*yfg1*, *yfg1* can be cloned into the XbaI site of pMGX-*yfg2*. Note that this gives the identical final vector system. This added flexibility enabling access to the same system from different cloning strategies is highly advantageous.

An important limitation of this methodology is that the proteins encoded at the 3′ end of the polycistronic transcript typically are expressed at lower levels than the proteins encoded at the 5′ end of the transcript, and this effect is more pronounced the longer the transcript is. This means that changing the order of the genes of interest in the synthetic operon can impact the relative expression levels of the proteins that they encode, providing a mechanism for fine-tuning relative expression levels.

In summary, the pMGX system provides a reliable method for the co-expression of multiple proteins from a single plasmid in *E. coli*, which can be used for a variety of synthetic biology applications and for biochemical pathway characterization.

Disclosures

The authors have nothing to disclose.

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