CLONING OF WHEAT GERM EUKARYOTIC INITIATION FACTOR eIF2

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Abstract

Eukaryotic Initiation Factor 2 (eIF2) is a protein complex found in eukaryotes involved in the initiation of translation. eIF2 consists of three non-identical subunits (α, β, and γ) and is required for the translation of virtually all mRNA transcripts. eIF2 forms a ternary complex with GTP and Met-tRNA$_i^{Met}$, which then associates with the 40S ribosomal subunit. This 43S preinitiation complex scans the mRNA transcript for the AUG initiation codon, at which time eIF2's GTP is hydrolyzed to GDP and eIF2-GDP dissociates from the 40S subunit. A guanine nucleotide exchange factor called eIF2B is required to reform eIF2-GTP, which can now facilitate a new round of translation initiation.

In many eukaryotic systems, eIF2 has been documented as a site of regulation of translation via phosphorylation at a conserved serine residue on the α subunit. These studies have found that phosphorylated eIF2 (eIF2[αP]) binds eIF2B with a much higher affinity than non-phosphorylated eIF2. Since eIF2B is typically present at less than half the cellular concentration of eIF2, the result of this strong binding is sequestration of eIF2B and inhibition of its nucleotide exchange activity. Because only eIF2-GTP can bind Met-tRNA$_i^{Met}$, the result is inhibition of eIF2's ternary complex formation, and thus inhibition of protein synthesis.

Most studies exploring this mechanism of translational control have focused on yeast and mammalian systems. However, in higher plants, regulation of translation by phosphorylation of eIF2α has not been shown to occur in vivo to date. Although homologous genes of all five eIF2B subunits have been identified in plants, the eIF2B protein complex has not yet been isolated from plants. Similarly, though a gene with homology to an eIF2α-kinase from yeast (*gcn2*) has been identified in plants,

phosphorylation of eIF2 in a plant translational lysate system has not yet been shown to inhibit translation. The difference of the binding affinity of eIF2 for GDP and GTP is not nearly as severe as that found in other eukaryotic systems, suggesting that a recycling factor (eIF2B) and phosphorylation of eIF2α may play a less critical regulatory role in plants.

There are significant differences between plants and animals in their responses to environmental stress, and therefore methods of translational regulation may also be different. In order to explore the extent of regulation via phosphorylation of eIF2α in plants, and to uncover other plant-specific regulation on eIF2, it is necessary to be able to express the eIF2 complex in a manipulable bacterial system. This will allow mutagenesis of the three subunits to probe structure and function. This thesis reports the use of recombinant methods to construct a single expression vector containing all three genes of eIF2 from *T. aestivum* (wheat), with the goal of subsequent expression of eIF2α, β, and γ as a functional complex in *E. coli*.

Background

Transcriptional vs Translational Regulation

A cell is defined in its function and in its identity by the constellation of proteins it produces, maintains, and secretes. The eukaryotic cell devotes a large portion of its total energy expenditure into the selective synthesis of proteins. For these reasons, protein expression is extensively regulated.

It is economically sensible for a cell to control any process at the initial step in order to preserve its limited energy and materials and, in fact, regulation of gene transcription is the most vastly prevalent and documented type of regulation in the entire pathway from gene to protein. Transcriptional control has the additional property of being highly selective, determining the specific array of proteins that the cell produces, thus conferring identity on to the cell.

However, there are several instances in which a cell might benefit by exhibiting control at the level of translation initiation. By regulating this step, a cell can respond more immediately to a particular physiological stimulus. If the cell senses an environmental stress, it can step down the rate of translation in order to preserve resources until the stress is removed and materials return to abundance. For example, virus-infected cells and cells subjected to a heat-shock treatment both exhibit translational repression. Additionally, some special systems such as early-stage embryos and reticulocytes both rely heavily on translational control, as there is an absence of any gene transcription (Mathews *et al.* 2000).

Regulation at the level of translation tends to have broad and even global effects, affecting the translation of a specified group of mRNAs, or the entire transcriptome

present in the cell cytoplasm. For this reason translational control often reveals something about the total rate of protein synthesis, which in turn can be a measure of the rate of metabolic activity, growth, and reproduction of a cell. Thus, it is important in theory and in practice to understand the mechanisms of translational control, for this is to understand what kinds of cues can increase or decrease protein synthesis and cell growth. The applications – from agricultural yield maximization to cancer therapy – are boundless.

The phenomenon of regulation at the level of translation is most widely appreciated and documented in eukaryotes (Pestova *et al.* 2007). This likely results from the spatial and temporal separation of transcription and translation in eukaryotes, which makes translational control much more necessary and useful. Translational control is most often implemented during the initiation phase of translation, by affecting the activity of an initiation factor via phosphorylation or another mechanism. These two observations warrant a detailed account of eukaryotic initiation of translation, in order to discuss the key sites and mechanisms targeted by translational regulators.

The Mechanism of Eukaryotic Translation Initiation

Eukaryotic translation initiation involves the assembly of the mRNA and Met-$tRNA_i^{Met}$ on the ribosome, followed by ribosomal scanning of the mRNA to locate the start codon. This process heavily depends on thirteen eukaryotic translation initiation factors known as eIFs (Figure 1). Before initiation can begin, the two subunits of the ribosome, usually found in association, must be separated from each other in order to allow the build up of the preinitiation complex on the 40S small ribosomal subunit. This

is accomplished by eIF3, eIF1, and eIF1A, which bind the 40S subunit and may hinder its interaction with the larger subunit (Hershey and Merrick 2000). eIF3 is a large complex that consists of thirteen polypeptides. eIF1 and eIF1A are both single polypeptide chains. It is not clear whether these three initiation factors remain associated with the 40S ribosomal subunit throughout initiation or whether they dissociate at some intermediate step. In any case, they have several important roles in later stages of initiation.

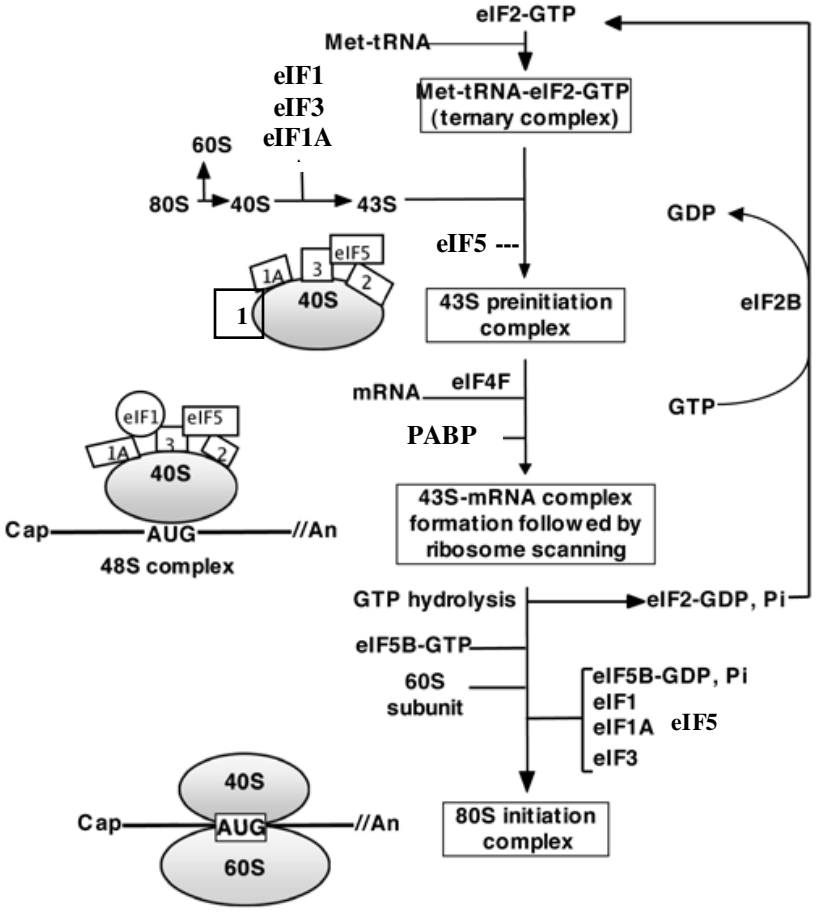

Figure 1. Mechanism of Eukaryotic Translation Initiation (Adapted from www.scielo.cl).

The first step of initiation is the formation of the ternary complex (consisting of eIF2, GTP, and Met-tRNA$_i^{Met}$) whose function is to deliver the Met-tRNA$_i^{Met}$ to the 40S

ribosomal subunit. eIF2 is a heterotrimeric protein complex consisting of α, β, and γ subunits. Of these, it is eIF2γ that binds Met-tRNA$_i^{Met}$ (assisted by eIF2β) via a particular A:U base pair (A1:U72) of the tRNA$_i^{Met}$'s acceptor stem (Pestova *et al.* 2007). eIF2γ also binds GTP and is the catalytic subunit with GTPase activity. It is important to note that eIF2-GDP cannot complex with Met-tRNA$_i^{Met}$, and hydrolysis of GTP bound to eIF2 causes a decreased affinity between eIF2γ and Met-tRNA$_i^{Met}$ (Hinnebush 2000). After the ternary complex is formed, an association between eIF3 and eIF2 helps bring the ternary complex to the 40S ribosomal subunit (Hershey and Merrick 2000). eIF5, a GTPase-activating protein (GAP) specific to eIF2, is also recruited at this time via interactions with eIF2β and eIF3.

At this stage the 40S ribosomal subunit, ternary complex, and all other associated translation factors are known collectively as the 43S preinitiation complex. The next step is the association of the 43S preinitiation complex with the mRNA transcript, facilitated by the eIF4 group of initiation factors and poly-A binding protein (PABP). eIF4F, also known as the cap-binding complex, is made up of three proteins in mammals: eIF4E, eIF4G, and eIF4A. eIF4E recognizes and binds the 7-methyl-guanosine cap at the 5' end of the mRNA (Hershey and Merrick 2000). This interaction is enhanced by binding of eIF4E by eIF4G, a large protein that plays important structural and stabilizing roles by causing conformational changes in the mRNA as well as protein factors that it binds. eIF4G also binds eIF4A, an ATP-dependant RNA helicase, effectively localizing the assembly of translation factors at the 5' end and promoting the helicase activity. Additional factors eIF4B and eIF4H increase the helicase's processivity. PABP, by binding the 3' poly-A tail and also the cap binding complex, creates a circular mRNA,

adding to the mRNA's stability (Hershey and Merrick 2000). Although it is known that eIF4G binds eIF3 and therefore that this interaction must assist the 43S preinitiation complex in loading onto the mRNA, it is not known whether the 43S complex initially associates exactly on or somewhat downstream of the 5' cap and associated factors. In the absence of any secondary structure in the 5' leader of the mRNA transcript, the 43S preinitiation complex is able to associate with the mRNA and identify the initiation codon without the presence of eIF4F, eIF4B, or PABP; however, these factors become required if the 5' UTR is even slightly structured (Pestova *et al* 2007).

It is important to note here that most members of the cap-binding complex are targets of translational control. eIF4H, eIF4B, eIF4E, eIF4G, and PABP can all be phosphorylated at several locations. Generally, the degree of phosphorylation positively correlates with the rate of translation (Gallie 2007).

These two steps have accomplished the binding of the Met-tRNA$_i^{Met}$ and the mRNA to the 40S ribosome. Although there is some evidence that these events occur in the order indicated, theoretically the mRNA may bind first, or else they may be occuring at more or less the same time (Hershey 2000). The initiation complex is now ready to scan the mRNA for the initiation codon. This process requires eIF4F unless there is no secondary structure present in the 5' leader. During scanning eIF4F maintains its association with eIF3, dissociating with the cap and causing the 5' leader end to feed out of the looped mRNA.

As noted above, the 43S preinitiation complex loads onto the mRNA at the 5' end, assisted by associations with the cap-binding complex. The 43S preinitiation complex then moves downstream along the mRNA until it encounters an AUG codon in

favorable context (Kozak 2002). The interaction between the mRNA AUG start codon and the $tRNA_i^{Met}$ UAC anticodon is the primary facilitator of initiation site recognition, at which point GTP is hydrolyzed (Pestova *et al* 2007). This linear scanning mechanism is supported by experiments showing that if an AUG is inserted upstream of the natural start codon, initiation will begin at the newly inserted AUG sequence (Kozak 2002). eIF1 is critical to the fidelity of the codon-anticodon interaction, preventing partial pairing between an incorrect mRNA codon with the $tRNA_i^{Met}$ anticodon which would result in false recognition and premature initiation of polypeptide synthesis (Pestova *et al* 2007). eIF1 also checks that the AUG start codon is in a good context on the mRNA, which entails an A or G at the -3 position of the mRNA, a G at the +4 position, and a 5' leader of at least 8 ribonucleotides. eIF2 and eIF5 have also been implicated in the fidelity of start codon recognition, based on evidence that eIF2β interacts directly with the mRNA and that mutations in eIF2 and eIF5 have been shown to allow an upstream UUG to start translation instead (Pestova *et al* 2007).

When the start site is recognized, the binding energy between the start codon and the $tRNA_i^{Met}$ anticodon pair causes the complex to stall, and the 40S ribosome switches from an open conformation to a closed conformation involving the rearrangement of the Met-$tRNA_i^{Met}$ and the mRNA. At this point, eIF5 stimulates eIF2γ to hydrolyze its bound GTP, causing a weakened interaction between Met-$tRNA_i^{Met}$ and eIF2. The energy from this GTP hydrolysis was initially thought to cause all factors to immediately dissociate (Hershey and Merrick 2000); however, this hypothesis has now been reinvestigated to show that rather than immediate dissociation, the factors are displaced from their original positions but remain associated (Pestova 2007). A factor called eIF5B

(a G protein) is then recruited and facilitates the displacement of all other translation factors. Finally, eIF5B itself dissociates using the energy of its own bound GTP, leaving the mRNA transcript and Met-tRNA$_i^{Met}$ attached to the 40S small ribosomal subunit, now with a bare interaction face which can readily associate with the 60S large ribosomal subunit to form the active 80S ribosome.

At this point, initiation is now complete, and elongation of the amino acid chain commences via the functioning of the active ribosome and elongation factors. However, in order to begin a new round of translation initiation, eIF2, currently associated with GDP after start-site dependent hydrolysis stimulated by eIF5, must be recycled to its active state, eIF2-GTP. As noted above, only eIF2-GTP can bind the Met-tRNA$_i^{Met}$ in order to form the ternary complex. The guanine exchange activity is carried out by eIF2B, a heteropentameric guanine nucleotide exchange factor (GEF) specific to eIF2.

Translational Control via eIF2α Phosphorylation

In response to certain environmental stresses, eIF2α-kinases are activated and phosphorylate eIF2α (yielding eIF2[αP]) at a conserved residue: Ser-51 (Hinnebush 2000). When phosphorylated at this position, the binding affinity between eIF2 and eIF2B increases greatly, so that eIF2 acts as an inhibitor instead of a substrate. Because the cellular concentration of eIF2 is typically two to five times as high as the cellular concentration of eIF2B (Hinnebush 2000), the effect of this strong binding is that all eIF2B becomes bound in non-functional complexes. This sequestration of eIF2B occurs even when only a fraction of the eIF2 molecules present are phosphorylated. The sequestration and inhibition of eIF2B prevents eIF2 recycling, which in turn causes a

global inhibition of translation in the cell (Figure 2).

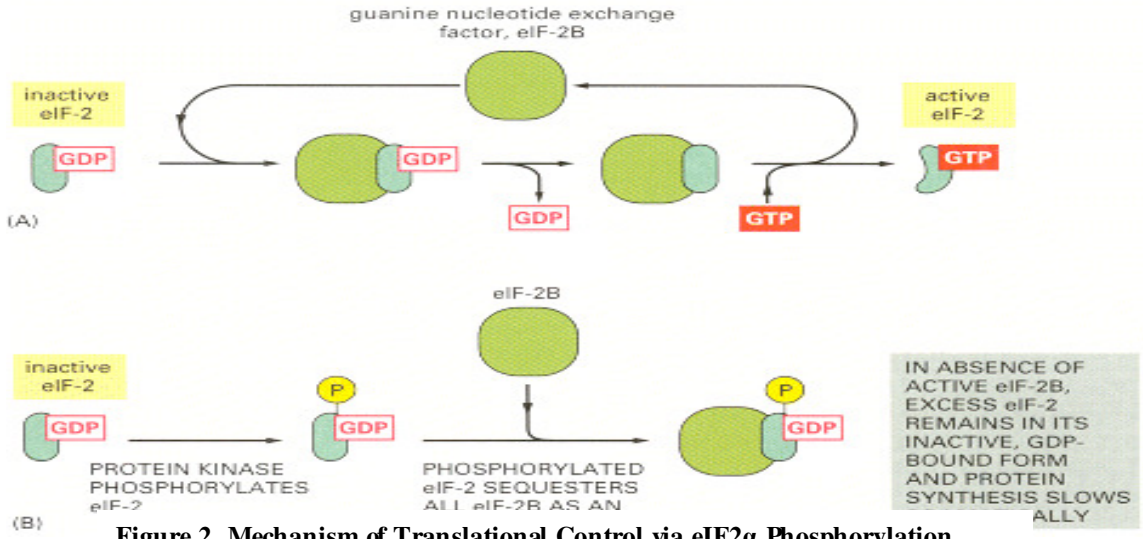

Figure 2. Mechanism of Translational Control via eIF2α Phosphorylation. From Albert's Molecular Biology of the Cell, 5th ed.

This recycling mechanism is a central point of translation inhibition, as has been demonstrated mainly in mammalian and yeast systems. There are four well characterized eIF2α-kinases studied to date (Dever *et al.* 2007). Protein Kinase R (PKR) is activated in response to viral infection via a binding domain for dsRNA; it is found in all vertebrates. PKR-like ER kinase (PERK) is present in the ER lumen of invertebrates and vertebrates and is activated in response to ER stress, as part of the unfolded protein response. GCN2 is activated in response to amino acid starvation by binding uncharged tRNAs; it has been found in all eukaryotes. Heme-regulated kinase (HRI), found in vertebrates, some yeasts, and some insects, is repressed by heme binding and activated in the absence of heme. In response to these kinases, the downstream effects in all four cases are the same: inhibition of GDP/GTP recycling by eIF2B and repression of translation (Dever *et al.* 2007).

In order to understand the nature of the interaction between eIF2 and eIF2B, it is necessary to first examine the structure and functional role of their subunits. The functions of eIF2's three subunits have been noted above as their role in translation initiation arises but are repeated here for detail and clarity. eIF2α is primarily a regulatory subunit, containing the site of phosphorylation (Serine-51) causing translational inhibition. Yeast eIF2α contains other serine residues (positions 292, 294, and 301) that are phosphorylated by Casein Kinase II (Pestova *et al.* 2007), and plant eIF2α is also phosphorylated by CK2 (Dennis 2008). However, these have not been linked directly to translational inhibition. eIF2γ has GTPase activity and binds both GTP (through a G-domain) and Met-tRNA$_i^{Met}$, which it recognizes through the A1:U72 base pair in the tRNA$_i^{Met}$'s acceptor stem. eIF2β contains three strands of lysine residues, known as K-boxes, near its amino-terminal domain that serve as the binding site for eIF5 and eIF2B. eIF2β also assists in Met-tRNA$_i^{Met}$ binding and interacts directly with the mRNA transcript, suggesting it plays a role in AUG start-codon recognition (Hinnebush 2000).

eIF2B has five subunits, three of which are regulatory (α, β, and δ) and two of which are catalytic for the GEF activity (mainly ε; stimulated and enhanced by γ). The essential interaction between the two translation factors occurs between eIF2Bε and eIF2β (Hinnebush 2000). A sequential mechanism for the guanine exchange reaction has been proposed, which suggests an intermediate quaternary complex which initially consists of eIF2B-GTP and eIF2-GDP, and subsequently rearranges to eIF2B-GDP and eIF2-GTP. The regulatory subunits of eIF2B (primarily α; stabilized by β and δ) form a pocket in which the phosphorylated arm of eIF2[αP] can settle; it is this interaction that

creates high-affinity binding leading to eIF2B inhibition and translational repression. This model is supported by experiments involving mutations near eIF2α's Ser-51 that block translational inhibition by eIF2α phosphorylation: these mutations do not block phosphorylation; rather, they weaken the interaction between eIF2[αP] and eIF2B, thus preventing sequestration of eIF2B (Hinnebush 2000).

Translational Control in Plants

Translational control in plants is an important area of study for many reasons, including applications to agriculture as well as comparative studies across eukaryotes. These comparative studies are important for understanding the evolutionary modification of translational control to suit the particular lifestyles of plants, animals, and fungi. In addition, the plant translational apparatus has benefits as a choice model for general eukaryotic translation because of easy manipulability from the molecular to the organismal level. Because of their autotrophic, sedentary lifestyle, plants have developed various molecular responses to environmental stress, whereas animals can respond to a given number of stresses simply by moving or finding another food source (Gallie 2007). Specifically, two plant species are typically used to study translational control: *Arabidopsis thaliana*, because of its sequenced genome and familiarity; and *Triticum aestivum* (wheat) because of its immediate agricultural significance, and because of the ability to easily produce an *in vitro* translation system from wheat germ.

All eukaryotic translation initiation factors discussed above arose before the divergence of the eukaryotic kingdoms and are present in animals, plants, and fungi. Most likely these initiation factors are linked to the evolutionary shift from translation

mediated by the Shine-Dalgarno sequence (prokaryotic) to 5' 7-methyl guanosine cap-dependant translation initiation (eukaryotic). Although the mechanism of translation initiation, elongation, and termination in plants is largely equatable with these processes in all other eukaryotes, it is uncertain how similar are the mechanisms of translational control between plants and the other eukaryotic kingdoms. Here the idiosyncrasies of the plant translational apparatus are presented.

The details and control of the cap-binding complex in plants differs from that in yeast and mammalian systems. Plants possess two isoforms of the cap-binding complex. The first is named eIF4F, composed of eIF4E and eIF4G; the second is named eIFiso4F, composed of eIFiso4E and eIFiso4G (Browning 1996). Both complexes combine with eIF4A. Plant eIF4F shares more sequence homology with mammalian eIF4F, whereas eIFiso4F has a slightly further evolutionary distance (Browning 1996). There exists a phosphorylated form of eIF4E/iso4E that is specific to plants and only active in rapidly growing root and shoot tips of young plants. In addition to this phosphorylated form of eIF4E, plants also express nCBP, a novel cap binding protein that shares similarity in sequence and function to eIF4E/iso4E. Because the cap-binding complex, and specifically eIF4E/iso4E, is responsible for binding and recognizing the mRNA transcript, the existence of several isoforms and phosphorylation states of eIF4E in plants suggests a mechanism of selective translation of separate classes of mRNA by the separate isoforms of the cap-binding complex.

Plant eIF4E/iso4E lack the conserved Ser-209 that in other systems affect its cap-binding affinity. In addition, 4E-BP, which inhibits the association of 4E with 4G, has not been isolated in plants. All of these findings demonstrate repeatedly that the

mechanism of the cap binding complex's activity and regulation differs significantly between plants and other eukaryotic systems, though many details remain to be uncovered.

Is Regulation via eIF2α Phosphorylation Functional in Plants?

There is evidence that the regulation of eIF2 activity via phosphorylation of eIF2α's Ser-51 residue may not be active in plants, or that it may not lead to as severe an inhibition of translation as occurs in other eukaryotic systems (Browning 2004). First, it has been shown that the dissociation constant for eIF2-GTP is only ten times higher than that for eIF2-GDP in plants (Shaikhin *et al* 1991). In contrast, the mammalian system shows as much as a 100-fold difference in the dissociation constant between the two binary complexes. As the dissociation constant measures the propensity for dissociation, this suggests that plant eIF2-GDP may be able to dissociate spontaneously, and therefore that the recycling factor eIF2B may not be as critical to translation initiation (Shaikhin *et al* 1991). This is further supported by the fact that though gene homologs of all five subunits of the recycling factor eIF2B have been identified in plant genomes, the eIF2B protein complex has not been isolated in plants, and whether it is expressed and functioning in plants remains to be shown.

Second, although one eIF2α-kinase (*gcn2)* gene is present and expressed in several plant species (Zhang *et al* 2008), it has not yet been shown to decrease the rate of translation in plants. The fact that plants do not possess *gcn4* in their genome, nor any other eIF2α-kinase gene suggests that eIF2α phosphorylation may not play a significant regulatory role in plants (Browning 2004). Since plants can synthesize all amino acids

from a *de novo* pathway, it is not logical that translational inhibition via amino acid starvation would be active in plants. In addition, four different phosphorylated isoforms of eIF2β have been found in plants, two of which are phosphorylated by Casein Kinase 2 (Dennis 2008). This suggests that it may be possible that the plant translational system may be regulated via eIF2 phosphorylation, but at another amino acid residue, and through a different mechanism.

Thus, it has not been shown conclusively that eIF2α phosphorylation occurs, is required, or is sufficient to cause translational inhibition in plants. The evidence collected to date is not fully consistent and does not convincingly show that this mechanism of regulation is or is not functional and active in plant systems. In order to resolve these differences, further study needs to be conducted. This study contributes to the clarification of this problem by constructing a single bacterial expression vector containing coding sequences of all three subunits of eIF2 from *T. aestivum* (wheat germ), in order to test the expression of eIF2α, β, and γ as a functional complex in *E. coli*. Once this is completed, manipulation and detailed characterization of wheat eIF2 may be conducted.

Experimental Methods

Gene Design

Amino acid sequences for wheat eIF2α, eIF2β, and eIF2γ were collected from NCBI (Appendix A). These amino acid sequences were reverse translated into DNA sequences (DNAworks® program at http://helixweb.nih.gov/dnaworks) to remove intronic sequences, adjust codon bias for expression in *E.coli*, and remove particular restriction sites via silent mutation. The codon usage tables for *E.coli* and *T.aestivum* (Appendix E) demonstrate the severity of the difference of codon bias of the two organisms; therefore, in order to optimize expression in *E.coli*, nearly all codons had to be adjusted. Additionally, prefix and suffix DNA sequences were added to each gene that included a mirror of the restriction site complement found in the BioBrick vector that surround the insertion region. The prefix of each gene also contains a Shine-Dalgarno prokaryotic ribosome binding site. Finally, in each gene a specific "backdoor" restriction site was engineered via silent mutation (http://mobyle.pasteur.fr/cgi-bin/portal.py?form=silent). After this manipulation, the length of the coding sequences were 1093 base pairs for eIF2α, 886 base pairs for eIF2β, and 1611 base pairs for eIF2γ. In order to ensure fidelity in the synthetic amplification process, eIF2γ was constructed in two parts: γ1 and γ2 (Figure 3, Appendix B).

The DNAworks® program generated a set of 5' to 3' overlapping 60-bp oligos spanning the entire length of the gene (both strands), allowing for gene synthesis via overlap extension PCR (Appendix C). Each set of oligos (Invitrogen) was combined in a mix so that each oligo (original concentration 100 μM) was diluted to a final concentration of 1 μM at a final volume of 200 μL.

PCR

Overlap Extension PCR: A PCR reaction was set up using 1 µL of the oligo mix, 5 µL 10X KOD® reaction buffer, 5 µL 2 mM dNTPs, 3 µL 25 mM MgSO4, 1 µL 1 U/µL KOD® HotStart® Polymerase, and 35 µL sterile dH_2O. All PCR reagents were purchased from EMD Chemicals, Novagen®. These reactions were thermocycled under the following conditions: 95°C for 2 minutes, followed by 20 cycles (denaturation at 95°C for 20 seconds, primer annealing at 58°C for 10 seconds, and elongation at 70°C for 15 seconds), and finally 70°C for 2 minutes. In overlap extension PCR, each overlapping region functions as a primer for the adjacent oligos, so that PCR products of all different lengths and positions along the gene are created. Out of this mixture of PCR products, some full gene sequence product is also present.

Gene Amplification PCR: To amplify the full gene sequence, a second PCR reaction was set up with 1 µL of the overlap extension PCR reaction, 5 µL 10X KOD® reaction buffer, 5 µL 2 mM dNTPs, 3 µL 25 mM MgSO4, 1 µL 20 mM 5' forward primer (Appendix D), 1 µL 20 mM 3' reverse primer (Appendix D), 1 µL 1U/µL KOD® HotStart Polymerase®, 33 µL sterile dH_2O. The thermal cycling conditions were as previously described, using a total of 30 cycles instead of 20.

RE Digestion

Double digest reactions consisted of 2.5 µL 20,000U/µL of each RE (New England Biolabs, Inc.®), 5 µL of the appropriate 10X NEBuffer™, (http://www.neb.com/nebecomm/DoubleDigestCalculator .asp), and 3-5 µg of plasmid in a 50 µL volume. Triple digests consisted of 2 µL 20,000 U/µL of each RE, 6 µL of the appropriate 10X

NEBuffer™ , and 3-5 µg of plasmid in a 60 µL volume. The reactions were incubated at 37ºC overnight. Vector digests were phosphatase-treated by adding 1 µL 5000U/µL Antarctic Phosphatase (NEB.®), 6 µL 10X Antarctic Phosphatase Reaction Buffer, and sterile water to a final volume of 60 µL. After adding the phosphatase, incubation at 37ºC was continued for 15 minutes, followed by 20 minutes at 65ºC for restriction enzyme heat inactivation.

Gel Purification

All PCR products or digested miniprepped plasmid products were separated on a 1% agarose gel, excised under UV visualization, and purified using the GenElute™ Gel Extraction Kit according to the manufacturer's instructions (Sigma-Aldrich®).

Ligation

For blunt-end ligations, 25 ng of Zero Blunt™ TOPO™ (Invitrogen) vector was combined with each gel-purified PCR product in a 100:1 molar ratio of PCR product insert to vector; to this was added 1 µL 400,000U/µL T4® DNA ligase (NEB®), 1 µL 10X T4® DNA ligase buffer, and sterile water to bring the final volume to 10 µL. Reactions were incubated for one hour at 16ºC.

For ligations involving restriction-digested plasmids, 50 ng of the digested BioBrick or pET22bb vector was combined with each digested insert in a 3:1 molar ratio of insert to vector, and incubated at 16ºC overnight.

Transformation

For transformation into Top10® cells (Invitrogen), 2 μL of the ligation reaction was added to thawed Top10® cells (100 μL). The cells were set on ice for 30 minutes, heat shocked in a water bath at 42℃ for 90 seconds and returned to ice for two minutes. 100 μL SOC broth was added and the cells were gently shaken at 37℃ for one hour; the entire volume was plated on agar plates containing LB and kanamycin when using Zero Blunt™, or ampicillin when using BioBrick or pet22bb, and incubated at 37℃ for 16-18 hours. For transformation into BL21® cells (Invitrogen), 1 μL of miniprepped plasmid was added to the cells; 500 μL of SOC broth was added and 50 μL was plated.

Screening

From each plate of transformed cells, 10 colonies were picked with a sterile pipette tip and immersed in a 25 μL PCR reaction containing 2.5 μL 10X Taq DNA Polymerase Reaction Buffer, 0.5 μL 10 mM dNTPs, 0.15 μL 5U/μL Taq DNA Polymerase (EMD Chemicals, Novagen®), and 1.25 μL of 20 μM 5' and 3' vector-specific primers (Zero Blunt-specific primers M13F and M13R, BioBrick-specific primers VF2 and VR, or pet22bb-specific primers T7pro and T7term (Appendix D). When colonies containing large inserts were screened (over 2 kb), an oligo was selected from the middle region of the coding sequence and in the reverse orientation, diluted to 20 μM, and used as a primer along with a vector-specific forward primer in order to decrease the length of the amplified region to about 500 bp and thereby ensure efficient amplification). After immersion in the PCR reaction, each picked colony was then immediately streaked onto replica LB-agar plates containing 30 μg/mL of kanamycin

(when using Zero Blunt) or ampicillin (when using BioBrick or pet22bb), and the tip was then ejected into a 5 mL culture of LB containing 100 µg/mL kanamycin or ampicillin. The "colony PCR" reactions were thermocycled according to the following parameters: 94ºC for 5 minutes, 35 cycles (denaturation at 94ºC for 30 seconds, primer annealing at 55ºC for 30 seconds, elongation at 72ºC for 90 seconds), and 72ºC for 5 minutes. The replica plates and cultures were incubated at 37ºC for 16-18 hours. The results of the PCR were run on a 1% agarose gel. The cultures corresponding to clones displaying an inserted sequence of the correct size were miniprepped via the GenElute™ Plasmid Miniprep Kit (Sigma-Aldrich®) and were sequenced with plasmid-specific primers for confirmation.

Results

Gene Synthesis

Complete coding sequences for eIF2α, eIF2β, eIF2γ1, and eIF2γ2 were designed and prepared through overlap extension PCR. The products of the second PCR reaction that uses 5' and 3' end primers specific to each subunit (Appendix C) are shown below (Figure 3).

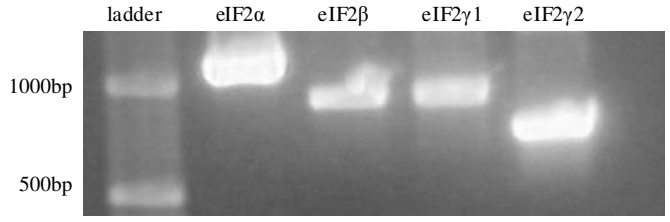

Figure 3. Confirmation Gel of Synthesized Coding Sequences. eIF2α 1093 bp; eIF2β 886 bp; eIF2γ1 886 bp; eIF2γ2 725 bp.

Each PCR product was ligated into the Zero Blunt™ vector and transformed into *E. Coli* strain Top10® competent cells. This strain of cells was chosen because of its susceptibility to the death gene *ccdB* which expresses in Zero Blunt™ vectors that have re-ligated without any insert. Because Zero Blunt™ confers kanamycin resistance, only transformed cells with inserts should be able to establish colonies on the LB-kanamycin plate. Colonies were screened (Figure 4), and true positives were miniprepped and sequenced (Appendix F). For eIF2α and eIF2β, complete correct clones were not identified. Two separate clones were chosen, one with a correct 5' end and one with a correct 3' end, for both eIF2α and eIF2β. For both eIF2γ1 and eIF2γ2, complete correct clones were identified (Appendix F).

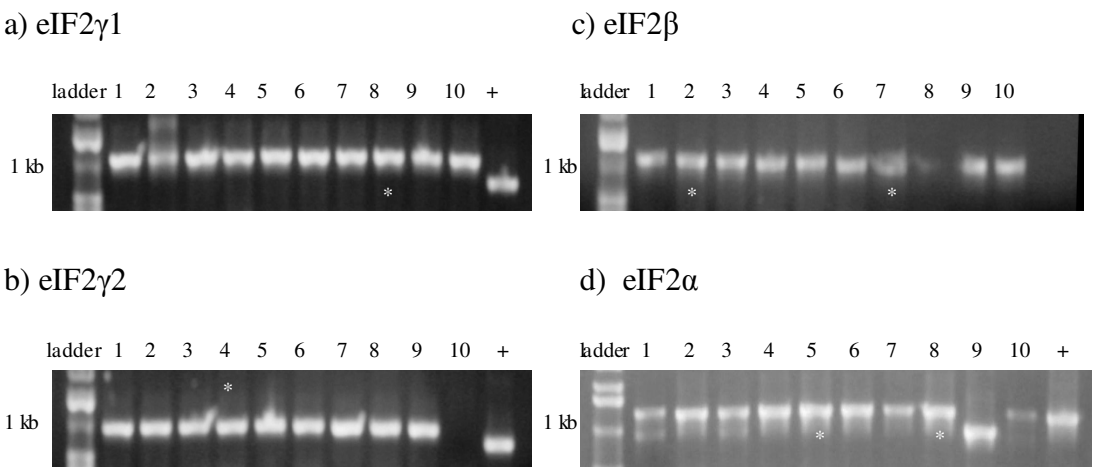

Figure 4. Colony PCR confirmation of insert-containing Zero Blunt plasmids using M13F and M13R primers. The + indicates a positive control in which a sequence-confirmed colony transformed with the Zero Blunt vector containing an insert was picked. Note there was no positive control run on image (c) because it is part of the same gel as image (d). Asterisks indicate colonies with correct sequencing results. For eIF2β and eIF2α, separate colonies were chosen with correct 5' and 3' ends because a complete correct clone was not identified. In c), eIF2β -7 has a correct 5' end, and eIF2β -2 has a correct 3' end. In d), eIF2α -8 has a correct 5' end, and eIF2α -5 has a correct 3' end.

Cloning Strategy

Overview--

1. Move correct 3' end of eIF2α, correct 5' end of eIF2β, and eIF2γ1 from Zero Blunt vectors into BioBrick vectors.
2. Move correct 5' end of eIF2α in Zero Blunt to BioBrick vector containing correct 3' end of eIF2α.
3. Move correct 3' end of eIF2β in Zero Blunt to BioBrick vector containing correct 5' end of eIF2β.
4. Use the eIF2γ1-BioBrick plasmid as a recipient vector for eIF2γ2 insert.
5. Use the eIF2α-BioBrick plasmid as a recipient vector for eIF2γ insert.
6. Use the eIF2αγ -BioBrick plasmid as a recipient vector for eIF2β insert.
7. Move the eIF2βαγ insert into pET22bb vector for protein expression studies.

Figure 5. Overview of Cloning Strategy

The three Zero Blunt plasmids that individually contained eIF2α-5 (correct 3' end), eIF2β-7 (correct 5' end), and eIF2γ1-8 were digested with EcoRI and PstI. The BioBrick vector was also digested with EcoRI and PstI; a BioBrick vector with an insert

was used so that a visible band would separate on the gel and confirm the vector was cut to completion. Each insert was ligated into the digested BioBrick vector and transformed. Colonies were screened by PCR and plasmids from positive colonies were isolated and sequenced. As shown in Figure 6, each subunit was engineered with a "backdoor" restriction cut site near the middle of the gene. This "backdoor" allows replacement of a portion of the DNA sequence if needed.

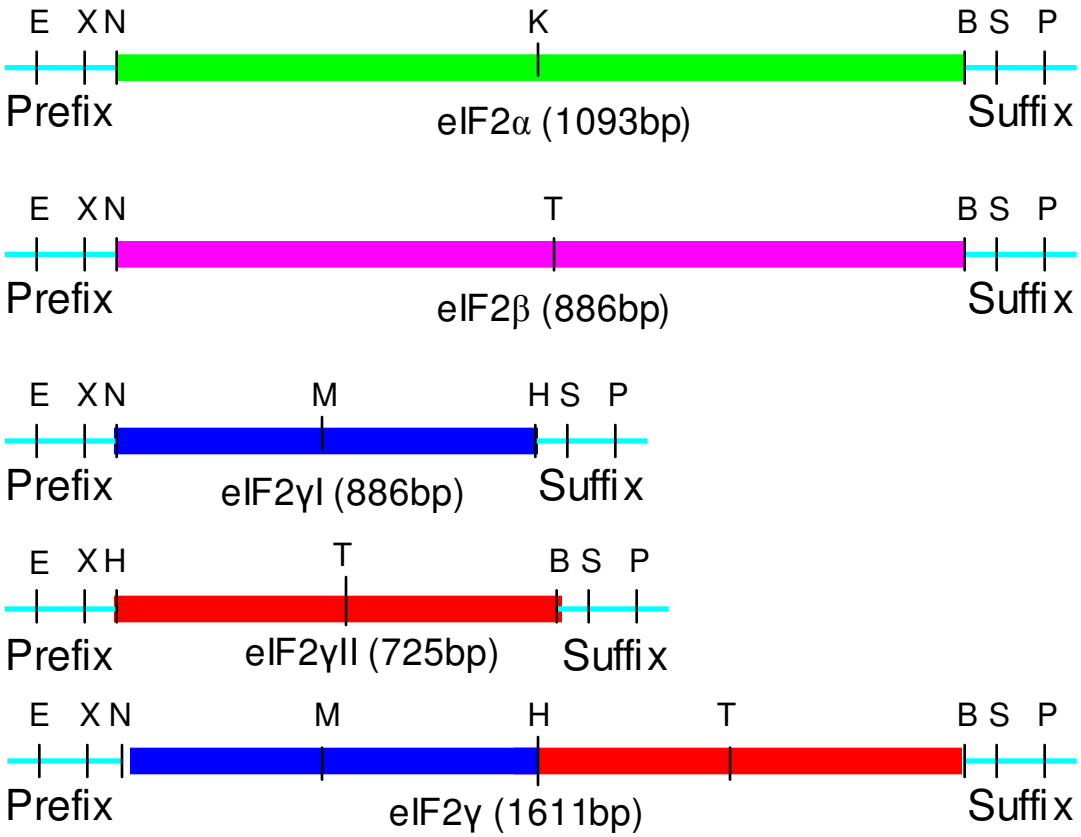

Figure 6. Gene assembly diagrams showing restriction sites, as designed and synthesized via the DNAworks program, read from left to right as 5' to 3'. Figures are not to scale. Abbreviations: E-EcoR1; X-Xba1; N-Nde1; B-BamH1; S-Spe1; P-Pst1; K-Kpn1; T-Sal1; M-Mlu1; H-HindIII. Schematics are adapted from those provided by Dr. Grace Choy PhD

To create full length correct-sequence constructs, the correct 3' end clone of eIF2α now in BioBrick (eIF2α-5) and the correct 5' end clone of eIF2α still in Zero Blunt™ (eIF2α-8) were each digested with KpnI and XbaI. The BioBrick vector band

containing the correct 3' end of eIF2α (eIF2α-5) was used as the recipient for the KpnI/XbaI fragment digested out of the Zero Blunt vector that contains the correct 5' end (eIF2α-8). In the same manner, the correct 5' end clone of eIF2β now in BioBrick (eIF2β-7) and the correct 3' end clone of eIF2β still in Zero Blunt™ (eIF2β-2) were each digested with BamHI and SalI, and the defective 3' end of eIF2β-7 was replaced by the correct 3' end of eIF2β-2. To join eIF2γ1 and γ2, as shown in Figure 3, eIF2γ1 in BioBrick and eIF2γ2 in Zero Blunt™ were each digested with HindIII and SpeI. After ligating, the resulting BioBrick plasmids now containing complete eIF2α, eIF2β, and eIF2γ coding regions were digested with restriction enzymes to confirm the correct size insert is obtained (Figure 7) and were sequenced (Appendix F).

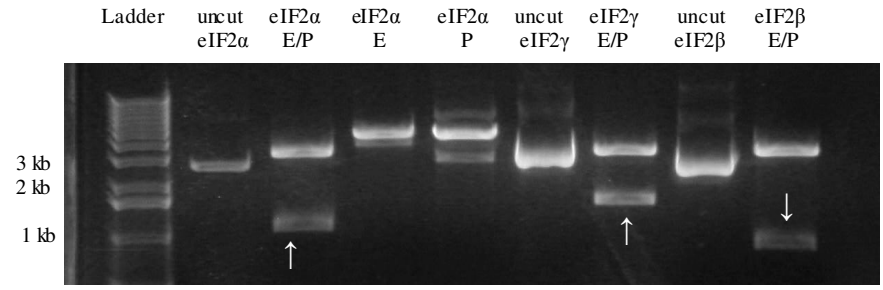

Figure 7. RE Digests with EcoRI and PstI on single-subunit plasmids in the BioBrick vector to confirm presence and correct size of insert. Expected sizes: eIF2α 1093 bp, eIF2γ 1611 bp, and eIF2β 886 bp. Abbreviations: E-EcoRI; P-PstI.

The BioBrick vector was developed at MIT (Knight 2003) and has a unique complement of restriction sites that allows the assembly of several genes in one vector via repeated restriction enzyme digests, ligations, and transformations. To place all the subunit coding regions into an operon for expression, the BioBrick vector containing eIF2α was used as the recipient for the eIF2γ coding region. Briefly, the eIF2α-BioBrick

plasmid was digested with SpeI and PstI, and the eIF2γ-BioBrick plasmid was digested with XbaI and PstI. The BioBrick vector is designed with XbaI and SpeI restriction sites on either side of the insertion region. These enzymes produce complementary overhangs that will ligate when mixed; however, the sites have different internal base pairs (those immediately adjacent to the overhang) and will no longer be cleaved by either enzyme (Figure 8). The joined parts are now a new "BioBrick" and may be joined to another DNA fragment using the same restriction enzymes.

XbaI: T/CTAGA	SpeI: A/CTAGT	After Ligation: TCTAGT
AGATC/T	TGATC/A	AGATCA

Figure 8. The figure shows the cut sites of XbaI and SpeI, and indicates the sequence resulting from a ligation reaction. All sequences are read 5' to 3' on top, 3' to 5' on bottom.

The eIF2γ insert was ligated into the eIF2α-BioBrick plasmid and correct clones were identified via screening and confirmed via restriction enzyme digestion (Figure 9).

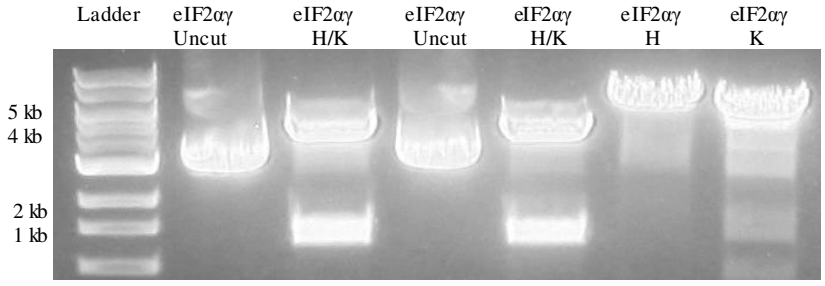

Figure 9. RE Digests with HindIII (eIF2γ backdoor enzyme) and KpnI (eIF2α backdoor enzyme) on eIF2αγ in the BioBrick vector to confirm presence and correct size of insert. Abbreviations: H – HindIII; K – KpnI.

In the same way, the eIF2αγ-BioBrick plasmid was used as the recipient for the eIF2β insert. The eIF2αγ-BioBrick was digested with EcoRI and XbaI, and the eIF2β-

BioBrick plasmid was digested with EcoRI and SpeI. The eIF2β insert was ligated into the eIF2αγ-BioBrick plasmid and resulting colonies were screened (Figure 10) and sequenced (Appendix F).

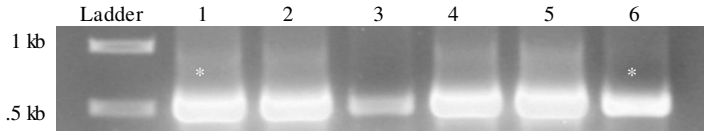

Figure 10. Colony PCR screening on eIF2βαγ in BioBrick using VF2 and an oligo from the middle of the eIF2β coding region. Asterisks indicate colonies with correct sequencing results used to continue cloning.

A schematic of the completed eIF2 operon in the BioBrick vector is shown below (Figure 11).

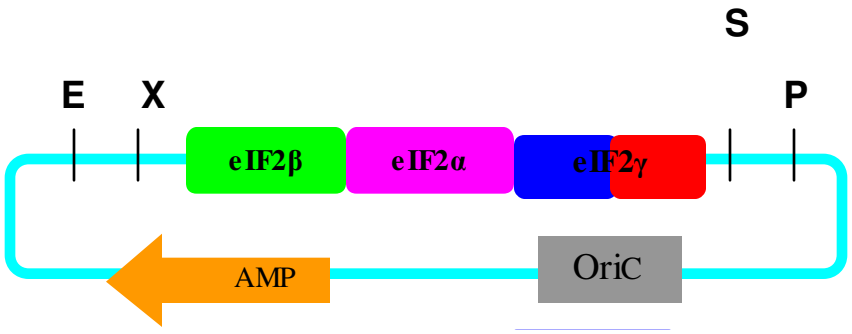

Figure 11. Completed eIF2βαγ operon in BioBrick. Each subunit contains its own Shine-Dalgarno ribosome binding site at its 5' end.

To m ector, the eIF2βαγ-BioBrick plasmid and the pET22bb vector were both digested with EcoRI, PstI, and XhoI. XhoI was added to cut the BioBrick vector backbone into three parts of around 1 kb, so that it would be easily separated from the eIF2βαγ insert (3.6 kb). The eIF2βαγ insert was ligated into the pET22bb vector and transformed into Top10 cells.

The resulting colonies were screened (Figure 12) and sequenced (Appendix F). The eIF2βαγ-pET22bb plasmid was transformed into BL21 for expression.

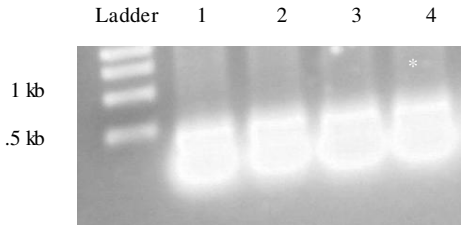

Figure 12. Colony PCR screening on eIF2βαγ in pET22bb using T7pro and an oligo from the middle of the eIF2β coding region. Asterisk indicates colony with correct sequencing results.

Discussion

Through the research experiments detailed here, plasmids have been constructed carrying coding sequences for wheat germ eIF2α, eIF2β, and eIF2γ individually in the BioBrick vector. Additionally, all three coding sequences have been assembled adjacent to one another in an operon in the BioBrick vector and transferred to the pET22bb vector for expression of the operon in *E. coli*. A discussion of the difficulties, summary of the strategies, and introduction to the initial stages of expression analysis follows in this section.

Difficulties were encountered at several steps throughout the gene-assembly process. Several of the double digest reactions called for a specific combination of restriction enzymes that did not share an optimal buffer. In these cases, the amount ratios of the two enzymes were adjusted from 1:1 to 3:2, using more of the enzyme that had lower performance in the chosen buffer. Another difficulty was obtaining sufficient recovery of the pET22bb plasmid from bacterial cultures because of its low copy number. In order to overcome this, the standard manufacture's protocol was adjusted so that elution volume was reduced to nearly half the recommended volume, and the incubation time (with the elution solution on the column) was increased to five minutes. A final difficulty of note was encountered in the colony PCR protocol. In order for the reaction to occur, the cells need to be broken open. It was found that this can be accomplished manually by rubbing the pipette tip against the wall of the PCR tube.

Though technical problems in individual protocols were faced, the overall strategies and procedures of the cloning strategy were successful. By designing DNA sequences from reverse-translated wheat eIF2 amino acid sequences and adjusting codons

to match usage in *E. coli,* the resulting DNA sequences encode identical proteins to those found in wild type wheat germ, and at the same time have optimal expression in *E. coli*. The engineering of "backdoor" restriction sites into the middle of each DNA sequence by silent mutation enabled the piecing together of partial correct clones, removing the need to screen and sequence large numbers of transformed colonies in search of a correct clone. This ability to pair 5' and 3' correct clones proved useful in the case of eIF2β and eIF2α, though it turned out to be unnecessary in the case of eIF2γ1 and eIF2γ2. The complement of restriction sites in the BioBrick plasmid itself allowed for the assembly of the wheat eIF2β, α, and γ coding sequences into a single operon by sequential digestion and ligation reactions.

Initial studies of protein expression have been conducted on the eIF2βαγ operon in the pET22bb vector in BL21 cells, including analysis of SDS-PAGE gel by Coomassie Stain and Western Blot. These results cannot yet be reported because of their inconclusive nature. The background of *E. coli* proteins seen on the Coomassie stain does not allow for distinctive bands to be seen, and the Western Blot is also not meaningful due to low-activity antibodies. Therefore, further testing needs to be completed before experimental data can be interpreted or reported. A partial protein purification will be done prior to repeating the SDS-PAGE gel analysis in order to add confidence to and simplify the interpretation of Coomassie Stain and Western Blot results. Expression of all three subunits of the eIF2 operon is expected, and in that light a brief discussion of future studies is presented in the following section.

Future Directions

It is the intent to express the fully functional eIF2 protein complex in *E. coli*. If it can be confirmed that all three subunits are expressed and are soluble, there are several further directions in which to take this research.

The purification of the expressed eIF2 protein complex from *E. coli* would be attempted. If this is successful, the function of the purified eIF2 complex can be assayed. This would include measuring the ability of eIF2 to support polypeptide synthesis in eIF2-depleted wheat germ extracts (Benkowski *et al.* 1995) or to bind [^{35}S]Met-tRNA$_i^{Met}$ or [3H]GTP as a measure of ternary complex formation.

After the activity of the expressed eIF2 is established, an exogenous eIF2α-kinase, perhaps from a yeast or mammalian system, may be used to determine whether or not eIF2α-phosphorylation causes translation in a wheat germ extract to be significantly hindered. In addition, we can explore other possible mechanisms of translational control via eIF2 in plant systems by introducing mutations via site-directed mutagenesis that may shed light on eIF2 function.

In conclusion, the completion of the current research project detailed in this thesis will allow for the study of a number of aspects of the mechanism and regulation of eIF2 function in wheat. This will add to the scientific body of knowledge concerning translational regulation in plants, which has significant implications for agricultural biotechnology as well as for comparative analyses of the mechanisms of protein synthesis across the kingdoms of life.

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Appendix A – Wheat germ eIF2 Amino Acid Sequences

eIF2α:

MANLECRMYEPRFPEVDAAVMIQVKHIADMGAYVSLLEYNNVEGMILFSELSRRRIRSISSLIKVGRQEPAIVLR
VDRDKGYIDLSKRRVSEEEARSCEDKYNKSKLVHSIMRHVAETLEIDLEPIYQRIGWPLYRKYGHAFEAFKLIVA
DPDAILDVLTYEERETGPDGQEVTKVVPAVTPEIKETLVQNIRRRMTPQPLKIRADVEMKCFQFDGVLHIKQAMR
KAEAAGNTNCPVKIKLVAPPLYVLTTQTLDKDQGISVLTDAVKACTAEIEKHKGKLVVKEAPRAVSEREDKLLNA
QLDTLVEQNAEVAGDDDSEDEEDTGMGDIDLTNSGVHAD

eIF2β:

MADEEQMERKEEATEIAPFDPTKKKKKKKVVIQDPADEVDKLAEKTEGLSVTESGEASFVGLKKKKKKLVELDPS
LVEAGDGEDTLDDQVGEDEQGEGIVLGGATQYPWEGTDRDYKYDELLGRVFNILRENNPDLAGDRRRTVMRPPQV
LREGTKKTVFVNFMDLCKTMHRQPEHVMMFLLAEMGTSGSLDGQQRLVIKGRFAPKNFEAILRRYINEYVICNGC
KSPDTILSKENRLFFLRCEQCGSSRSVAPIKAGFVAQVGRRKAGT

eIF2γ:

MARRGLMEQDLTKLDVTKLHPLSPEVISRQATINIGTIGHVAHGKSTVVKAISGVQTVRFKNELERNITIKLGYA
NAKIYKCEDDRCPRPMCYKAYGSGKEDTPACDVPGFENTRMKLLRHVSFVDCPGHDILMATMLNGAAIMDGALLL
IAANESCPQPQTSEHLAAVEIMRLQHLIILQNKIDLIQESAAMNQHEAIQKFIQGTIAEGAPVVPISAQLKYNID
VICEYIIKKIPIPENFTSPPNMIVIRSFDVNKPGSE
FTSPPNMIVIRSFDVNKPGSEVDEIRGGVAGGSILRGVLRVNQNIEVRPGIVMKDESGNIKCTPIYSRIVSLYAE
QNELQFAVPGGLIGVGTTMDPTLTRADRLVGQVLGEIGSLPDVFVELEINFFLLRRLLGVRTKGTEKAGKVSKLT
KGEILMLNIGSMSTGARVVAVKNDLAKLQLTAPVCTSKGEKVALSRRVEKHWRLIGWGQIQAGATLEVPPCPL

Appendix B – Wheat germ eIF2 DNA sequences.

These were modified using DNAworks to adjust codon bias for expression in E.coli, and remove particular restriction sites via silent mutation. Additionally a prefix and suffix (in red) were added to each gene that included a mirror of BioBrick restriction site complement and an IRES element in the prefix. Note that here eIF2γ is artificially broken up into two parts for the sake of efficient and accurate cloning. It is pieced back together at a later stage.

eIF2α:

GAATTCGCGGCCGCTTCTAGAGATTAAAGAGGAGAAATACCATATGGCGAACCTGGAATGCCGTATGTATGAACC
GCGTTTTCCCGAAGTGGATGCTGCGGTGATGATTCAGGTGAAACATATTGCGGATATGGGCGCGTATGTGAGCCT
GCTGGAATATAACAATGTGGAAGGCATGATTCTGTTTTCCGAGCTGAGCCGTCGCCGCATTCGGAGCATAAGCAG
CCTGATAAAAGTGGGTCGTCAAGAACCGGCGATAGTTCTTCGTGTGGATCGTGATAAAGGCTATATTGACTTGTC
CAAGCGCCGTGTTTCCGAAGAAGAAGCGCGTAGCTGCGAGGATAAATACAATAAGAGCAAGCTGGTCCATAGCAT
TATGCGTCACGTTGCTGAGACCCTGGAGATTGATCTGGAACCGATCTATCAGCGGATTGGCTGGCCGCTGTATCG
TAAATACGGCCACGCATTTGAAGCGTTCAAGCTGATTGTTGCGGACCCTGACGCCATATTAGACGTGCTGACCTA
TGAAGAGCGTGAAACCGGGCCAGATGGCCAAGAAGTGACCAAAGTGGTACCAGCAGTCACCCCGGAAATAAAGGA
AACCCTGGTGCAGAACATTCGTCGTCGGATGACACCACAACCGCTGAAAATTCGTGCGGACGTGGAAATGAAATG
CTTTCAGTTTGATGGCGTGCTGCATATCAAACAGGCGATGCGTAAGGCGGAGGCTGCGGGCAATACCAATTGTCC
GGTAAAGATTAAGTTAGTGGCGCCTCCACTGTATGTACTGACCACCCAGACACTGGACAAAGATCAGGGCATTAG
CGTTTTAACCGATGCGGTCAAAGCGTGCACTGCGGAAATCGAAAAACATAAGGGCAAACTGGTGGTGAAAGAAGC
CCCACGCGCGGTGTCAGAACGTGAAGATAAACTGCTGAATGCCCAACTGGATACTCTGGTAGAACAAAATGCGGA
AGTCGCAGGCGACGATGATAGCGAGGACGAAGAGGATACCGGCATGGGCGACATCGATCTGACCAATAGCGGCGT
GCATGCGGATTAATAAGGATCCTACTAGTAGCGGCCGCTGCAG

eIF2β:

GAATTCGCGGCCGCTTCTAGAGATTAAAGAGGAGAAATACCATATGGCGGACGAAGAACAGATGGAACGTAAGGA
AGAAGCGACCGAGATTGCGCCGTTTGACCCGACTAAGAAGAAGAAGAAGAAGAAAGTGGTGATTCAGGACCCAGC
GGATGAAGTGGATAAATTGGCCGAAAAGACCGAAGGCCTGAGCGTGACCGAAAGCGGCGAGGCGAGCTTTGTGGG
CTTGAAGAAAAAGAAAAAGAAGTTAGTAGAACTGGACCCCAGCCTTGTCGAAGCCGGTGATGGCGAAGATACCCT
GGACGATCAGGTGGGCGAGGATGAACAAGGAGAGGGCATTGTGCTGGGCGGTGCCACCCAGTATCCGTGGGAAGG
AACCGATCGTGATTACAAATATGATGAGCTGTTGGGCCGTGTGTTCAATATTCTGCGCGAGAATAATCCAGACCT
GGCGGGAGACCGTCGACGTACCGTGATGCGTCCACCGCAGGTCTTGCGTGAAGGCACCAAAAAAACCGTGTTTGT
GAATTTCATGGATCTGTGCAAAACCATGCATCGTCAGCCGGAACATGTGATGATGTTTCTGCTTGCGGAAATGGG
CACCAGCGGCAGCCTGGATGGGCAGCAGCGTCTGGTCATTAAAGGCCGCTTCGCTCCCAAGAATTTTGAAGCGAT
CCTGCGGCGTTACATCAATGAATATGTGATTTGCAATGGCTGCAAGAGCCCGGATACCATTCTGAGCAAAGAGAA
TCGTCTGTTTTTCCTGCGTTGCGAACAGTGCGGCAGTAGCCGTAGCGTGGCGCCGATTAAAGCCGGGTTCGTTGC
CCAGGTAGGCCGTCGTAAGGCGGGCACCTAATAAGGATCCTACTAGTAGCGGCCGCTGCAG

eIF2γ1:

GAATTCGCGGCCGCTTCTAGAGATTAAAGAGGAGAAATACCATATGGCGCGTCGGGGACTGATGGAACAGGATCT
TACCAAATTGGATGTCACAAAACTGCATCCGCTGAGCCCGGAAGTGATTAGCCGTCAGGCCACGATTAACATAGG
GACCATTGGCCATGTGGCGCATGGCAAAAGCACTGTGGTTAAGGCTATTAGCGGGGTGCAGACCGTGCGTTTCAA
GAATGAATTGGAGCGTAACATAACCATTAAACTGGGCTATGCGAACGCGAAAATCTACAAATGCGAAGATGATCG
TTGCCCGCGTCCCATGTGCTACAAAGCGTATGGCAGCGGTAAAGAGGATACCCCTGCGTGCGATGTCCCGGGTTT
TGAGAATACGCGTATGAAACTGTTGCGCCATGTTAGCTTTGTGGATTGCCCTGGTCATGATATTTTGATGGCGAC
CATGCTGAACGGAGCCGCTATTATGGATGGCGCCCTGTTGCTGATTGCGGCAAATGAATCTTGCCCTCAGCCGCA
GACCAGCGAACATCTGGCTGCGGTGGAAATTATGCGTCTTCAGCACCTGATAATTCTTCAGAATAAGATTGATCT
GATTCAGGAATCCGCAGCGATGAATCAACATGAGGCGATTCAAAAGTTCATTCAAGGCACCATAGCGGAGGGCGC
TCCAGTGGTTCCGATTAGCGCGCAGTTGAAGTATAACATCGACGTAATTTGCGAATACATCATAAAGAAGATTCC
GATCCCTGAACGTAACTTTACCAGCCCACCGAATATGATTGTTATTCGAAGCTTCGATGTGAATAAACCGGGCAG
TGAAGTGGATGAAATTCGTGGCGGCGTGGCTGGGGGTTCCTACTAGTAGCGGCCGCTGCAG

eIF2γ2:

GAATTCGCGGCCGCTTCTAGAGCTTTACCAGCCCACCGAATATGATTGTTATTCGAAGCTTCGATGTGAATAAAC
CGGGCAGTGAAGTGGATGAAATTCGTGGCGGCGTGGCTGGGGGTTCCATTTTACGTGGCGTGTTGCGGGTGAACC
AGAACATTGAAGTGCGTCCGGGCATTGTGATGAAAGACGAATCGGGGAACATAAAGTGCACGCCCATTTATAGCC
GTATTGTGAGCTTATATGCGGAACAAAATGAGCTGCAATTCGCGGTGCCTGGGGGCCTGATTGGGGTGGGCACGA
CTATGGACCCGACCCTGACTCGTGCGGATCGTCTGGTGGGCCAAGTGCTGGGAGAAATTGGTAGCCTGCCGGATG
TCTTTGTTGAATTAGAGATTAACTTTTTTCAGCTGCGTCGTCTGCTGGGTGTTCGGACCAAAGGGACCGAGAAAG
CGGGGAAAGTGAGCAAATTGACCAAGGGGGAAATTCTGATGTTAAACATCGGAAGCATGAGCACCGGAGCGCGTG
TGGTTGCGGTCAAAAATGACCTGGCGAAGTTACAGTTAACAGCCCCAGTATGTACTTCGAAAGGCGAGAAGGTAG
CGCTGTCCCGTCGTGTGGAAAAGCATTGGCGTCTGATCGGTTGGGGCCAGATTCAAGCTGGAGCTACCCTGGAAG
TGCCGCCGTGCCCGCTGTAATAAGGATCCTACTAGTAGCGGCCGCTGCAG

Appendix C- DNAworks-generated Overlapping Oligos

Note again that here eIF2γ is artificially broken up into two parts for the sake of efficient and accurate cloning. It is pieced back together at a later stage. In each gene a "backdoor" restriction site was engineered via silent mutation (bolded, with silent mutation in red and recognizing enzyme noted to the side).

eIF2α:

```
 1    ATGGCGAACCTGGAATGCCGTATGTATGAACCGCGTTTTCCCG    43
 2    TCCGCAATATGTTTCACCTGAATCATCACCGCAGCATCCACTTCGGGAAAACGCGGTTCA    60
 3    TCAGGTGAAACATATTGCGGATATGGGCGCGTATGTGAGCCTGCTGGAATATAACAATGT    60
 4    GGCGACGGCTCAGCTCGGAAAACAGAATCATGCCTTCCACATTGTTATATTCCAGCAGGC    60
 5    AGCTGAGCCGTCGCCGCATTCGGAGCATAAGCAGCCTGATAAAAGTGGGTCGTCAAGAAC    60
 6    TAGCCTTTATCACGATCCACACGAAGAACTATCGCCGGTTCTTGACGACCCACTTTTATC    60
 7    TGTGGATCGTGATAAAGGCTATATTGACTTGTCCAAGCGCCGTGTTTCCGAAGAAGAAGC    60
 8    GACCAGCTTGCTCTTATTGTATTTATCCTCGCAGCTACGCGCTTCTTCTTCGGAAACACG    60
 9    TAAATACAATAAGAGCAAGCTGGTCCATAGCATTATGCGTCACGTTGCTGAGACCCTGGA    60
10    AGCGGCCAGCCAATCCGCTGATAGATCGGTTCCAGATCAATCTCCAGGGTCTCAGCAACG    60
11    GGATTGGCTGGCCGCTGTATCGTAAATACGGCCACGCATTTGAAGCGTTCAAGCTGATTG    60
12    CATAGGTCAGCACGTCTAATATGGCGTCAGGGTCCGCAACAATCAGCTTGAACGCTTCAA    60
13    CATATTAGACGTGCTGACCTATGAAGAGCGTGAAACCGGGCCAGATGGCCAAGAAGTGAC    60
14    GGTTTCCTTTATTTCCGGGGTGACTGCTGGTACCACTTTGGTCACTTCTTGGCCATCTGG    60  kpnI
15    ACCCCGGAAATAAAGGAAACCCTGGTGCAGAACATTCGTCGTCGGATGACACCACAACCG    60
16    AAACTGAAAGCATTTCATTTCCACGTCCGCACGAATTTTCAGCGGTTGTGGTGTCATCCG    60
17    GTGGAAATGAAATGCTTTCAGTTTGATGGCGTGCTGCATATCAAACAGGCGATGCGTAAG    60
18    TTAATCTTTACCGGACAATTGGTATTGCCCGCAGCCTCCGCCTTACGCATCGCCTGTTTG    60
19    ATACCAATTGTCCGGTAAAGATTAAGTTAGTGGCGCCTCCACTGTATGTACTGACCACCC    60
20    CGGTTAAAACGCTAATGCCCTGATCTTTGTCCAGTGTCTGGGTGGTCAGTACATACAGTG    60
21    GGGCATTAGCGTTTTAACCGATGCGGTCAAAGCGTGCACTGCGGAAATCGAAAAACATAA    60
22    CACCGCGCGTGGGGCTTCTTTCACCACCAGTTTGCCCTTATGTTTTTCGATTTCCGCAGT    60
23    CCCCACGCGCGGTGTCAGAACGTGAAGATAAACTGCTGAATGCCCAACTGGATACTCTGG    60
24    CTCGCTATCATCGTCGCCTGCGACTTCCGCATTTTGTTCTACCAGAGTATCCAGTTGGGC    60
25    GGCGACGATGATAGCGAGGACGAAGAGGATACCGGCATGGGCGACATCGATCTGACCAAT    60
26    ATCCGCATGCACGCCGCTATTGGTCAGATCGATGTCGC      38
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eIF2β:

```
 1    ATGGCGGACGAAGAACAGATGGAACGTAAGGAAGAAGCGACCGAGATTGCGCCGTTTGA    59
 2    TCCTGAATCACCACTTTCTTCTTCTTCTTCTTCTTAGTCGGGTCAAACGGCGCAATCTCG    60
 3    AGAAGAAAGTGGTGATTCAGGACCCAGCGGATGAAGTGGATAAATTGGCCGAAAAGACCG    60
 4    CAAAGCTCGCCTCGCCGCTTTCGGTCACGCTCAGGCCTTCGGTCTTTTCGGCCAATTTAT    60
 5    GGCGAGGCGAGCTTTGTGGGCTTGAAGAAAAAGAAAAAGAAGTTAGTAGAACTGGACCCC    60
 6    TCCAGGGTATCTTCGCCATCACCGGCTTCGACAAGGCTGGGGTCCAGTTCTACTAACTTC    60
 7    ATGGCGAAGATACCCTGGACGATCAGGTGGGCGAGGATGAACAAGGAGAGGGCATTGTGC    60
 8    ACGATCGGTTCCTTCCCACGGATACTGGGTGGCACCGCCCAGCACAATGCCCTCTCCTTG    60
 9    TGGGAAGGAACCGATCGTGATTACAAATATGATGAGCTGTTGGGCCGTGTGTTCAATATT    60
10    GTCGACGGTCTCCCGCCAGGTCTGGATTATTCTCGCGCAGAATATTGAACACACGGCCCA    60  SalI
11    GCGGGAGACCGTCGACGTACCGTGATGCGTCCACCGCAGGTCTTGCGTGAAGGCACCAAA    60  SalI
12    CATGGTTTTGCACAGATCCATGAAATTCACAAACACGGTTTTTTTGGTGCCTTCACGCAA    60
13    ATGGATCTGTGCAAAACCATGCATCGTCAGCCGGAACATGTGATGATGTTTCTGCTTGCG    60
14    ACGCTGCTGCCCATCCAGGCTGCCGCTGGTGCCCATTTCCGCAAGCAGAAACATCATCAC    60
15    GGATGGGCAGCAGCGTCTGGTCATTAAAGGCCGCTTCGCTCCCAAGAATTTTGAAGCGAT    60
16    CATTGCAAATCACATATTCATTGATGTAACGCCGCAGGATCGCTTCAAAATTCTTGGGAG    60
17    CATCAATGAATATGTGATTTGCAATGGCTGCAAGAGCCCGGATACCATTCTGAGCAAAGA    60
18    TGCCGCACTGTTCGCAACGCAGGAAAAACAGACGATTCTCTTTGCTCAGAATGGTATCCG    60
19    TGCGAACAGTGCGGCAGTAGCCGTAGCGTGGCGCCGATTAAAGCCGGGTTCGTTGCCCAG    60
20    GGTGCCCGCCTTACGACGGCCTACCTGGGCAACGAACCCG    40
```

eIF2γ1:

```
 1    ATGGCGCGTCGGGGACTGATGGAACAGGATCTTACCAAATTGGATGTCACAAAACTGCA    59
 2    ATCGTGGCCTGACGGCTAATCACTTCCGGGCTCAGCGGATGCAGTTTTGTGACATCCAAT    60
 3    GCCGTCAGGCCACGATTAACATAGGGACCATTGGCCATGTGGCGCATGGCAAAAGCACTG    60
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 4   TCTTGAAACGCACGGTCTGCACCCCGCTAATAGCCTTAACCACAGTGCTTTTGCCATGCG   60
 5   CAGACCGTGCGTTTCAAGAATGAATTGGAGCGTAACATAACCATTAAACTGGGCTATGCG   60
 6   GCGGGCAACGATCATCTTCGCATTTGTAGATTTTCGCGTTCGCATAGCCCAGTTTAATGG   60
 7   AAGATGATCGTTGCCCGCGTCCCATGTGCTACAAAGCGTATGGCAGCGGTAAAGAGGATA   60
 8   ATACGCGTATTCTCAAAACCCGGGACATCGCACGCAGGGGTATCCTCTTTACCGCTGCCA   60    MluI
 9   CGGGTTTTGAGAATACGCGTATGAAACTGTTGCGCCATGTTAGCTTTGTGGATTGCCCTG   60    MluI
10   AGCGGCTCCGTTCAGCATGGTCGCCATCAAAATATCATGACCAGGGCAATCCACAAAGCT   60
11   GCTGAACGGAGCCGCTATTATGGATGGCGCCCTGTTGCTGATTGCGGCAAATGAATCTTG   60
12   TCCACCGCAGCCAGATGTTCGCTGGTCTGCGGCTGAGGGCAAGATTCATTTGCCGCAATC   60
13   CATCTGGCTGCGGTGGAAATTATGCGTCTTCAGCACCTGATAATTCTTCAGAATAAGATT   60
14   TTGATTCATCGCTGCGGATTCCTGAATCAGATCAATCTTATTCTGAAGAATTATCAGGTG   60
15   ATCCGCAGCGATGAATCAACATGAGGCGATTCAAAAGTTCATTCAAGGCACCATAGCGGA   60
16   TTATACTTCAACTGCGCGCTAATCGGAACCACTGGAGCGCCCTCCGCTATGGTGCCTTGA   60
17   TAGCGCGCAGTTGAAGTATAACATCGACGTAATTTGCGAATACATCATAAAGAAGATTCC   60
18   TATTCGGTGGGCTGGTAAAGTTACGTTCAGGGATCGGAATCTTCTTTATGATGTATTCGC   60
19   CTTTACCAGCCCACCGAATATGATTGTTATTCGAAGCTTCGATGTGAATAAACCGGGCAG   60    HindIII
20   GGAACCCCCAGCCACGCCGCCACGAATTTCATCCACTTCACTGCCCGGTTTATTCACATC   60
```

eIF2γ2:

```
19   CTTTACCAGCCCACCGAATATGATTGTTATTCGAAGCTTCGATGTGAATAAACCGGGCAG   60    HindIII
20   GGAACCCCCAGCCACGCCGCCACGAATTTCATCCACTTCACTGCCCGGTTTATTCACATC   60
21   CGTGGCTGGGGGTTCCATTTTACGTGGCGTGTTGCGGGTGAACCAGAACATTGAAGTGCG   60
22   TTTATGTTCCCCGATTCGTCTTTCATCACAATGCCCGGACGCACTTCAATGTTCTGGTTC   60
23   AGACGAATCGGGGAACATAAAGTGCACGCCCATTTATAGCCGTATTGTGAGCTTATATGC   60
24   GGCCCCCAGGCACCGCGAATTGCAGCTCATTTTGTTCCGCATATAAGCTCACAATACGGC   60
25   GGTGCCTGGGGGCCTGATTGGGGTGGGCACGACTATGGACCCGACCCTGACTCGTGCGGA   60
26   CCGGCAGGCTACCAATTTCTCCCAGCACTTGGCCCACCAGACGATCCGCACGAGTCAGGG   60
27   AAATTGGTAGCCTGCCGGATGTCTTTGTTGAATTAGAGATTAACTTTTTTCAGCTGCGTC   60    SalI
28   GCTTTCTCGGTCCCTTTGGTCCGAACACCCAGCAGACGACGCAGCTGAAAAAAGTTAATC   60    SalI
29   CCAAAGGGACCGAGAAAGCGGGGAAAGTGAGCAAATTGACCAAGGGGGAAATTCTGATGT   60
30   CAACCACACGCGCTCCGGTGCTCATGCTTCCGATGTTTAACATCAGAATTTCCCCCTTGG   60
31   GGAGCGCGTGTGGTTGCGGTCAAAAATGACCTGGCGAAGTTACAGTTAACAGCCCCAGTA   60
32   CGACGGGACAGCGCTACCTTCTCGCCTTTCGAAGTACATACTGGGGCTGTTAACTGTAAC   60
33   TAGCGCTGTCCCGTCGTGTGGAAAAGCATTGGCGTCTGATCGGTTGGGGCCAGATTCAAG   60
34   CAGCGGGCACGGCGGCACTTCCAGGGTAGCTCCAGCTTGAATCTGGCCCCAACC   54
```

Appendix D: Primers

EIF2•-F:
5′-GAATTCGCGGCCGCTTCTAGAGATTAAAGAGGAGAAATACCATATGGCGAACCTGGAATGCCG-3′

EIF2•-R:
5′-CTGCAGCGGCCGCTACTAGTAGGATCCTTATTAATCCGCATGCACGCCGCTAT-3′

EIF2•-F:
5′-GAATTCGCGGCCGCTTCTAGAGATTAAAGAGGAGAAATACCATATGGCGGACGAAGAACAGAT-3′

EIF2•-R:
5′-CTGCAGCGGCCGCTACTAGTAGGATCCTTATTAGGTGCCCGCCTTACGACGGC-3′

EIF2•I-F:
5′-GAATTCGCGGCCGCTTCTAGAGATTAAAGAGGAGAAATACCATATGGCGCGTCGGGGACTGAT-3′

EIF2•I-R:
5′-CTGCAGCGGCCGCTACTAGTAGGAACCCCCAGCCACGCCGC-3′

EIFGII-F:
5′-GAATTCGCGGCCGCTTCTAGAGCTTTACCAGCCCACCGAATA-3′

EIFGII-R:
5′-CTGCAGCGGCCGCTACTAGTAGGATCCTTATTACAGCGGGCACGGCGGCACTT-3′

M13F:
5′—GTAAAACGACGGCCAGT—3′

M13R:
5′—AACAGCTATGACCATG—3′

VF2:
5′—TGCCACCTGACGTCTAAGAA—3′

VR:
5′—ATTACCGCCTTTGAGTGAGC—3′

T7pro:
5′—TAATACGACTCACTATAGGG —3′

T7term:
5′—GCTAGTTATTGCTCAGCGG —3′

Appendix E – Codon Usage Tables

For each species is listed the three nucleotide codon, the single letter abbreviation for the amino acid it encodes, and the codon percent usage. The percent usage is determined by how often the codon appears in the coding regions of the species' genome. (adapted from Codon Usage Database at http://www.kazusa.or.jp/codon/)

Triticum aestivum

TTT F 0.31	TCT S 0.16	TAT Y 0.29	TGT C 0.30
TTC F 0.69	TCC S 0.25	TAC Y 0.71	TGC C 0.70
TTA L 0.05	TCA S 0.12	TAA * 0.00	TGA * 1.00
TTG L 0.18	TCG S 0.22	TAG * 0.00	TGG W 1.00
CTT L 0.14	CCT P 0.15	CAT H 0.40	CGT R 0.05
CTC L 0.31	CCC P 0.27	CAC H 0.60	CGC R 0.26
CTA L 0.07	CCA P 0.27	CAA Q 0.53	CGA R 0.02
CTG L 0.26	CCG P 0.32	CAG Q 0.47	CGG R 0.23
ATT I 0.26	ACT T 0.20	AAT N 0.3	AGT S 0.04
ATC I 0.52	ACC T 0.33	AAC N 0.63	AGC S 0.22
ATA I 0.23	ACA T 0.16	AAA K 0.16	AGA R 0.02
ATG M 1.00	ACG T 0.31	AAG K 0.84	AGG R 0.41
GTT V 0.17	GCT A 0.17	GAT D 0.49	GGT G 0.14
GTC V 0.29	GCC A 0.29	GAC D 0.51	GGC G 0.51
GTA V 0.14	GCA A 0.22	GAA E 0.38	GGA G 0.07
GTG V 0.40	GCG A 0.32	GAG E 0.62	GGG G 0.28

E. coli

TTT F 0.59	TCT S 0.17	TAT Y 0.60	TGT C 0.47
TTC F 0.41	TCC S 0.14	TAC Y 0.40	TGC C 0.53
TTA L 0.15	TCA S 0.15	TAA X 0.60	TGA X 0.31
TTG L 0.13	TCG S 0.13	TAG X 0.09	TGG W 1.00
CTT L 0.12	CCT P 0.19	CAT H 0.59	CGT R 0.34
CTC L 0.10	CCC P 0.14	CAC H 0.41	CGC R 0.34
CTA L 0.04	CCA P 0.21	CAA Q 0.34	CGA R 0.07
CTG L 0.46	CCG P 0.47	CAG Q 0.66	CGG R 0.12
ATT I 0.49	ACT T 0.19	AAT N 0.52	AGT S 0.17
ATC I 0.37	ACC T 0.38	AAC N 0.48	AGC S 0.23
ATA I 0.14	ACA T 0.19	AAA K 0.73	AGA R 0.08
ATG M 1.00	ACG T 0.24	AAG K 0.27	AGG R 0.05
GTT V 0.29	GCT A 0.19	GAT D 0.64	GGT G 0.34
GTC V 0.20	GCC A 0.26	GAC D 0.36	GGC G 0.35
GTA V 0.17	GCA A 0.24	GAA E 0.67	GGA G 0.15
GTG V 0.34	GCG A 0.31	GAG E 0.33	GGG G 0.16

Appendix F – Sequencing Data

For each sequence, the correct coding sequences as presented in Appendix C are listed as 'Query' (top line), and the actual sequenced sample as 'Sbjct' (bottom line). The sequences were aligned using the NCBI BLAST specialized nucleotide alignment tool: (http://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch&PROG_DEF=blastn&BLAST_PROG_DEF=megaBlast&SHOW_DEFAULTS=on&BLAST_SPEC=blast2seq&LINK_LOC=align2seq). For Sequences 1-4, the engineered 'backdoor' restriction site is noted in red, and only the 3' or 5' end of that sequence was used, as indicated. Primers used for sequencing are noted in ().

Sequence 1: eIF2α in Zero Blunt, Correct 5' end (M13F)

```
Query  79    GAATTCGCGGCCGCTTCTAGAGATTAAAGAGGAGAAATACCATATGGCGAACCTGGAATG  138
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1     GAATTCGCGGCCGCTTCTAGAGATTAAAGAGGAGAAATACCATATGGCGAACCTGGAATG  60

Query  139   CCGTATGTATGAACCGCGTTTTCCCGAAGTGGATGCTGCGGTGATGATTCAGGTGAAACA  198
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61    CCGTATGTATGAACCGCGTTTTCCCGAAGTGGATGCTGCGGTGATGATTCAGGTGAAACA  120

Query  199   TATTGCGGATATGGGCGCGTATGTGAGCCTGCTGGAATATAACAATGTGGAAGGCATGAT  258
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121   TATTGCGGATATGGGCGCGTATGTGAGCCTGCTGGAATATAACAATGTGGAAGGCATGAT  180

Query  259   TCTGTTTTCCGAGCTGAGCCGTCGCCGCATTCGGAGCATAAGCAGCCTGATAAAAGTGGG  318
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181   TCTGTTTTCCGAGCTGAGCCGTCGCCGCATTCGGAGCATAAGCAGCCTGATAAAAGTGGG  240

Query  319   TCGTCAAGAACCGGCGATAGTTCTTCGTGTGGATCGTGATAAAGGCTATATTGACTTGTC  378
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241   TCGTCAAGAACCGGCGATAGTTCTTCGTGTGGATCGTGATAAAGGCTATATTGACTTGTC  300

Query  379   CAAGCGCCGTGTTTCCGAAGAAGAAGCGCGTAGCTGCGAGGATAAATACAATAAGAGCAA  438
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301   CAAGCGCCGTGTTTCCGAAGAAGAAGCGCGTAGCTGCGAGGATAAATACAATAAGAGCAA  360

Query  439   GCTGGTCCATAGCATTATGCGTCACGTTGCTGAGACCCTGGAGATTGATCTGGAACCGAT  498
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361   GCTGGTCCATAGCATTATGCGTCACGTTGCTGAGACCCTGGAGATTGATCTGGAACCGAT  420

Query  499   CTATCAGCGGATTGGCTGGCCGCTGTATCGTAAATACGGCCACGCATTTGAAGCGTTCAA  558
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421   CTATCAGCGGATTGGCTGGCCGCTGTATCGTAAATACGGCCACGCATTTGAAGCGTTCAA  480

Query  559   GCTGATTGTTGCGGACCCTGACGCCATATTAGACGTGCTGACCTATGAAGAGCGTGAAAC  618
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  481   GCTGATTGTTGCGGACCCTGACGCCATATTAGACGTGCTGACCTATGAAGAGCGTGAAAC  540

Query  619   CGGGCCAGATGGCCAAGAAGTGACCAAAGTGGTACCAGCAGTCACCCCGGAAATAAAGGA  678
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  541   CGGGCCAGATGGCCAAGAAGTGACCAAAGTGGTACCAGCAGTCACCCCGGAAATAAAGGA  600

Query  679   AACCCTGGTGCAGAACATTCGTCGTCGGA-CACACCACAACCGCTGAAAATTCGTGCGGA  737
             |||||||||||||||||||||||||||| ||||||||||||||||||||||||||||||
Sbjct  601   AACCCTGGTGCAGAACATTCGTCGTCGGATGACACCACAACCGCTGAAAATTCGTGCGGA  660

Query  738   CGTGGAAATGAAATGCTTTCAGTTTGATGG-G--C---ATATCAAACAGGCGATGCGTAA  791
             |||||||||||||||||||||||||||| |  |    ||||||||||||||||||||||
Sbjct  661   CGTGGAAATGAAATGCTTTCAGTTTGATGGCGTGCTGCATATCAAACAGGCGATGCGTAA  720

Query  792   GGCGGAGGCTGCGGGCAATACCAATTGTCCGGTAAAGATTAAGTTAGTGGCGCCTCCACT  851
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  721   GGCGGAGGCTGCGGGCAATACCAATTGTCCGGTAAAGATTAAGTTAGTGGCGCCTCCACT  780
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Query  852   GTATGTACTGACCACCCAGACACTGGACAAAGATCAGGGCATTAGCGTTTTAACCGATGC  911
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  781   GTATGTACTGACCACCCAGACACTGGACAAAGATCAGGGCATTAGCGTTTTAACCGATGC  840

Query  912   GGTCAAAGCGTGCACTGCGGAAATCGAAAAACATANGG-CAAACTGGTGGTGAAAGAAGC  970
             |||||||||||||||||||||||||||||||||| || ||||||||||||||||||||||
Sbjct  841   GGTCAAAGCGTGCACTGCGGAAATCGAAAAACATAAGGGCAAACTGGTGGTGAAAGAAGC  900

Query  971   CCCACGCGCGGNGTCAGAACGTGAAGATAAACTGCTGAATGCCCAACTGGNTACTCTGGT  1030
             |||||||||| |||||||||||||||||||||||||||||||||| |||||||||
Sbjct  901   CCCACGCGCGGTGTCAGAACGTGAAGATAAACTGCTGAATGCCCAACTGGATACTCTGGT  960

Query  1031  AGA-CAAA-TGNNGAAGTCGCNNNN-ACGATGATAGCNNNGnnnan-anGNTACCNGCAT  1086
             ||| |||| ||  |||||||||     ||||||||||   |   |  | | |||| ||||
Sbjct  961   AGAACAAAATGCGGAAGTCGCAGGCGACGATGATAGCGAGGACGAAGAGGATACCGGCAT  1020

Query  1087  GGGCGACNTCGATCTGANNA-TANCGGCGTGCATG  1120
             ||||||| |||||||||  | || ||||||||||
Sbjct  1021  GGGCGACATCGATCTGACCAATAGCGGCGTGCATG  1055
```

Sequence 2: eIF2α in Zero Blunt, Correct 3' end (M13R)

```
Query  80    CTGCAGCGGCCGCTACTAGTAGGATCCTTATTAATCCGCATGCACGCCGCTATTGGTCAG  139
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1093  CTGCAGCGGCCGCTACTAGTAGGATCCTTATTAATCCGCATGCACGCCGCTATTGGTCAG  1034

Query  140   ATCGATGTCGCCCATGCCGGTATCCTCTTCGTCCTCGCTATCATCGTCGCCTGCGACTTC  199
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1033  ATCGATGTCGCCCATGCCGGTATCCTCTTCGTCCTCGCTATCATCGTCGCCTGCGACTTC  974

Query  200   CGCATTTTGTTCTACCAGAGTATCCAGTTGGGCATTCAGCAGTTTATCTTCACGTTCTGA  259
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  973   CGCATTTTGTTCTACCAGAGTATCCAGTTGGGCATTCAGCAGTTTATCTTCACGTTCTGA  914

Query  260   CACCGCGCGTGGGGCTTCTTTCACCACCAGTTTGCCCTTATGTTTTTCGATTTCCGCAGT  319
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  913   CACCGCGCGTGGGGCTTCTTTCACCACCAGTTTGCCCTTATGTTTTTCGATTTCCGCAGT  854

Query  320   GCACGCTTTGACCGCATCGGTTAAAACGCTAATGCCCTGATCTTTGTCCAGTGTCTGGGT  379
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  853   GCACGCTTTGACCGCATCGGTTAAAACGCTAATGCCCTGATCTTTGTCCAGTGTCTGGGT  794

Query  380   GGTCAGTACATACAGTGGAGGCGCCACTAACTTAATCTTTACCGGACAATTGGTATTGCC  439
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  793   GGTCAGTACATACAGTGGAGGCGCCACTAACTTAATCTTTACCGGACAATTGGTATTGCC  734

Query  440   CGCAGCCTCCGCCTTACGCATCGCCTGTTTGATATGCAGCACGCCATCAAACTGAAAGCA  499
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  733   CGCAGCCTCCGCCTTACGCATCGCCTGTTTGATATGCAGCACGCCATCAAACTGAAAGCA  674

Query  500   TTTCATTTCCACGTCCGCACGAATTTTCAGCGGTTGTGGTGTCATCCGACGACGAATGTT  559
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  673   TTTCATTTCCACGTCCGCACGAATTTTCAGCGGTTGTGGTGTCATCCGACGACGAATGTT  614

Query  560   CTGCACCAGGGTTTCCTTTATTTCCGGGGTGACTGCTGGTACCACTTTGGTCACTTCTTG  619
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  613   CTGCACCAGGGTTTCCTTTATTTCCGGGGTGACTGCTGGTACCACTTTGGTCACTTCTTG  554

Query  620   GCCATCTGGCCCGGTTTCACGCTCTTCATAGGTCAGCACGTCTAATATGGCGTCAGG-TC  678
             |||||||||||||||||||||||||||||||||||||||||||||||||||||||| ||
Sbjct  553   GCCATCTGGCCCGGTTTCACGCTCTTCATAGGTCAGCACGTCTAATATGGCGTCAGGGTC  494

Query  679   CGCAACAATCAGCTTGAACGCTTCAAATGCGTGGCCGTATTTACGATACAGCGGCCAGCC  738
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  493   CGCAACAATCAGCTTGAACGCTTCAAATGCGTGGCCGTATTTACGATACAGCGGCCAGCC  434

Query  739   AATCCGCTGATAGATCGGTTCCAGATCAATCTCCAGGGTCTCAGCAACGTGACGCATAAT  798
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  433   AATCCGCTGATAGATCGGTTCCAGATCAATCTCCAGGGTCTCAGCAACGTGACGCATAAT  374
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Query  799   GCTATGGACCAGCTTGCTCTTAT-GTATTTATCCTCGCAGCTACGCGCTTCTTCTTCGGA  857
             ||||||||||||||||||||||| ||||||||||||||||||||||||||||||||||||
Sbjct  373   GCTATGGACCAGCTTGCTCTTATTGTATTTATCCTCGCAGCTACGCGCTTCTTCTTCGGA  314

Query  858   AACACGGCGCTTGGACAAGTCAATATAGCCTTTATCACGATCCACACGAAGAACTATCGC  917
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  313   AACACGGCGCTTGGACAAGTCAATATAGCCTTTATCACGATCCACACGAAGAACTATCGC  254

Query  918   CGGTTCTTGACGACCCACTTTTATCNNN-TGCTTATGCTCCGAATGCGGCGANGGNTCAG  976
             |||||||||||||||||||||||||    |||||||||||||||||||||| || ||||
Sbjct  253   CGGTTCTTGACGACCCACTTTTATCAGGCTGCTTATGCTCCGAATGCGGCGACGGCTCAG  194

Query  977   CTCGNAAAACAGAATCATGCCTTCNACATTGTNATATTNCAGC  1019
             |||| |||||||||||||||||| ||||||| ||||| ||||
Sbjct  193   CTCGGAAAACAGAATCATGCCTTCCACATTGTTATATTCCAGC  151
```

Sequence 3: eIF2β in Zero Blunt, Correct 5' end (M13F)

```
Query  81    CTGCAGCGGCCGCTACTAGTAGGATCCTTATTAGGTGCCCGCCTTACGACGGCCTACCTG  140
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  886   CTGCAGCGGCCGCTACTAGTAGGATCCTTATTAGGTGCCCGCCTTACGACGGCCTACCTG  827

Query  141   GGCAACGAACCCGGCTTTAATCGGCGCCACGCTA-GGCTACTGCCGCACTGTTCGCAACG  199
             |||||||||||||||||||||||||||||||||| ||||||||||||||||||||||||
Sbjct  826   GGCAACGAACCCGGCTTTAATCGGCGCCACGCTACGGCTACTGCCGCACTGTTCGCAACG  767

Query  200   CA-GAAAAACAGACGATTCTCTTTGCTCAGAATGGTATCCGGGCTCTTGCAGCCATTGCA  258
             || ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  766   CAGGAAAAACAGACGATTCTCTTTGCTCAGAATGGTATCCGGGCTCTTGCAGCCATTGCA  707

Query  259   AATCACATATCATTGATGTAACGCCGCAGGATCGCTTCAAAATTCTTGGGAGCGAAGCG  317
             ||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  706   AATCACATATTCATTGATGTAACGCCGCAGGATCGCTTCAAAATTCTTGGGAGCGAAGCG  647

Query  318   GCCTTTAATGACCAGACGCTGCTGCCCATCCAGGCTGCCGCTGGTGCCCATTTCCGCAAG  377
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  646   GCCTTTAATGACCAGACGCTGCTGCCCATCCAGGCTGCCGCTGGTGCCCATTTCCGCAAG  587

Query  378   CAGAAACATCATCACATGTTCCGGCTGACGATGCATGGTTTTGCACAGATCCATGAAATT  437
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  586   CAGAAACATCATCACATGTTCCGGCTGACGATGCATGGTTTTGCACAGATCCATGAAATT  527

Query  438   CACAAACACGGttttttGGTGCCTTCACGCAAGACCTGCGGTGGACGCATCA---TACG  494
             ||||||||||||||||||||||||||||||||||||||||||||||||||   ||||
Sbjct  526   CACAAACACGGTTTTTTTGGTGCCTTCACGCAAGACCTGCGGTGGACGCATCACGGTACG  467

Query  495   TCGACGGTCTCCCGCCAGGTCTGGATTATTCTCGCGCAGAATATTGAACACACGGCCCAA  554
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  466   TCGACGGTCTCCCGCCAGGTCTGGATTATTCTCGCGCAGAATATTGAACACACGGCCCAA  407

Query  555   CAGCTCATCATATTTGTAATCACGATCGGTTCCTTCCCACGGATACTGGGTGGCACCGCC  614
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  406   CAGCTCATCATATTTGTAATCACGATCGGTTCCTTCCCACGGATACTGGGTGGCACCGCC  347

Query  615   CAGCACAATGCCCTCTCCTTGTTCATCCTCGCCCACCTGATCGTCCAGGGTATCTTCGCC  674
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  346   CAGCACAATGCCCTCTCCTTGTTCATCCTCGCCCACCTGATCGTCCAGGGTATCTTCGCC  287

Query  675   ATCACCGGCTTCGACAAGGCTGGGGTCCAGTTCTACTAACTTCTTTTTCTTTTTCTTCAA  734
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  286   ATCACCGGCTTCGACAAGGCTGGGGTCCAGTTCTACTAACTTCTTTTTCTTTTTCTTCAA  227

Query  735   GCCCACAAAGCTCGCCTCGCCGCTTTCGGTCACGCTCAGGCCTTCGGTCTTTTCGGCCAA  794
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  226   GCCCACAAAGCTCGCCTCGCCGCTTTCGGTCACGCTCAGGCCTTCGGTCTTTTCGGCCAA  167

Query  795   TTTATCCACTTCATCCGCTGGGTCCTGAATCACCActttcttcttcttcttcttcttAGT  854
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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Sbjct  166  TTTATCCACTTCATCCGCTGGGTCCTGAATCACCACTTTCTTCTTCTTCTTCTTCTTAGT  107

Query  855  CGGGTCAAACGGCGCAATCTCGGTCGCTTCTTCCTTACGTTCCATCTGTTCTTCGTCCGC  914
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  106  CGGGTCAAACGGCGCAATCTCGGTCGCTTCTTCCTTACGTTCCATCTGTTCTTCGTCCGC  47

Query  915  CATATGG  921
            |||||||
Sbjct  46   CATATGG  40
```

Sequence 4: eIF2β in Zero Blunt, Correct 3' end (M13R)

```
Query  101  CTGCAGCGGCCGCTACTAGTAGGATCCTTATTAGGTGCCCGCCTTACGACGGCCTACCTG  160
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  886  CTGCAGCGGCCGCTACTAGTAGGATCCTTATTAGGTGCCCGCCTTACGACGGCCTACCTG  827

Query  161  GGCAACGAACCCGGCTTTAATCGGCGCCACGCTACGGCTACTGCCGCACTGTTCGCAACG  220
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  826  GGCAACGAACCCGGCTTTAATCGGCGCCACGCTACGGCTACTGCCGCACTGTTCGCAACG  767

Query  221  CAGGAAAAACAGACGATTCTCTTTGCTCAGAATGGTATCCGGGCTCTTGCAGCCATTGCA  280
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  766  CAGGAAAAACAGACGATTCTCTTTGCTCAGAATGGTATCCGGGCTCTTGCAGCCATTGCA  707

Query  281  AATCACATATTCATTGATGTAACGCCGCAGGATCGCTTCAAAATTCTTGGGAGCGAAGCG  340
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  706  AATCACATATTCATTGATGTAACGCCGCAGGATCGCTTCAAAATTCTTGGGAGCGAAGCG  647

Query  341  GCCTTTAATGACCAGACGCTGCTGCCCATCCAGGCTGCCGCTGGTGCCCATTTCCGCAAG  400
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  646  GCCTTTAATGACCAGACGCTGCTGCCCATCCAGGCTGCCGCTGGTGCCCATTTCCGCAAG  587

Query  401  CAGAAACATCATCACATGTTCCGGCTGACGATGCATGGTTTTGCACAGATCCATGAAATT  460
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  586  CAGAAACATCATCACATGTTCCGGCTGACGATGCATGGTTTTGCACAGATCCATGAAATT  527

Query  461  CACAAACACGGttttttttGGTGCCTTCACGCAAGACCTGCGGTGGACGCATCACGGTACG  520
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  526  CACAAACACGGTTTTTTTGGTGCCTTCACGCAAGACCTGCGGTGGACGCATCACGGTACG  467

Query  521  TCGACGGTCTCCCGCCAGGTCTGGATTATTCTCGCGCAGAATATTGAACACACGGCCCAA  580
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  466  TCGACGGTCTCCCGCCAGGTCTGGATTATTCTCGCGCAGAATATTGAACACACGGCCCAA  407

Query  581  CAGCTCATCATATTTGTAATCACGATCGGTTC-TTCCCACGGATACTGGGTG-CACCGCC  638
            |||||||||||||||||||||||||||||||| |||||||||||||||||| |||||||
Sbjct  406  CAGCTCATCATATTTGTAATCACGATCGGTTCCTTCCCACGGATACTGGGTGGCACCGCC  347

Query  639  CAGCACA-TGCCCTCTCCTTGTTCATCCTCGCCCACCTGATCGTCCAGGGTATCTTCGCC  697
            ||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  346  CAGCACAATGCCCTCTCCTTGTTCATCCTCGCCCACCTGATCGTCCAGGGTATCTTCGCC  287

Query  698  ATCACCGGCTTCGACAAGGCTGGGGTCCAGTTCTACTAACTTCTTTTTCTTTTTCTTCAA  757
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  286  ATCACCGGCTTCGACAAGGCTGGGGTCCAGTTCTACTAACTTCTTTTTCTTTTTCTTCAA  227

Query  758  GCCCACAAAGCTCGCCTCGCCGCTTTCGGTCACGCTCAGGCCTTCGGTCTTTTCGGCCAA  817
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  226  GCCCACAAAGCTCGCCTCGCCGCTTTCGGTCACGCTCAGGCCTTCGGTCTTTTCGGCCAA  167

Query  818  TTTATCCACTTCATCCGCTGGGTCCTGAATCACCActttcttcttcttcttcttcttAGT  877
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  166  TTTATCCACTTCATCCGCTGGGTCCTGAATCACCACTTTCTTCTTCTTCTTCTTCTTAGT  107

Query  878  CGGGTCAAACGGCGCAATCTCGGTCGCTTCTTCCTTACGTTCCATCTGTTCTTCGTCCGC  937
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  106  CGGGTCAAACGGCGCAATCTCGGTCGCTTCTTCCTTACGTTCCATCTGTTCTTCGTCCGC  47

Query  938  CATATGGTATTTCTCCTCTTTAATCTC  964
            |||||||||||||||||||||||||||
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Sbjct  46   CATATGGTATTTCTCCTCTTTAATCTC  20
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Sequence 5: eIF2γ1 in Zero Blunt, Correct Complete Clone (M13F)

```
Query  1    GAATTCGCGGCCGCTTCTAGAGATTAAAGAGGAGAAATACCATATGGCGCGTCGGGGACT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  78   GAATTCGCGGCCGCTTCTAGAGATTAAAGAGGAGAAATACCATATGGCGCGTCGGGGACT  137

Query  61   GATGGAACAGGATCTTACCAAATTGGATGTCACAAAACTGCATCCGCTGAGCCCGGAAGT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  138  GATGGAACAGGATCTTACCAAATTGGATGTCACAAAACTGCATCCGCTGAGCCCGGAAGT  197

Query  121  GATTAGCCGTCAGGCCACGATTAACATAGGGACCATTGGCCATGTGGCGCATGGCAAAAG  180
            |||||||||||||||||||||||||||||||||||||||||||||||| ||||||||||
Sbjct  198  GATTAGCCGTCAGGCCACGATTAACATAGGGACCATTGGCCATGTGGCG-ATGGCAAAAG  256

Query  181  CACTGTGGTTAAGGCTATTAGCGGGGTGCAGACCGTGCGTTTCAAGAATGAATTGGAGCG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  257  CACTGTGGTTAAGGCTATTAGCGGGGTGCAGACCGTGCGTTTCAAGAATGAATTGGAGCG  316

Query  241  TAACATAACCATTAAACTGGGCTATGCGAACGCGAAAATCTACAAATGCGAAGATGATCG  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  317  TAACATAACCATTAAACTGGGCTATGCGAACGCGAAAATCTACAAATGCGAAGATGATCG  376

Query  301  TTGCCCGCGTCCCATGTGCTACAAAGCGTATGGCAGCGGTAAAGAGGATACCCCTGCGTG  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  377  TTGCCCGCGTCCCATGTGCTACAAAGCGTATGGCAGCGGTAAAGAGGATACCCCTGCGTG  436

Query  361  CGATGTCCCGGGTTTTGAGAATACGCGTATGAAACTGTTGCGCCATGTTAGCTTTGTGGA  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  437  CGATGTCCCGGGTTTTGAGAATACGCGTATGAAACTGTTGCGCCATGTTAGCTTTGTGGA  496

Query  421  TTGCCCTGGTCATGATATTTTGATGGCGACCATGCTGAACGGAGCCGCTATTATGGATGG  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  497  TTGCCCTGGTCATGATATTTTGATGGCGACCATGCTGAACGGAGCCGCTATTATGGATGG  556

Query  481  CGCCCTGTTGCTGATTGCGGCAAATGAATCTTGCCCTCAGCCGCAGACCAGCGAACATCT  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  557  CGCCCTGTTGCTGATTGCGGCAAATGAATCTTGCCCTCAGCCGCAGACCAGCGAACATCT  616

Query  541  GGCTGCGGTGGAAATTATGCGTCTTCAGCACCTGATAATTCTTCAGAATAAGATTGATCT  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  617  GGCTGCGGTGGAAATTATGCGTCTTCAGCACCTGATAATTCTTCAGAATAAGATTGATCT  676

Query  601  GATTCAGGAATCCGCAGCGATGAATCAACATGAGGCGATTCAAAAGTTCATTCAAGGCAC  660
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  677  GATTCAGGAATCCGCAGCGATGAATCAACATGAGGCGATTCAAAAGTTCATTCAAGGCAC  736

Query  661  CATAGCGGAGGGCGCTCCAGTGGTTCCGATTAGCGCGCAGTTGAAGTATAACATCGACGT  720
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  737  CATAGCGGAGGGCGCTCCAGTGGTTCCGATTAGCGCGCAGTTGAAGTATAACATCGACGT  796

Query  721  AATTTGCGAATACATCATAAAGAAGATTCCGATCCCTGAACGTAACTTTACCAGCCCACC  780
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  797  AATTTGCGAATACATCATAAAGAAGATTCCGATCCCTGAACGTAACTTTACCAGCCCACC  856

Query  781  GAATATGATTGTTATTCGAAGCTTCGATGTGAATAAACCGGGCAGTGAAGTGGATGAAAT  840
             |||||||||||||||||||||||||||||||||||||||||||||||||||| |||||||
Sbjct  857  GAATATGATTGTTATTCGAAGCTTCGATGTGAATAAACCGGGCAGTGAAGTG-ATGAAAT  915

Query  841  TCGTGGCGGCGTGGCTGGGGGTTCCTACTAGTAGCGGCCGCTGCAG  886
            |||||||||||||| |||||||||||||||||||||||||||||||
Sbjct  916  TCGTGGCGGCGTGGNTGGGGGTTCCTACTAGTAGCGGCCGCTGCAG  961
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Sequence 6: eIF2γ2 in Zero Blunt, Complete Correct Clone (M13F)

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Query  1    GAATTCGCGGCC-GCTTCTAGAGCTTTACCAGCCCACCGAATATGATTGTTATTCGAAGC  59
            |||||||||||| ||||||||||||||||||||||||||||||||||||||||||||||
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Sbjct  79   GAATTCGCGGCCGGCTTCTAGAGCTTTACCAGCCCACCGAATATGATTGTTATTCGAAGC  138

Query  60   TTCGATGTGAATAAACCGGGCAGTGAAGTGGATGAAATTCGTGGCGGCGTGGCTGGGGGT  119
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  139  TTCGATGTGAATAAACCGGGCAGTGAAGTGGATGAAATTCGTGGCGGCGTGGCTGGGGGT  198

Query  120  TCCATTTTACGTGGCGTGTTGCGGGTGAACCAGAACATTGAAGTGCGTCCGGGCATTGTG  179
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  199  TCCATTTTACGTGGCGTGTTGCGGGTGAACCAGAACATTGAAGTGCGTCCGGGCATTGTG  258

Query  180  ATGAAAGACGAATCGGGGAACATAAAGTGCACGCCCATTTATAGCCGTATTGTGAGCTTA  239
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  259  ATGAAAGACGAATCGGGGAACATAAAGTGCACGCCCATTTATAGCCGTATTGTGAGCTTA  318

Query  240  TATGCGGAACAAAATGAGCTGCAATTCGCGGTGCCTGGGGGCCTGATTGGGGTGGGCACG  299
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  319  TATGCGGAACAAAATGAGCTGCAATTCGCGGTGCCTGGGGGCCTGATTGGGGTGGGCACG  378

Query  300  ACTATGGACCCGACCCTGACTCGTGCGGATCGTCTGGTGGGCCAAGTG-CTGGGAGAAAT  358
            |||||||||||||||||||||||||||||||||||||||||||||||| ||||||||||
Sbjct  379  ACTATGGACCCGACCCTGACTCGTGCGGATCGTCTGGTGGGCCAAGTGCCTGGGAGAAAT  438

Query  359  TGGTAGCCTGCCGGATGTCTTTGTTGAATTAGAGATTAACTTTTTTCAGCTGCGTCGTCT  418
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  439  TGGTAGCCTGCCGGATGTCTTTGTTGAATTAGAGATTAACTTTTTTCAGCTGCGTCGTCT  498

Query  419  GCTGGGTGTTCGGACCAAAGGGACCGAGAAAGCGGGGAAAGTGAGCAAATTGACCAAGGG  478
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  499  GCTGGGTGTTCGGACCAAAGGGACCGAGAAAGCGGGGAAAGTGAGCAAATTGACCAAGGG  558

Query  479  GGAAATTCTGATGTTAAACATCGGAAGCATGAGCACCGGAGCGCGTGTGGTTGCGGTCAA  538
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  559  GGAAATTCTGATGTTAAACATCGGAAGCATGAGCACCGGAGCGCGTGTGGTTGCGGTCAA  618

Query  539  AAATGACCTGGCGAAGTTACAGTTAACAGCCCCAGTATGTACTTCGAAAGGCGAGAAGGT  598
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  619  AAATGACCTGGCGAAGTTACAGTTAACAGCCCCAGTATGTACTTCGAAAGGCGAGAAGGT  678

Query  599  AGCGCTGTCCCGTCGTGTGGAAAAGCATTGGCGTCTGATCGGTTGGGGCCAGATTCAAGC  658
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  679  AGCGCTGTCCCGTCGTGTGGAAAAGCATTGGCGTCTGATCGGTTGGGGCCAGATTCAAGC  738

Query  659  TGGAGCTACCCTGGAAGTGCCGCCGTGCCCGCTGTAATAAGGATCCTACTAGTAGCGGCC  718
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  739  TGGAGCTACCCTGGAAGTGCCGCCGTGCCCGCTGTAATAAGGATCCTACTAGTAGCGGCC  798

Query  719  GCTGCAG  725
            |||||||
Sbjct  799  GCTGCAG  805
```

Sequence 7: Complete eIF2α in BioBrick (VF2)

```
Query  1     GAATTCGCGGCCGCTTCTAGAGATTAAAGAGGAGAAATACCATATGGCGAACCTGGAATG  60
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  89    GAATTCGCGGCCGCTTCTAGAGATTAAAGAGGAGAAATACCATATGGCGAACCTGGAATG  148

Query  61    CCGTATGTATGAACCGCGTTTTCCCGAAGTGGATGCTGCGGTGATGATTCAGGTGAAACA  120
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  149   CCGTATGTATGAACCGCGTTTTCCCGAAGTGGATGCTGCGGTGATGATTCAGGTGAAACA  208

Query  121   TATTGCGGATATGGGCGCGTATGTGAGCCTGCTGGAATATAACAATGTGGAAGGCATGAT  180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  209   TATTGCGGATATGGGCGCGTATGTGAGCCTGCTGGAATATAACAATGTGGAAGGCATGAT  268

Query  181   TCTGTTTTCCGAGCTGAGCCGTCGCCGCATTCGGAGCATAAGCAGCCTGATAAAAGTGGG  240
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  269   TCTGTTTTCCGAGCTGAGCCGTCGCCGCATTCGGAGCATAAGCAGCCTGATAAAAGTGGG  328

Query  241   TCGTCAAGAACCGGCGATAGTTCTTCGTGTGGATCGTGATAAAGGCTATATTGACTTGTC  300
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            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  329  TCGTCAAGAACCGGCGATAGTTCTTCGTGTGGATCGTGATAAAGGCTATATTGACTTGTC  388

Query  301  CAAGCGCCGTGTTTCCGAAGAAGAAGCGCGTAGCTGCGAGGATAAATACAATAAGAGCAA  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  389  CAAGCGCCGTGTTTCCGAAGAAGAAGCGCGTAGCTGCGAGGATAAATACAATAAGAGCAA  448

Query  361  GCTGGTCCATAGCATTATGCGTCACGTTGCTGAGACCCTGGAGATTGATCTGGAACCGAT  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  449  GCTGGTCCATAGCATTATGCGTCACGTTGCTGAGACCCTGGAGATTGATCTGGAACCGAT  508

Query  421  CTATCAGCGGATTGGCTGGCCGCTGTATCGTAAATACGGCCACGCATTTGAAGCGTTCAA  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  509  CTATCAGCGGATTGGCTGGCCGCTGTATCGTAAATACGGCCACGCATTTGAAGCGTTCAA  568

Query  481  GCTGATTGTTGCGGACCCTGACGCCATATTAGACGTGCTGACCTATGAAGAGCGTGAAAC  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  569  GCTGATTGTTGCGGACCCTGACGCCATATTAGACGTGCTGACCTATGAAGAGCGTGAAAC  628

Query  541  CGGGCCAGATGGCCAAGAAGTGACCAAAGTGGTACCAGCAGTCACCCCGGAAATAAAGGA  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  629  CGGGCCAGATGGCCAAGAAGTGACCAAAGTGGTACCAGCAGTCACCCCGGAAATAAAGGA  688

Query  601  AACCCTGGTGCAGAACATTCGTCGTCGGATGACACCACAACCGCTGAAAATTCGTGCGGA  660
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  689  AACCCTGGTGCAGAACATTCGTCGTCGGATGACACCACAACCGCTGAAAATTCGTGCGGA  748

Query  661  CGTGGAAATGAAATGCTTTCAGTTTGATGGCGTGCTGCATATCAAACAGGCGATGCGTAA  720
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  749  CGTGGAAATGAAATGCTTTCAGTTTGATGGCGTGCTGCATATCAAACAGGCGATGCGTAA  808

Query  721  GGCGGAGGCTGCGGGCAATACCAATTGTCCGGTAAAGATTAAGTTAGTGGCGCCTCCACT  780
             |||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  809  GGCGGANGCTGCGGGCAATACCAATTGTCCGGTAAAGATTAAGTTAGTGGCGCCTCCACT  868

Query  781  GTATGTACTGACCACCCAGACACTGGACAAAGATCAGGGCATTAGCGTTTTAACCGATGC  840
            |||||||||||||||||||| |||||||||||| |||||||||||||||||||||||||||
Sbjct  869  GTATGTACTGACCACCCAGACNCTGGACAAAGATCNGGGCATTAGCGTTTTAACCGATGC  928

Query  841  GGTCAAAGCGTGCACTGCGGAAATCGAAAAACATAAGGGCAAACTGG-TGGTGAAAGAAG  899
            |||||||||||||||||||||||||||||||||||     ||||||| ||||||||||||
Sbjct  929  GGTCAAAGCGTGCACTGCGGAAATCGAAAAACATANNNN-AAACTGGGTGGTGAAAGAAG  987

Query  900  CCCCACGCGCGGTGTCAGAACGTGAAGATAAACTGCTGAATGCCCAACT  948
            ||||||||||||||||||  ||||||| || |||||||||| || |||
Sbjct  988  CCCCACGCGCGGTGTCAGAN-GTGAAGANAA-CTGCTGAATGNCCNACT  1034
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Sequence 8: Complete eIF2α in BioBrick (VR)

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Query  165   ATGTGGAAGGCATGATTCTGTTTTCCGAGCTGAGCCGTCGCCGCATTCGGAGCATAAGCA  224
             ||||||| |||||| ||||||||| ||||||||||||||| |||||||| ||||||||||
Sbjct  1049  ATGTGGANGGCATGNTTCTGTTTTNCGAGCTGAGCCGTCGNCGCATTCGNAGCATAAGCA  990

Query  225   GCCTGATAAAAGTGGGTCGTCAAGAACCGGCGATAGTTCTTCGTGTGGATCGTGATAAAG  284
              |||||||||||||||||||| |||||||||||||||||||||||||||||||||||
Sbjct  989   -NNNGATAAAAGTGGGTCGTCAAGANCCGGCGATAGTTCTTCGTGTGGATCGTGATAAAG  931

Query  285   GCTATATTGACTTGTCCAAGCGCCGTGTTTCCGAAGAAGAAGCGCGTAGCTGCGAGGATA  344
              |||||||||||||  ||||||||||||||| |||||||||||||||||||||||||||||
Sbjct  930   GCTATATTGACTTNNCCAAGCGCCGTGTTTCNGAAGAAGAAGCGCGTAGCTGCGAGGATA  871

Query  345   AATACAATAAGAGCAAGCTGGTCCATAGCATTATGCGTCACGTTGCTGAGACCCTGGAGA  404
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  870   AATACAATAAGAGCAAGCTGGTCCATAGCATTATGCGTCACGTTGCTGAGACCCTGGAGA  811

Query  405   TTGATCTGGAACCGATCTATCAGCGGATTGGCTGGCCGCTGTATCGTAAATACGGCCACG  464
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  810   TTGATCTGGAACCGATCTATCAGCGGATTGGCTGGCCGCTGTATCGTAAATACGGCCACG  751

Query  465   CATTTGAAGCGTTCAAGCTGATTGTTGCGGACCCTGACGCCATATTAGACGTGCTGACCT  524
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            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  750  CATTTGAAGCGTTCAAGCTGATTGTTGCGGACCCTGACGCCATATTAGACGTGCTGACCT  691

Query  525  ATGAAGAGCGTGAAACCGGGCCAGATGGCCAAGAAGTGACCAAAGTGGTACCAGCAGTCA  584
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  690  ATGAAGAGCGTGAAACCGGGCCAGATGGCCAAGAAGTGACCAAAGTGGTACCAGCAGTCA  631

Query  585  CCCCGGAAATAAAGGAAACCCTGGTGCAGAACATTCGTCGTCGGATGACACCACAACCGC  644
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  630  CCCCGGAAATAAAGGAAACCCTGGTGCAGAACATTCGTCGTCGGATGACACCACAACCGC  571

Query  645  TGAAAATTCGTGCGGACGTGGAAATGAAATGCTTTCAGTTTGATGGCGTGCTGCATATCA  704
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  570  TGAAAATTCGTGCGGACGTGGAAATGAAATGCTTTCAGTTTGATGGCGTGCTGCATATCA  511

Query  705  AACAGGCGATGCGTAAGGCGGAGGCTGCGGGCAATACCAATTGTCCGGTAAAGATTAAGT  764
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  510  AACAGGCGATGCGTAAGGCGGAGGCTGCGGGCAATACCAATTGTCCGGTAAAGATTAAGT  451

Query  765  TAGTGGCGCCTCCACTGTATGTACTGACCACCCAGACACTGGACAAAGATCAGGGCATTA  824
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  450  TAGTGGCGCCTCCACTGTATGTACTGACCACCCAGACACTGGACAAAGATCAGGGCATTA  391

Query  825  GCGTTTTAACCGATGCGGTCAAAGCGTGCACTGCGGAAATCGAAAAACATAAGGGCAAAC  884
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  390  GCGTTTTAACCGATGCGGTCAAAGCGTGCACTGCGGAAATCGAAAAACATAAGGGCAAAC  331

Query  885  TGGTGGTGAAAGAAGCCCCACGCGCGGTGTCAGAACGTGAAGATAAACTGCTGAATGCCC  944
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  330  TGGTGGTGAAAGAAGCCCCACGCGCGGTGTCAGAACGTGAAGATAAACTGCTGAATGCCC  271

Query  945  AACTGGATACTCTGGTAGAACAAAATGCGGAAGTCGCAGGCGACGATGATAGCGAGGACG  1004
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  270  AACTGGATACTCTGGTAGAACAAAATGCGGAAGTCGCAGGCGACGATGATAGCGAGGACG  211

Query  1005 AAGAGGATACCGGCATGGGCGACATCGATCTGACCAATAGCGGCGTGCATGCGGATTAAT  1064
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  210  AAGAGGATACCGGCATGGGCGACATCGATCTGACCAATAGCGGCGTGCATGCGGATTAAT  151

Query  1065 AAGGATCCTACTAGTAGCGGCCGCTGCAG  1093
            |||||||||||||||||||||||||||||
Sbjct  150  AAGGATCCTACTAGTAGCGGCCGCTGCAG  122
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Sequence 9: Complete eIF2β in BioBrick (VF2)

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Query  40   CCATATGGCGGACGAAGAACAGATGGAACGTAAGGAAGAAGCGACCGAGATTGCGCCGTT  99
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  103  CCATATGGCGGACGAAGAACAGATGGAACGTAAGGAAGAAGCGACCGAGATTGCGCCGTT  162

Query  100  TGACCCGACTaagaagaagaagaagaagaaagTGGTGATTCAGGACCCAGCGGATGAAGT  159
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  163  TGACCCGACTAAGAAGAAGAAGAAGAAAGTGGTGATTCAGGACCCAGCGGATGAAGT  222

Query  160  GGATAAATTGGCCGAAAAGACCGAAGGCCTGAGCGTGACCGAAAGCGGCGAGGCGAGCTT  219
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  223  GGATAAATTGGCCGAAAAGACCGAAGGCCTGAGCGTGACCGAAAGCGGCGAGGCGAGCTT  282

Query  220  TGTGGGCTTGAAGAAAAAGAAAAAGAAGTTAGTAGAACTGGACCCCAGCCTTGTCGAAGC  279
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  283  TGTGGGCTTGAAGAAAAAGAAAAAGAAGTTAGTAGAACTGGACCCCAGCCTTGTCGAAGC  342

Query  280  CGGTGATGGCGAAGATACCCTGGACGATCAGGTGGGCGAGGATGAACAAGGAGAGGGCAT  339
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  343  CGGTGATGGCGAAGATACCCTGGACGATCAGGTGGGCGAGGATGAACAAGGAGAGGGCAT  402

Query  340  TGTGCTGGGCGGTGCCACCCAGTATCCGTGGGAAGGAACCGATCGTGATTACAAATATGA  399
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  403  TGTGCTGGGCGGTGCCACCCAGTATCCGTGGGAAGGAACCGATCGTGATTACAAATATGA  462

Query  400  TGAGCTGTTGGGCCGTGTGTTCAATATTCTGCGCGAGAATAATCCAGACCTGGCGGGAGA  459
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            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  463  TGAGCTGTTGGGCCGTGTGTTCAATATTCTGCGCGAGAATAATCCAGACCTGGCGGGAGA  522

Query  460  CCGTCGACGTACCGTGATGCGTCCACCGCAGGTCTTGCGTGAAGGCACCaaaaaaaCCGT  519
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  523  CCGTCGACGTACCGTGATGCGTCCACCGCAGGTCTTGCGTGAAGGCACCAAAAAAACCGT  582

Query  520  GTTTGTGAATTTCATGGATCTGTGCAAAACCATGCATCGTCAGCCGGAACATGTGATGAT  579
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  583  GTTTGTGAATTTCATGGATCTGTGCAAAACCATGCATCGTCAGCCGGAACATGTGATGAT  642

Query  580  GTTTCTGCTTGCGGAAATGGGCACCAGCGGCAGCCTGGATGGGCAGCAGCGTCTGGTCAT  639
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  643  GTTTCTGCTTGCGGAAATGGGCACCAGCGGCAGCCTGGATGGGCAGCAGCGTCTGGTCAT  702

Query  640  TAAAGGCCGCTTCGCTCCCAAGAATTTTGAAGCGATCCTGCGGCGTTACATCAATGAATA  699
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  703  TAAAGGCCGCTTCGCTCCCAAGAATTTTGAAGCGATCCTGCGGCGTTACATCAATGAATA  762

Query  700  TGTGATTTGCAATGGCTGCAAGAGCCCGGATACCATTCTGAGCAAAGAGAATCGTCTGTT  759
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  763  TGTGATTTGCAATGGCTGCAAGAGCCCGGATACCATTCTGAGCAAAGAGAATCGTCTGTT  822

Query  760  TTTCCTGCGTTGCGAACAGTGCGGCAGTAGCCGTAGCGTGGCGCCGATTAAAGCCGGG-T  818
             |||||||||||||||||||||||||||||||||||||||||||| ||||||||| || |
Sbjct  823  TTTCCTGCGTTGCGAACAGTGCGGCAGTAGCCGTAGCGTGGCGCCNATTAAAGCCNGGNT  882

Query  819  TCGTTGCCCAGGTAGGCCGTCGTAAGGCGGGCACCTAATAAGGATCCTACTAGTAGCGGC  878
             |||||| |||||||||||||||||||| |||||||||||||||||||||||||||||||
Sbjct  883  TCGTTGNCCAGGTAGGCCGTCGTAAGGNGGGCACCTAATAAGGATCCTACTAGTAGCGGN  942

Query  879  CGCTGCAG  886
            ||||||||
Sbjct  943  CGCTGCAG  950
```

Sequence 10: Complete eIF2β in BioBrick (VR)

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Query  40   CCATATGGCGGACGAAGAACAGATGGAACGTAAGGAAGAAGCGACCGAGATTGCGCCGTT  99
            ||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  970  CCATATGGCGGACGANGAACAGATGGAACGTAAGGAAGAAGCGACCGAGATTGCGCCGTT  911

Query  100  TGACCCGACTaagaagaagaagaagaagaaagTGGTGATTCAGGACCCAGCGGATGAAGT  159
            ||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  910  TGACCNGACTAAGAAGAAGAAGAAGAAAGTGGTGATTCAGGACCCAGCGGATGAAGT  851

Query  160  GGATAAATTGGCCGAAAAGACCGAAGGCCTGAGCGTGACCGAAAGCGGCGAGGCGAGCTT  219
            |||||||||||||||||||| |||||| ||||||||||||||||||||||||||||||||
Sbjct  850  GGATAAATTGGCCGAAAAGACNGAAGGCNTGAGCGTGACCGAAAGCGGCGAGGCGAGCTT  791

Query  220  TGTGGGCTTGAAGAAAAAGAAAAAGAAGTTAGTAGAACTGGACCCCAGCCTTGTCGAAGC  279
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  790  TGTGGGCTTGAAGAAAAAGAAAAAGAAGTTAGTAGAACTGGACCCCAGCCTTGTCGAAGC  731

Query  280  CGGTGATGGCGAAGATACCCTGGACGATCAGGTGGGCGAGGATGAACAAGGAGAGGGCAT  339
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  730  CGGTGATGGCGAAGATACCCTGGACGATCAGGTGGGCGAGGATGAACAAGGAGAGGGCAT  671

Query  340  TGTGCTGGGCGGTGCCACCCAGTATCCGTGGGAAGGAACCGATCGTGATTACAAATATGA  399
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  670  TGTGCTGGGCGGTGCCACCCAGTATCCGTGGGAAGGAACCGATCGTGATTACAAATATGA  611

Query  400  TGAGCTGTTGGGCCGTGTGTTCAATATTCTGCGCGAGAATAATCCAGACCTGGCGGGAGA  459
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  610  TGAGCTGTTGGGCCGTGTGTTCAATATTCTGCGCGAGAATAATCCAGACCTGGCGGGAGA  551

Query  460  CCGTCGACGTACCGTGATGCGTCCACCGCAGGTCTTGCGTGAAGGCACCaaaaaaaCCGT  519
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  550  CCGTCGACGTACCGTGATGCGTCCACCGCAGGTCTTGCGTGAAGGCACCAAAAAAACCGT  491
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Query  520  GTTTGTGAATTTCATGGATCTGTGCAAAACCATGCATCGTCAGCCGGAACATGTGATGAT  579
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  490  GTTTGTGAATTTCATGGATCTGTGCAAAACCATGCATCGTCAGCCGGAACATGTGATGAT  431

Query  580  GTTTCTGCTTGCGGAAATGGGCACCAGCGGCAGCCTGGATGGGCAGCAGCGTCTGGTCAT  639
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  430  GTTTCTGCTTGCGGAAATGGGCACCAGCGGCAGCCTGGATGGGCAGCAGCGTCTGGTCAT  371

Query  640  TAAAGGCCGCTTCGCTCCCAAGAATTTTGAAGCGATCCTGCGGCGTTACATCAATGAATA  699
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  370  TAAAGGCCGCTTCGCTCCCAAGAATTTTGAAGCGATCCTGCGGCGTTACATCAATGAATA  311

Query  700  TGTGATTTGCAATGGCTGCAAGAGCCCGGATACCATTCTGAGCAAAGAGAATCGTCTGTT  759
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  310  TGTGATTTGCAATGGCTGCAAGAGCCCGGATACCATTCTGAGCAAAGAGAATCGTCTGTT  251

Query  760  TTTCCTGCGTTGCGAACAGTGCGGCAGTAGCCGTAGCGTGGCGCCGATTAAAGCCGGGTT  819
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  250  TTTCCTGCGTTGCGAACAGTGCGGCAGTAGCCGTAGCGTGGCGCCGATTAAAGCCGGGTT  191

Query  820  CGTTGCCCAGGTAGGCCGTCGTAAGGCGGGCACCTAATAAGGATCCTACTAGTAGCGGCC  879
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  190  CGTTGCCCAGGTAGGCCGTCGTAAGGCGGGCACCTAATAAGGATCCTACTAGTAGCGGCC  131

Query  880  GCTGCAG  886
            |||||||
Sbjct  130  GCTGCAG  124
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Sequence 11: Complete eIF2γ in BioBrick (VF2)

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Query  866   CTTTACCAGCCCACCGAATATGATTGTTATTCGAAGCTTCGATGTGAATAAACCGGGCAG  925
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  824   CTTTACCAGCCCACCGAATATGATTGTTATTCGAAGCTTCGATGTGAATAAACCGGGCAG  765

Query  926   TGAAGTGGATGAAATTCGTGGCGGCGTGGCTGGGGGTTCCATTTTACGTGGCGTGTTGCG  985
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  764   TGAAGTGGATGAAATTCGTGGCGGCGTGGCTGGGGGTTCCATTTTACGTGGCGTGTTGCG  705

Query  986   GGTGAACCAGAACATTGAAGTGCGTCCGGGCATTGTGATGAAAGACGAATCGGGGAACAT  1045
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  704   GGTGAACCAGAACATTGAAGTGCGTCCGGGCATTGTGATGAAAGACGAATCGGGGAACAT  645

Query  1046  AAAGTGCACGCCCATTTATAGCCGTATTGTGAGCTTATATGCGGAACAAAATGAGCTGCA  1105
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  644   AAAGTGCACGCCCATTTATAGCCGTATTGTGAGCTTATATGCGGAACAAAATGAGCTGCA  585

Query  1106  ATTCGCGGTGCCTGGGGGCCTGATTGGGGTGGGCACGACTATGGACCCGACCCTGACTCG  1165
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  584   ATTCGCGGTGCCTGGGGGCCTGATTGGGGTGGGCACGACTATGGACCCGACCCTGACTCG  525

Query  1166  TGCGGATCGTCTGGTGGGCCAAGTGCTGGGAGAAATTGGTAGCCTGCCGGATGTCTTTGT  1225
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  524   TGCGGATCGTCTGGTGGGCCAAGTGCTGGGAGAAATTGGTAGCCTGCCGGATGTCTTTGT  465

Query  1226  TGAATTAGAGATTAACTTTTTTCAGCTGCGTCGTCTGCTGGGTGTTCGGACCAAAGGGAC  1285
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  464   TGAATTAGAGATTAACTTTTTTCAGCTGCGTCGTCTGCTGGGTGTTCGGACCAAAGGGAC  405

Query  1286  CGAGAAAGCGGGGAAAGTGAGCAAATTGACCAAGGGGGAAATTCTGATGTTAAACATCGG  1345
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  404   CGAGAAAGCGGGGAAAGTGAGCAAATTGACCAAGGGGGAAATTCTGATGTTAAACATCGG  345

Query  1346  AAGCATGAGCACCGGAGCGCGTGTGGTTGCGGTCAAAAATGACCTGGCGAAGTTACAGTT  1405
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  344   AAGCATGAGCACCGGAGCGCGTGTGGTTGCGGTCAAAAATGACCTGGCGAAGTTACAGTT  285

Query  1406  AACAGCCCCAGTATGTACTTCGAAAGGCGAGAAGGTAGCGCTGTCCCGTCGTGTGGAAAA  1465
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  284   AACAGCCCCAGTATGTACTTCGAAAGGCGAGAAGGTAGCGCTGTCCCGTCGTGTGGAAAA  225
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Query  1466  GCATTGGCGTCTGATCGGTTGGGGCCAGATTCAAGCTGGAGCTACCCTGGAAGTGCCGCC  1525
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  224   GCATTGGCGTCTGATCGGTTGGGGCCAGATTCAAGCTGGAGCTACCCTGGAAGTGCCGCC  165

Query  1526  GTGCCCGCTGTAATAAGGATCCTACTAGTAGCGGCCGCTGCAG  1568
             |||||||||||||||||||||||||||||||||||||||||||
Sbjct  164   GTGCCCGCTGTAATAAGGATCCTACTAGTAGCGGCCGCTGCAG  122


Query  496   TGCGGCAAATGAATCTTGCCCTCAGCCGCAGACCAGCGAACATCTGGCTGCGGTGGAAA  554
             ||||||||| ||||  || |||||||   | ||||||||| |||| |||||||||| ||
Sbjct  1088  TGCGGCAAANGAAT-NTG-CCTCAGCNNNCNGACCAGCGANCATCNGGCTGCGGTGG-AA  1032

Query  555   TTATGCGTCTTCAGCACCTGATAATTCTTCAGAATAAGATTGATCTGATTCAGGAATCCG  614
              |||||||  |||||    |||      |||||||||| |||| ||||||||||||||
Sbjct  1031  NTATGCGT-NTCAGCNNNNGAT-NNNNNTCAGAATAAGA-TGATNTGATTCAGGAATCCG  975

Query  615   CAGCGATGAATCAACATGAGGCGATTCAAAAGTTCATTCAAGGCACCATAGCGGAGGGCG  674
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  974   CAGCGATGAATCAACATGAGGCGATTCAAAAGTTCATTCAAGGCACCATAGCGGAGGGCG  915

Query  675   CTCCAGTGGTTCCGATTAGCGCGCAGTTGAAGTATAACATCGACGTAATTTGCGAATACA  734
              ||||||  | ||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  914   CTCCAG-NGNTCCGATTAGCGCGCAGTTGAAGTATAACATCGACGTAATTTGCGAATACA  856

Query  735   TCATAAAGAAGATTCCGATCCCTGAACGTAACTTTACCAGCCCACCGAATATGATTGTTA  794
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  855   TCATAAAGAAGATTCCGATCCCTGAACGTAACTTTACCAGCCCACCGAATATGATTGTTA  796

Query  795   TTCGAAGCTTCGATGTGAATAAACCGGGCAGTGAAGTGGATGAAATTCGTGGCGGCGTGG  854
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  795   TTCGAAGCTTCGATGTGAATAAACCGGGCAGTGAAGTGGATGAAATTCGTGGCGGCGTGG  736

Query  855   CTGGGGGTTCCCTTT  869
             |||||||||||| |||
Sbjct  735   CTGGGGGTTCCATTT  721
```

Sequence 12: Complete eIF2αγ in BioBrick (VF2)

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Query  1    GAATTCGCGGCCGCTTCTAGAGATTAAAGAGGAGAAATACCATATGGCGAACCTGGAATG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  86   GAATTCGCGGCCGCTTCTAGAGATTAAAGAGGAGAAATACCATATGGCGAACCTGGAATG  145

Query  61   CCGTATGTATGAACCGCGTTTTCCCGAAGTGGATGCTGCGGTGATGATTCAGGTGAAACA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  146  CCGTATGTATGAACCGCGTTTTCCCGAAGTGGATGCTGCGGTGATGATTCAGGTGAAACA  205

Query  121  TATTGCGGATATGGGCGCGTATGTGAGCCTGCTGGAATATAACAATGTGGAAGGCATGAT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  206  TATTGCGGATATGGGCGCGTATGTGAGCCTGCTGGAATATAACAATGTGGAAGGCATGAT  265

Query  181  TCTGTTTTCCGAGCTGAGCCGTCGCCGCATTCGGAGCATAAGCAGCCTGATAAAAGTGGG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  266  TCTGTTTTCCGAGCTGAGCCGTCGCCGCATTCGGAGCATAAGCAGCCTGATAAAAGTGGG  325

Query  241  TCGTCAAGAACCGGCGATAGTTCTTCGTGTGGATCGTGATAAAGGCTATATTGACTTGTC  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  326  TCGTCAAGAACCGGCGATAGTTCTTCGTGTGGATCGTGATAAAGGCTATATTGACTTGTC  385

Query  301  CAAGCGCCGTGTTTCCGAAGAAGAAGCGCGTAGCTGCGAGGATAAATACAATAAGAGCAA  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  386  CAAGCGCCGTGTTTCCGAAGAAGAAGCGCGTAGCTGCGAGGATAAATACAATAAGAGCAA  445

Query  361  GCTGGTCCATAGCATTATGCGTCACGTTGCTGAGACCCTGGAGATTGATCTGGAACCGAT  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  446  GCTGGTCCATAGCATTATGCGTCACGTTGCTGAGACCCTGGAGATTGATCTGGAACCGAT  505

Query  421  CTATCAGCGGATTGGCTGGCCGCTGTATCGTAAATACGGCCACGCATTTGAAGCGTTCAA  480
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            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  506  CTATCAGCGGATTGGCTGGCCGCTGTATCGTAAATACGGCCACGCATTTGAAGCGTTCAA  565

Query  481  GCTGATTGTTGCGGACCCTGACGCCATATTAGACGTGCTGACCTATGAAGAGCGTGAAAC  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  566  GCTGATTGTTGCGGACCCTGACGCCATATTAGACGTGCTGACCTATGAAGAGCGTGAAAC  625

Query  541  CGGGCCAGATGGCCAAGAAGTGACCAAAGTGGTACCAGCAGTCACCCCGGAAATAAAGGA  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  626  CGGGCCAGATGGCCAAGAAGTGACCAAAGTGGTACCAGCAGTCACCCCGGAAATAAAGGA  685

Query  601  AACCCTGGTGCAGAACATTCGTCGTCGGATGACACCACAACCGCTGAAAATTCGTGCGGA  660
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  686  AACCCTGGTGCAGAACATTCGTCGTCGGATGACACCACAACCGCTGAAAATTCGTGCGGA  745

Query  661  CGTGGAAATGAAATGCTTTCAGTTTGATGGCGTGCTGCATATCAAACAGGCGATGCGTAA  720
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  746  CGTGGAAATGAAATGCTTTCAGTTTGATGGCGTGCTGCATATCAAACAGGCGATGCGTAA  805

Query  721  GGCGGAGGCTGCGGGCAATACCAATTGTCCGGTAAAGATTAAGTTAGTGGCGCCTCCACT  780
             |||||||  ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  806  GGCGGAGNNTGCGGGCAATACCAATTGTCCGGTAAAGATTAAGTTAGTGGCGCCTCCACT  865

Query  781  GTATGTACTGACCACCCAGACACTGGACAAAGATCAGGGCATTAGCGTTTTAACCGATGC  840
            |||||||||||||||||||||||||||| ||||||     ||||||||||||||||||||
Sbjct  866  GTATGTACTGACCACCCAGACACTGGACNAAGATCNNNN-ATTAGCGTTTTAACCGATGC  924

Query  841  GGTCAAAGCGTGCACTGCGGAAATCGAAAAACATAAGGGCAAACTGGTGGTGAA  894
            |||||||||||||||||||||||||||||||||||| ||| ||||||||||||
Sbjct  925  GGTCAAAGCGTGCACTGCGGAAATCGAAAAACATAAGG-CAA-CTGGTGGTGAA  976
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Sequence 13: Complete eIF2αγ in BioBrick (VR)

```
Query  1883  CTTTACCAGCCCACCGAATATGATTGTTATTCGAAGCTTCGATGTGAATAAACCGGGCAG  1942
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  821   CTTTACCAGCCCACCGAATATGATTGTTATTCGAAGCTTCGATGTGAATAAACCGGGCAG  762

Query  1943  TGAAGTGGATGAAATTCGTGGCGGCGTGGCTGGGGGTTCCATTTTACGTGGCGTGTTGCG  2002
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  761   TGAAGTGGATGAAATTCGTGGCGGCGTGGCTGGGGGTTCCATTTTACGTGGCGTGTTGCG  702

Query  2003  GGTGAACCAGAACATTGAAGTGCGTCCGGGCATTGTGATGAAAGACGAATCGGGGAACAT  2062
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  701   GGTGAACCAGAACATTGAAGTGCGTCCGGGCATTGTGATGAAAGACGAATCGGGGAACAT  642

Query  2063  AAAGTGCACGCCCATTTATAGCCGTATTGTGAGCTTATATGCGGAACAAAATGAGCTGCA  2122
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  641   AAAGTGCACGCCCATTTATAGCCGTATTGTGAGCTTATATGCGGAACAAAATGAGCTGCA  582

Query  2123  ATTCGCGGTGCCTGGGGGCCTGATTGGGGTGGGCACGACTATGGACCCGACCCTGACTCG  2182
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  581   ATTCGCGGTGCCTGGGGGCCTGATTGGGGTGGGCACGACTATGGACCCGACCCTGACTCG  522

Query  2183  TGCGGATCGTCTGGTGGGCCAAGTGCTGGGAGAAATTGGTAGCCTGCCGGATGTCTTTGT  2242
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  521   TGCGGATCGTCTGGTGGGCCAAGTGCTGGGAGAAATTGGTAGCCTGCCGGATGTCTTTGT  462

Query  2243  TGAATTAGAGATTAACTTTTTTCAGCTGCGTCGTCTGCTGGGTGTTCGGACCAAAGGGAC  2302
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  461   TGAATTAGAGATTAACTTTTTTCAGCTGCGTCGTCTGCTGGGTGTTCGGACCAAAGGGAC  402

Query  2303  CGAGAAAGCGGGGAAAGTGAGCAAATTGACCAAGGGGGAAATTCTGATGTTAAACATCGG  2362
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  401   CGAGAAAGCGGGGAAAGTGAGCAAATTGACCAAGGGGGAAATTCTGATGTTAAACATCGG  342

Query  2363  AAGCATGAGCACCGGAGCGCGTGTGGTTGCGGTCAAAAATGACCTGGCGAAGTTACAGTT  2422
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  341   AAGCATGAGCACCGGAGCGCGTGTGGTTGCGGTCAAAAATGACCTGGCGAAGTTACAGTT  282

Query  2423  AACAGCCCCAGTATGTACTTCGAAAGGCGAGAAGGTAGCGCTGTCCCGTCGTGTGGAAAA  2482
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                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  281   AACAGCCCCAGTATGTACTTCGAAAGGCGAGAAGGTAGCGCTGTCCCGTCGTGTGGAAAA  222

Query  2483  GCATTGGCGTCTGATCGGTTGGGGCCAGATTCAAGCTGGAGCTACCCTGGAAGTGCCGCC  2542
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  221   GCATTGGCGTCTGATCGGTTGGGGCCAGATTCAAGCTGGAGCTACCCTGGAAGTGCCGCC  162

Query  2543  GTGCCCGCTGTAATAAGGATCCTACTAGTAGCGGCCGCTGCAG  2585
              |||||||||||||||||||||||||||||||||||||||||||
Sbjct  161   GTGCCCGCTGTAATAAGGATCCTACTAGTAGCGGCCGCTGCAG  119

Query  1612  TGATCTGATTCAGGAATCCGCAGCGATGAATCAACATGAGGCGATTCAAAAGTTCATTCA  1671
              |||| ||| |||||||||| ||||||||||||  |||||  ||||||||||||||||||||
Sbjct  988   TGATNTGA-TCAGGAATCNGCAGCGATGAAT-NACATGNNGCGATTCAAAAGTTCATTCA  931

Query  1672  AGGCACCATAGCGGAGGGCGCTCCAGTGGTTCCGATTAGCGCGCAGTTGAAGTATAACAT  1731
               |||| ||||||||||||||  |||||||| ||||||||||||||||||||||||||||
Sbjct  930   AGGCNCCATAGCGGAGGGCGNNCCAGTGGTTNCGATTAGCGCGCAGTTGAAGTATAACAT  871

Query  1732  CGACGTAATTTGCGAATACATCATAAAGAAGATTCCGATCCCTGAACGTAACTTTACCAG  1791
              |||||| | |||||||||||||||||||||||||||||   | ||||||||||||||||
Sbjct  870   CGACGT-ANTTGCGAATACATCATAAAGAAGATTCCGA-NNCNGAACGTAACTTTACCAG  813

Query  1792  CCCACCGAATATGATTGTTATTCGAAGCTTCGATGTGAATAAACCGGGCAGTGAAGTGGA  1851
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  812   CCCACCGAATATGATTGTTATTCGAAGCTTCGATGTGAATAAACCGGGCAGTGAAGTGGA  753

Query  1852  TGAAATTCGTGGCGGCGTGGCTGGGGGTTCCCTTT  1886
              |||||||||||||||||||||||||||||||| |||
Sbjct  752   TGAAATTCGTGGCGGCGTGGCTGGGGGTTCCATTT  718
```

Sequence 14: Complete eIF2βαγ in BioBrick (VF2)

```
Query  2592  CCATATGGCGGACGAAGAACAGATGGAACGTAAGGAAGAAGCGACCGAGATTGCGCCGTT  2651
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  97    CCATATGGCGGACGAAGAACAGATGGAACGTAAGGAAGAAGCGACCGAGATTGCGCCGTT  156

Query  2652  TGACCCGACTaagaagaagaagaagaagaaagTGGTGATTCAGGACCCAGCGGATGAAGT  2711
              ||||||||||||||||||||||||||||||||||||||||||   ||||||  |||||||||
Sbjct  157   TGACCCGACTAAGAAGAAGAAGAAGAAGAAAGTGGTGATTNNNGACCCANNGGATGAAGT  216

Query  2712  GGATAAATTGGCCGAAAAGACCGAAGGCCTGAGCGTGACCGAAAGCGGCGAGGCGAGCTT  2771
              ||||||||||||||||||||  ||||| ||||  |||||||||||| || |||| ||| ||
Sbjct  217   GGATAAATTGGCCGAAAAGANNGAAGGNCTGANNGTGACCGAAAGNGGNGAGGNGAGNTT  276

Query  2772  TGTGGGCTTGAAGAAAAAGAAAAAGAAGTTAGTAGAACTGGACCCCAGCCTTGTCGAAGC  2831
              ||||||||||||||||||||||||||||||||||||||| ||||||||   ||||||||||||
Sbjct  277   TGTGGGCTTGAAGAAAAAGAAAAAGAAGTTAGTAGAANTGGACCCCCNNCTTGTCGAAGC  336

Query  2832  CGGTGATGGCGAAGATACCCTGGACGATCAGGTGGGCGAGGATGAACAAGGAGAGGGCAT  2891
              |||||||||||||||||| |||||||||| ||||||||||||||||||||||||||||||||
Sbjct  337   CGGTGATGGCGAAGATACNCTGGACGATCNGGTGGGCGAGGATGAACAAGGAGAGGGCAT  396

Query  2892  TGTGCTGGGCGGTGCCACCCAGTATCCGTGGGAAGGAACCGATCGTGATTACAAATATGA  2951
              |||||||||||||||| |||||||||||||||||||||||||||||||||| |||||||
Sbjct  397   TGTGCTGGGCGGTGCCNCCCAGTATCCGTGGGAAGGAACCGATCGTGATTACNAATATGA  456

Query  2952  TGAGCTGTTGGGCCGTGTGTTCAATATTCTGCGCGAGAATAATCCAGACCTGGCGGGAGA  3011
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  457   TGAGCTGTTGGGCCGTGTGTTCAATATTCTGCGCGAGAATAATCCAGACCTGGCGGGAGA  516

Query  3012  CCGTCGACGTACCGTGATGCGTCCACCGCAGGTCTTGCGTGAAGGCACCaaaaaaaCCGT  3071
              |||||  |||||||||||||||||  |||||||||||||||||||||||||  ||||||||||
Sbjct  517   CCGTCNACGTACCGTGATGCGTCCNCCGCAGGTCTTGCGTGAAGGCACCNAAAAAACCGT  576

Query  3072  GTTTGTGAATTTCATGGATCTGTGCAAAACCATGCATCGTCAGCCGGAACATGTGATGAT  3131
              |||||||||||||||||||||||||||||||||||  |||||  ||||||||||||||||||
Sbjct  577   GTTTGTGAATTTCATGGATCTGTGCAAAACCATGCNTCGTCNGCCGGAACATGTGATGAT  636

Query  3132  GTTTCTGCTTGCGGAAATGGGCACCAGCGGCAGCCTGGATGGGCAGCAGCGTCTGGTCAT  3191
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            |||||||||||||||||||||||||||||| |||||||||||||||||| ||||||| |
Sbjct  637  GTTTCTGCTTGCGGAAATGGGCACCAGCGGCNGCCTGGATGGGCAGCAGCNTCTGGTCNT  696

Query  3192 TAAAGGCCGCTTCGCTCCCAAGAATTTTGAAGCGATCCTGCGGCGTTACATCAATGAATA  3251
            |||||||| |||| ||||| ||||||||||||||||||||| ||||||| || |||||||
Sbjct  697  TAAAGGCCNCTTCNCTCCCNAGAATTTTGAAGCGATCCTGCNGCGTTACNTCNATGAATA  756

Query  3252 TGTGATTTGCAATGGCTGCAAGAGCCCGGATAC-CATTCTGAGCAAAGAGAATCGTCTGT  3310
            |||||||||||||||||| |||||||||||| | |||||||| ||||||||| ||||| 
Sbjct  757  TGTGATTTGCAATGGCTGCNAGAGCCCGGATACNCNTTCTGAGCNAAGAGAATCNTCTGT  816

Query  3311 TTTTCCTGCGTTGCGAACAGTGCGGCAGTAGCCGTAGCGTGGCGCCGATTAAAGCCG-GG  3369
            |||||||||||||| |||||||||||||||||||||||||     ||||||| |  ||
Sbjct  817  TTTTCCTGCGTTGCNAACAGTGCGGCAGTAGCCGTAGCGTGGNNNNNNATTAAAGNCNNGG  876

Query  3370 TTCGTTGCCCAGGTAGGCCGTCGTAAGGCGGGCACCTAATA  3410
            |||||||||| |||||| | |  ||||| |||| |||||||
Sbjct  877  TTCGTTGCCCNGGTAGGNCNTNNTAAGGNGGGCNCCTAATA  917
```

Sequence 15 Complete eIF2βαγ in BioBrick (VR)

```
Query  1883 CTTTACCAGCCCACCGAATATGATTGTTATTCGAAGCTTCGATGTGAATAAACCGGGCAG  1942
            |||||||| ||||| ||||||||||||||| ||||||| || | ||||||||||||||| 
Sbjct  826  CTTTACCANCCCACNGAATATGATTGTTATTNGAAGCTTNGANGNGAATAAACCGGGCAG  767

Query  1943 TGAAGTGGATGAAATTCGTGGCGGCGTGGCTGGGGGTTCCATTTTACGTGGCGTGTTGCG  2002
            ||||| ||| |||||| | ||||| | |||||||||||||||||||||||||||||||| |
Sbjct  766  TGAAGNGGANGAAATTNGNGGCGGNGNGGCTGGGGGTTCCATTTTACGTGGCGTGTTGNG  707

Query  2003 GGTGAACCAGAACATTGAAGTGCGTCCGGGCATTGTGATGAAAGACGAATCGGGGAACAT  2062
            |||||||||||||| |||| ||||||||||||||||||||||||||||||||||||||||
Sbjct  706  GGTGAACCAGAACATNGAAGNGCGTCCGGGCATTGTGATGAAAGACGAATCGGGGAACAT  647

Query  2063 AAAGTGCACGCCCATTTATAGCCGTATTGTGAGCTTATATGCGGAACAAAATGAGCTGCA  2122
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  646  AAAGTGCACGCCCATTTATAGCCGTATTGTGAGCTTATATGCGGAACAAAATGAGCTGCA  587

Query  2123 ATTCGCGGTGCCTGGGGGCCTGATTGGGGTGGGCACGACTATGGACCCGACCCTGACTCG  2182
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  586  ATTCGCGGTGCCTGGGGGCCTGATTGGGGTGGGCACGACTATGGACCCGACCCTGACTCG  527

Query  2183 TGCGGATCGTCTGGTGGGCCAAGTGCTGGGAGAAATT-GGTAGCCTGCCGGATGTCTTTG  2241
            |||||||||||||||||||||||||||||||||||| |||||||||||   | ||||||
Sbjct  526  TGCGGATCGTCTGGTGGGCCAAGTGCTGGGAGAAATTNGGTAGCCTGCNNNANGTCTTTN  467

Query  2242 TTGAATTAGAGATTAACTTTTTTCAGCTGCGTCGTCTGCTGGGTGTTCGGACCAAAGGGA  2301
            |||||  |||||||||||||||  |   ||||||    |   |||| |||||||| |
Sbjct  466  NNNAATTANNGATTAACTTTTTTCANNTNNNTCGTCTNNNNGNNNTTCGNACCAAAGGNA  407

Query  2302 CCGAGAAAGCGGGGAAAGTGAGCAAATTGACCAAGGGGGAAATTCTGATGTTAAACATCG  2361
            || ||||||||| ||||    | |||||| |||||||||||||| | |  ||||||||||
Sbjct  406  CCNAGAAAGCGGNGAAANNNANCAAATTNACCAAGGGGGAAATTNTNANNTTAAACATCG  347

Query  2362 GAAGCATGAGCACCGGAGCGCGTGTGGTTGCGGTCAAAAATGACCTGGCGAAGTTACAGT  2421
            |||||  ||||||  ||||||  |||||||||||||||| |||||  ||||||||||
Sbjct  346  NAAGCANNAGCACCNNAGCGCGNNTGGTTGCGGTCAAAAATNACCTGNNGAAGTTACAGT  287

Query  2422 TAACAGCCCCAGTATGTACTTCGAAAGGCGAGAAGGTAGCGCTGTCCCGTCGTGTGGAAA  2481
            |||||||||||||||||||||||||| |||||||||||||||||||||||||||||||||
Sbjct  286  TAACAGCCCCAGTATGTACTTCGAAAGNCGAGAAGGTAGCGCTGTCCCGTCGTGTGGAAA  227

Query  2482 AGCATTGGCGTCTGATCGGTTGGGGCCAGATTCAAGCTGGAGCTACCCTGGAAGTGCCGC  2541
            |||||||||||||| |||| |||||||||||||||||||||||||||||||||||||||||
Sbjct  226  AGCATTGGCGTCTNATCGNTTGGGGCCAGATTCAAGCTGGAGCTACCCTGGAAGTGCCGC  167

Query  2542 CGTGCCCGCTGGAAT  2556
            ||| ||||||| |||
Sbjct  166  CGTNCCCGCTGTAAT  152
```

```
Query  3406  TAATAAGGATCCTACTAGTAGCGGCCGCTGCAG  3438
             ||||||||||||||||||||||||||||||||
Sbjct  155   TAATAAGGATCCTACTAGTAGCGGCCGCTGCAG  123
```

Sequence 16: Complete eIF2βαγ in pet22bb (T7pro)

```
Query  2592  CCATATGGCGGACGAAGAACAGATGGAACGTAAGGAAGAAGCGACCGAGATTGCGCCGTT  2651
             ||||||||| ||||||||||||||||||||||||||||||        ||||||||||||
Sbjct  35    CCATATGGNGGACGAAGAACAGATGGAACGTAAGGAAGAANNNNNNNNNGATTGCGCCGTT  94

Query  2652  TGACCCGACTaagaagaagaagaagaagaaagTGGTGATTCAGGACCCAGCGGATGAAGT  2711
             |||||||||||||||||||||||| ||||||||||||||||||||||||||||||||||
Sbjct  95    TGACCCGACTAAGAAGAAGAAGAANAAGAAAGTGGTGATTCAGGACCCAGCGGATGAAGT  154

Query  2712  GGATAAATTGGCCGAAAAGACCGAAGGCCTGAGCGTGACCGAAAGCGGCGAGGCGAGCTT  2771
             ||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  155   GGATAAATTGGCCGATAAGACCGAAGGCCTGAGCGTGACCGAAAGCGGCGAGGCGAGCTT  214

Query  2772  TGTGGGCTTGAAGAAAAAGAAAAAGAAGTTAGTAGAACTGGACCCCAGCCTTGTCGAAGC  2831
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  215   TGTGGGCTTGAAGAAAAAGAAAAAGAAGTTAGTAGAACTGGACCCCAGCCTTGTCGAAGC  274

Query  2832  CGGTGATGGCGAAGATACCCTGGACGATCAGGTGGGCGAGGATGAACAAGGAGAGGGCAT  2891
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  275   CGGTGATGGCGAAGATACCCTGGACGATCAGGTGGGCGAGGATGAACAAGGAGAGGGCAT  334

Query  2892  TGTGCTGGGCGGTGCCACCCAGTATCCGTGGGAAGGAACCGATCGTGATTACAAATATGA  2951
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  335   TGTGCTGGGCGGTGCCACCCAGTATCCGTGGGAAGGAACCGATCGTGATTACAAATATGA  394

Query  2952  TGAGCTGTTGGGCCGTGTGTTCAATATTCTGCGCGAGAATAATCCAGACCTGGCGGGAGA  3011
             |||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||
Sbjct  395   TGAGCTGTTGGGCCGTGTGTTCAATATTCTGCGCGAGAATAATCCAAACCTGGCGGGAGA  454

Query  3012  CCGTCGACGTACCGTGATGCGTCCACCGCAGGTCTTGCGTGAAGGCACCaaaaaaaCCGT  3071
             ||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  455   CCGTCCACGTACCGTGATGCGTCCACCGCAGGTCTTGCGTGAAGGCACCAAAAAAACCGT  514

Query  3072  GTTTGTGAATTTCATGGATCTGTGCAAAACCATGCATCGTCAGCCGGAACATGTGATGAT  3131
             |||||||||||||||||||||||| | || | ||  |   |  |  ||||||||||||||
Sbjct  515   GTTTGTGAATTTCATGGATCTGTGNANAANCNTGNNTNNNCNNCNNGAACATGTGATGAN  574

Query  3132  GTTTCTGCTTGCGGAAATGGG  3152
             |||||||  |||||||| |||
Sbjct  575   GTTTCTGNNTGCGGAAANGGG  595
```

Sequence 17: Complete eIF2βαγ in pet22bb (T7term)

```
Query  1883  CTTTACCAGCCCACCGAATATGATTGTTATTCGAAGCTTCGATGTGAATAAACCGGGCAG  1942
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  766   CTTTACCAGCCCACCGAATATGATTGTTATTCGAAGCTTCGATGTGAATAAACCGGGCAG  707

Query  1943  TGAAGTGGATGAAATTCGTGGCGGCGTGGCTGGGGGTTCCATTTTACGTGGCGTGTTGCG  2002
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  706   TGAAGTGGATGAAATTCGTGGCGGCGTGGCTGGGGGTTCCATTTTACGTGGCGTGTTGCG  647

Query  2003  GGTGAACCAGAACATTGAAGTGCGTCCGGGCATTGTGATGAAAGACGAATCGGGGAACAT  2062
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  646   GGTGAACCAGAACATTGAAGTGCGTCCGGGCATTGTGATGAAAGACGAATCGGGGAACAT  587

Query  2063  AAAGTGCACGCCCATTTATAGCCGTATTGTGAGCTTATATGCGGAACAAAATGAGCTGCA  2122
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  586   AAAGTGCACGCCCATTTATAGCCGTATTGTGAGCTTATATGCGGAACAAAATGAGCTGCA  527

Query  2123  ATTCGCGGTGCCTGGGGGCCTGATTGGGGTGGGCACGACTATGGACCCGACCCTGACTCG  2182
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  526   ATTCGCGGTGCCTGGGGGCCTGATTGGGGTGGGCACGACTATGGACCCGACCCTGACTCG  467
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Query  2183  TGCGGATCGTCTGGTGGGCCAAGTGCTGGGAGAAATTGGTAGCCTGCCGGATGTCTTTGT  2242
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  466   TGCGGATCGTCTGGTGGGCCAAGTGCTGGGAGAAATTGGTAGCCTGCCGGATGTCTTTGT  407

Query  2243  TGAATTAGAGATTAACTTTTTTCAGCTGCGTCGTCTGCTGGGTGTTCGGACCAAAGGGAC  2302
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  406   TGAATTAGAGATTAACTTTTTTCAGCTGCGTCGTCTGCTGGGTGTTCGGACCAAAGGGAC  347

Query  2303  CGAGAAAGCGGGGAAAGTGAGCAAATTGACCAAGGGGGAAATTCTGATGTTAAACATCGG  2362
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  346   CGAGAAAGCGGGGAAAGTGAGCAAATTGACCAAGGGGGAAATTCTGATGTTAAACATCGG  287

Query  2363  AAGCATGAGCACCGGAGCGCGTGTGGTTGCGGTCAAAAATGACCTGGCGAAGTTACAGTT  2422
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  286   AAGCATGAGCACCGGAGCGCGTGTGGTTGCGGTCAAAAATGACCTGGCGAAGTTACAGTT  227

Query  2423  AACAGCCCCAGTATGTACTTCGAAAGGCGAGAAGGTAGCGCTGTCCCGTCGTGTGGAAAA  2482
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  226   AACAGCCCCAGTATGTACTTCGAAAGGCGAGAAGGTAGCGCTGTCCCGTCGTGTGGAAAA  167

Query  2483  GCATTGGCGTCTGATCGGTTGGGGCCAGATTCAAGCTGGAGCTACCCTGGAAGTGCCGCC  2542
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  166   GCATTGGCGTCTGATCGGTTGGGGCCAGATTCAAGCTGGAGCTACCCTGGAAGTGCCGCC  107

Query  2543  GTGCCCGCTGGAAT  2556
             |||||||||| |||
Sbjct  106   GTGCCCGCTGTAAT  93


Query  1439  TGCCCTGGTCATGATATTTTGATGGCGACCATGCTGAACGGAGCCGCTATTATGGATGGC  1498
              |||||| |||  |||  |||||||||| ||||||||| | |||||| |  |||||||| |
Sbjct  1102  TGCCCT-GTCNNGATNNTTTGATGGCGNCCATGCTGANC-GAGCCGNT-NTATGGATGNC  1046

Query  1499  GCCCTGTTGCTGATTGCGGCAAATGAATCTTGCCCTCAGCCGCAGACCAGCGAACATCTG  1558
              | || |||||||  ||  ||||| ||||| |     ||||||||||||||||||||||||
Sbjct  1045  G-CCNGTTGCTG-NTGNNGCAAANGAATC-T-NNNNCAGCCGCAGACCAGCGAACATCTG  990

Query  1559  GCTGCGGTGGAAATTATGCGTCTTCAGCACCTGATAATTCTTCAGAATAAGATTGATCTG  1618
              |||||||||||||| |||||| ||||||  ||||||||||||||||||||||||||||||
Sbjct  989   GCTGCGGTGGAAATNATGCGTNTTCAGC-NCTGATAATTCTTCAGAATAAGATTGATCTG  931

Query  1619  ATTCAGGAATCCGCAGCGATGAATCAACATGAGGCGATTCAAAAGTTCATTCAAGGCACC  1678
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  930   ATTCAGGAATCCGCAGCGATGAATCAACATGAGGCGATTCAAAAGTTCATTCAAGGCACC  871

Query  1679  ATAGCGGAGGGCGCTCCAGTGGTTCCGATTAGCGCGCAGTTGAAGTATAACATCGACGTA  1738
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  870   ATAGCGGAGGGCGCTCCAGTGGTTCCGATTAGCGCGCAGTTGAAGTATAACATCGACGTA  811

Query  1739  ATTTGCGAATACATCATAAAGAAGATTCCGATCCCTGAACGTAACTTTACCAGCCCACCG  1798
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  810   ATTTGCGAATACATCATAAAGAAGATTCCGATCCCTGAACGTAACTTTACCAGCCCACCG  751

Query  1799  AATATGATTGTTATTCGAAGCTTCGATGTGAATAAACCGGGCAGTGAAGTGGATGAAATT  1858
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  750   AATATGATTGTTATTCGAAGCTTCGATGTGAATAAACCGGGCAGTGAAGTGGATGAAATT  691

Query  1859  CGTGGCGGCGTGGCTGGGGGTTCCCTTT  1886
             |||||||||||||||||||||||| |||
Sbjct  690   CGTGGCGGCGTGGCTGGGGGTTCCATTT  663


Query  3406  TAATAAGGATCCTACTAGTAGCGGCCGCTGCAG  3438
             ||||||||||||||||||||||||||||||||
Sbjct  96    TAATAAGGATCCTACTAGTAGCGGCCGCTGCAG  64
```