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Appendix I – General Protocols

# Appendix I.A.i – Normal Plasmid Prep

Using **QIAGEN QIAprep Spin Miniprep Kit**

1. Pellet 1-5mL bacterial overnight culture by centrifuge at >8000rpm (6800xg) for 3 minutes at room temperature
2. Resuspend pelleted bacterial cells in 250uL Buffer P1 and transfer to a microcentrifuge tube
3. Add 250uL Buffer P2 and mix thoroughly by inverting the tube 6x until the solution becomes clear. Do not allow lysis to occur for longer than 5 minutes.
4. Add 350uL Buffer N3 and mix immediately and thoroughly by inverting 6x.
5. Place tube in microcentrifuge and spin at 13,000rpm for 10 min
6. Apply the supernatant from step 5 to QIAprep spin column by pipetting. Centrifuge 30-60 seconds and discard flow-through.
7. Wash spin column w/ 500uL Buffer PB, centrifuge 30-60 seconds, discard flow-through.
8. Wash spin column by adding 750uL Buffer PE, centrifuge 30-60s, discard flow-through.
9. Centrifuge 1 min to remove residual wash buffer.
10. Place spin column in a clean, labelled 1.5mL microcentrifuge tube. Add 50uL Buffer EB, let stand 1 min, centrifuge 1 min.
11. Use immediately or store in -20C.

# Appendix I.A.ii – Plasmid Prep for pACYC184

1. Pellet whole overnight culture volume (20-50mL) bacterial overnight culture by centrifuge at >8000rpm (6800xg) for 10 minutes at room temperature
2. While centrifuging, place Buffer EB in warm water bath
3. Resuspend pelleted bacterial cells in 250uL Buffer P1, leaving in large centrifuge tube.
4. Add 250uL Buffer P2 and mix thoroughly by inverting the tube 6x until the solution becomes clear. Do not allow lysis to occur for longer than 5 minutes.
5. Add 350uL Buffer N3 and mix immediately and thoroughly by inverting 6x.
6. Place tube in centrifuge and spin at 13,000rpm for 10 min
7. Pipette ~750mL of supernatant into QIAprep spin column. Centrifuge 30-60 seconds and discard flow-through.
8. Repeat Step 6 until all supernatant has been processed.
9. Wash spin column w/ 500uL Buffer PB, centrifuge 30-60 seconds, discard flow-through.
10. Wash spin column by adding 750uL Buffer PE, centrifuge 30-60s, discard flow-through.
11. Centrifuge 1 min to remove residual wash buffer.
12. Place spin column in a clean, labelled 1.5mL microcentrifuge tube. Add 30uL pre-warmed Buffer EB, let stand 3 min, centrifuge 1 min.
13. Use immediately or store in -20C.

**Notes:** This procedure may “over-load” the spin column. It fortunately gave a high plasmid yield in this experiment. Caution should be taken to extend it to other systems.

# Appendix I.A.iii – Gel Electrophoresis and Gel Extraction

**Solution Prep**:

5x Tris-borate (TBE) – 1L

54g Tris base

27.5g boric acid

3.722g EDTA (20mL 0.5M EDTA)

To volume H2O

**Gel Prep**:

1. Add 5mL 5x TBE buffer to 250mL Erlenmeyer flask (labeled with EtBr)
2. Add 45mL MilliQ-H2O
3. Add 0.5g routine-use agarose
4. Heat on hot plate, taking care to not boil over
   1. While it is heating:
5. Tape reservoir well with two layers of masking tape to form a “wall” on open sides of reservoir. Do not block comb insertion site. Start from bottom of reservoir and fold up to make base. Then add a double layer. Seal tightly
6. **Reservoir Prep**
7. Add 60uL EtBr or SYBR®Safe to center
8. Add 30mL 5x TBE buffer
9. Add 270mL MilliQ-H2O
10. Swirl to mix well without contacting solution
11. Select comb by number of wells and wideness desired
12. Once solution is boiling and turns clear, remove from heat
13. Allow to cool to touch, add 10uL of 5mg/mL EtBr (or SYBR® Safe)
14. Swirl contents and pour into taped reservoir well carefully to not entrap air bubbles
15. Insert comb straight and gently down
16. Allow to set until solidified
17. Remove tape and insert well into reservoir where wells are toward the negative end

**Sample Loading:**

1. Prepare samples with DNA dye in approximately a 1:4 sample:dye ratio.
2. Load samples with pipette taking care to not stab well, overflow well, or produce air bubbles.
3. Load appropriate ladder into a blank well

**Gel Operation:**

1. Set to “low range”
2. Determine voltage for how long/how specific run needs to be
   1. 120V for 1 hour sufficient for separation of very different sized bands, slower and lesser voltage for greater separation
3. Once separated, remove gel and observe with UV light

**Gel Extraction**: Using QIAGEN’s QIAquick Gel Extraction Kit

1. Excise DNA fragment from gel with sharp razor
2. Weigh gel slice in a colorless tube. If gel is above 400uL try to cut off unnecessary agarose to get it below this mass.
3. Add 3 volumes Buffer QG to 1 volume gel (100mg gel = 100uL)
4. Incubate at 50C until complete dissolution, vortexing to speed up process.
5. Add 1 volume isopropanol to sample and mix.
6. Place QIAquick spin column in a 2mL collection tube.
7. Pipette 750uL sample into column and centrifuge 1 minute. Discard flow-through.
8. Repeat Step 7 until all sample processed.
9. Add 500uL Buffer QG to clumn, centrifuge 1 min, discard flow-through, replace to column.
10. Wash with 750uL Buffer PE and centrifuge 1 min, discard flow-through, replace to column
11. Centrifuge column 1 min to rid any remaining wash buffer.
12. Place in clean 1.5mL centrifuge tube.
13. Add 50uL EB Buffer, wait up to 4 minutes, and centrifuge 1 min for eluted DNA.

# Appendix I.B – Polymerase Chain Reactions

For all PCRs, the same ratio of reagents were prepared in PCR tubes. The sub-sections then outline the PCR operational programs that were successful for each. VentR had a higher temperature and shorter length for denature than other polymerases used. The annealing temperature was found from NEB’s Tm calculator by the primers used.

**Reagents Prepared:**

|  |  |
| --- | --- |
| **Component** | **Volume** |
| 10x ThermoPol Reaction Buffer | 5uL |
| 10mM dNTPs | 1uL |
| 10uM Frwd Primer | 2.5uL |
| 10uM Rvse Primer | 2.5uL |
| DNA Template | 0.5uL |
| VentR Polymerase | 0.5uL |
| H2O | 38uL |

**PCR Operation Programs**

1. **Amplification of: GFP-LVA from iGEM (DNA Template)**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Step** | **Temp** | **Time** |
|  | Initial Denature | 95°C | 2-5 min |
| X 30 Cycles | Denature | 95°C | 15 sec |
| Anneal | 59°C | 30 sec |
| Elongation | 72°C | 30 sec |
|  | Final Elongation | 72°C | 5 min |
| Hold | 4°C | ∞ |

1. **Amplification of both GenAVERT’s 54bp and 168bp αs from pACYC184-GFP-LVA (DNA Template)**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Step** | **Temp** | **Time** |
|  | Initial Denature | 95°C | 2-5 min |
| X 30 Cycles | Denature | 95°C | 15 sec |
| Anneal | 52.5°C | 30 sec |
| Elongation | 72°C | 30 sec |
|  | Final Elongation | 72°C | 5 min |
| Hold | 4°C | ∞ |

**Note:** These Tm were much lower than what ApE predicted. Chose to listen to the NEB Tm predictor for use with these reagents.

1. **Amplification of SFold predicted αs pACYC184-GFP-LVA (DNA Template)**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Step** | **Temp** | **Time** |
|  | Initial Denature | 95°C | 2-5 min |
| X 30 Cycles | Denature | 95°C | 15 sec |
| Anneal | 55°C | 30 sec |
| Elongation | 72°C | 30 sec |
|  | Final Elongation | 72°C | 5 min |
| Hold | 4°C | ∞ |

**Note:** These Tm were much lower than what ApE predicted. Chose to listen to the NEB Tm predictor for use with these reagents.

1. **Amplification of pBAD18/Kan with pBAD18/Kan (DNA Template)**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Step** | **Temp** | **Time** |
|  | Initial Denature | 95°C | 2-5 min |
| X 30 Cycles | Denature | 95°C | 15 sec |
| Anneal | 56°C | 30 sec |
| Elongation | 72°C | 5.66 min |
|  | Final Elongation | 72°C | 8 min |
| Hold | 4°C | ∞ |

**Note:** The longer elongation time was due to the recommendation of elongation of 1min/kb. The length of the plasmid required this long interval. The final elongation was also increased to ensure the last round was complete before the reaction was ceased.

# Appendix I.C – Restriction Enzyme Digestions

**Notes**: Buffers, digestion time, and enzyme volumes were chosen by the enzyme pair’s compatibility. New England Biolabs’ Double Digestion Finder was used for recommendations <https://www.neb.com/tools-and-resources/interactive-tools/double-digest-finder>

1. Double Digest with **EcoRI** and **NcoI**

Purpose: To make sticky ends of GFP-LVA extracted from iGEM and of pACYC184

Protocol: To microcentrifuge tubes add:

|  |  |  |
| --- | --- | --- |
| DNA Template | pACYC184 | GFP-LVA PCR |
| Template Volume | 5uL | 20uL |
| NEB 3.1 Buffer | 5uL | 5uL |
| NcoI | 1uL | 1uL |
| EcoRI | 2uL | 2uL |
| H2O | 37uL | 22uL |

* Place in 37°C hot water bath for 5 minutes
* Quench with 10uL loading dye

1. Double Digest with **HindIII** and **EagI-HF**

Purpose: To replace Tetracycline gene on pACYC184 with GFP-LVA

Protocol: To PCR tubes add:

|  |  |  |
| --- | --- | --- |
| DNA Template | pACYC184 | GFP-LVA PCR |
| Template Volume | 5uL | 15uL |
| NEB 2.1 Buffer | 5uL | 5uL |
| HindIII | 1uL | 1uL |
| H2O | 33uL | 28uL |

* Place in thermocycler for 50min at 37°C
* Open, add 1uL EagI-HF, place back at 37°C for 10 more minutes
* Heat inactivate at 80°C for 20 minutes

1. Double Digest with **ScaI** and **NcoI**

Purpose: To insert antisense into modified pBAD18/Kan backbone

Protocol: To PCR tubes add:

|  |  |  |
| --- | --- | --- |
| DNA Template | pBAD18 PCR | Antisense PCR |
| Template Volume | 15uL | 15uL |
| NEB CutSmart Buffer | 5uL | 5uL |
| ScaI-HF | 1uL | 1uL |
| NcoI-HF | 1uL | 1uL |
| H2O | 28uL | 28uL |

* Place in thermocycler for 10min at 37°C
* Heat inactivate at 80°C for 20 minutes

# Appendix I.D – Plasmid Ligation

**Would NOT recommend 10 minute ligation, overnight is best**

Ratios matter – because each plasmid prep/PCR amplification may have different yields, these exact amounts may not work in the future, but the ones that worked are listed here:

In PCR tubes add:

|  |  |  |
| --- | --- | --- |
| Which Ligation: | 168bp, 54bp | SFold |
| PCR Insert | 6uL | 4uL |
| Plasmid Backbone | 6uL | 8uL |
| Ligation Buffer | 2uL | 2uL |
| Water | 6uL | 6uL |
| T4 Ligase | 1uL | 1uL |

Set thermocycler to 22°C and let run overnight. Set up overnight culture of cells to be transformed with ligation the next day

# Appendix I.E.i – Chemical Transformation

**Prep:**

* Prepare 100mM sterile CaCl2 (either from 1M stock or fresh)
* Antibiotic Selection LB Plates (25ug/mL Tetracycline, 25ug/mL Kanamycin,

30ug/mL Chloramphenicol)

* Ice bucket – place labelled microcentrifuge tubes (# of ligations + 1 for control) and CaCl2

solution on ice

* PBS
* SOC Media
* Shaker on at 37°C – prewarm SOC media
* Water bath on at 42°C

**Procedure:**

1. Start an overnight cell culture at 225rpm, 30°C
2. In the morning subculture 1:50 (1mL overnight culture:49mL fresh LB media) in a 250mL flask at 225rpm, 37°C
3. ~4 hours later spin cells down in 50mL centrifuge tubes at 20°C, 4000rpm for 10 min
4. Decant supernatant
5. Add ~10mL PBS and resuspend cells
6. Centrifuge again for 10 min at 20°C, 4000rpm
7. Decant supernatant
8. Add ~10mL COLD CaCl2 and resuspend
9. Add 50uL cells to each of the microcentrifuge tubes
10. Add 10uL ligation to respective tube, adding only the 50uL cells to the “C” control

\*\*Normally 1.5-2uL ligation. In troubleshooting, this volume was increased and was successful. Possibly not recommended for other systems.

1. Allow to sit on ice for 5 minutes

\*\*This allows ligation to adhere to cell surface

1. Place in hot water bath of 42°C for 45 seconds
2. Immediately move to ice for 2 minutes
3. Get prewarmed SOC ready, at 2 minutes add 1mL SOC to microcentrifuge tubes
4. Cap and immediately put into shaker at 37°C, 250rpm for 30-40min

\*\*Decreased from 1 hour to not lose plasmid

1. Place agar plates in hood to warm to room temperature and label “L1” “L2” “C” etc
2. Plate 500uL of each onto selection plates, spread thoroughly and allow to dry

\*\*For a readily transformed system this volume may be too much and produce a lawn than colonies. This wasn’t an issue here, but if a lawn was grown it would have to be spread to achieve a genetic isolate colony.

1. Place plates upside down in 37°C incubator overnight

# Appendix I.E.ii – Electroporation

**Material Prep:**

* Sterile 10% glycerol solution
* Prewarmed SOC Media
* Antibiotic selection plates (25ug/mL Tet or Kan, 30ug/mL Chloram)
* Ice bucket with electroporation and centrifuge tubes chilled

**Competent Cell Prep:**

* Grow cells overnight
* Subculture in the morning 1:100 volume (5mL O/N + 495 mL LB) at 300rpm, 37°C
* Grow until OD600 of 0.4
* Prepare 10% sterile glycerol solution and place on ice while wait
* At 0D600 of 0.4, pour into 10 chilled 50mL centrifuge tubes and sit on ice for 20 min
* Centrifuge 20min, 4°C, 4000rpm
* Keeping on ice, resuspend in ~8-9mL cold 10% glycerol solution
* Combine 5 samples together to have 2 tubes left
* Centrifuge 20 min, 4°C, 4000 rpm
* Resuspend in ~20mL glycerol and combine to 1 tube
* Centrifuge 20 min, 4°C, 4000 rpm
* Resuspend ~20mL
* Centrifuge 15 min, 4°C, 4000 rpm
* Resuspend in 750uL glycerol. Use or freeze immediately

**Electroporation:**

1. In cold microcentrifuge tubes, add 40uL competent cells with 2-5uL plasmid (ligation or control)
2. Incubate on ice 1 min
3. Transfer to cold 0.2cm electroporation cuvette
4. Program electroporator dependent on plasmid:
   1. If plasmid – no salt issues – set to “Ec3”
   2. If ligation – possible salt from ligation buffer, to minimize arcing – set to “Ec2”
5. Insert cuvette into electroporator and pulse once
6. Remove from chamber and immediately add 1mL prewarmed SOC and resuspend cells
7. Transfer to microfuge tube, incubate in shaker at 37°C, 225rpm, for 45 minutes
8. Spin down in microcentrifuge and discard ½ supernatant
9. Resuspend cells, pour on selection plates
10. Incubate plates upside down overnight

# Appendix I.F. – Fluorescence Measurement

**Note:** This is a 2-day process. The first day

**At 9:00 A.M.** inoculate a freezer stab into 10mL LB with the appropriate antibiotic selection:

1. DH5α
2. DH5α+ pACYC184-GFP-LVA
3. DH5α+ pACYC184-GFP-LVA + pBAD18/Kan-αs

Place in shaker at 300rpm, 37C for 8 hours

**At 7:00 P.M.** in 8 250mL flasks, subculture 2.5mL of the following into 47.5mL fresh media with appropriate antibiotics. Continue shaking at 300rpm, 37C

1. DH5α
2. DH5α+ pACYC184-GFP-LVA

3, 4, 5 DH5α+ pACYC184-GFP-LVA + pBAD18/Kan-αs (Labelled “I” = Induced)

6,7,8 DH5α+ pACYC184-GFP-LVA + pBAD18/Kan-αs (Labelled “U” = Uninduced)

**At 9:30 P.M. (2.5 hours later)** Add spectinomycin stock to all flasks for final concentration of 300ug/mL

**The next day:**

**Prep:**

1. Weigh out 0.5g arabinose into 6 boats
2. Get 8 cuvettes ready
3. Label 8 microcentrifuge tubes 1-8
4. Turn spectrophotometer on and run “Bastern” test
5. Blank with LB media
6. Turn Qubit on and plug USB in with Fluorimeter Raw Mode program (**Appendix V.B.**)

**At 7:00A.M.** **begin:**

1. Take 1mL samples of all flasks into lined cuvettes
2. Add 0.5g arabinose (to make 2%) to flasks 1-5 (**DO NOT ADD TO 6, 7, or 8)**
3. Measure and record 0D600 of each sample
4. Pipette 125uL of each sample into the labelled microcentrifuge tube for the flask
5. Spin tubes at 13000rpm on microcentrifuge for 2 min
6. Resuspend in 500uL PBS
7. Spin tubes at 13000rpm on microcentrifuge for 4.5 min
8. Discard supernatant
9. Resuspend in 500uL PBS
10. Pipette sample into Qubit tube
11. Measure on Qubit with Raw Mode program in **Appendix V.B.**
12. Record raw fluorescence for each flask and divide by OD600 to get normalized fluorescence in Excel at the “time” of sample extraction
13. Repeat steps (1,3-12) every 45 minutes until conclusion of experimental collection

**Appendix II –Sequences**

# Appendix II.A – Primers

The primers are listed by their desired purpose. Restriction enzyme cut sites are highlighted within the sequence and the corresponding enzyme is listed in the properties if applicable to the primer. The properties of melting temperature (Tm) and G/C content as determined by A Plasmid Editor (ApE) are also listed as they were success criteria in primer determination.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Purpose** | **Direction** | **Sequence** | **Properties** |
| i | **GFP-LVA Sequencing Primers** | Forward | ATGCGTAAAGGAGAAGAACTTTTC | 55°C Tm, 38% G/C |
| Reverse | AGCTACTAAAGCGTAGTTTTCG | 55°C Tm, 32% G/C |
| ii | **GFP-LVA Cloning Primers into Chloramphenicol** | Forward | GAGGAGGAATTCATGCGTAAAGGAGAAGAACTTTTC  **EcoRI** | 55°C Tm, 38% G/C |
| Reverse | GAGGAGCCATGGTTATTAAGCTACTAAAGCGTAGTTTTCG  **NcoI** | 55°C Tm, 32% G/C |
| iii | **GFP-LVA Cloning Primers to replace Tet** | Forward | GAGGAGAAGCTTTAATGCGGTAGTTTATCACAGTTAAATTGCT  AACGCAGTCAGGCACCGTGTATGCGTAAAGGAGAAGAACTTT  TCACTGG **HindIII** | 61°C Tm, 41% G/C |
| Reverse | GAGGAGCGGCCGCTCTAGTAGAGAGCGTTCACCGACAAAC  **EagI-HF** | 62°C Tm, 50% G/C |
| iv | **GFP-LVA Sequencing Primer in pACYC184** | Forward | CATGTTTGACAGCTTATCATCG | 53°C Tm, 41% G/C |
| Reverse | GCGAGAAGAATCATAATGGGG | 54°C Tm, 48% G/C |
| v | **54bp αs Cloning Primers** | Forward | GAGGAGCCATGGAAGTTAACTTTGATTCCATTCTTTTGTTTGTC  **NcoI** | 58°C Tm, 28% G/C |
| Reverse | GAGGAGAGTACTACACAATGTATACATCATGGCAGAC  **ScaI** | 57°C Tm, 40% G/C |
| vi | **168bp αs Cloning Primers** | Forward | GAGGAGCCATGGCTTCAATGTTGTGTCTAATTTTGAAGTTAA  CTTTGATTCC **NcoI** | 61°C Tm, 30% G/C |
| Reverse | GAGGAGAGTACTATACCCTTGTTAATAGAATCGAGTTAAAAG  GTATTG **ScaI** | 59°C Tm, 31% G/C |
| vii | **S-Fold αs Cloning Primers** | Forward | GAGGAGCCATGGGGGATCTTTCGAAAGGGCAGATTG  **NcoI** | 60°C Tm, 50% G/C |
| Reverse | GAGGAGAGTACTTACCAGACAACCATTACCTGTCCAC  **ScaI** | 60°C Tm, 48% G/C |
| viii | **pBAD18/Kan Cloning Primers** | Transcript  Start | GAGGAGCCATGGCAGTAGAGAGTTGCGATAAAAAGCGTC  **NcoI** | 59°C Tm, 44% G/C |
| Termin.  End | GAGGAGAGTACTTGCCTGGCGGCAGTAGC  **ScaI** | 61°C Tm, 71% G/C |

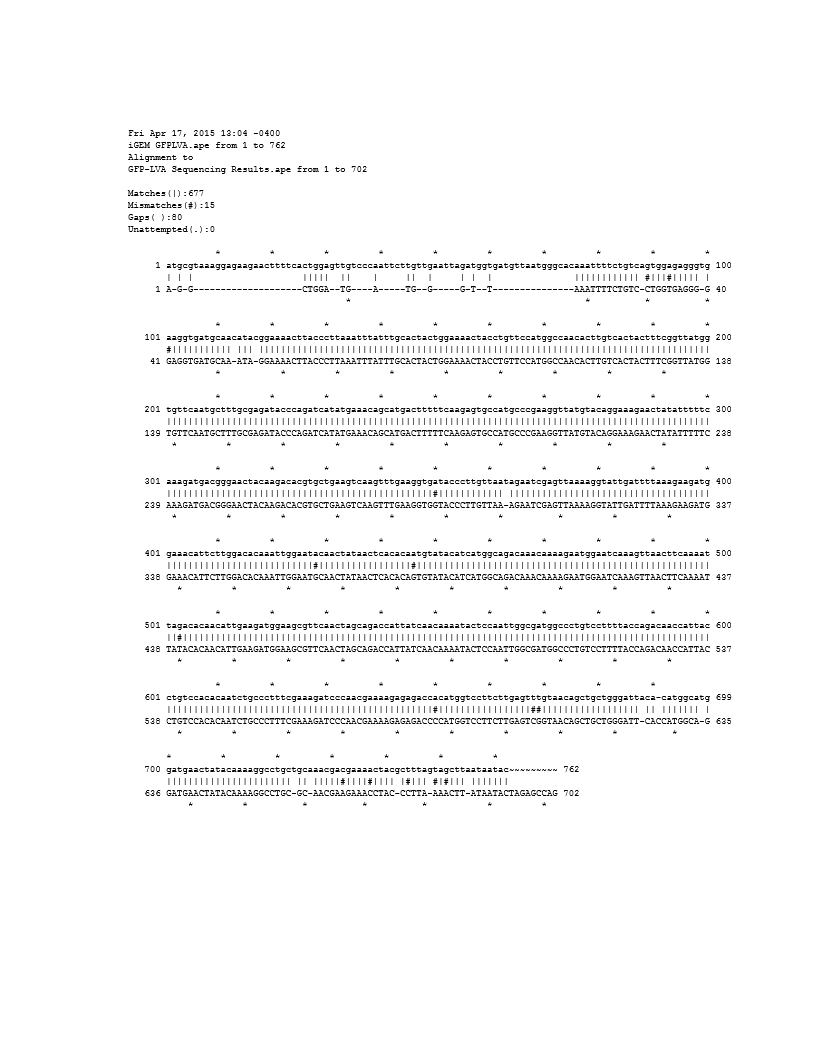
# Appendix II.B – Sequencing Results

Note: All Sequencing Center files and formats can be found at: <http://biotek.mcb.uconn.edu/dnaseq/Srivastava/Andrea/> They are summarized here as text with alignments in ApE to the template submitted.

**Appendix II.B.i – GFP-LVA Sequence Confirmation**

Forward Sequencing Nucleotides:AGGCTGGATGATGGGTTAAATTTTCTGTCCTGGTGAGGGGGAGGTGATGCAAATAGGAAAACTTACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTTCCATGGCCAACACTTGTCACTACTTTCGGTTATGGTGTTCAATGCTTTGCGAGATACCCAGATCATATGAAACAGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAAAGAACTATATTTTTCAAAGATGACGGGAACTACAAGACACGTGCTGAAGTCAAGTTTGAAGGTGGTACCCTTGTTAAAGAATCGAGTTAAAAGGTATTGATTTTAAAGAAGATGGAAACATTCTTGGACACAAATTGGAATGCAACTATAACTCACACAGTGTATACATCATGGCAGACAAACAAAAGAATGGAATCAAAGTTAACTTCAAAATTATACACAACATTGAAGATGGAAGCGTTCAACTAGCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCCACACAATCTGCCCTTTCGAAAGATCCCAACGAAAAGAGAGACCCCATGGTCCTTCTTGAGTCGGTAACAGCTGCTGGGATTCACCATGGCAGGATGAACTATACAAAAGGCCTGCGCAACGAAGAAACCTACCCTTAAAACTTATAATACTAGAGCCAG

Alignment to iGEM Sequence:



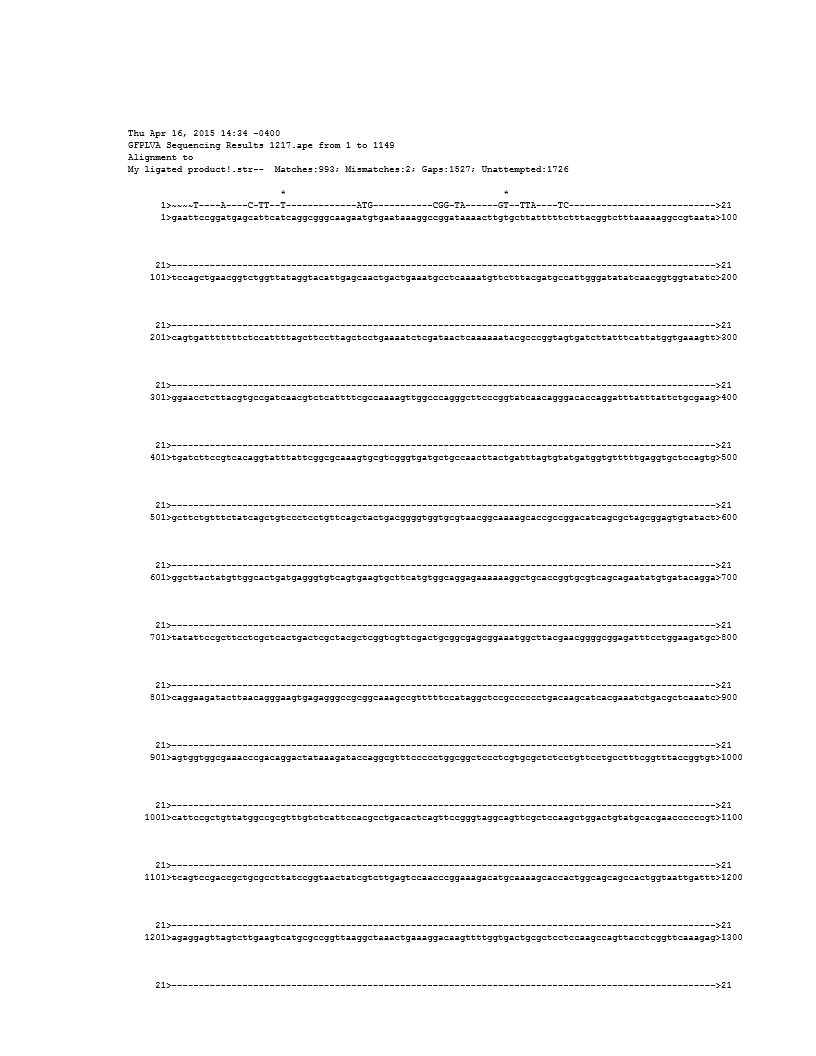
**Summary:** 677 (|) Matches, 15 (#) Mismatches, 80 ( ) Gaps, 0 (.) Unattempted

**Appendix II.B.ii – GFP-LVA inserted into pACYC184 Confirmation**

Forward Sequencing Nucleotides

TACTTTATGCGGTAGTTTATCCAGTTAAATTGCTAACGCAGTCAGGCACCGTGTATGCGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACATACGGAAAACTTACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTTCCATGGCCAACACTTGTCACTACTTTCGGTTATGGTGTTCAATGCTTTGCGAGATACCCAGATCATATGAAACAGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAAAGAACTATATTTTTCAAAGATGACGGGAACTACAAGACACGTGCTGAAGTCAAGTTTGAAGGTGGTACCCTTGTTAATAGAATCGAGTTAAAAGGTATTGATTTTAAAGAAGATGGAAACATTCTTGGACACAAATTGGAATACAACTATAACTCACACAATGTATACATCATGGCAGACAAACAAAAGAATGGAATCAAAGTTAACTTCAAAATTAGACACAACATTGAAGATGGAAGCGTTCAACTAGCAGACCATTATCAACAAAATAGTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCCACACAATCTGCCCTTTCGAAAGATCCCAACGAAAAGAGAGACCACATGGTCCTTCTTGAGTTTGTAACAGCTGCTGGGATTACACATGGCATGGATGAACTATACAAAAGGCCTGCTGCAAACGACGAAAACTACGCTTTAGTAGCTTAATAATACTAGAGCCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCTACTAGAGCGGCCGACGCGCTGGGCTACGTCTTGCTGGCGTTCGCGACGCGAGGCTGGATGGCCTTCCCCATTATGATTCTTCTCGCTTCCCGGCGGCATCCGGGATGCCCCGCGTTTGCAGGGCCATGCTGTCCAGGCCAGGGTTAGGATGAACGAACCCATCAGGGGACCAGCTTCCAAAGGAATCCGCTCGGCGCCTCTTAACCAGCCCCTAAACCTTTTCGTATTCCATTGTGGCGACACACG

Alignment to Expected Cloned pACYC184-GFP-LVA Sequence:



**Summary:** 993 Matches, 2 Mismatches, 1527 Gaps, 1726 Unattempted

**Appendix III – Antisense Prediction Results**

# Appendix III.A. – GenAVERT

**GFP-LVA Input:** guuuaucacaguuaaauugcuaacgcagucaggcaccguguaugcguaaaggagaagaacuuuucacuggaguugucccaauucuuguugaauuagauggugauguuaaugggcacaaauuuucugucaguggagagggugaaggugaugcaacauacggaaaacuuacccuuaaauuuauuugcacuacuggaaaacuaccuguuccauggccaacacuugucacuacuuucgguuaugguguucaaugcuuugcgagauacccagaucauaugaaacagcaugacuuuuucaagagugccaugcccgaagguuauguacaggaaagaacuauauuuuucaaagaugacgggaacuacaagacacgugcugaagucaaguuugaaggugauacccuuguuaauagaaucgaguuaaaagguauugauuuuaaagaagauggaaacauucuuggacacaaauuggaauacaacuauaacucacacaauguauacaucauggcagacaaacaaaagaauggaaucaaaguuaacuucaaaauuagacacaacauugaagauggaagcguucaacuagcagaccauuaucaacaaaauacuccaauuggcgauggcccuguccuuuuaccagacaaccauuaccuguccacacaaucugcccuuucgaaagaucccaacgaaaagagagaccacaugguccuucuugaguuuguaacagcugcugggauuacacauggcauggaugaacuauacaaaaggccugcugcaaacgacgaaaacuacgcuuuaguagcuuaauaauacuagagccaggcaucaaauaaaacgaaaggcucagucgaaagacugggccuuucguuuuaucuguuguuugucggugaacgcucucuacuagag

**GenAVERT Output:**

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

5'-3' VOLATILE REGION, WITH RANK:

1

ACAAUGUAUACAU

REGION BEGINS AT BASE:

484

REGION LENGTH:

13

3'-5' ANTISENSE

UGUUACAUAUGUA

5'-3' ANTISENSE

AUGUAUACAUUGU

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5'-3' VOLATILE REGION, WITH RANK:

2

ACACAAUGUAUACAUCAUGGCAGACAAACAAAAGAAUGGAAUCAAAGUUAACUU

REGION BEGINS AT BASE:

482

REGION LENGTH:

54

3'-5' ANTISENSE

UGUGUUACAUAUGUAGUACCGUCUGUUUGUUUUCUUACCUUAGUUUCAAUUGAA

5'-3' ANTISENSE

AAGUUAACUUUGAUUCCAUUCUUUUGUUUGUCUGCCAUGAUGUAUACAUUGUGU

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5'-3' VOLATILE REGION, WITH RANK:

3

AUACCCUUGUUAAUAGAAUCGAGUUAAAAGGUAUUGAUUUUAAAGAAGAUGGAAACAUUCUUGGACACAAAUUGGAAUACAACUAUAACUCACACAAUGUAUACAUCAUGGCAGACAAACAAAAGAAUGGAAUCAAAGUUAACUUCAAAAUUAGACACAACAUUGAAG

REGION BEGINS AT BASE:

391

REGION LENGTH:

168

3'-5' ANTISENSE

UAUGGGAACAAUUAUCUUAGCUCAAUUUUCCAUAACUAAAAUUUCUUCUACCUUUGUAAGAACCUGUGUUUAACCUUAUGUUGAUAUUGAGUGUGUUACAUAUGUAGUACCGUCUGUUUGUUUUCUUACCUUAGUUUCAAUUGAAGUUUUAAUCUGUGUUGUAACUUC

5'-3' ANTISENSE

CUUCAAUGUUGUGUCUAAUUUUGAAGUUAACUUUGAUUCCAUUCUUUUGUUUGUCUGCCAUGAUGUAUACAUUGUGUGAGUUAUAGUUGUAUUCCAAUUUGUGUCCAAGAAUGUUUCCAUCUUCUUUAAAAUCAAUACCUUUUAACUCGAUUCUAUUAACAAGGGUAU

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5'-3' VOLATILE REGION, WITH RANK:

4

UGAAGUCAAGUUUGAAGGUGAUACCCUUGUUAAUAGAAUCGAGUUAAAAGGUAUUGAUUUUAAAGAAGAUGGAAACAUUCUUGGACACAAAUUGGAAUACAACUAUAACUCACACAAUGUAUACAUCAUGGCAGACAAACAAAAGAAUGGAAUCAAAGUUAACUUCAAAAUUAGACACAACAUUGAAGAUGGAAGCGUUCAACUAGCAGACCAUUAUCAACAAAAUACUCCAAUUGGCGAUGGCCCUGUCCUUUUACCAGACAACCAUUACCUGUCCACACAAUCUGCCCUUUCGAAAGAUC

REGION BEGINS AT BASE:

371

REGION LENGTH:

302

3'-5' ANTISENSE

ACUUCAGUUCAAACUUCCACUAUGGGAACAAUUAUCUUAGCUCAAUUUUCCAUAACUAAAAUUUCUUCUACCUUUGUAAGAACCUGUGUUUAACCUUAUGUUGAUAUUGAGUGUGUUACAUAUGUAGUACCGUCUGUUUGUUUUCUUACCUUAGUUUCAAUUGAAGUUUUAAUCUGUGUUGUAACUUCUACCUUCGCAAGUUGAUCGUCUGGUAAUAGUUGUUUUAUGAGGUUAACCGCUACCGGGACAGGAAAAUGGUCUGUUGGUAAUGGACAGGUGUGUUAGACGGGAAAGCUUUCUAG

5'-3' ANTISENSE

GAUCUUUCGAAAGGGCAGAUUGUGUGGACAGGUAAUGGUUGUCUGGUAAAAGGACAGGGCCAUCGCCAAUUGGAGUAUUUUGUUGAUAAUGGUCUGCUAGUUGAACGCUUCCAUCUUCAAUGUUGUGUCUAAUUUUGAAGUUAACUUUGAUUCCAUUCUUUUGUUUGUCUGCCAUGAUGUAUACAUUGUGUGAGUUAUAGUUGUAUUCCAAUUUGUGUCCAAGAAUGUUUCCAUCUUCUUUAAAAUCAAUACCUUUUAACUCGAUUCUAUUAACAAGGGUAUCACCUUCAAACUUGACUUCA

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5'-3' VOLATILE REGION, WITH RANK:

5

CGUGCUGAAGUCAAGUUUGAAGGUGAUACCCUUGUUAAUAGAAUCGAGUUAAAAGGUAUUGAUUUUAAAGAAGAUGGAAACAUUCUUGGACACAAAUUGGAAUACAACUAUAACUCACACAAUGUAUACAUCAUGGCAGACAAACAAAAGAAUGGAAUCAAAGUUAACUUCAAAAUUAGACACAACAUUGAAGAUGGAAGCGUUCAACUAGCAGACCAUUAUCAACAAAAUACUCCAAUUGGCGAUGGCCCUGUCCUUUUACCAGACAACCAUUACCUGUCCACACAAUCUGCCCUUUCGAAAGAUC

REGION BEGINS AT BASE:

366

REGION LENGTH:

307

3'-5' ANTISENSE

GCACGACUUCAGUUCAAACUUCCACUAUGGGAACAAUUAUCUUAGCUCAAUUUUCCAUAACUAAAAUUUCUUCUACCUUUGUAAGAACCUGUGUUUAACCUUAUGUUGAUAUUGAGUGUGUUACAUAUGUAGUACCGUCUGUUUGUUUUCUUACCUUAGUUUCAAUUGAAGUUUUAAUCUGUGUUGUAACUUCUACCUUCGCAAGUUGAUCGUCUGGUAAUAGUUGUUUUAUGAGGUUAACCGCUACCGGGACAGGAAAAUGGUCUGUUGGUAAUGGACAGGUGUGUUAGACGGGAAAGCUUUCUAG

5'-3' ANTISENSE

GAUCUUUCGAAAGGGCAGAUUGUGUGGACAGGUAAUGGUUGUCUGGUAAAAGGACAGGGCCAUCGCCAAUUGGAGUAUUUUGUUGAUAAUGGUCUGCUAGUUGAACGCUUCCAUCUUCAAUGUUGUGUCUAAUUUUGAAGUUAACUUUGAUUCCAUUCUUUUGUUUGUCUGCCAUGAUGUAUACAUUGUGUGAGUUAUAGUUGUAUUCCAAUUUGUGUCCAAGAAUGUUUCCAUCUUCUUUAAAAUCAAUACCUUUUAACUCGAUUCUAUUAACAAGGGUAUCACCUUCAAACUUGACUUCAGCACG

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5'-3' VOLATILE REGION, WITH RANK:

6

GACGGGAACUACAAGACACGUGCUGAAGUCAAGUUUGAAGGUGAUACCCUUGUUAAUAGAAUCGAGUUAAAAGGUAUUGAUUUUAAAGAAGAUGGAAACAUUCUUGGACACAAAUUGGAAUACAACUAUAACUCACACAAUGUAUACAUCAUGGCAGACAAACAAAAGAAUGGAAUCAAAGUUAACUUCAAAAUUAGACACAACAUUGAAGAUGGAAGCGUUCAACUAGCAGACCAUUAUCAACAAAAUACUCCAAUUGGCGAUGGCCCUGUCCUUUUACCAGACAACCAUUACCUGUCCACACAAUCUGCCCUUUCGAAAGAUC

REGION BEGINS AT BASE:

348

REGION LENGTH:

325

3'-5' ANTISENSE

CUGCCCUUGAUGUUCUGUGCACGACUUCAGUUCAAACUUCCACUAUGGGAACAAUUAUCUUAGCUCAAUUUUCCAUAACUAAAAUUUCUUCUACCUUUGUAAGAACCUGUGUUUAACCUUAUGUUGAUAUUGAGUGUGUUACAUAUGUAGUACCGUCUGUUUGUUUUCUUACCUUAGUUUCAAUUGAAGUUUUAAUCUGUGUUGUAACUUCUACCUUCGCAAGUUGAUCGUCUGGUAAUAGUUGUUUUAUGAGGUUAACCGCUACCGGGACAGGAAAAUGGUCUGUUGGUAAUGGACAGGUGUGUUAGACGGGAAAGCUUUCUAG

5'-3' ANTISENSE

GAUCUUUCGAAAGGGCAGAUUGUGUGGACAGGUAAUGGUUGUCUGGUAAAAGGACAGGGCCAUCGCCAAUUGGAGUAUUUUGUUGAUAAUGGUCUGCUAGUUGAACGCUUCCAUCUUCAAUGUUGUGUCUAAUUUUGAAGUUAACUUUGAUUCCAUUCUUUUGUUUGUCUGCCAUGAUGUAUACAUUGUGUGAGUUAUAGUUGUAUUCCAAUUUGUGUCCAAGAAUGUUUCCAUCUUCUUUAAAAUCAAUACCUUUUAACUCGAUUCUAUUAACAAGGGUAUCACCUUCAAACUUGACUUCAGCACGUGUCUUGUAGUUCCCGUC

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5'-3' VOLATILE REGION, WITH RANK:

7

UUUCAAAGAUGACGGGAACUACAAGACACGUGCUGAAGUCAAGUUUGAAGGUGAUACCCUUGUUAAUAGAAUCGAGUUAAAAGGUAUUGAUUUUAAAGAAGAUGGAAACAUUCUUGGACACAAAUUGGAAUACAACUAUAACUCACACAAUGUAUACAUCAUGGCAGACAAACAAAAGAAUGGAAUCAAAGUUAACUUCAAAAUUAGACACAACAUUGAAGAUGGAAGCGUUCAACUAGCAGACCAUUAUCAACAAAAUACUCCAAUUGGCGAUGGCCCUGUCCUUUUACCAGACAACCAUUACCUGUCCACACAAUCUGCCCUUUCGAAAGAUC

REGION BEGINS AT BASE:

338

REGION LENGTH:

335

3'-5' ANTISENSE

AAAGUUUCUACUGCCCUUGAUGUUCUGUGCACGACUUCAGUUCAAACUUCCACUAUGGGAACAAUUAUCUUAGCUCAAUUUUCCAUAACUAAAAUUUCUUCUACCUUUGUAAGAACCUGUGUUUAACCUUAUGUUGAUAUUGAGUGUGUUACAUAUGUAGUACCGUCUGUUUGUUUUCUUACCUUAGUUUCAAUUGAAGUUUUAAUCUGUGUUGUAACUUCUACCUUCGCAAGUUGAUCGUCUGGUAAUAGUUGUUUUAUGAGGUUAACCGCUACCGGGACAGGAAAAUGGUCUGUUGGUAAUGGACAGGUGUGUUAGACGGGAAAGCUUUCUAG

5'-3' ANTISENSE

GAUCUUUCGAAAGGGCAGAUUGUGUGGACAGGUAAUGGUUGUCUGGUAAAAGGACAGGGCCAUCGCCAAUUGGAGUAUUUUGUUGAUAAUGGUCUGCUAGUUGAACGCUUCCAUCUUCAAUGUUGUGUCUAAUUUUGAAGUUAACUUUGAUUCCAUUCUUUUGUUUGUCUGCCAUGAUGUAUACAUUGUGUGAGUUAUAGUUGUAUUCCAAUUUGUGUCCAAGAAUGUUUCCAUCUUCUUUAAAAUCAAUACCUUUUAACUCGAUUCUAUUAACAAGGGUAUCACCUUCAAACUUGACUUCAGCACGUGUCUUGUAGUUCCCGUCAUCUUUGAAA

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5'-3' VOLATILE REGION, WITH RANK:

8

AGAACUAUAUUUUUCAAAGAUGACGGGAACUACAAGACACGUGCUGAAGUCAAGUUUGAAGGUGAUACCCUUGUUAAUAGAAUCGAGUUAAAAGGUAUUGAUUUUAAAGAAGAUGGAAACAUUCUUGGACACAAAUUGGAAUACAACUAUAACUCACACAAUGUAUACAUCAUGGCAGACAAACAAAAGAAUGGAAUCAAAGUUAACUUCAAAAUUAGACACAACAUUGAAGAUGGAAGCGUUCAACUAGCAGACCAUUAUCAACAAAAUACUCCAAUUGGCGAUGGCCCUGUCCUUUUACCAGACAACCAUUACCUGUCCACACAAUCUGCCCUUUCGAAAGAUC

REGION BEGINS AT BASE:

327

REGION LENGTH:

346

3'-5' ANTISENSE

UCUUGAUAUAAAAAGUUUCUACUGCCCUUGAUGUUCUGUGCACGACUUCAGUUCAAACUUCCACUAUGGGAACAAUUAUCUUAGCUCAAUUUUCCAUAACUAAAAUUUCUUCUACCUUUGUAAGAACCUGUGUUUAACCUUAUGUUGAUAUUGAGUGUGUUACAUAUGUAGUACCGUCUGUUUGUUUUCUUACCUUAGUUUCAAUUGAAGUUUUAAUCUGUGUUGUAACUUCUACCUUCGCAAGUUGAUCGUCUGGUAAUAGUUGUUUUAUGAGGUUAACCGCUACCGGGACAGGAAAAUGGUCUGUUGGUAAUGGACAGGUGUGUUAGACGGGAAAGCUUUCUAG

5'-3' ANTISENSE

GAUCUUUCGAAAGGGCAGAUUGUGUGGACAGGUAAUGGUUGUCUGGUAAAAGGACAGGGCCAUCGCCAAUUGGAGUAUUUUGUUGAUAAUGGUCUGCUAGUUGAACGCUUCCAUCUUCAAUGUUGUGUCUAAUUUUGAAGUUAACUUUGAUUCCAUUCUUUUGUUUGUCUGCCAUGAUGUAUACAUUGUGUGAGUUAUAGUUGUAUUCCAAUUUGUGUCCAAGAAUGUUUCCAUCUUCUUUAAAAUCAAUACCUUUUAACUCGAUUCUAUUAACAAGGGUAUCACCUUCAAACUUGACUUCAGCACGUGUCUUGUAGUUCCCGUCAUCUUUGAAAAAUAUAGUUCU

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5'-3' VOLATILE REGION, WITH RANK:

9

AAGAACUAUAUUUUUCAAAGAUGACGGGAACUACAAGACACGUGCUGAAGUCAAGUUUGAAGGUGAUACCCUUGUUAAUAGAAUCGAGUUAAAAGGUAUUGAUUUUAAAGAAGAUGGAAACAUUCUUGGACACAAAUUGGAAUACAACUAUAACUCACACAAUGUAUACAUCAUGGCAGACAAACAAAAGAAUGGAAUCAAAGUUAACUUCAAAAUUAGACACAACAUUGAAGAUGGAAGCGUUCAACUAGCAGACCAUUAUCAACAAAAUACUCCAAUUGGCGAUGGCCCUGUCCUUUUACCAGACAACCAUUACCUGUCCACACAAUCUGCCCUUUCGAAAGAUC

REGION BEGINS AT BASE:

326

REGION LENGTH:

347

3'-5' ANTISENSE

UUCUUGAUAUAAAAAGUUUCUACUGCCCUUGAUGUUCUGUGCACGACUUCAGUUCAAACUUCCACUAUGGGAACAAUUAUCUUAGCUCAAUUUUCCAUAACUAAAAUUUCUUCUACCUUUGUAAGAACCUGUGUUUAACCUUAUGUUGAUAUUGAGUGUGUUACAUAUGUAGUACCGUCUGUUUGUUUUCUUACCUUAGUUUCAAUUGAAGUUUUAAUCUGUGUUGUAACUUCUACCUUCGCAAGUUGAUCGUCUGGUAAUAGUUGUUUUAUGAGGUUAACCGCUACCGGGACAGGAAAAUGGUCUGUUGGUAAUGGACAGGUGUGUUAGACGGGAAAGCUUUCUAG

5'-3' ANTISENSE

GAUCUUUCGAAAGGGCAGAUUGUGUGGACAGGUAAUGGUUGUCUGGUAAAAGGACAGGGCCAUCGCCAAUUGGAGUAUUUUGUUGAUAAUGGUCUGCUAGUUGAACGCUUCCAUCUUCAAUGUUGUGUCUAAUUUUGAAGUUAACUUUGAUUCCAUUCUUUUGUUUGUCUGCCAUGAUGUAUACAUUGUGUGAGUUAUAGUUGUAUUCCAAUUUGUGUCCAAGAAUGUUUCCAUCUUCUUUAAAAUCAAUACCUUUUAACUCGAUUCUAUUAACAAGGGUAUCACCUUCAAACUUGACUUCAGCACGUGUCUUGUAGUUCCCGUCAUCUUUGAAAAAUAUAGUUCUU

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5'-3' VOLATILE REGION, WITH RANK:

10

UUAUGUACAGGAAAGAACUAUAUUUUUCAAAGAUGACGGGAACUACAAGACACGUGCUGAAGUCAAGUUUGAAGGUGAUACCCUUGUUAAUAGAAUCGAGUUAAAAGGUAUUGAUUUUAAAGAAGAUGGAAACAUUCUUGGACACAAAUUGGAAUACAACUAUAACUCACACAAUGUAUACAUCAUGGCAGACAAACAAAAGAAUGGAAUCAAAGUUAACUUCAAAAUUAGACACAACAUUGAAGAUGGAAGCGUUCAACUAGCAGACCAUUAUCAACAAAAUACUCCAAUUGGCGAUGGCCCUGUCCUUUUACCAGACAACCAUUACCUGUCCACACAAUCUGCCCUUUCGAAAGAUC

REGION BEGINS AT BASE:

314

REGION LENGTH:

359

3'-5' ANTISENSE

AAUACAUGUCCUUUCUUGAUAUAAAAAGUUUCUACUGCCCUUGAUGUUCUGUGCACGACUUCAGUUCAAACUUCCACUAUGGGAACAAUUAUCUUAGCUCAAUUUUCCAUAACUAAAAUUUCUUCUACCUUUGUAAGAACCUGUGUUUAACCUUAUGUUGAUAUUGAGUGUGUUACAUAUGUAGUACCGUCUGUUUGUUUUCUUACCUUAGUUUCAAUUGAAGUUUUAAUCUGUGUUGUAACUUCUACCUUCGCAAGUUGAUCGUCUGGUAAUAGUUGUUUUAUGAGGUUAACCGCUACCGGGACAGGAAAAUGGUCUGUUGGUAAUGGACAGGUGUGUUAGACGGGAAAGCUUUCUAG

5'-3' ANTISENSE

GAUCUUUCGAAAGGGCAGAUUGUGUGGACAGGUAAUGGUUGUCUGGUAAAAGGACAGGGCCAUCGCCAAUUGGAGUAUUUUGUUGAUAAUGGUCUGCUAGUUGAACGCUUCCAUCUUCAAUGUUGUGUCUAAUUUUGAAGUUAACUUUGAUUCCAUUCUUUUGUUUGUCUGCCAUGAUGUAUACAUUGUGUGAGUUAUAGUUGUAUUCCAAUUUGUGUCCAAGAAUGUUUCCAUCUUCUUUAAAAUCAAUACCUUUUAACUCGAUUCUAUUAACAAGGGUAUCACCUUCAAACUUGACUUCAGCACGUGUCUUGUAGUUCCCGUCAUCUUUGAAAAAUAUAGUUCUUUCCUGUACAUAA

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5'-3' VOLATILE REGION, WITH RANK:

11

UUAUGUACAGGAAAGAACUAUAUUUUUCAAAGAUGACGGGAACUACAAGACACGUGCUGAAGUCAAGUUUGAAGGUGAUACCCUUGUUAAUAGAAUCGAGUUAAAAGGUAUUGAUUUUAAAGAAGAUGGAAACAUUCUUGGACACAAAUUGGAAUACAACUAUAACUCACACAAUGUAUACAUCAUGGCAGACAAACAAAAGAAUGGAAUCAAAGUUAACUUCAAAAUUAGACACAACAUUGAAGAUGGAAGCGUUCAACUAGCAGACCAUUAUCAACAAAAUACUCCAAUUGGCGAUGGCCCUGUCCUUUUACCAGACAACCAUUACCUGUCCACACAAUCUGCCCUUUCGAAAGAUC

REGION BEGINS AT BASE:

314

REGION LENGTH:

359

3'-5' ANTISENSE

AAUACAUGUCCUUUCUUGAUAUAAAAAGUUUCUACUGCCCUUGAUGUUCUGUGCACGACUUCAGUUCAAACUUCCACUAUGGGAACAAUUAUCUUAGCUCAAUUUUCCAUAACUAAAAUUUCUUCUACCUUUGUAAGAACCUGUGUUUAACCUUAUGUUGAUAUUGAGUGUGUUACAUAUGUAGUACCGUCUGUUUGUUUUCUUACCUUAGUUUCAAUUGAAGUUUUAAUCUGUGUUGUAACUUCUACCUUCGCAAGUUGAUCGUCUGGUAAUAGUUGUUUUAUGAGGUUAACCGCUACCGGGACAGGAAAAUGGUCUGUUGGUAAUGGACAGGUGUGUUAGACGGGAAAGCUUUCUAG

5'-3' ANTISENSE

GAUCUUUCGAAAGGGCAGAUUGUGUGGACAGGUAAUGGUUGUCUGGUAAAAGGACAGGGCCAUCGCCAAUUGGAGUAUUUUGUUGAUAAUGGUCUGCUAGUUGAACGCUUCCAUCUUCAAUGUUGUGUCUAAUUUUGAAGUUAACUUUGAUUCCAUUCUUUUGUUUGUCUGCCAUGAUGUAUACAUUGUGUGAGUUAUAGUUGUAUUCCAAUUUGUGUCCAAGAAUGUUUCCAUCUUCUUUAAAAUCAAUACCUUUUAACUCGAUUCUAUUAACAAGGGUAUCACCUUCAAACUUGACUUCAGCACGUGUCUUGUAGUUCCCGUCAUCUUUGAAAAAUAUAGUUCUUUCCUGUACAUAA

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5'-3' VOLATILE REGION, WITH RANK:

12

GUUUAUCACAGUUAAAUUGCUAACGCAGUCAGGCACCGUGUAUGCGUAAAGGAGAAGAACUUUUCACUGGAGUUGUCCCAAUUCUUGUUGAAUUAGAUGGUGAUGUUAAUGGGCACAAAUUUUCUGUCAGUGGAGAGGGUGAAGGUGAUGCAACAUACGGAAAACUUACCCUUAAAUUUAUUUGCACUACUGGAAAACUACCUGUUCCAUGGCCAACACUUGUCACUACUUUCGGUUAUGGUGUUCAAUGCUUUGCGAGAUACCCAGAUCAUAUGAAACAGCAUGACUUUUUCAAGAGUGCCAUGCCCGAAGGUUAUGUACAGGAAAGAACUAUAUUUUUCAAAGAUGACGGGAACUACAAGACACGUGCUGAAGUCAAGUUUGAAGGUGAUACCCUUGUUAAUAGAAUCGAGUUAAAAGGUAUUGAUUUUAAAGAAGAUGGAAACAUUCUUGGACACAAAUUGGAAUACAACUAUAACUCACACAAUGUAUACAUCAUGGCAGACAAACAAAAGAAUGGAAUCAAAGUUAACUUCAAAAUUAGACACAACAUUGAAGAUGGAAGCGUUCAACUAGCAGACCAUUAUCAACAAAAUACUCCAAUUGGCGAUGGCCCUGUCCUUUUACCAGACAACCAUUACCUGUCCACACAAUCUGCCCUUUCGAAAGAUC

REGION BEGINS AT BASE:

1

REGION LENGTH:

672

3'-5' ANTISENSE

CAAAUAGUGUCAAUUUAACGAUUGCGUCAGUCCGUGGCACAUACGCAUUUCCUCUUCUUGAAAAGUGACCUCAACAGGGUUAAGAACAACUUAAUCUACCACUACAAUUACCCGUGUUUAAAAGACAGUCACCUCUCCCACUUCCACUACGUUGUAUGCCUUUUGAAUGGGAAUUUAAAUAAACGUGAUGACCUUUUGAUGGACAAGGUACCGGUUGUGAACAGUGAUGAAAGCCAAUACCACAAGUUACGAAACGCUCUAUGGGUCUAGUAUACUUUGUCGUACUGAAAAAGUUCUCACGGUACGGGCUUCCAAUACAUGUCCUUUCUUGAUAUAAAAAGUUUCUACUGCCCUUGAUGUUCUGUGCACGACUUCAGUUCAAACUUCCACUAUGGGAACAAUUAUCUUAGCUCAAUUUUCCAUAACUAAAAUUUCUUCUACCUUUGUAAGAACCUGUGUUUAACCUUAUGUUGAUAUUGAGUGUGUUACAUAUGUAGUACCGUCUGUUUGUUUUCUUACCUUAGUUUCAAUUGAAGUUUUAAUCUGUGUUGUAACUUCUACCUUCGCAAGUUGAUCGUCUGGUAAUAGUUGUUUUAUGAGGUUAACCGCUACCGGGACAGGAAAAUGGUCUGUUGGUAAUGGACAGGUGUGUUAGACGGGAAAGCUUUCUAG

5'-3' ANTISENSE

GAUCUUUCGAAAGGGCAGAUUGUGUGGACAGGUAAUGGUUGUCUGGUAAAAGGACAGGGCCAUCGCCAAUUGGAGUAUUUUGUUGAUAAUGGUCUGCUAGUUGAACGCUUCCAUCUUCAAUGUUGUGUCUAAUUUUGAAGUUAACUUUGAUUCCAUUCUUUUGUUUGUCUGCCAUGAUGUAUACAUUGUGUGAGUUAUAGUUGUAUUCCAAUUUGUGUCCAAGAAUGUUUCCAUCUUCUUUAAAAUCAAUACCUUUUAACUCGAUUCUAUUAACAAGGGUAUCACCUUCAAACUUGACUUCAGCACGUGUCUUGUAGUUCCCGUCAUCUUUGAAAAAUAUAGUUCUUUCCUGUACAUAACCUUCGGGCAUGGCACUCUUGAAAAAGUCAUGCUGUUUCAUAUGAUCUGGGUAUCUCGCAAAGCAUUGAACACCAUAACCGAAAGUAGUGACAAGUGUUGGCCAUGGAACAGGUAGUUUUCCAGUAGUGCAAAUAAAUUUAAGGGUAAGUUUUCCGUAUGUUGCAUCACCUUCACCCUCUCCACUGACAGAAAAUUUGUGCCCAUUAACAUCACCAUCUAAUUCAACAAGAAUUGGGACAACUCCAGUGAAAAGUUCUUCUCCUUUACGCAUACACGGUGCCUGACUGCGUUAGCAAUUUAACUGUGAUAAAC

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5'-3' VOLATILE REGION, WITH RANK:

13

GUUUAUCACAGUUAAAUUGCUAACGCAGUCAGGCACCGUGUAUGCGUAAAGGAGAAGAACUUUUCACUGGAGUUGUCCCAAUUCUUGUUGAAUUAGAUGGUGAUGUUAAUGGGCACAAAUUUUCUGUCAGUGGAGAGGGUGAAGGUGAUGCAACAUACGGAAAACUUACCCUUAAAUUUAUUUGCACUACUGGAAAACUACCUGUUCCAUGGCCAACACUUGUCACUACUUUCGGUUAUGGUGUUCAAUGCUUUGCGAGAUACCCAGAUCAUAUGAAACAGCAUGACUUUUUCAAGAGUGCCAUGCCCGAAGGUUAUGUACAGGAAAGAACUAUAUUUUUCAAAGAUGACGGGAACUACAAGACACGUGCUGAAGUCAAGUUUGAAGGUGAUACCCUUGUUAAUAGAAUCGAGUUAAAAGGUAUUGAUUUUAAAGAAGAUGGAAACAUUCUUGGACACAAAUUGGAAUACAACUAUAACUCACACAAUGUAUACAUCAUGGCAGACAAACAAAAGAAUGGAAUCAAAGUUAACUUCAAAAUUAGACACAACAUUGAAGAUGGAAGCGUUCAACUAGCAGACCAUUAUCAACAAAAUACUCCAAUUGGCGAUGGCCCUGUCCUUUUACCAGACAACCAUUACCUGUCCACACAAUCUGCCCUUUCGAAAGAUC

REGION BEGINS AT BASE:

1

REGION LENGTH:

672

3'-5' ANTISENSE

CAAAUAGUGUCAAUUUAACGAUUGCGUCAGUCCGUGGCACAUACGCAUUUCCUCUUCUUGAAAAGUGACCUCAACAGGGUUAAGAACAACUUAAUCUACCACUACAAUUACCCGUGUUUAAAAGACAGUCACCUCUCCCACUUCCACUACGUUGUAUGCCUUUUGAAUGGGAAUUUAAAUAAACGUGAUGACCUUUUGAUGGACAAGGUACCGGUUGUGAACAGUGAUGAAAGCCAAUACCACAAGUUACGAAACGCUCUAUGGGUCUAGUAUACUUUGUCGUACUGAAAAAGUUCUCACGGUACGGGCUUCCAAUACAUGUCCUUUCUUGAUAUAAAAAGUUUCUACUGCCCUUGAUGUUCUGUGCACGACUUCAGUUCAAACUUCCACUAUGGGAACAAUUAUCUUAGCUCAAUUUUCCAUAACUAAAAUUUCUUCUACCUUUGUAAGAACCUGUGUUUAACCUUAUGUUGAUAUUGAGUGUGUUACAUAUGUAGUACCGUCUGUUUGUUUUCUUACCUUAGUUUCAAUUGAAGUUUUAAUCUGUGUUGUAACUUCUACCUUCGCAAGUUGAUCGUCUGGUAAUAGUUGUUUUAUGAGGUUAACCGCUACCGGGACAGGAAAAUGGUCUGUUGGUAAUGGACAGGUGUGUUAGACGGGAAAGCUUUCUAG

5'-3' ANTISENSE

GAUCUUUCGAAAGGGCAGAUUGUGUGGACAGGUAAUGGUUGUCUGGUAAAAGGACAGGGCCAUCGCCAAUUGGAGUAUUUUGUUGAUAAUGGUCUGCUAGUUGAACGCUUCCAUCUUCAAUGUUGUGUCUAAUUUUGAAGUUAACUUUGAUUCCAUUCUUUUGUUUGUCUGCCAUGAUGUAUACAUUGUGUGAGUUAUAGUUGUAUUCCAAUUUGUGUCCAAGAAUGUUUCCAUCUUCUUUAAAAUCAAUACCUUUUAACUCGAUUCUAUUAACAAGGGUAUCACCUUCAAACUUGACUUCAGCACGUGUCUUGUAGUUCCCGUCAUCUUUGAAAAAUAUAGUUCUUUCCUGUACAUAACCUUCGGGCAUGGCACUCUUGAAAAAGUCAUGCUGUUUCAUAUGAUCUGGGUAUCUCGCAAAGCAUUGAACACCAUAACCGAAAGUAGUGACAAGUGUUGGCCAUGGAACAGGUAGUUUUCCAGUAGUGCAAAUAAAUUUAAGGGUAAGUUUUCCGUAUGUUGCAUCACCUUCACCCUCUCCACUGACAGAAAAUUUGUGCCCAUUAACAUCACCAUCUAAUUCAACAAGAAUUGGGACAACUCCAGUGAAAAGUUCUUCUCCUUUACGCAUACACGGUGCCUGACUGCGUUAGCAAUUUAACUGUGAUAAAC

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5'-3' VOLATILE REGION, WITH RANK:

14

GUUUAUCACAGUUAAAUUGCUAACGCAGUCAGGCACCGUGUAUGCGUAAAGGAGAAGAACUUUUCACUGGAGUUGUCCCAAUUCUUGUUGAAUUAGAUGGUGAUGUUAAUGGGCACAAAUUUUCUGUCAGUGGAGAGGGUGAAGGUGAUGCAACAUACGGAAAACUUACCCUUAAAUUUAUUUGCACUACUGGAAAACUACCUGUUCCAUGGCCAACACUUGUCACUACUUUCGGUUAUGGUGUUCAAUGCUUUGCGAGAUACCCAGAUCAUAUGAAACAGCAUGACUUUUUCAAGAGUGCCAUGCCCGAAGGUUAUGUACAGGAAAGAACUAUAUUUUUCAAAGAUGACGGGAACUACAAGACACGUGCUGAAGUCAAGUUUGAAGGUGAUACCCUUGUUAAUAGAAUCGAGUUAAAAGGUAUUGAUUUUAAAGAAGAUGGAAACAUUCUUGGACACAAAUUGGAAUACAACUAUAACUCACACAAUGUAUACAUCAUGGCAGACAAACAAAAGAAUGGAAUCAAAGUUAACUUCAAAAUUAGACACAACAUUGAAGAUGGAAGCGUUCAACUAGCAGACCAUUAUCAACAAAAUACUCCAAUUGGCGAUGGCCCUGUCCUUUUACCAGACAACCAUUACCUGUCCACACAAUCUGCCCUUUCGAAAGAUC

REGION BEGINS AT BASE:

1

REGION LENGTH:

672

3'-5' ANTISENSE

CAAAUAGUGUCAAUUUAACGAUUGCGUCAGUCCGUGGCACAUACGCAUUUCCUCUUCUUGAAAAGUGACCUCAACAGGGUUAAGAACAACUUAAUCUACCACUACAAUUACCCGUGUUUAAAAGACAGUCACCUCUCCCACUUCCACUACGUUGUAUGCCUUUUGAAUGGGAAUUUAAAUAAACGUGAUGACCUUUUGAUGGACAAGGUACCGGUUGUGAACAGUGAUGAAAGCCAAUACCACAAGUUACGAAACGCUCUAUGGGUCUAGUAUACUUUGUCGUACUGAAAAAGUUCUCACGGUACGGGCUUCCAAUACAUGUCCUUUCUUGAUAUAAAAAGUUUCUACUGCCCUUGAUGUUCUGUGCACGACUUCAGUUCAAACUUCCACUAUGGGAACAAUUAUCUUAGCUCAAUUUUCCAUAACUAAAAUUUCUUCUACCUUUGUAAGAACCUGUGUUUAACCUUAUGUUGAUAUUGAGUGUGUUACAUAUGUAGUACCGUCUGUUUGUUUUCUUACCUUAGUUUCAAUUGAAGUUUUAAUCUGUGUUGUAACUUCUACCUUCGCAAGUUGAUCGUCUGGUAAUAGUUGUUUUAUGAGGUUAACCGCUACCGGGACAGGAAAAUGGUCUGUUGGUAAUGGACAGGUGUGUUAGACGGGAAAGCUUUCUAG

5'-3' ANTISENSE

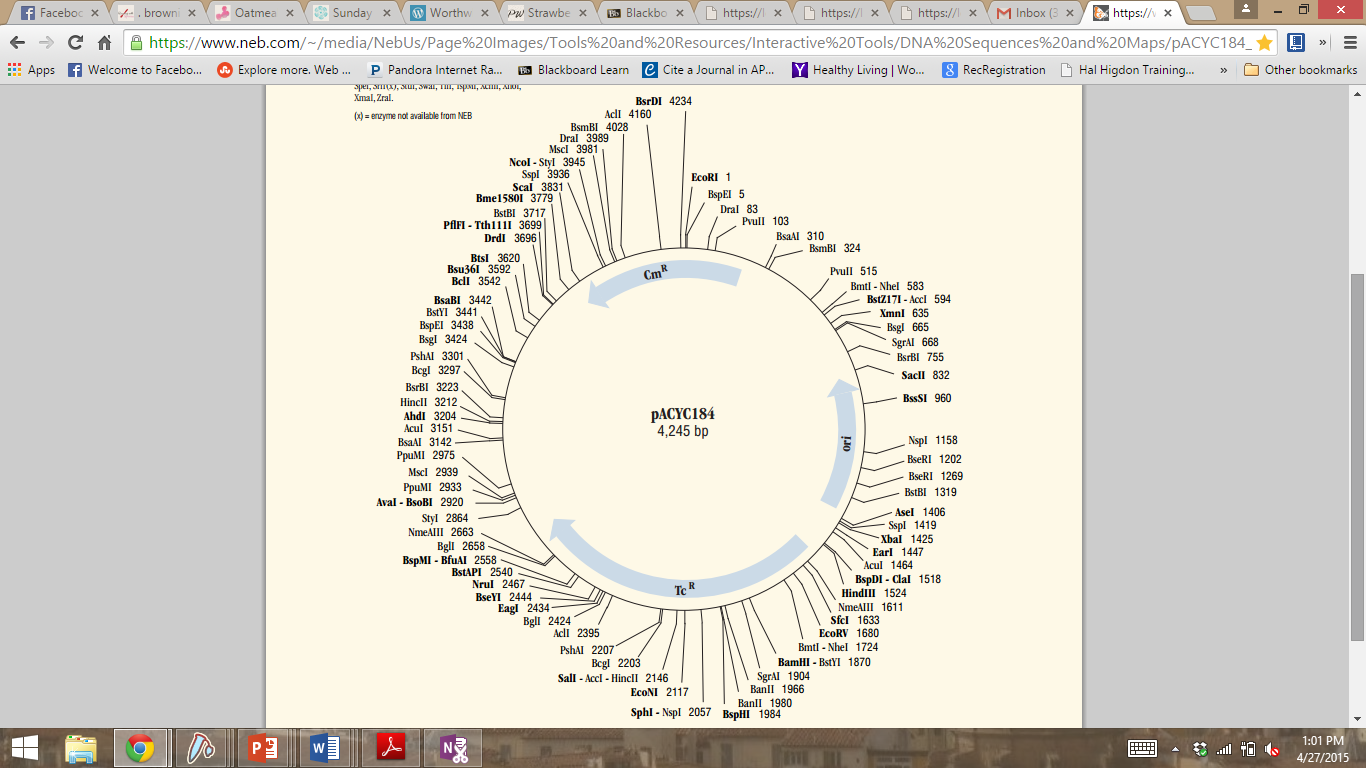
GAUCUUUCGAAAGGGCAGAUUGUGUGGACAGGUAAUGGUUGUCUGGUAAAAGGACAGGGCCAUCGCCAAUUGGAGUAUUUUGUUGAUAAUGGUCUGCUAGUUGAACGCUUCCAUCUUCAAUGUUGUGUCUAAUUUUGAAGUUAACUUUGAUUCCAUUCUUUUGUUUGUCUGCCAUGAUGUAUACAUUGUGUGAGUUAUAGUUGUAUUCCAAUUUGUGUCCAAGAAUGUUUCCAUCUUCUUUAAAAUCAAUACCUUUUAACUCGAUUCUAUUAACAAGGGUAUCACCUUCAAACUUGACUUCAGCACGUGUCUUGUAGUUCCCGUCAUCUUUGAAAAAUAUAGUUCUUUCCUGUACAUAACCUUCGGGCAUGGCACUCUUGAAAAAGUCAUGCUGUUUCAUAUGAUCUGGGUAUCUCGCAAAGCAUUGAACACCAUAACCGAAAGUAGUGACAAGUGUUGGCCAUGGAACAGGUAGUUUUCCAGUAGUGCAAAUAAAUUUAAGGGUAAGUUUUCCGUAUGUUGCAUCACCUUCACCCUCUCCACUGACAGAAAAUUUGUGCCCAUUAACAUCACCAUCUAAUUCAACAAGAAUUGGGACAACUCCAGUGAAAAGUUCUUCUCCUUUACGCAUACACGGUGCCUGACUGCGUUAGCAAUUUAACUGUGAUAAAC

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**Appendix IV – Plasmid Maps**

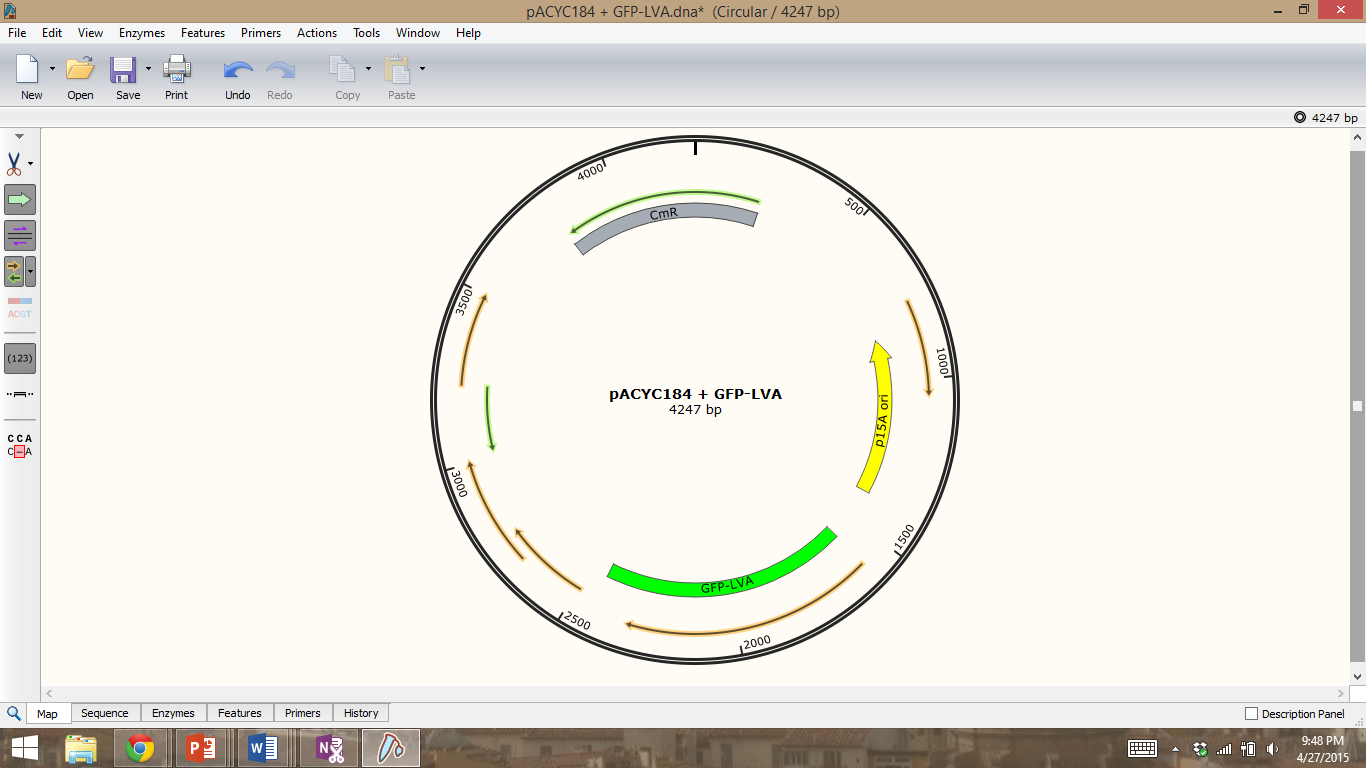
# Appendix IV.A. –Purchased pACYC184 Map

**This data was supplied by New England Biolabs as product E. coli K12 ER2420/pACYC184**



# Appendix IV.B. – Constructed pACYC184-GFP-LVA Map

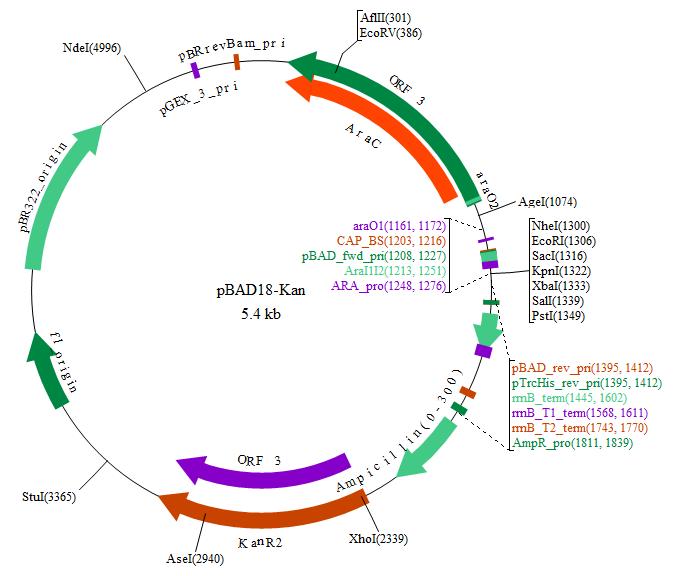
**This map was constructed using SnapGene Viewer**



# Appendix IV.C. – Purchased pBAD/18Kan Map for Antisense Insertion

**This plasmid was supplied by ATCC ® Product Number 87397**

**This map was supplied from BioVisual Tech, Inc.**

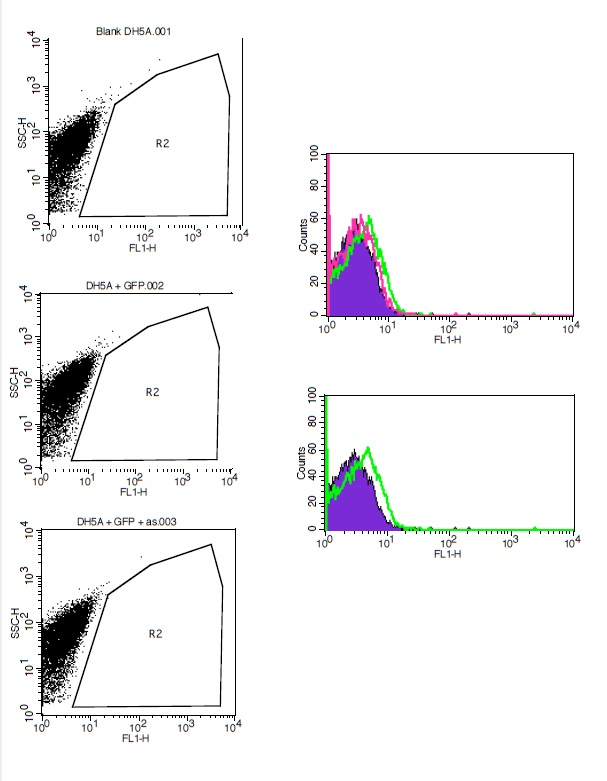


**Appendix V – Miscellaneous**

# Appendix V.A. –FACS Results

**Notation:** “Blank” = DH5α cells without plasmid

“GFP” = DH5α cells with GFP-LVA in iGEM’s plasmid



# Appendix V.B. – Qubit Raw Mode Program

**This is the program written for fluorescence quantification of the GFP-LVA protein**

