

# The Amplification and Cloning of *Staphylococcus aureus* purD

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## Abstract

Our lab is researching the biochemical action of the enzyme formylglycinamide ribonucleotide amidotransferase (FGARAT). The substrate for this enzyme, however, is not available for purchase; instead we intend to produce it enzymatically from a substrate that is available. Of the three enzymes needed, my research focuses on glycinamide ribonucleotide (GAR) synthetase (purD). The gene for this protein was amplified by PCR using *Staphylococcus aureus* genomic DNA. Next, I cloned the PCR product into a Topo 2.1 TA vector (Invitrogen). From here, the gene was sub-cloned into a pET 29a expression vector (Novagen). Initial expression experiments analyzed by SDS-PAGE, as well as a series of restriction enzyme digests revealed that the GenBank entry for my clone was incorrectly annotated, using a downstream start codon. New primers were designed from the corrected sequence, and the correct-length fragment was amplified. The new fragment was then cloned into a Topo 2.1 TA vector (Invitrogen). Future study will focus on sub-cloning the new fragment into a pET expression vector, from which GAR synthetase can be expressed and purified.

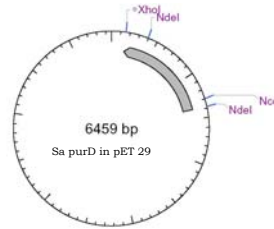


Figure 2. Map of Sa purD in pET 29

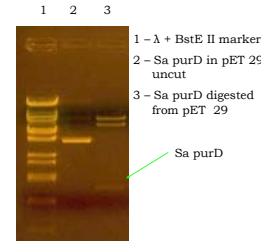


Figure 3. Sa purD in pET 29 Digested with NcoI/XhoI

## Current Work

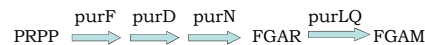
- New primers designed based on corrected sequence
- Full length gene amplified with PCR, cloned into pCR 2.1
- Currently cloning into pET expression vectors

Sa purD Start NcoI  
 5'-gggggcatggatgtattagaattgggtgc  
 5'-atggatgtattagaattgggtgctgggagcagaacatgcacttga...  
 3'-tacctacataatctttaaccacgaccctgctctgtacgtgaacgt...  
 Sa purD Stop XhoI  
 5'-...tatgctcatgacattgcaataaagcactacaacttaataa  
 3'-...atagcagtactgtaacgcttattttagatgttgaattatt  
 3'-cgatgtgtgaattatttagctcgggggg

Figure 6. Primers used to amplify full length Sa purD

## Background

•To produce FGAR for biochemical studies, the products of purF, purD, and purN are required:



## Project Goals

- Amplify Sa purD
- Clone Sa purD into a pET vector
- Induce expression of Sa purD
- Purify the Sa PurD enzyme

atgaagaacgcgctgctgctaattcgaaagccagccacatggac  
 M K E T A A A K F E R Q H M D  
 agccagatctgggtaccctcggtccacggttccatggacct  
 S P D L G T L V P R Q S M G P  
 atagctgaagtacacactgaaattcagaatcctaatcctcaa...  
 I A E V H T E I S E S N H Q  
 ...tatgctcatgacattgcaataaagcactacaacttaataa  
 Y R H D I A N K A L Q L K \*

S-tag Thrombin cleavage purD

Figure 4. Fusion protein sequence produced from Sa purD in pET 29a  
 407 residues, MW=44.89 kDa

## Protein Methods

- Transformed Sa purD into Escherichia coli BL21 expression strain
- Induced expression with IPTG
- Initial protein expression experiments were inconclusive

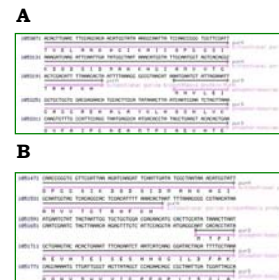


Figure 5. Identification of incorrect annotation in genome sequence

Panel A: Genomic sequence showing correct start codon annotation of purD gene.

Panel B: Genomic sequence showing incorrect start codon annotation of purD gene.

Panel C: Multiple sequence alignments showing correct purD gene and other homologous gene. Incorrectly annotated Sa purD is listed second.

Accession	Score	E	Accession	SI	Protein Description
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