Cloning and sequencing of GAPC in *Carex appalachia*

Zane Billings and Erin Thompson

Biology Department, Western Carolina University, Cullowhee, NC 28723

Abstract

GAPDH (Glyceraldehye-3-Phosphate Dehydrogenase) is a "house-keeping" gene that is essential for glycolysis in plants. Due to the essential nature of the GAPDH products, the gene is conserved across most life, including plants. *Carex appalachia*, or Appalachian sedge, is a plant native to Western North Carolina and the Appalachian mountain range. Scientific research is not commonly conducted on native plants, so sequencing native plant genomes is important for both cultural preservation and potential applications to ecological restoration in damaged Appalachian biomes. In this series of experiments, gDNA was extracted from live *C. appalachia* samples, purified, inserted into plasmid vectors and amplified in *E. coli*, before the gene was sequenced and bioinformatics analysis was performed. BLASTing aligned contig sequence data with the NCBI genomic reference database confirmed similarity to the GAPC gene in several other plant species.

Figure 1: The *C. appalachia* plant that was used for gDNA extraction.

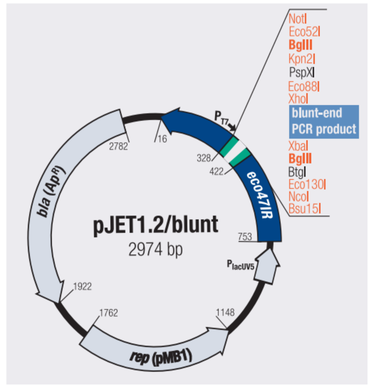

Figure 2: The PJET 1.2 vector[2] that was used for transformation into *E. coli*, showing salient DNA features.

Introduction

The purpose of this experiment is to duplicate a portion of the GAPDH gene from a plant sample. GAPC is one of the GAPDH genes which specifically codes for the cytosolic GAPDH protein. GAPC is a key enzyme in the process of glycolysis and is essential for cells to survive. Due to its essential nature, GAPC is highly conserved across most of life, including the higher plants such as *Carex appalachia*, a sedge native to Western North Carolina (family: Cyperaceae).

Native plants are typically not as well studied as common model organisms, so expanding the available genomic database with native plant DNA sequences both improves the genomic database and allows for future research into native plants, including potential applications in medicine and drug discovery, as well as in reconstruction of phylogenies. Genomic DNA was extracted from a live specimen of *C. Appalachia*, and the GAPC gene was amplified utilizing PCR. The gene was ligated into a vector and transformed into bacteria so it could be amplified for sequencing and bioinformatics analysis, to compare the extracted DNA with a genomic reference database and determine the similarity between the extracted DNA and GAPC genes of other plants.

DNA Extraction

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Initial PCR

↓

Nested PCR

↓

Ligation

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Transformation

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Plasmid Purification

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Restriction Enzyme Digestion

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DNA Sequencing

Results

Initial PCR

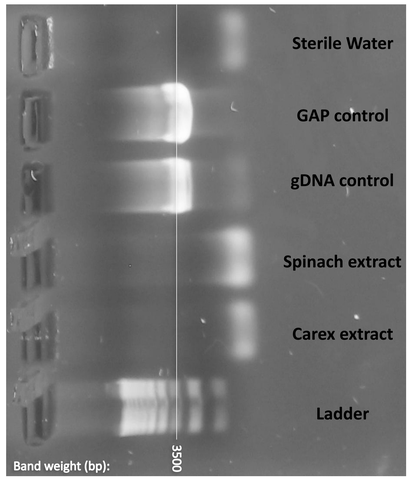

Figure 3: Electrophoresis results of the initial PCR experiment, utilizing degenerate primers. The lack of banding in wells 2 & 3 (from the ladder) indicates that PCR was not successful in producing a GAPC product from the extracted samples.

Nested PCR

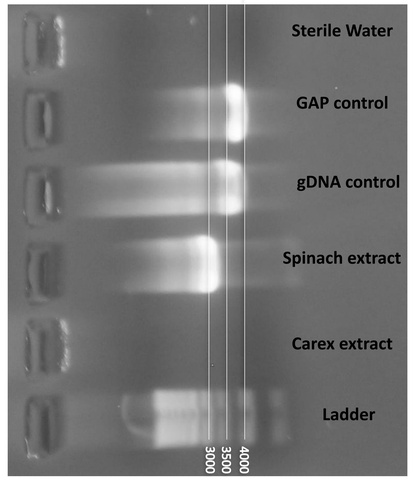

Figure 4: Electrophoresis results of the nested PCR experiment. The nested PCR was successful in duplicating the spinach GAPC gene (lane 3 from the ladder), but not the carex gene, since no banding is seen in the carex lane.

Digestion PCR

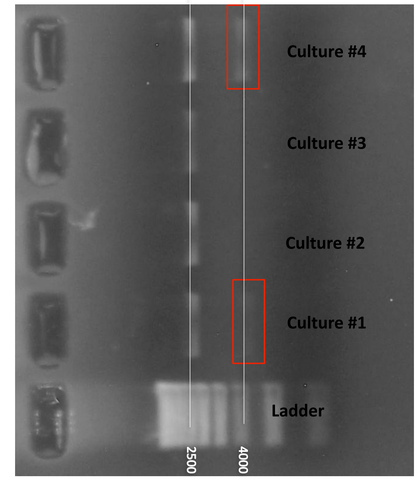

Figure 5: Electrophoresis results for plasmids extracted from miniprep *E. coli* cultures. From the gel we observed that miniprep colonies 1 & 4 (from the ladder) were successful recombinants expressing the desired plasmid.

Table 1: Comparison of the top 3 BLAST hits for the aligned contig sequence, showing bit-score (a measure of relative quality), grade, pairwise identity, and the specific gene that is being compared.

Bit-score	Grade	% Pairwise identity	Species	Gene
176.213	71.5%	74.5%	Asparagus officinalis	GAPC-2
170.803	60.3%	95.4%	Setaria italica	GAPC-2
165.393	59.7%	94.4%	Zea mays	GAPC-1

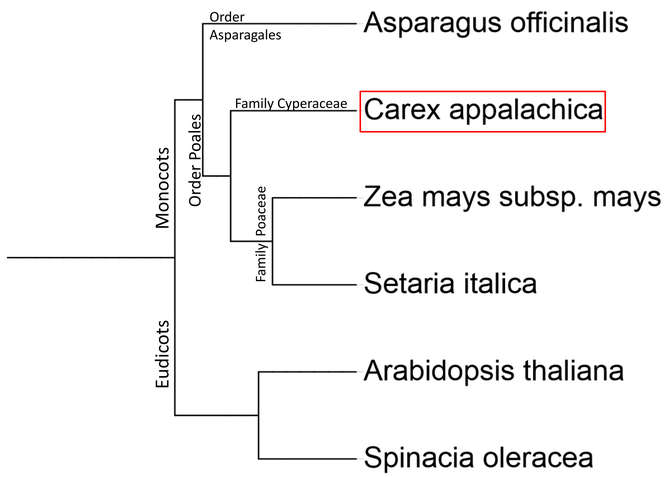

Figure 6: Taxonomic comparison of *C. Appalachia* contig BLAST top matches, including Arabidopsis and spinach as references.

Conclusions and Future Work

The sample produced BLAST matches for GAPC genes from several different plants, including members of the same order, as well as a top results from a different order in the Monocot clade. Results also showed up for some Eudicots, although these matches were not as strong, and no homology to *Arabidopsis thaliana* was detected.

Overall, bioinformatics results indicate that the cloning and sequencing of GAPC from *C. appalachia* was successful. In the future it may be possible to replicate different genes from *C. appalachia*, leading to a more complete knowledge of native plants.

Acknowledgments

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References

1. Beyer, Harris, Hickman, & Rundle. Cell and molecular biology lab manual; Gene cloning and sequencing supplemental manual. 2018.
2. Michaelson, BK. *Transformation of Escherichia coli increases 260-fold upon inactivation of T4 DNA Ligase.* Anal biochem. 1995;225(1):172-174. https://doi.org/10.1006/abio.1995.1130