

What is in your supplements? Streamlining sequencing methods for accurate results.



Whitney Inman

URSA Fall 2022

University of Alaska Fairbanks

Abstract

Importance of DNA Sequencing in the Supplement Industry:

The rapidly growing supplement industry in the United States provides the potential for unchecked quality control and ingredient regulation. In the supplement industry, lax quality standards and cost cutting substitutions test consumer confidence and safety. Mislabeling plant ingredients can cause prescription drug interaction as well as allergic reactions.

Goals:

- Identifying species present using DNA sequencing
- Increasing sequencing accuracy through
 - Reducing costs by avoiding error
 - Good lab and personal hygiene
 - Awareness of environmental contamination

The Principles of Genetics class at UAF uses Illumina sequencing, this protocol teaches hands on lab skills and data interpretation for approximately 20 students per semester. Improving the protocols of the primers and clean up methods used can reduce costs, increase accuracy, and reduce cross contamination in the UAF lab as well as in other industries.

Acknowledgements

This project would not be possible without the help and guidance from my mentor, Professor Diana Wolf and supporting biology professor Dr. Naoki Takebayashi. Sierra Tyssedal and Amanda Rust were supportive in the creation of this poster in proofreading as well as being moral support throughout the time I spent in the UAF Core lab. Special thanks to Martin DeBourge for video editing and audio files.

Materials and Methods

Preparation:

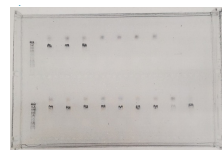
- Bleach all high contact surfaces
- Test reagents for contamination
- Test in-house made PCR beads

Caution:

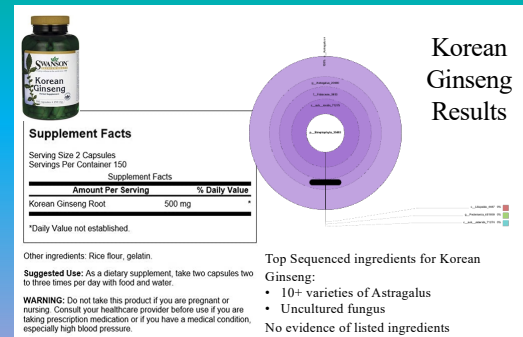
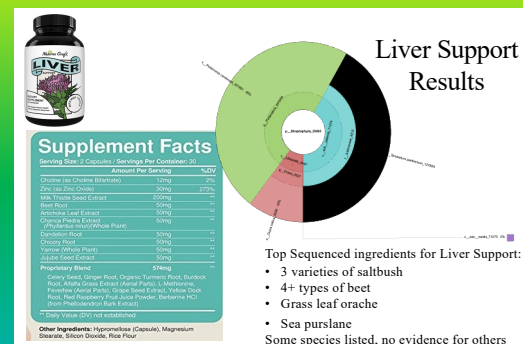
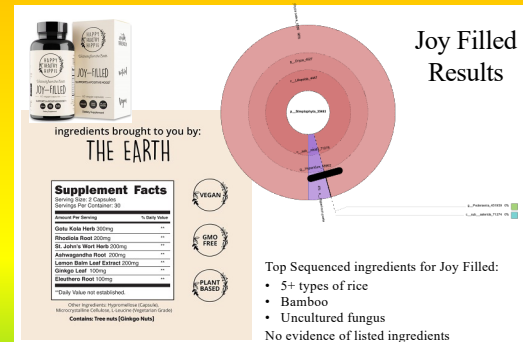
- If gel indicates contamination, STOP and retest reagents

Procedure:

- **DNA extraction** of eleven supplements using the Genra Puregene Tissue Kit
- **PCR 1:**
 - to isolate and amplify the ITS2 region
 - **internal indices** were added with the goal of tracing contamination between samples
- Agarose gel electrophoresis to verify DNA segments amplified are desired size and to verify reagents contain no contamination
- **PCR 2:** Using the KAPA HiFi ReadyMix kit to add adaptors used for attachment to the PCR1 product and **external indices** needed to identify separate supplements
- Agarose gel electrophoresis to verify DNA segments amplified are the desired size, and to verify reagents contain no contamination
- DNA sequencing by the UAF DNA core lab on an Illumina MiSeq DNA sequencer.
- **Metagenomic analysis** to identify the plant species provided by Naoki Takebayashi.
- Compare the internal and external indices to identify the performance of experimental internal indices and contamination rate.



*Figure 1 – PCR2 agarose gel electrophoresis indicates no contamination and proper segment size for desired amplification.



Conclusion

Increasing accuracy and reducing cost in genetic sequencing provides an accurate method for supplement ingredient accountability.

Contamination prevention is important for reliable results, this can be accomplished with:

- Thorough lab and personal hygiene
- Awareness of environmental contamination such as pollen or dust
- Step wise analysis of potential contamination sources
- Additional indices may help trace where contamination arose

The internal and external indices matched for 98% of reads, indicating low contamination rates.

Although internal indices could potentially increase error due to reduced sequencing overlap requirements, our results show that it can enhance external indices for contaminant tracing.

Recommendations

Accurate DNA sequencing:

- Adds to the genomic databases
- Reduces costs and errors
- Allows for a reliable method of ingredient identification
- Prevents contamination

Supplement Industry needs to improve:

- Quality standards
- Health and safety warnings
- Properly labeling ingredients
- Legislation for ingredient labeling and quality
- More ethical standards, supervision, and enforcement by the FDA