The Case of the Mystery Frogs

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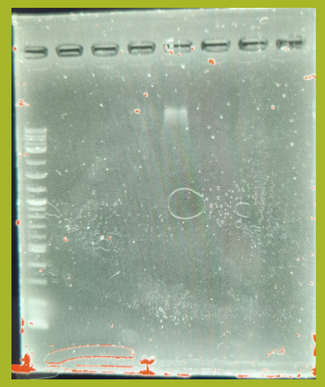
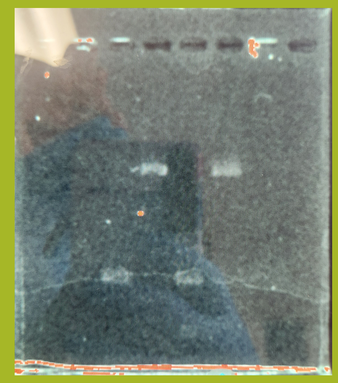
Intro/Background:

Biologists in Arizona are working to relocate the endangered Chiricahua Leopard frogs due to heavy droughts and immense livestock grazing. The Chiricahua Leopard frogs have a similar appearance to other species of leopard frogs. This makes it difficult to determine which frogs need to be relocated. Our hope is to genetically identify these frogs to help biologists relocate the correct species.



First Gel Sequence



Second Gel Sequence

Summary:

We obtained samples of an unknown frog from a project in Arizona. Our group extracted the DNA, prepared our samples for PCR reactions, then started the process of gel electrophoresis. Once we had a ladder and good band readings we prepared and sent our samples off to be sequenced by a company called GENEWIZ. All of this was done to discover what type of frog these samples were from and determine if this species was endangered.

Materials/Methods:

- Dissection and DNA extraction
  - Primers: ControlP-H, CytbA-L, R012s 460 Reverse, R012s 216 Forward.
- PCR and gel electrophoresis- to separate the DNA products and analyze them.
  - PCR conditions: Bio mix red and approximately 8 hours in a PCR machine.
  - Gel materials: Agarose and Tris-acetate-EDTA (TAE buffer).
- DNA sequencing
  - PCR samples with R012s 216 Forward and CytbA-L primers.

Sequencing results:

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☑	Rana yavapaiensis isolate LVT4561 mitochondrial control region, partial sequence	Lithobates yavapaiensis	1386	2310	84%	0.0	95.50%	1046	AF343778.1
☑	Rana yavapaiensis isolate LVT4575 mitochondrial control region, partial sequence	Lithobates yavapaiensis	1386	2310	84%	0.0	95.50%	1046	AF343777.1

Sort by description

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☑	Lithobates yavapaiensis isolate Pop_MRHS 12S ribosomal RNA, tRNA-Val, and 16S ribosomal RNA genes…	Lithobates yava…	329	329	24%	6e-85	96.88%	2587	MF537017.1
☑	Lithobates yavapaiensis isolate Pop_AS 12S ribosomal RNA, tRNA-Val, and 16S ribosomal RNA genes, co…	Lithobates yava…	329	329	24%	6e-85	96.88%	2587	MF537016.1

Conclusion:

Our DNA sequences that we sent to GENEWIZ were successful! With these results, we discovered that our frog samples were most similar to a few species of lowland leopard frogs (*Lithobates yavapainesis* and *Rana yavapaiensis*). With this information, we can send our findings back to the Arizona Game and Fish Department. Biologists there can then use our results to compare them with those of other Chiricahua Leopard frogs in the area. This will help them keep track of the number of frogs they have in the area and determine which frogs in the area are endangered species.

Cited Sources:

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Chiricahua leopard frog. (n.d.). https://www.biologicaldiversity.org/species/amphibians/Chiricahua_leopard_frog/index.html

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