# The Fecal Position: Tracking Progressive DNA Repeat Expansion via Stool DNA Extraction

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## Introduction

### Friedrich Ataxia

Friedreich Ataxia is a progressive DNA repeat expansion disease. This disease is caused by expansion of the GAA●TTC tract in the frataxin gene. The subsequent low levels of frataxin lead to muscle weakness and is eventually fatal.

### Mouse Model

Examining DNA repeat expansion in a mouse model requires sacrificing the mouse and taking samples of internal organs. Currently there is no reliable non-lethal way to monitor the expansion of the GAA●TTC tract in the frataxin gene in mice. The obvious non-lethal targets, such as ears, tails, and blood do not have levels of repeat expansion comparable to internal organs (Figure 1).

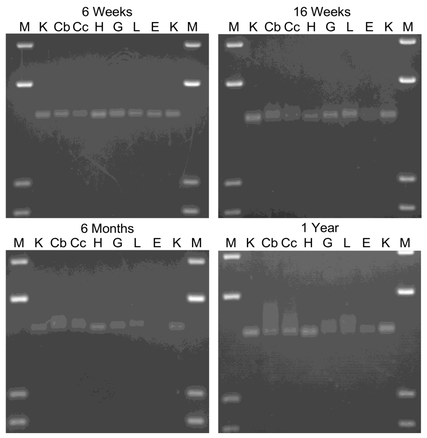


**Figure 1: DNA repeat expansion in multiple tissues over different time frames;** Tissues analyzed include Kidney (K), Cerebellum (Cb), Cerebrum (Cc), Heart (H), Gastrocnemius (G), Liver (L), and Ear (E).

### Stool as a Model for Repeat Expansion

Stool DNA poses a unique challenge due to the degradation of its components, high lipid content, and high level of bacterial DNA contamination. DNA isolation was first attempted with the Norgen BioTek Stool DNA Isolation Kit, then attempted with a "home-made" method. We theorize that DNA from stool will show comparable expansion to the liver DNA since stool samples DNA from the intestine.

Stool DNA has recently been shown to be sufficient for monitoring repeat expansion in other mouse models, but not yet attempted with Friedrich Ataxia mice[1].

## Norgen BioTek Stool DNA Isolation Kit

### Initially unable to amplify DNA

This kit was chosen due to its success amplifying DNA in other repeat expansion mouse models[1].

Many rounds of PCR were conducted with modifications of the number of cycles, amount of templet DNA, number of fecal pellets, age of mouse, and type of primers. No modifications yielded reliable bands for mouse beta actin PCR primers or primers specific for the expanded GAA●TTC tract in the frataxin transgene. After extensive trials with this DNA extraction method, the decision was made to develop a DNA extraction method initially adapted from Hart et al[2].

## "Home-made" DNA Extraction Method

**Stool Collection**

Place Mouse in autoclaved Caged → Discard First Fecal Pellet → Collect 2nd and 3rd Fecal Pellet

**Cell Lysis**

Aliquot 1 mL Lysis Buffer → Add beads and homogenize in Bullet Blender → Incubate at 70° C for 20 minutes and vortex every 5 minutes

**Precipitation of Nucleic Acids**

Centrifuge and Save Supernatant → Wash with 100% isopropanol → Wash with 100% Ethanol

**Removal of RNA, Protein, and Purification**

Incubate overnight in Proteinase K Buffer, Proteinase K, and RNAse A. Centrifuge and save Supernatant → Add Phenol:Chloroform, mixture, centrifuge, and save supernatant → Wash with Ethanol and resuspend in 1 x TE

## Results

### Mouse actin primers confirm the presence of mouse DNA




**Figure 2: Mouse Actin Primers confirm presence of mouse DNA.** Stool Samples were taken from ten mice. Two ear samples were used as a control. The 4 week mice are lanes 4,8,9, and 10. The 29 week mice are lanes 1,2,3, and 6. The >1 year mice are lanes 5 and 7.

- Utilized Mouse Actin Primers with expected base pair length of 138 bp.
- If the DNA is highly degraded, it is expected to degrade around a nucleosome which is 146 base pairs long. The size band amplified by these mouse beta actin primers is expected to still be present since it is smaller than the number of base pairs surrounding a mouse nucleosome.
- This confirms that there is mouse DNA in the samples.

### Amplification of the GAA●TTC tract in the frataxin transgene.

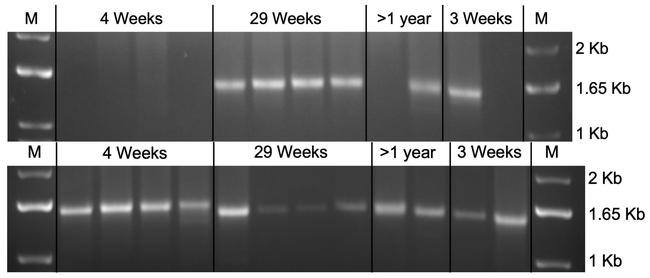


**Figure 3: Amplification of the GAA●TTC tract in the frataxin transgene.** Samples were diluted 1:5 (above) and not diluted (below) to assess the predictive value of the PCR with mouse beta actin primers. 3 week mice are ear samples used as a control.

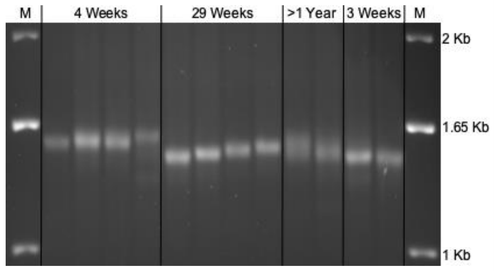


**Figure 4: Amplification of the GAA●TTC tract in the frataxin transgene.** Diluted and undiluted samples were run on the same gel to assess the ability to depict different size bands. 3 Week mice are ear samples used as a control.

- PCR amplification of the GAA●TTC tract in the frataxin transgene successfully shows distinctive bands.
- The mouse beta actin primer PCR is predictive of the necessity for dilutions of templet DNA. This supports the hypothesis that too much DNA was inhibiting the mouse actin primer PCR in some cases.

## Results

### Expansion in the Stool Samples

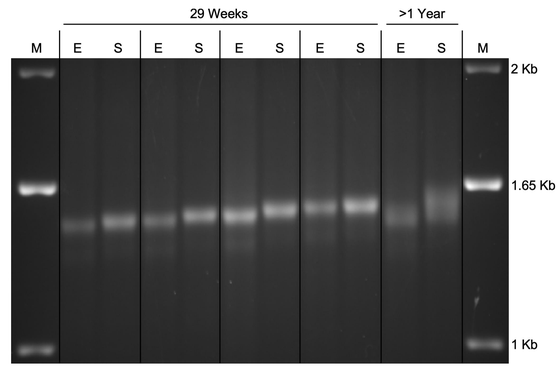


**Figure 5: Expansion in the Stool Samples.** Samples of ears (E) taken at three weeks are compared to samples of stool (S) from the same mouse at a later age.

- Stool Samples show DNA repeat expansion when compared to ear samples taken at three weeks of age.
- The oldest mouse shows more expansion than the younger mice.
- The oldest mouse is the father of the five younger mice. He has the lowest number of repeats in the three-week ear sample.

## Conclusions

### DNA repeat expansion was demonstrated

- Stool DNA poses a unique challenge due to the degradation of its components and high lipid content. For use in future projects, the "home-made" DNA extraction method is reliable.
- Mouse beta actin primers were predictive of the necessity for a dilution in the subsequent primers for the GAA●TTC tract in the frataxin gene.
- Nested PCR yielded the most reliable results.
- This protocol demonstrates expansion levels in as few as 29 weeks.

## Future Directions

### Longitudinal study of repeat expansion is now possible

- This method of DNA extraction and amplification can be used to monitor interventions over time.
- It is possible to conduct a longitudinal study showing expansion of the GAA●TTC tract in the frataxin gene and the subsequent halting of this expansion upon intervention.
- Further analysis is necessary to determine how sensitive this protocol is and how much differences in expansion can be identified in younger mice.

## Sources

1. Zhao, Xiaonan, et al. "Stool Is a Sensitive and Noninvasive Source of DNA for Monitoring Expansion in Repeat Expansion Disease Mouse Models." *Disease Models & Mechanisms*, vol. 15, no. 5, May 2022, p. dmm049453. *PubMed Central.*
2. Hart, Marcia L., et al. "Comparative Evaluation of DNA Extraction Methods from Feces of Multiple Host Species for Downstream Next-Generation Sequencing." *PLOS ONE*, vol. 10, no. 11, Nov. 2015, p. e0143334. *PLoS Journals.*

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