Pilot experiment for biomarker detection in buccal cells using Mawi's iSWAB Protein (ISWAB-P-1200) non-invasive sample collection kit.

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Materials and Methods

- Buccal samples collected using mawi's iSWAB – Protein (ISWAB-P-1200) per package instructions
- 1 male, 1 female subject
- Mouths were rinsed 2X with water
- Swabbing was performed 30 min after rinse
- Protein lysates processed 4 hours post-collection
- Samples were vortexed briefly
- Samples were centrifuged 1000x g, 1 min, 25°C
- Supernatant was transferred to a fresh 1.5 mL tube
- Aliquots were removed for Bradford assay
- Protein sample buffer was added to remaining sample
- Samples were boiled 3 min, 95°C
- Samples were loaded onto 4-12% Bis-Tris PAGE gels (50 µg/sample)
- Proteins were transferred to nitrocellulose membrane
- Membrane was stained with Ponceau S. following transfer
- Blots were probed for proteins of interest (Frataxin and GAPDH)

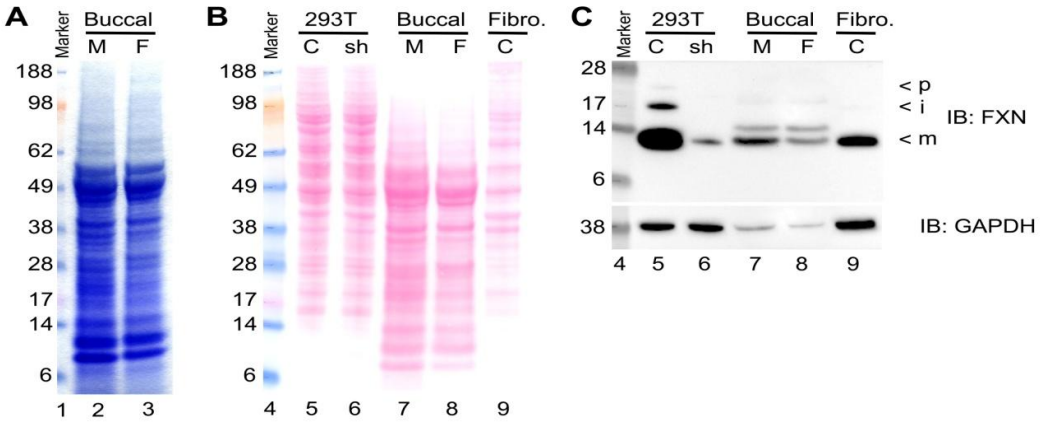

Figure 1. Western blot analysis of the mitochondrial protein Frataxin in buccal cell samples. Buccal cells were collected from 2 individuals (1 male, 1 female) using the Mawi iSWAB-Protein-1200 kit per manufacturers' recommendation. Samples were processed 4 hours post-collection, and 50 ug per sample were resolved by denaturing PAGE. **(A)** Lanes 1 - 3 were stained with Coommassie Brilliant Blue, which illustrates the wide molecular weight range of proteins represented in the lysate **(A)**. Lanes 4 - 9 were transferred to nitrocellulose and stained with Ponceau S. **(B)**, then immunoblotted for detection of Frataxin (precursor-p, intermediate-i, mature-m) and GAPDH **(C)**.

Lanes 2 - 3 and 7 - 8 - Mawi iSWAB-Protein lysates collected from 2 individuals
Lanes 5 and 6 - whole cell lysates prepared from 293T cells expressing a control (C) or FXN-specific shRNA (sh)
Lane 9 - whole cell lysate prepared from a control fibroblast cell line

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