Isohelix StoolFix kit stabilizes Faecal Microbiome DNA for 2 Months at Room Temperature

Introduction

Studies investigating individual gut microbiomes require the purification of microbial DNA from faecal samples. To ensure accurate results when sequencing, it is important that these samples are collected and stabilised correctly.

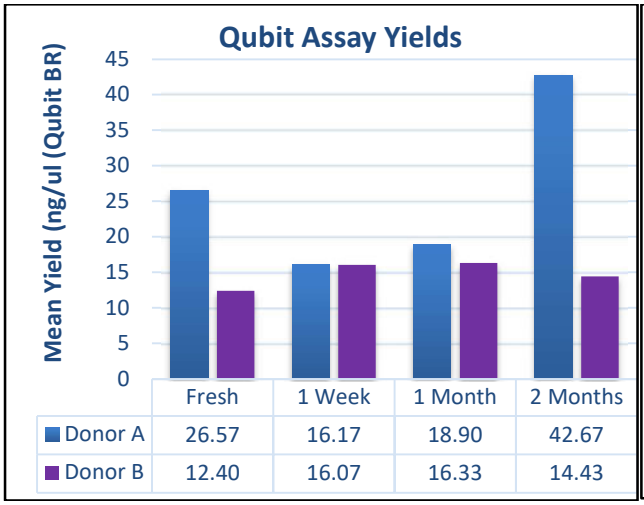
Isohelix™ DNA swabs and stabilization chemistries have recently been used in the non-invasive collection and preservation of faecal samples[1], and in the analysis of changes to individual microbiome diversity in disease states such as cervical cancer[2]. With the StoolFix stabilisation solution, this study aims to demonstrate the more general suitability of Isohelix products to stabilize DNA in Faecal samples for analysis of the gut microbiome.

Methods & Materials

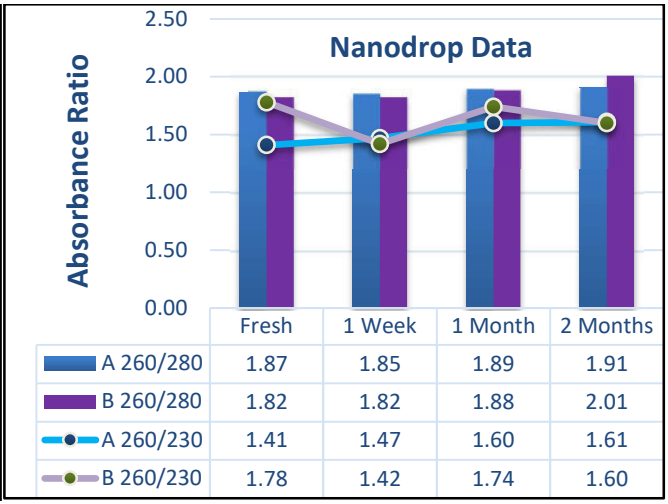
Isohelix swabs were used to collect faecal samples from two donors (A & B). Using a single stool sample for each, samples were collected by brushing lightly with each face of the swab. Following this, swabs were placed in StoolFix tubes and vortexed thoroughly. In total, twelve swabs were collected from each donor, which were then divided randomly into four sets of three. One set per donor were designated as a Fresh sample control, while the rest were incubated for a specified time at 37°C for the real-time equivalent of 1 Week, 1 Month, & 2 Months by accelerated ageing.

Following the incubation period, DNA samples were purified using the Isohelix™ BuccalFix-Plus Kit. Purified samples were then assessed for yield, purity, & quality via Nanodrop, Qubit BR DNA assay & 1.0% Agarose gel. Real-Time PCR reactions were then prepared for each sample isolated in the stability studies, alongside appropriate controls & *E. coli* gDNA standard. 1ng of sample DNA was used per reaction. The PCR primers chosen were from a region of the 16S gene that cover a broad range of bacterial genera.

Results

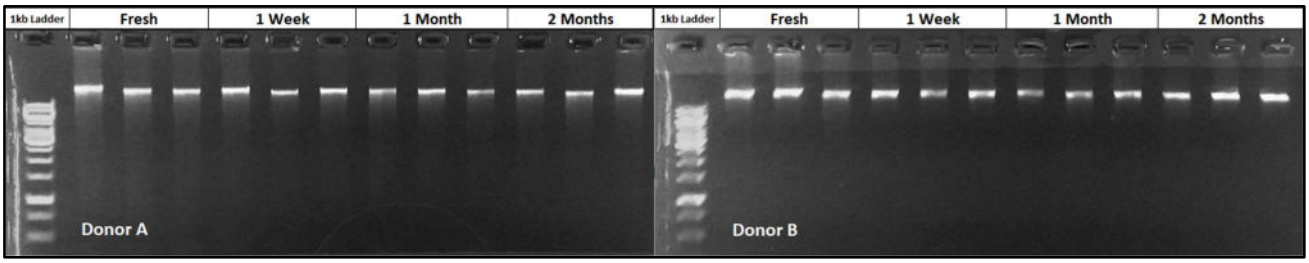


	Fresh	1 Week	1 Month	2 Months
Donor A	26.57	16.17	18.90	42.67
Donor B	12.40	16.07	16.33	14.43

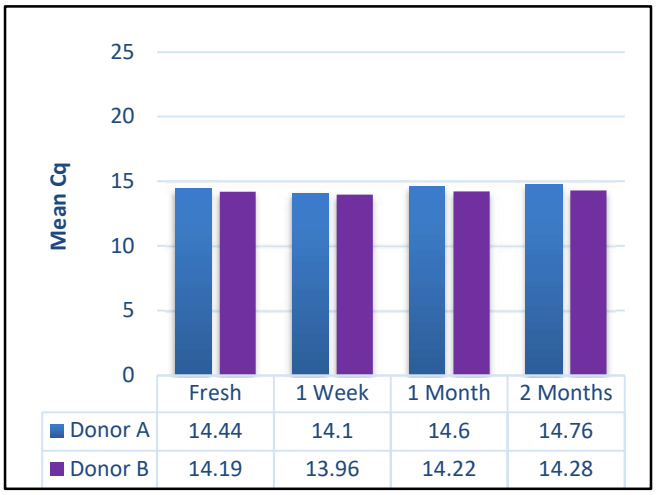


	Fresh	1 Week	1 Month	2 Months
A 260/280	1.87	1.85	1.89	1.91
B 260/280	1.82	1.82	1.88	2.01
A 260/230	1.41	1.47	1.60	1.61
B 260/230	1.78	1.42	1.74	1.60

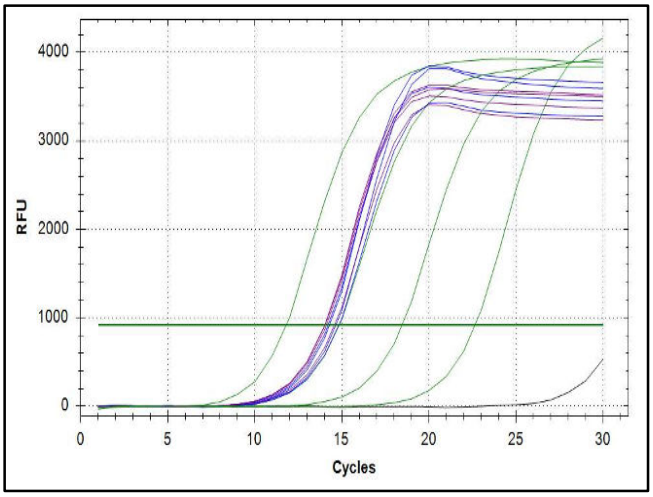
- Mean DNA concentrations of samples measured by Qubit show consistent yields across time points, with no signs of degradation between Fresh and Two Month samples.
- Nanodrop assay of mean A260/280 & A260/230 purity ratios show that, on average, samples maintain their high purity compared to Fresh samples over the two month testing period.



- 1.0% Agarose gel of 1ng/µl DNA purified from faecal samples stored in StoolFix solution, ranging from fresh samples to the real-time equivalent of 2 Months, display a single clear high molecular weight band for each sample, indicating high quality whole DNA.



	Fresh	1 Week	1 Month	2 Months
Donor A	14.44	14.1	14.6	14.76
Donor B	14.19	13.96	14.22	14.28



- Amplification data of 16S Real-Time PCR of samples, displaying mean Cq threshold data and the amplification plot of the tested samples, alongside a standard curve produced using serial dilutions of *E.coli* control gDNA.
- These data show that samples collected using Isohelix swabs and preserved in StoolFix amplify and maintain consistent Cq thresholds over the two month testing period. These samples could then be used for downstream 16S sequencing applications.

Conclusions

- **Isohelix swabs, combined with StoolFix stabilization buffer, can be effectively used to collect and preserve microbial DNA from faecal samples.**
- **Samples collected are stable at room temperature for up to two months while maintaining ideal yields, purity, & high quality. This emulates conditions *in vivo*, where there may be delays between sample collection and processing.**
- **16S Real-Time PCR of these samples demonstrate that Isohelix collection & preservation methods give reliable, consistent results.**

References:

1. Bourgeois, S *et al*. Improving cost-efficiency of faecal genotyping: New tools for elephant species. PLoS ONE 14(1): e0210811 (2019). https://doi.org/10.1371/journal.pone.0210811
2. Sims, TT *et al*. Gut microbial diversity and genus-level differences identified in cervical cancer patients versus healthy controls. Gynecologic Oncology 155(2): 237-244 (2019). https://doi.org/10.1016/j.ygyno.2019.09.002