OMNIgene®·GUT

OMNIgene®·GUT accurately captures and stabilizes the human fecal dsDNA virome

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Introduction

The human gut is inhabited by a tremendous variety of microorganisms that collectively form the gut microbiome. This diverse community is dominated by hundreds of bacterial species, while fungal, archaeal and viral species are also present but in lower relative abundance. The human virome remains poorly characterized and varies greatly between individuals. Double-stranded (ds) DNA bacteriophages of the *Siphoviridae*, *Podoviridae* and *Myoviridae* families are some of the main components of the human intestinal virome, and they are well known to play an important role in influencing the gut bacterial community by infecting their host and/or promoting horizontal gene transfer.[1,2] Moreover, recent evidence suggests that crAssphage-like bacteriophages are also highly abundant in the human gut.[3,4] In this technical note, we provide evidence that OMNIgene®·GUT stool collection devices are able to accurately capture and stabilize human gut viral dsDNA. In combination with our past data, OMNIgene·GUT collected samples are thus suitable for microbiome studies focusing on the characterization of both viral and bacterial profiles.

Materials and methods

Sample collection and storage

Fresh fecal samples from 6 crAssphage positive healthy adult donors were collected in 3 OMNIgene·GUT devices per donor. Baseline DNA extractions from fresh samples as well as OMNIgene·GUT collected samples were performed immediately using QIAamp® PowerFecal® Pro DNA Kit (QIAGEN®). OMNIgene·GUT collected samples were then incubated at room temperature for 60 days, at 50°C for 3 days or subjected to 6 cycles of freeze/thaw (with room temperature holds in between) to simulate shipping conditions. DNA extractions were performed after each of these treatments and compared to baseline. Alternatively, due to the lack of other viral signatures shared by a high enough proportion of donors, fresh fecal samples from 5 healthy adult donors were spiked with purified intact T3/T5 bacteriophages and collected in 3 OMNIgene·GUT devices. A bacteriophage-permissive *E. coli* strain was also co-spiked into these samples in order to ensure substantial presence of the phages' host. Samples were then processed using same methodology as the crAssphage positive fecal samples.

Real-time PCR and long-range PCR assays

Levels of crAssphage, T3 and T5 bacteriophages were measured in real-time PCR assays using 10 ng total DNA as template and primer pairs specific for each of these bacteriophages. A crAssphage-specific long-range PCR assay was also used to assess DNA stability in our chemistry (i.e., presence of high molecular weight DNA). Long-range PCR amplification of the crAssphage genome was performed with a different set of specific primers using LongAmp® *Taq* polymerase (NEB) and 50 ng of total DNA as template.

Results

OMNIgene·GUT captures the human virome at the point of collection without introducing a bias

We tested the ability of OMNIgene·GUT devices to capture and stabilize an endogenous virus such as crAssphage (sensu stricto). The relative levels of crAssphage were compared by real-time PCR in fresh and OMNIgene·GUT stabilized stool samples at baseline (T_0) and showed no significant differences (Figure 1). Similar results were seen with stool samples spiked with either T5 (*Siphoviridae* family) or T3 (*Podoviridae* family) bacteriophages (Figure 1 and data not shown). Taken together this demonstrates the ability of OMNIgene·GUT devices to capture viral profiles with no bias from the sample stabilization technology.

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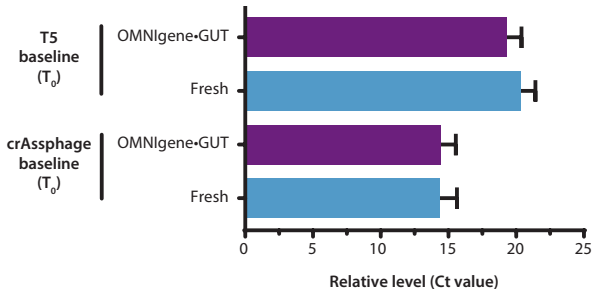

***Figure 1**: Relative levels of the crAssphage and T5 bacteriophages in fresh stool compared to OMNIgene•GUT collected samples at baseline (T_0). Total DNA was extracted from each sample using the QIAamp PowerFecal Pro DNA kit. Real-time PCR analysis was performed with primers specific for each bacteriophage using 10 ng total DNA as a template. No significant differences were seen using paired one-way ANOVA. n=5 for spiking experiments (T5 bacteriophage) and n=6 for crAssphage-positive samples.*

OMNIgene•GUT stabilizes viral DNA during transport and up to 60 days at room temperature

The ability of the OMNIgene•GUT kits to stabilize viral DNA during simulated transport and storage at room temperature for extended periods of time was tested by subjecting fecal samples collected in OMNIgene•GUT to elevated temperatures (50°C for 3 days), freezing (6 cycles of freeze/thaw interspersed with holds at room temperature) or long-term storage at room temperature (up to 60 days). For each of these conditions, total DNA was extracted and relative levels of the bacteriophages were measured by real-time PCR and compared to baseline levels (T_0). All of the simulated shipping and storage conditions tested had no impact on the relative levels of crAssphage or T5/T3 bacteriophages in OMNIgene•GUT collected samples (Figure 2 and data not shown), highlighting the ability of our chemistry to stabilize viral profiles over time. Importantly, extended incubation of the unstabilized samples at room temperature (14 days) led to a large increase in T5 bacteriophage levels following infection/lysis of the bacterial host. This demonstrates the need for proper stabilization to accurately measure gut viral profiles (Figure 2). No changes in crAssphage levels were observed in unstabilized samples; this is likely due to the fact that its host is predicted to be a member of the obligate anaerobic genus *Bacteroides*, and would not be expected to grow and/or survive in aerobic conditions.

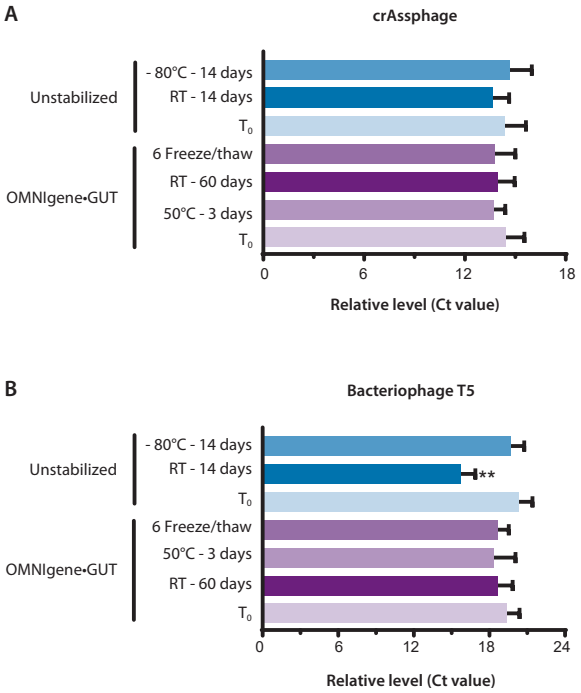

***Figure 2**: OMNIgene•GUT stabilizes crAssphage (A) and T5 bacteriophage (B) during simulated shipping conditions and storage at room temperature for 60 days. Total DNA was extracted from each sample using the QIAamp PowerFecal Pro DNA kit and real-time PCR analysis was performed with primers specific for crAssphage or T5 phage using 10 ng total DNA as a template. No significant differences were seen using paired one-way ANOVA for crAssphage (n=6) or T5 bacteriophage (n=5). However, a significant change was seen for samples left unstabilized, **p<0.01 paired one-way ANOVA.*

The presence of high molecular weight viral DNA was also assessed in these samples using a long-range PCR assay specific for a 9.5 kb fragment of the crAssphage genome. A high molecular weight PCR band was observed in OMNIgene•GUT stabilized samples at baseline, but also following incubation at room temperature for extended periods of time (60 days), subjected to high temperatures (50°C for 3 days) or 6 cycles of freeze/thaw (Figure 3). This indicates that our chemistry not only stabilizes viral profiles but also maintains the integrity of high molecular weight viral DNA.

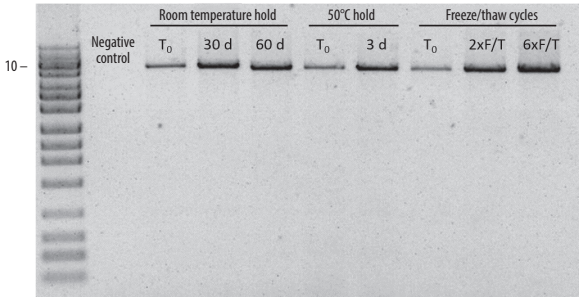

***Figure 3**: OMNIgene•GUT protects high molecular weight viral DNA during simulated shipping conditions and storage at room temperature for 60 days. Total DNA was extracted from each sample using the QIAamp PowerFecal Pro DNA kit and a crAssphage specific long-range PCR assay was performed using 50 ng total DNA as a template. Presence of a 9.5 kb PCR band indicates the presence of high molecular weight viral DNA in the sample. Data from a representative donor is shown here.*

Conclusions

Using representative viruses from some the main bacteriophage families of the human gut virome (crAssphage-like, *Siphoviridae-T5* and *Podoviridae-T3*), we demonstrate that OMNIgene•GUT devices capture and stabilize the viral profiles:

- No detectable bias in viral levels are introduced at the point-of-collection.
- High molecular weight double-stranded viral DNA is stabilized in our chemistry for up to 60 days at room temperature or when subjected to harsher simulated shipping conditions (high temperature or multiple freeze/thaw).

OMNIgene•GUT devices are therefore highly suitable for gut virome studies focusing on dsDNA viruses.

References

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4 Yutin N. et al. Discovery of an expansive bacteriophage family that includes the most abundant viruses from the human gut. *Nat. Microbiol*. 3:38–46. (2018)

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MK-AN-00035 Issue 1/2019-01