**CrAssphage summary**

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**Methods:**

Inflow sewerage samples were provided from Dunedin, Invercargill, Christchurch and Greymouth, which are all located in the South Island of New Zealand.

Primers were ordered from IDT and used in PCR following the protocol provided.

Forward and reverse primers were used for sequencing of all products at the Massey Genome Service, Massey University, New Zealand.

**Results:**

The samples from Dunedin and Greymouth gave bands of the correct sizes for all three pairs of primers (gels can be provided if required).

Invercargill and Christchurch gave no bands. We then repeated this as per the advised protocol and also in a protocol where we increased the template material 2-fold. Whereas the controls with the standard amount of template worked, nothing worked with double template (including controls). Invercargill and Christchurch still gave no bands under any condition.

All products from Dunedin and Greymouth were sequenced. Products B and C gave good reads in both directions and were automatically assembled in geneious. For all sequences there are quite a few places where the consensus is mixed due to a SNP at that point of the population. In some cases, the consensus base is shown, even though there might be a mix at that position (see raw reads).

We have provided the 12 sequencing reads and the 4 consensus files for product B and C for these populations. Product A gave good sequence with the reverse primer and some good sequence with the F primer. However, even after doing this 3-4 times, the F primer gives nice sequence for ~230 bp then becomes mixed.

Visual analysis of the sanger reads of product A from both locations suggest that there is a population of two different sequences (*see sequence below* – end of good sequence in bold uppercase). This short region has a difference of 6 extra bp in one of the populations and a bit of variation in that area. It then appears that the sequences are then similar to crassphage again. This insertion in some member of the PCR population lead to “jumbled” sequence after this point. It would be possible for us to clone this to resolve this issue if you are particularly interested or if you think it is very important. However, if you are not so worried, we would rather not do it.

Product A two read population…

**GTTATTTATAAA** aaanttaatggncntgaacctttatggt gtagaagtgttgaaagttttaaaagtcattttagtgatgctaaagttgaagg...

**GTTATTTATAAA**catnaagctagngnatctgatattttatggtggtgtagaattgttgaaagttttaaaagtcattttagtgatgctaaagttgaagg...