**DRAFT PROTOCOL**

**Extraction of DNA from RUSITEC liquid and solid samples**

Background:

This protocol was selected because Vaidya *et al.* (2018) showed very similar results when comparing a soil DNA extraction kit with other DNA extraction methods, such as repeated bead beating (Yu and Morrison, 2004) and PQIAmini (Zoetendal *et al.,* 2006), using liquid and solid (fibrous) samples from the cow rumen. The soil DNA extraction kit is the most convenient in terms of time and equipment. There are a few modifications we have made compared to what was tested by Vaidya *et al.* First, we are using the PowerSoil (or PowerSoil Pro) kit, as this kit has led to high quality sequencing data in recent RUSITEC projects in the Mann group at the VetMed (ex. Wetzels *et al.*, 2018). Secondly, we have eliminated the liquid nitrogen freezing and grinding (mortal and pestle) step from Vaidya *et al.* for the solid samples, as this was done to facilitate weighing, but we found this to not be a problem with the regular frozen samples and still recovered sufficient DNA.

An additional upcoming modifications may be to add a PBS washing step to the solid fraction extraction protocol. This modifications will depend on how the DMD disappearance is done.

Protocols:

Liquid fraction:

1. Pellet the cells from 1mL of the liquid sample by centrifuging at 8000 rpm for 5 min.
2. The cell pellet is then used as the starting material for the PowerSoil kit by resuspending in Solution C1 (CD1 in PowerSoil Pro) and transferring to a PowerBead (or PowerBead Pro) tube.
3. Follow the instructions on the PowerSoil (or PowerSoil Pro) kit with the exception of the final elution step.
4. For elution, use 50µl 70°C DEPC water, instead of solution C6, and centrifuge at 10000 rpm for 1 min.
5. Freeze the purified DNA at 20°C.

Solid fraction:

1. Weigh out 0.2 g of the solid (fibrous) fraction. This is best done when the samples are still frozen, as you can cut or break of small pieces more easily.
2. The 0.2 g sample is then used as the starting material for the PowerSoil (or Power Soil Pro)
3. Follow the instructions on the PowerSoil (or PowerSoil Pro) kit with the exception of the final elution step.
4. For elution, use 50µl 70°C DEPC water, instead of solution C6, and centrifuge at 10000 rpm for 1 min.
5. Freeze the purified DNA at -20°C.

Solid fraction with washing (testing / in consideration):

1. Weigh out 0.2 g of the solid (fibrous) fraction. This is best done when the samples are still frozen, as you can cut or break of small pieces more easily.
2. In a 2 mL Epi tube, add 1mL of 4°C PBS and vortex briefly.
3. Centrifuge at 8000 rpm for 30 seconds.
4. Remove the supernatant and transfer to a clean, labelled Epi tube.
5. Repeat steps 2-4 two additional times, for a total of 3 washes.
6. Store all supernatant samples at -20°C. These will later be pooled and included during DNA extraction of negative controls.
7. The pellet from the original 0.2 g sample is then used as the starting material for the PowerSoil (or Power Soil Pro)
8. Follow the instructions on the PowerSoil (or PowerSoil Pro) kit with the exception of the final elution step.
9. For elution, use 50µl 70°C DEPC water, instead of solution C6, and centrifuge at 10000 rpm for 1 min.
10. Freeze the purified DNA at -20°C.