**2017 Hjelmso - Evaluation of Methods for the Concentration and Extraction of Viruses from Sewage in the Context of Metagenomic Sequencing**

* They test out four concentration methods and four extraction kits to do wastewater genomics
* Methods/results
  + Collected sewage and spiked it with norovirus
  + Concentrated virions in four ways \*all treated with OmniCleave endonuclease to remove extracellular DNA/RNA and further purified to remove nucleases and inhibitors -> all concentration methods end up being very different
    - Protein precipitation with PEG: precipitate out viruses form filtered supernatant with PEG and NaCl
    - Organic flocculation with skim milk flocculation (SMF) and filtration: flocculated skim milk added to sewage, virus ends up in pellet after incubation in acidic solution, resuspend viruses in neutral buffer prior to storing
    - Monolithic absorption filtration (MAF): use custom charged filters to trop the viruses from the wastewater, then ellute out the viruses from the filter.
    - Glass wool filtration (GW): glass wool is packed in PVC tube, sewage passes through, viruses get stuck, elute viruses
  + Extraction methods
    - NUC (Macherey-Nagel, Du ̈ren, Germany) -> clusters away from other kits
    - QIA (Qiagen, Valen- cia CA, USA)
    - MIN (BioMerieux, Herlev, Denmark)
    - POW (MO BIO, Carlsbad, CA, USA).
  + qRT-PCR/qPCR.
    - Made DNA with SuperScript III
    - 2nd strand DNA synthesis withKlenoe Fragment exo-polymerase
    - dsDNA amplified with AmpliTaq Gold
    - Clean and concentrate PCR product
    - Prepare next generation sequencing library with Nextera XT DNA Library Preparation kit
  + Bioinfomatics
    - MGmapper tool, cutadapt quality control, compare sequences to NCBI database (70% similarity)