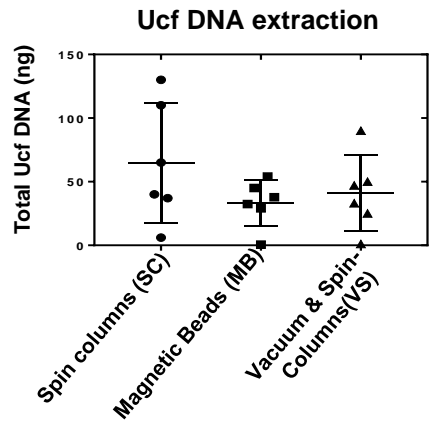


Different methods showed similar average yields for urinary cell-free DNA (Ucf DNA).

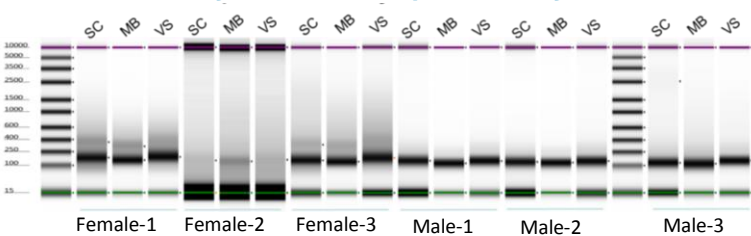
- First morning First void urine samples (FMMV)
- Picoquant analysis for cell-free DNA recovery.



Colli-Pee device (Novosanis)

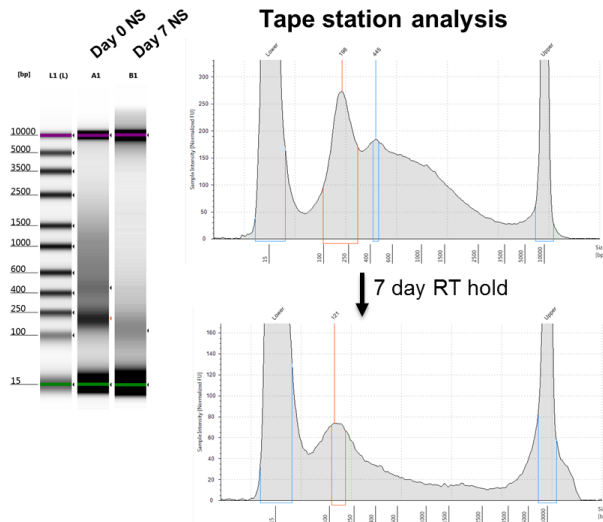
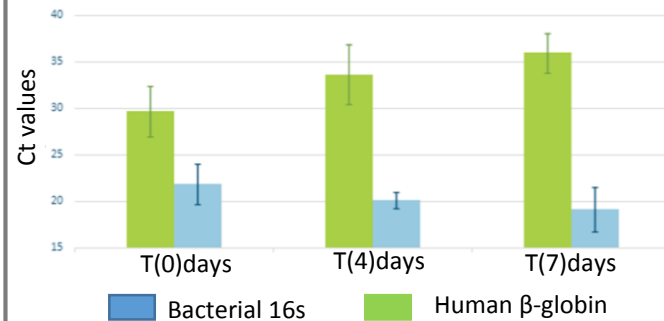


Urinary cell-free DNA profile analysis



Average size of cell free DNA was found to be shorter in both SC (~137 bp) and MB (~125 bp) extraction when compared with VS (~150 bp).

Bacterial overgrowth/lysis & loss in quantity and profile of human cell-free DNA in unstabilized urine samples



Principles for stabilization

Lytic

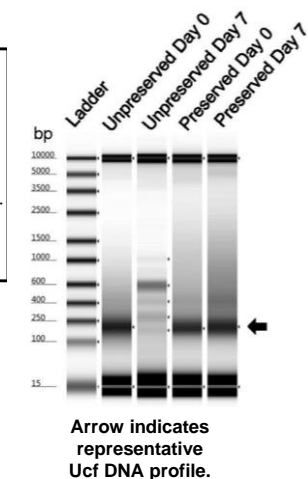
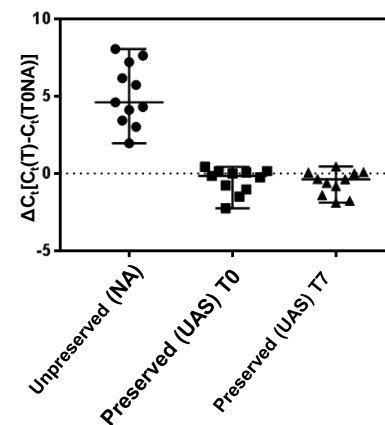
- Pros**
- Simple principle. Typically use of chaotropes & detergent containing solution.
 - Effectively preserves total DNA and RNA in urine at ambient temperatures.
 - Inhibits microbial growth during long-term (cold-free) storage of urine samples
- Cons**
- Requires up-front separation of extracellular & intracellular fraction (e.g. centrifugation); hence not ideal for home sampling kit.
 - Not suitable for effective preparation of intact cellular pellets for downstream processing (e.g. isolation of total bacterial DNA from the pellets for microbiome studies, cellular RNA for transcriptomic etc..)

Non-lytic

- Inhibits microbial growth during long-term (cold-free) storage of urine samples
- Preserve both cellular and extracellular fractions (DNA, RNA).
- Can also preserve intact extracellular bodies (exosomes, microvesicles).
- Complex and difficult principle, but effective for separation of intra and extra cellular space.

DNA Genotek's proprietary urinary analyte stabilizer (UAS) preserves detection of Ucf-DNA for 7 days at RT.

Ucf DNA content



- First morning/ random First void urine samples with and without UAS
- QiaAmp circulating NA kit based extraction
- Quantification: qPCR assay on β -globin gene.