

Supplementary Material 1. Extraction protocol of eDNA lysates at UIBK using the Qiagen BioSprint® 96 Workstation.

Depending on the amount of lysate, prepare several bind plates (each bind plate will hold 200 µL of lysate, adjust accordingly).

Create the master mix: 300 µL AL Buffer, 300 µL Isopropanol.

Add 30 µL MagAttract magnetic particles (vortex briefly) to each well in the first bind plate.

Add 200 µL lysate to each of the Bind plates. Fill up missing lysate volumes with TES buffer and note the lysate volume for each sample on laboratory metadata.

Fill the other blocks/plates with the following:

Reagent	BIOSPRINT code	Volume	Location
Master mix + Lysate	BIND PLATE (Lysate)	630 + 200 µL	S-Block
AW1 Buffer*	WASH 1+2	650 µL + 500 µL	S-Block + S-Block
AW2 Buffer*	WASH 3+4	500 µL + 500 µL	S-Block + S-Block
RNase free water + Tween 20**	WASH 5	500 µL	S-Block
1× TE-Buffer (or AE)	ELUTION	100 µL	Microplate
Rod Cover <input type="checkbox"/> <i>NEW</i>	ROD COVER		Microplate <input type="checkbox"/> <i>re-use</i>

*AW1 and AW2 Buffer-concentrates have to be diluted with ethanol.

**RNase free water=MilliQ water; add Tween 20 to a final concentration of 0.02%.

Put Rod cover into the first Bind plate, start the right “DNA uptake” program according to the number of Bind plates (2-7 are available). At the end of DNA uptake, the DNA and the Rod cover are in the last Bind plate.

Start the “eDNA extract” program, add the last bind plate with the rod cover and all other plates.

Put processed extracts into Eppendorf tubes®

Afterwards:

Carefully dispose of toxic waste.

Wash blocks/plates several times with water and subsequently incubate in a bleach-water bath. After 1 day, wash the blocks and plates in the dishwasher for reuse.

For further information, please refer to Qiagen (2012) BioSprint® 96 DNA Handbook. Available via Qiagen:

<https://www.qiagen.com/us/resources/resourcedetail?id=64902c5d-9c3c-4fe3-a3f7-668c4704d9eb&lang=en>