Bio299: Is the Succinate Dehydrogenase A gene transcribed in the virus PkV RF01?

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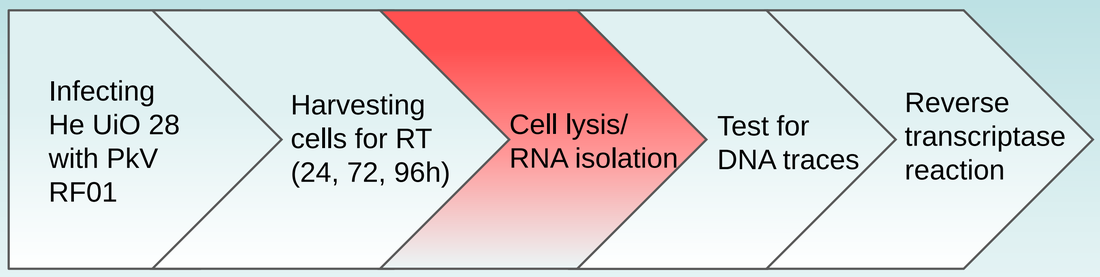
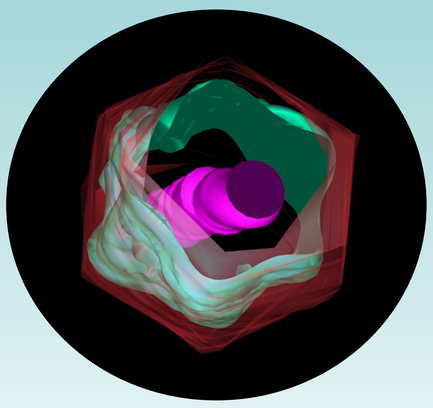
1 EXPERIMENTAL DESIGN

X Ambion cells to CT kit
X RNeasy Plant Mini Kit (Qiagen)
RNeasy Plus Universal Tissue Mini Kit (Qiagen)
In combination with TURBO DNA-free™ Kit (Ambion)



X – kits that did not work

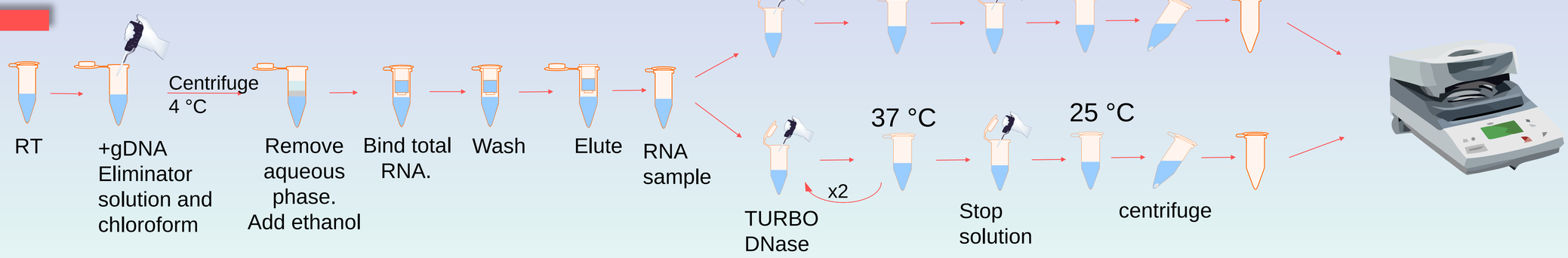
THE VIRUS



61 pictures, -60-60^{o}, image every 2^{0}

Structure of PkV RF01, determined by Cryo-tomography, showing an outer membrane (red), inner membrane consisting of a bunch of convoluted membranes (green) and a internal rod shaped core filled with dense material (pink) (Antje Hofgaard, UiO).

2 RNA ISOLATION



3 RESULTS

Yes!

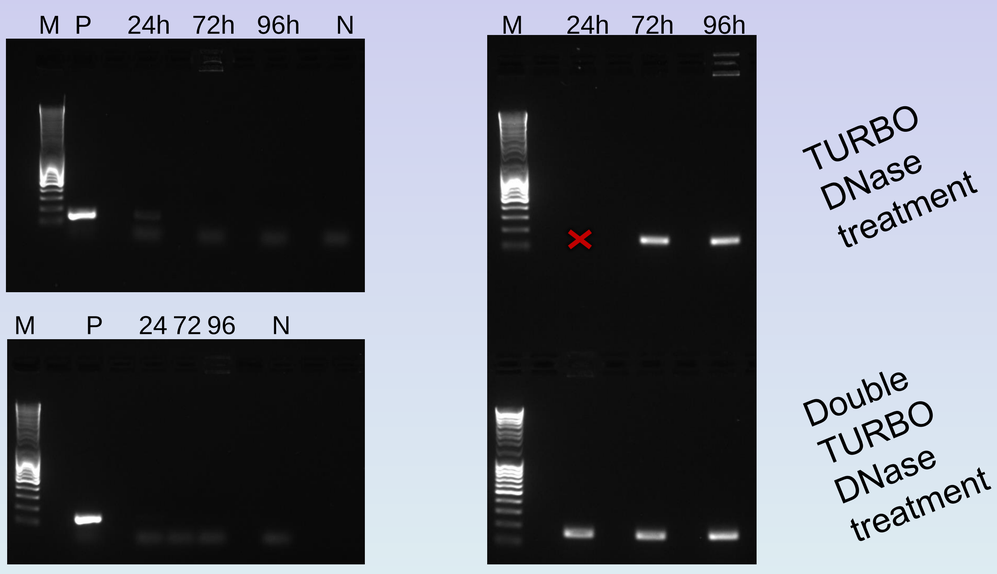


Figure 1. Results from Reverse transcriptase PCR. The kits were first optimized for the MCP control gene. The samples were subjected to RNA isolation followed by TURBO DNase treatment and RT-PCR with sdhA specific primers. A: control of DNA traces in samples harvested after 24h, 72h and 96h. B: RT of samples harvested after 72h and 96h (TURBO DNase treatment on top) and 24h, 72h and 96h (double TURBO DNase treatment).

BACKGROUND

Prymnesium kappa virus RF01 (PkVRF01) isolated from Raunefjorden, Norway. PkV RF01 infects *H. ericina* (the host used in this experiment) and has a capsid of ~310 nm. This is the largest algal virus particle ever reported. (Johannessen et al, 2015.)

Succinate dehydrogenase complex (SDH) is a membrane-bound protein complex composed of four subunits (A, B, C, D). The SDH complex catalyzes a step in both the citric acid cycle and oxidative phosphorylation, and is thus very important for energy metabolism. The A subunit in question contains a binding site for the succinate substrate. (Nelson et al, 2017).

Reverse transcriptase reaction (RT) is a method where a mRNA template is used to create complementary DNA (cDNA). The cDNA complementary to the mRNA of interest is then amplified in a polymerase chain reaction (PCR).

REFERENCES
1 Johannessen et al 2015, Virology doi: 10.1016/j.virol.2014.12.014.
2. Nelson, D.L., Lehninger, A.L. & Cox, M.M., 2017. Lehninger principles of biochemistry 7th int., New York: W.H. Freeman., p.719

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