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Project: iGEM_Munich2019 Shared Project
Authors: Theresa Keil

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RNA isolation out of lysed and unlysed VLP and exosomes

- starting samples V11 lysed cells
- V11 unlysed supernatant
- E26 lysed supernatant
- E26 lysed cells

two different RNA isolation techniques were tried out

1 RNeasy Mini Kit for samples
2 Trizol treatmen tfor samples E26 lysed supernatant + lysed cells V11 unlysed supernatant
Protocol for trizol treatment see
concentration: V11 unlysed supernatant 400 ng/µl
E26 lysed supernatant 426 ng/µl
E26 lysed cells 1520 ng/µl

samples purified via RNeasy Mini Kit were discarded because no RNA could be measured at nanodrop
The samples form Trizol treatment showed RNA yields in the range of measurements above
the samples from trizol treatment and aliquot of the in vitro transcription divided in two samples
-> are used for reverse transcription via super script II first strand synthesis supermix
procedure according to protocol
sample: ivT, VLPs, Exosomes, all from other exosome group transfected with constructs
4 samples and 4 no RT as negative control
added 1 µg of RNA to each sample

qPCR
setup of master mix: 2x MM 650 µl
10µM Primer fw: 6,5 µl
10 µM Primer rev: 19.5 µl
H2O: 494 µl
dilution serie in 10 steps form 5E0 copies/µl to 5E7 copies /µlof ivT cDNA
technical failure due to the well not being closed of by sealing foil