Green Lab Nanoparticle For 6 Well Cardiomyocyte Transfection v2 V.2

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Protocol status: Working
We use this protocol and it's working

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ABSTRACT

nanoparticle transfection

MATERIALS

Sodium acetate 25mM pH5
Polymer stock (100 ug/ul)
Desired media

Nanoparticle Synthesis

1 Defining Terms

- DNA refers to nucleic acid cargo encoding genes of interest, generally stored at a concentration of 1 ug/ul.
- Polymer refers to the Poly(beta-amino ester) (PBAE) that makes up the backbone of the nanoparticle.
- Nanoparticle refers to the final complexed polymer + DNA that is ready for transfection.

2 DNA Dilution

For 3 technical replicate wells of one condition in 6-well plate format, mix the following in microcentrifuge tube.

- 60 ul DNA at 1ug/ul
- 440 ul sodium acetate
- Total volume = 500 ul for 3 technical replicate wells per one condition

3 Polymer Dilution (7.2 mg.ml polymer concentration in NaAc)

For 3 technical replicate wells of one condition in 6-well plate format, mix the following in microcentrifuge tube.

- 40 ul polymer (e.g. 4-5-6)
- 460 ul sodium acetate
- Total volume = 500 ul for 3 technical replicate wells per one condition

4 Nanoparticle (DNA+polymer) Synthesis

Combine diluted DNA with diluted polymer

- Add 500 ul of diluted DNA to 500 ul diluted polymer (can freeze here at -80 at your own risk)
- Add 5000 ul of serum containing media to 1000 ul NP's for final volume master mix of 6000 ul per condition
- Incubate for 10 minutes

5 Transfection

- Change media from each well of 6 well plate with 2000 ul nanoparticle master mix (media + nanoparticles)
- Incubate for 24 hours or desired time
- Change media after desired transfection time.