Mar 29, 2019

Biolistic Transfection in *Euplotes crassus* (provisional)

In 1 collection

DOI
dx.doi.org/10.17504/protocols.io.zk6f4ze

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Protocol Citation: Angela Piersanti 2019. Biolistic Transfection in Euplotes crassus (provisional). **protocols.io https://dx.doi.org/10.17504/protocols.io.zk6f4ze**

Protocol status: In development
We are still developing and optimizing this protocol

Created: March 29, 2019

Last Modified: March 29, 2019

Protocol Integer ID: 21886

Keywords: Biolistic, golden nanoparticles, biolistic transfection in euplotes crassus, euplotes crassus, euplote, biolistic transfection, transfection

Troubleshooting

1 10^5 vegetative *Euplotes crassus* cells or 10^5 mating DP1 and DP3 *E. crassus* strains (50 h after mixing) were placed on a filter paper soaked with 10 mM HEPES pH 7.4.

2 Shooting conditions were set as follows in the Bio-Rad Biolistic PDS-1000/He Particle Delivery System : rupture disk 1550 psi, helium pressure 1750 psi, vacuum 26 inches Hg, gap distance ⅜ inches, 1st shelf.

3 They were shot with 0.6 µm or 1.6 µm golden nanoparticles coated with 1.25 µg of the construct containing G418 resistance gene.

4 The filter paper was then fold and placed into 50 ml of sea water. After the transfection more than 50% of the cells were alive.

5 The antibiotic selection started after 24 h; increasing concentrations of G418 were added every 4 days, from 1 mg/ml up to 10 mg/ml.