Biolistic Transfection in *Euplotes crassus* (provisional)

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In devel.

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Protist Research to Optimize Tools in Genetics (PROT-G)

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PROTOCOL STATUS

In development

We are still developing and optimizing this protocol

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.