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## Biolistic Transfection in *Euplotes crassus* (provisional)

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Angela Piersanti<sup>1</sup>

<sup>1</sup>University of Camerino

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Angela Piersanti

University of Camerino

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**Protocol status:** In development

**We are still developing and optimizing this protocol**

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## 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  $\frac{3}{8}$  inches, 1st shelf.
- 3 They were shot with 0.6  $\mu\text{m}$  or 1.6  $\mu\text{m}$  golden nanoparticles coated with 1.25  $\mu\text{g}$  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.