Jun 25, 2019

Cell deposition and drug sensitivity assay

Lev Tsypin[1], Aaron Turkewitz[2]

[1]California Institute of Technology, [2]University of Chicago

Working dx.doi.org/10.17504/protocols.io.336gqre

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

Lev Tsypin

ABSTRACT

This protocol describes how to deposit several cells of *S. robusta* in droplets on a petri dish, allowing the experimenter to track the growth of approximately single cell isolates with high throughput. This was used to determine drug sensitivity.

We found that in, liquid culture, *S. robusta* is sensitive to 10 ug/mL puromycin and 250 ug/mL glyphosate. One caveat to keep in mind is that *S. robusta* appears more tolerant to antibiotics when grown on agar plates, being insensitive to up to 20 ug/mL of puromycin, for example. We recommend selection in liquid culture.

MATERIALS

NAME	CATALOG #	VENDOR
Puromycin dihydrochloride	P9200	US Biological
Hygromycin B	h397	Phytotech Labs
Glyphosate	g345	Phytotech Labs

Measure density of floating cells in culture

1 Grow 100 mL volume of cells in a 175 cm2 flask for three to four days, until there is a substantial number of healthy, floating cells

2 Stand flask vertically and take a 15 mL volume of the floating culture (so as to not disturb the adherent cells) to measure cell density

3 Spin cells down at 6000xg to pellet them. Scrape and resuspend in 1 mL

4 Measure cell density using a hemocytometer. The target concentration for distributing cells is 400-500 floating cells/mL, so dilute as necessary. The volume needed is 2 mL, including any drugs you want to add.

Deposit cells in droplets on a petri dish

5 b. Take the 2 mL, containing the desired number of cells, in an Eppendorf tube.

6 Centrifuge using a personal mini-centrifuge for two minutes. At this speed (which I believe is 6000 rpm), many cells remain in the supernatant. Those are the cells we want. Do not disturb any pellet that forms from the centrifugation.

7 Using a Pipetteman, withdraw 40µl aliquots from near the top of the Eppendorf tube. Deposit 40µl drops in a 6x8 array in a Petri dish.

Count initial cells

8 Record the approximate number of cells deposited in each drop.

9 In our hands, this is ~4-10 cells per droplet on average

10 Wrap petri dish with parafilm and place in incubator

Assay

11 After three days of treatment, count cell numbers/determine cell viability in each droplet