



Apr 29, 2022

Antibiotic Sensitivity Assay for *Heterometopus palaeformis* (strain RAJCA)

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dx.doi.org/10.17504/protocols.io.x54v9y3mzg3e/v1

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

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Goal: To determine the optimal concentration of three antibiotics, Puromycin, Geneticin (G418) and Blasticidin, that will effectively inhibit the cells growth and division of *Heterometopus palaeformis*.

We examined the cells viability and growth rate in the presence of different concentrations of these antibiotics (Table 1, 2 &3). These antibiotics are routinely used for selecting the transformed eukaryotic cells. The choice of puromycin was based on previous transfection protocol on ciliate, *T. thermophila* (Iwamoto et al., 2014; *Gene*). The Geneticin (G418) was used by our group for selecting transformed *Bodo saltans* with plasmids carrying neomycin resistance gene (*Neo*).

Procedure:

- The experiments were carried in 5-ml Eppendorf tubes to cut down on the volume of the antibiotic that will be used.
- The tubes were filled with 4 ml cerophyl culture medium.
- Add the antibiotics at the final concentrations shown in the tables below.
- In total there were 6-7 tubes per assay, including the control with no antibiotic treatment.
- Transfer 1ml of cells from RAJCA culture at log phase to each of the tubes.
- Inspect the cells every couple of days under inverted microscope to check for any evidence of cell toxicity and count the number of live cells.
- The optimal dose of antibiotic for selection is the lowest one for which all cells have died after 7 to 12 days. A low dose is the concentration which has minimal effects on cells after 2 weeks of antibiotic treatment. Conversely, a high dose is a concentration which is highly toxic to cells within 1-3 days of starting antibiotic treatment.

Results:

A-Puromycin

Results indicate that puromycin at concentration of 300 µg/ml kills the entire population in 10 to 12 days. Using lower puromycin concentration (i. e., 200 µg/mL)

was inefficient as the cells maintained to grow for longer that two weeks.

Table 1

A	B
Puromycin ($\mu\text{g/ml}$)	Cells survival (days) Exp.1 – Exp.2
300	10-12
200	16-12
100	>16->16
50	>16->16
20	>16->16
5	>16->16
0	>16->16

B-Geneticin (G418)

G418 is an effective antibiotic to kill the *H. palaeformis* wild type cells at different concentrations. Our results show that G418 of concentration 20 $\mu\text{g/mL}$ kills the entire population of cells in approximately 10 days, which is considered enough time for the selection of resistant cells after transfection. We will use this antibiotic as a second choice if needed after the puromycin.

Table 2

Geneticin/ G418 ($\mu\text{g/ml}$)	Cells survival (days)
200	<4
100	<4
50	6
20	10
10	14
0	>16

C-Blasticidin

Blasticidin was supplemented at different concentrations to *H. palaeformis* cultures. However, our results show that blasticidin didn't have any lethal effect on the cells. Although the growth rate for the cells in presence of the blasticidin were lower than the control cultures (based on cell count). But cells remained alive and active for more than 2 weeks period at the highest concentration that we used (300 $\mu\text{g/ml}$). Therefore,

we excluded Blastidicin from our list.

Table 3

Blasticidin (µg/ml)	Cells survival (days)
300	>16
200	>16
100	>16
50	>16
10	>16
0	>16

DOI

[dx.doi.org/10.17504/protocols.io.x54v9y3mzg3e/v1](https://doi.org/10.17504/protocols.io.x54v9y3mzg3e/v1)

Fatma Gomaa, Johana Rotterova, Roxanne Berinate, Maria Pachiadaki, Virginia Edgcomb 2022. Antibiotic Sensitivity Assay for *Heterometopus palaeformis* (strain RAJCA). **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.x54v9y3mzg3e/v1>



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