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Optimizing the electroporation parameters for *Heterometopus palaeformis* (strain RAJCA)

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Cell viability after incubation with different electroporation buffers,
Phosphate buffered saline (PBS) is used as an electroporation buffer for many eukaryotic cells. However, we observed that the PBS has a detrimental effect on the cell, causing disruption in *H. palaeformis* cell membrane. We then tested the tolerance of *H. palaeformis* to three different electroporation buffers:
A-Gene Pulser Electroporation Buffer *(BIO-RAD, USA)*
B-Iso-osmolar Buffer *(Eppendorf, USA)*
C-Hypo-osmolar Buffer *(Eppendorf, USA)*
Cells were incubated in each buffer independently for 10 minutes and observed for their viability under inverted microscopy. For all the three tested buffers, we noticed that once buffer was added, the cells showed instant osmic shock, include loss of motility and relative shrinking in the cell membrane (Fig. 1). After 10 minutes incubation period in each buffer, cells were transferred to their Cerophyl culture medium to recover. We observed progressive recovery of the cells. Table 1 shows the time that took the cells to completely recovered and the percentages of the recovered cells after incubation at the three different electroporation buffers.

Table 1

Electroporation buffer	Duration of recovery	Percentage of the recovered cells
Gene Pulser	10 minutes	>90%
Iso-osmolar	10 minutes	70%
Hypo-osmolar	2-5 minutes	70%

Fig.1. Light microscopy images showing the effect of the different electroporation buffer on the *H. palaeformis* cell membrane; PBS has detrimental effect on the cell causing disruption of cell membrane.

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