

Dear Researcher,

Thank you for requesting the DNA plasmid for mammalian expression of Perceval, our fluorescent sensor of the ATP:ADP ratio.

The protein sequence is as published in Berg, Hung, and Yellen, *Nature Methods* 2009 Feb (see the supplement for details). The vector is GW1, a CMV-based vector originally from British Biotech. If a different vector is to be used, the full coding region (with a bit of 5' UTR) can be transferred as a 1.9kb HindIII - EcoRI fragment.

Things to note:

1. Perceval is pH sensitive, as described in our paper and the supplement. It is important to make a simultaneous measurement of pH, as otherwise a pH change can mimic a change in ATP:ADP.
2. The half-maximal response of Perceval occurs at an ATP:ADP ratio of ~ 0.5 . This is very low for most mammalian cells, so the current version is more suited to detect starvation than subtle changes in ATP:ADP. Bacteria and invertebrate cells probably have lower levels of ATP:ADP even in well-fed cells, and the sensor may show a better response to mild energy deprivation in these systems.
3. Perceval contains several cysteines. If you intend to use it in mitochondria or in highly-stressed cells, it may be important to check its sensitivity to ROS.

We hope that you find the sensor useful! We would appreciate hearing if you get any exciting results with Perceval, or if you have problems with it.

With best wishes,

Gary Yellen, Jim Berg, and Yin Pun Hung

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