Kaede – a photoconvertible fluorescent tracer (Jon Clarke, UCL, UK)

Kaede is a naturally fluorescent protein cloned from stony coral that appears to have no toxicity when expressed in zebrafish embryos. The native form of the protein fluoresces green, but on exposure to UV light (350-400nm) a cleavage event converts the protein to a shorter form that fluoresces bright red. The green to red conversion is irreversible and the red protein is very stable (we have detected it 4 days after photoconversion in the zebrafish forebrain). Kaede protein readily diffuses throughout cells thus labeling all cell processes such as axons and dendrites. These properties suggest that Kaede should be a very useful non-invasive tool for cell tracking experiments in the zebrafish embryo. Since photoconversion is efficient using a standard DAPI filter set, the only equipment needed is a standard epifluorescent microscope. Excitation with either FITC or TRITC filter blocks does not lead to photoconversion, thus allowing observation of the green and red fluorescent cells without further altering Kaede fluorescence. Photoconversion is also possible on a confocal microscope with a UV laser line.

Protocol

1. Inject Kaede RNA (50ng/ul) into an embryo at early cleavage stages. Anytime from the 1 to 128 cell stage is fine. The later the RNA is injected the more mosaic the expression of green Kaede will be and for some experiments this is a significant advantage.

2. Incubate embryos **in the dark** until appropriate stage. It's a good idea to wrap Petri dishes in baking foil to minimise exposure to UV light. It is good to be paranoid about this as you want to minimize background photoconversion.

3. Select embryos with the brightest green fluorescence and embed these in 0.5 – 1% low melting point agarose. Orient target area to the top. During this and all subsequent stages turn any room fluorescent lights off.

4. Using a FITC filter set locate the cells to be traced. Once the field of cells has been identified, reduce the diameter of diaphragm in the epifluorescence light path such that only the target cells are illuminated and in focus in the centre of the field of view. Switch to a DAPI filter block and photoconvert for approximately 2 minutes. As photoconversion proceeds the cells will appear pink under the DAPI filter set. The time required for complete green-to-red conversion is likely to depend on many variables, but is easily monitored by switching between DAPI, FITC and TRITC filter sets. A UV laser can also be used for photoconversion if one is available.

5. To minimize the number of cells exposed to the UV light you can insert a custom made pinhole into the UV light path. We use a homemade one that has a 0.2mm pinhole which can be used to photoconvert as few as 1 or 2 cells in favourable cases.

6. After photoconversion record position of target cells (preferably using a confocal microscope to get good 3D resolution) and then return embryos to incubate **in the dark** until appropriate stage. We have followed neurons for at least 4 days with good resolution but in proliferating cells the red protein will be diluted by division and so may not be detectable for so long.

7. Record eventual fate of target cells when appropriate. You can make multiple observations or even timelapse movies of these cells without further photoconversion as long as they are not exposed to UV light. Because embryos were injected with Kaede RNA even the photoconverted cells will continue to make the green form of the fluorescent protein, thus target cells are likely to be both green and red fluorescent.

Something to keep in mind:

When using a standard epifluorescence microscope to photoconvert Kaede, the cells most sharply in focus will undergo the most efficient photoconversion. However cells out of focus but still in the UV light path will also be photoconverted albeit to a lesser extent. This could be a source of error if not taken into account.

And there's now a Kaede transgenic – see Sato et al (2006)

References

Ando et al. (2002). An optical marker based on the UV-induced green-to-red photoconversion of a fluorescent protein. PNAS 99, 12651-12656.

Sato et al (2006) HuC:Kaede, a useful tool to label neural morphologies in networks in vivo. Genesis 44, 136-142