Observation of autofluorescence after clearing of whole roots

In order to visualize Casparian strips, roots were cleared as follows:

1. Vertically grown (in ½ MS-agar plates), 5-days old seedlings are incubated in 0.24 N HCl (prepared in 20% methanol), at 57°C for 15 minutes.
2. This solution is replaced with 7% NaOH in 60% ethanol and incubated at room temperature for 15 minutes.
3. Roots are rehydrated insubsequent baths of 5 minutes in 40%, 20% and 10% ethanol.
4. Roots are infiltrated in 5% ethanol and 25% glycerol for 15 minutes.
5. Samples are mounted in 50% glycerol for analysis under the microscope.

Microscopy:

Use a wide-field microscope with a standard GFP filter or a confocal microscope with excitation and emission frequencies for GFP (488 nm, 500–600 nm).

Remarks:

• Use 12-wells microtiter plates for incubations.
• Avoid squeezing roots, use featherweight forceps.

Adapted from: Malamy JE, Benfey PN, 1997. Organization and cell differentiation in lateral roots of *Arabidopsis thaliana*. Development 124:33–44.