Vital Staining of Bone

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Alizarin Red S staining was performed as described by Kimmel et al. (2010). Mineralized bone was vitally stained in five fish at 8 dpf in 1:1 deionized water and E3 embryo medium with 50µg/mL Alizarin Red S (JT Baker A475-03) in 6-well plates for 2 hours in the dark and rinsed with water (3x, 10 min.). Calcein staining was performed at 9 dpf as described in Du et al. (2001). A 0.2% calcein solution (Life Technologies C481) was created in deionized water and pH was restored to 7 by adding NaOH (1N) and HCl (1N). Embryos were stained in the calcein solution for 10 minutes in the dark and rinsed in deionized water (3x, 10min.). Embryos were then left in deionized water in the dark at room temperature for 2 hours before imaging. Alizarin red S and calcein staining solutions were made four days prior to staining.

Calcein
Excitation 495nm
Emission 515nm

Alizarin Red
Excitation 530-560
Emission 580