Paraffin sample processing for mouse embryonic heads

1. After dissecting the mouse embryonic head, wash the sample in 1XPBS.
2. Fix the samples in 10% Formalin O/N at RT or 4% PFA O/N at 4°C.
3. Wash the samples in 1XPBS.
    a. For samples younger the E14.5, proceed immediately to step 4 for dehydration.
    b. For samples older than the E14.5, decalcifiy in 10% EDTA at 4 °C first then proceed to step 4 for dehydration. The decalcification time is dependent on the stage of the embryo.
4. Dehydrate the samples in ascending concentration of ethanol solutions at RT following the order below. The time for each step depends on the age of the embryo.
    a) 30% EtOH
    b) 50% EtOH
    c) 70% EtOH
    d) 95% EtOH
    e) 100% EtOH
    f) 100% EtOH
5. Transfer the samples to Xylene in small GLASS VIALS. Leave the samples in Xylene until samples turn transparent (should not exceed 3hrs).
6. Transfer the samples to Xylene : paraffin wax 1:1 mix and incubate for 45min at 65 °C

7. Transfer the samples to 100% paraffin wax and incubate in the paraffin wax at 65 °C. Change the paraffin once every hour for a total of 4-6 times. For older embryonic stages, leave to incubate in the wax once overnight at 65 °C.
8. Embed the samples in paraffin wax.