Solutions

Fixation solution	1 % formaldehyde, 10 % DMSO, 2 mM EGTA pH 7.5, 0.1 % Tween20 in 1xPBS
PBT buffer	PBS and 0.1% Tween20
Postfix solution	1 % formaldehyde in PBT
Staining solution	5 μg/ml propidium iodide in 1xPBS
RNase solution	0.1 mg/ml RNase A, 1 % Tween20 in 1xPBS
methanol	
ethanol series	30%, 50%, 70%, 90%, 100%
xylene/ethanol	1:1 (v/v) mix of xylene and ethanol

Procedures

We use eppendorf tubes, and ~0.5 ml solutions are enough to process a couple of siliques. All procedures were done at room temperature except step 10.

1. Take siliques from plants and make small holes with a 27G needle through a longitudinal cut.
2. Fix siliques with the fixation solution for 30 min.
3. Wash fixed siliques 3 times with PBT buffer.
4. Incubate siliques in 100 % methanol, 2 min x 2.
5. Incubate siliques in 100 % ethanol, 2 min x 2.
6. Incubate siliques in xylene:ethanol (1:1 v/v), 30 min.
7. Incubate siliques in 100 % ethanol, 10 min x 2.
8. Rehydrate siliques by ethanol series (90, 70, 50, 30 % ethanol) 5 min each.
9. Wash siliques 2 times with PBT buffer, 10 min each.
10. Incubate siliques in RNase solution at 37 ºC for 90 ~ 120 min.
11. Wash siliques 2 times with PBT buffer, 10 min each.
12. Postfix siliques with the postfix solution for 15 min.
13. Wash siliques 2 times with PBT buffer.
14. Stain the samples with the Staining solution for 15 min.
15. Wash 2 times with 1xPBS.
16. Take seeds out from the siliques and mount the sample with 0.2 μg/ml propidium iodide in Vectashield (Vector Laboratories, H1300)