for distribution

The CUBIC clearing protocol with Reagent-1A (for whole mouse brain)

from Lab. for Synthetic Biology, RIKEN Quantitative Biology Center
Dept. of Systems Pharmacology, UTokyo Grad. Sch. of Med.

References for original methods and standard protocols:
Susaki EA *et al.* ***Cell*** 157, 726-739, 2014
Tainaka K *et al.* ***Cell*** 159, 911-924, 2014
Susaki EA *et al.* ***Nature Protocols*** ,10, 1709-1727, 2015

Further questions to: Drs. Hiroki R. Ueda (uedah-tky@umin.ac.jp) and
Etsuo A. Susaki (esusaki@m.u-tokyo.ac.jp)

Reagent-1A protocol:
enhanced fluorescence preservation

Although CUBIC reagents can preserve fluorescent protein signals (Susaki, ***Cell*** 2014), users may experience signal loss if they use a sample with lower fluorescent protein expressions. In this case, we propose this alternative protocol, particularly caring for fluorescent signal preservation.
We hope you to consider referring us in Acknowledgements and writing proper Material&Methods part when you publish the results because the protocol was unpublished (but mentioned in Susaki and Ueda, ***Cell Chemical Biology*** 2016, http://dx.doi.org/10.1016/j.chembiol.2015.11.009).

modified Sca/eCUBIC-1 (Reagent-1A)
10wt% Triton
5wt% NNNN-tetrakis (2-HP) ethylenediamine
10wt% Urea
Mix them in this order at RT, and add 1/200~ volume of 5 M NaCl (final 25 mM~)*
*NaCl is needed to repress an excess swelling of samples.

Day-0: Immerse an fixed whole mouse brain in 1/2-diluted Reagent-1A (~8 mL in 15 mL tube) for 6 h at RT with shaking, and replace to Reagent-1A (not diluted) (~12 mL in 30 mL tube), continue shaking at RT
Day-2: exchange the fresh media, place them at 37°C
Day-4: exchange the fresh media, continue shaking at 37°C
Day-6: exchange the fresh media, continue shaking at 37°C
Day-8~10: stop clearing, wash with PBS
Day-9~11: immerse in 1/2-diluted Reagent-2 (~8 mL) for at least 24 h at RT and then immerse in Reagent-2 (non-diluted, 15~20 mL) at RT for 2 days.

*1/2-diluted reagent-1A and reagent-2:
use distilled water and 1x PBS, respectively.
If deformation of the sample is visible, use 0.5x PBS or water for dilution of reagent-2.

During clearing, white matter is more visible in this reagent, but de-lipidation is progressing and you can clear sample at the same degree or more than the original protocol (Susaki, et al. ***Nature Protocols*** 2015).
If further clearing seems needed after Reagent-2 clearing, incubate in the Reagent-1A again for an additional few days at RT.