

Tobin lab 2015 CLARITY/PACT Protocol

Published in:

Cronan, M.R., Rosenberg, A.F., Oehlers, S.H., Saelens, J.W., Sisk, D.M., Smith, K.L., Lee, S., and Tobin, D.M. (2015). CLARITY and PACT-based imaging of adult zebrafish and mouse for whole-animal analysis of infections. *Dis Model Mech.* Oct 8. pii: dmm.021394.

Modified from two papers:

Chung, K., Wallace, J., Kim, S. Y., Kalyanasundaram, S., Andalman, A. S., Davidson, T. J., Mirzabekov, J. J., Zalocusky, K. A., Mattis, J., Denisin, A. K. et al. (2013). Structural and molecular interrogation of intact biological systems. *Nature* **497**, 332-337.

Yang, B., Treweek, J. B., Kulkarni, R. P., Deverman, B. E., Chen, C. K., Lubeck, E., Shah, S., Cai, L. and Gradinaru, V. (2014). Single-cell phenotyping within transparent intact tissue through whole-body clearing. *Cell* **158**, 945-958.

CLARITY and PACT clearing

General Notes: PACT is faster, but we think CLARITY might be a little better – it may keep the tissue a bit more together. Overall the smaller the tissue the better – for whole animal imaging it is best to image younger/smaller animals since they clear much faster and more completely. If you are particularly interested in certain organs, it can be useful to dissect the organs and PACT/CLARITY clear those directly. Muscle in particular doesn't always clear well because of the high lipid content and birefringence of muscle fibers, so pulling the organ out simplifies the clearing process since you don't have to worry about the muscle. Also, we think skin/scales probably scatter some light, so if you don't need the skin it could be helpful to skin the animal, although we haven't tested this extensively. Depending on the tissue needed, we will also remove head/tail to increase permeability of the various solutions.

CLARITY was performed by modifying the acrylamide concentration used in the protocol described in Chung et al 2013 to 1%.

1. Make 10 mL of CLARITY solution ON ICE per experimental condition This is: 4% PFA, 1% acrylamide, 0.05% bis-acrylamide and 0.25% 2,20-Azobis[2-(2-imidazolin-2-yl) propane]dihydrochloride (VA-044, temperature dependent initiator, Wako Chemicals

USA) in 1x phosphate buffered saline. (You can make more than you need and freeze the excess solution at -20° C – it'll last 3 months or more and you can just thaw the solution when you next need it). Euthanize your animals and incubate them for 3 days at 4 ° C in CLARITY solution (they can sit longer if necessary but we haven't extensively tested the effects of longer incubations).

2. After the 3-day incubation this hydrogel solution was overlaid with mineral oil in a 15mL tube (We see polymerization whether we use mineral oil or not, but mineral oil is supposed to help exclude oxygen which inhibits free-radical polymerization) and the hydrogel was polymerized by incubating for 3 hours in a 37° C water bath.
3. Make 8% SDS solution: for 500ml: 6.183g boric acid, MQ H₂O to 200ml, pH with NaOH to pH=8.5, MQ H₂O to 300ml then add 200ml of 20% SDS.
4. Remove tissue from excess hydrogel (put fish in a new tube, or, drain away as much gel as possible, can also run fish across a kimwipe) and incubate in 4% or 8% SDS in 200 mM Boric acid, pH 8.5 at 37 ° C on a nutator. Change this SDS solution every other day, clearing is achieved in 7-10 days. Longer may be better to remove all the lipids in the muscle but the skin starts to peel off at later time points. (The tissue will not be fully clear in SDS solution, but will further clear when put in refractive index matching media).
5. After clearing, wash the tissue for 2 days in PBS, 0.1% Triton X-100 at 37 ° C on a nutator (The Triton is necessary to remove excess SDS, which will otherwise precipitate in the fish, forming cloudy spots). Change solution daily.
6. Refractive index matching was achieved by incubating the cleared tissue in RIMS solution (Yang et al., 2014). (RIMS: 15ml of 20mM phosphate buffer pH 7.5, 20g Histodenz, 250ul Sodium Azide 10% solution).

PACT clearing was performed based on the approach of Yang et al 2014 except that the solutions were applied by soaking rather than perfusion-driven processes. This modification resulted in alterations in the timing of the incubation steps.

1. Briefly, zebrafish were fixed in 4% paraformaldehyde (PFA) for 2 days at 4°C (for small fish, around a month old, 2-3 hours at RT also works, or overnight in PFA at 4°C).
2. Fixed whole adult fish were incubated at 4°C for three days (again, 2 days minimum, a week or longer is fine) in the freshly made hydrogel monomer solution of A4P0 (4% acrylamide in PBS) supplemented with 0.25% VA-044. For example, use 32ml MQ H₂O, 4ml 10X PBS, 4ml 40% acrylamide (this should already be cold) and .100 g VA-044 initiator (also should be cold). Mix! Pour off the final PBS wash and replace with cold acrylamide solution.
3. A4P0-infused samples were incubated for 3 hours in a 37 ° C water bath to initiate tissue-hydrogel hybridization. (overlying here with mineral oil as noted in the CLARITY protocol may help polymerization).
4. Make 8% SDS solution: for 500ml: 200ml of 20% SDS, 6.183g boric acid, MQ H₂O to 400ml, pH with NaOH to pH=8.5, then MQ H₂O to 500ml)
5. Fish were next removed to clean 15ml conical tubes and incubated in 8% SDS in 200 mM Boric acid, pH 8.5 for 5 days at 37 ° C on a nutator, and SDS solution was changed every other day. For a small/juvenile fish clearing can be achieved in 1-3 days, for a large fish 5-7+ days of passive clearing may be necessary. We actually think longer is probably better since muscle is so hard to clear. However, at longer times, skin may start peeling off the fish after a while so this may be a problem.

6. Samples were then washed for 2 days in two changes of PBS, 0.1% Triton X-100 at 37 °C on nutator (as above, the Triton is critical to remove residual SDS in the tissue).
7. Incubated samples in RIMS imaging media (Yang et al., 2014) for a day at room temperature on a rotator (for very large fish this may take longer). Samples were stored in RIMS at room temperature. (RIMS: 15ml of 20mM phosphate buffer pH 7.5, 20g Histodenz, 250ul Sodium Azide 10% solution)