Supplementary Material

Optimization of GFP fluorescence preservation by a modified uDISCO clearing protocol

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**Supplementary Table 3: Troubleshooting table.**

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| **Problem** | **Possible reason** | **Solution** |
| The 100% tert-butanol solution is crystallized during clearing process | The room temperature is too low | Clear the samples with a 25°C water bath |
| Poor transparency of samples after clearing | Too much blood residue in the samples | Prolong the time of PBS perfusion |
| Cavity in the ventricle of a cleared brain | Bubble entered the ventricle during PBS/PFA perfusion | Check and drain the bubble in a conduit |
| Poor image quality when fluorescence imaging | Insufficient clearing | Extend the incubation time of each clearing step |
| Brain with strong spontaneous fluorescence | PFA post-fixation time is too long | Limit post-fixation time to 24 hours |
| Weak or no fluorescence signal | Negative fluorescence expression  Fluorescence quenching | Check the samples before clearing  Use freshly-prepared agents |
| Sample bleaching | Laser power is too strong | Decrease the laser power and increase the gain of the acquisition to compensate |