fixing ~~EM~~ protocol.

1. ~~Get MTSB(± 0.2% TritonX-100) out from the -20, and warm it up to RT temperature.~~
2. 3.5g paraformaldehyde into 90ml H2O; add 40ul 10M NaOH, dissolve by slightly heat (10s × 2); add 20ml 5XPBS (or 10ml 10XPBS), 1ml 4-fold diluted HCl to adjust pH=6.6~6.9. [200ml 1:4 (Hcl : ddH2o) Hcl to 50ml para-,
   Small scale: 0.35g paraformaldehyde into 9ml H2O; add 4ul 10M NaOH, dissolve by slightly heat (5s + 3s); add 2ml 5X PBS buffer, add 100ul diluted HCl (conc. HCl/ddH2O = ¼) to adjust pH=6.6~6.9.
3. 1X KB 100ml, + 1ml 20% TritonX-100; 1X KB 100ml.
   Small scale: 1X KB 10ml, + 100ul 20% TritonX-100.
   1XKB 100ml.
   NOTE: 1X KB can be prepared from 10X KB, and both of them are stored at 4°C.
4. Now, set the timer 7min and 5min. Take the cell dishes out; and pipette the medium carefully, and add 2ml paraformalduhyde to the dish for 7min. Remove the paraformaldehyde out carefully and add 2ml KB(+ 0.2% TritonX-100) for 5min. Move the coverslip out to KB.
5. The second way is to first add KB (+Triton) 90s and then add paraformaldehyde solution for 7min, and finally add KB.
6. Another two dishes, pipette the medium carefully, and add 2ml MTSB(+ 0.2% TritonX-100) for 1min. Remove the MTSB(+ 0.2% TritonX-100) out carefully and add 2ml MTSB for 2min. Remove the MTSB out carefully and add 2ml paraformaldehyde for 7min. Move the coverslip to KB.

The 3rd way to fix

7.