1. Thaw 2M nuclei
2. Add Yeast tRNA and RNase inhibitor to Hyb Buff Stock, keep on ice
3. Heat ddPadlock probes at 95 for 3 min, chill on ice
4. Prep the Hyb reaction
5. Hyb overnight
6. Next Day: Add RNase inhibitor to PBST, label as Nuclei Washing Buffer, keep on ice
7. Wash nuclei twice with 1000uL Nuclei Washing Buffer
8. Prep RT reaction mix
9. Resuspend nuclei with RT reaction mix
10. Split to 4 aliquots, each 75uL in 1.5mL Tubes
11. RT at 42 with Rotation for 5min, 30min, 60min and 120 min, with lid.
12. Add 1000uL NWB, spindown for 5 min.
13. Run Cleavage in 100uL each
14. Add 1000uL NWB, spindown for 5 min.
15. Prepare 500uL Ligation Mix,
16. Resuspend nucleis with 100 uL LM, incubate at 40 for 2 hours, then hold at 4C overnight.
17. Add 1000uL NWB, spindown for 5 min.
18. Run PCR to build library, either by 10x preamp+lib, or by one step PCR.