1. Thaw 3 tube of fixed cells
2. Permealization in 500 uL 0.01% Digitonin in PBS at RT for 10, 15, 20 minutes.
3. Add 1000uL PBS with RNAsinhibitor, mix and split each tube to two aliquots, spindwon for 5
4. Add Yeast tRNA and RNase inhibitor to Hyb Buff Stock, keep on ice
5. Prepare linear probe mix by mixing them in equal volume
6. Heat ddPadlock probes and **linear probes** at 95 for 3 min, chill on ice
7. Prep the Hyb reaction for both linear probes and padlock probes
8. Hyb overnight
9. Next Day: Add RNase inhibitor to PBS, label as Nuclei Washing Buffer, keep on ice
10. Wash nuclei twice with 1000uL Nuclei Washing Buffer
11. Prep RT reaction mix
12. Resuspend nuclei with RT reaction mix
13. RT at 42 with Rotation, 30minwith lid.
14. Add 1000uL NWB, spin down for 5 min.
15. Run Cleavage in 100uL each, 50C for 15min
16. Add 1000uL NWB, spindown for 5 min.
17. Prepare 500uL Ligation Mix,
18. Resuspend nucleis with 100 uL LM, incubate at 25 for 4 hours, then hold at 4C overnight.
19. Add 1000uL NWB, spindown for 5 min.
20. Run PCR to build library, either by 10x preamp+lib