1. Hyb following 10x protocol, use 200nM padlock probe mix, volume 50uL, 16 hours at least
2. Wash with PBS with RNase inhibitor
3. RT at 42C with for 5 min, volume: 150uL

5x RT buffer: 30

RRI: 10

Illumine ddNTP mix: 50uL

RT enzyme: 10uL

DDW: 50

1. Wash with PR2 from Miseq kits with RRI
2. Add cleavage mix (Illumina MiSeq reagent #4) and incubate at 50°C for 15 minutes.
3. Wash with PBS with RNase inhibitor
4. splitRligase Ligation, volume 100uL

|  |  |
| --- | --- |
| H2O | 70µl |
| 10X Reaction Buffer | 10 µl |
| RRI | 10 µl |
| SplintR Ligase | 10 µl |
| Total | 100µl |

1. Incubate for 60 minutes at 25C, then heat inactivation at 65, keep at 4C. [Pause]
2. Keep reaction on ice until needed (hrs) or store at -20.
3. PCR amplification