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| **Step 3:**  **Reverse Transcription and First Strand Synthesis**  Time:  2h | **Reverse Transcription and First Strand Synthesis**  Take 10µl RNA use as RT (+), add 9µL H2O to the rest 2µL and use it as RT (-). RT (-) is 1/5 of RT (+).   |  |  |  |  |  | | --- | --- | --- | --- | --- | |  | Single cell | 1000 cells |  |  | | Reagent | Volume (µl) |  |  |  | | RNA | 4 | 10 |  |  | | Superscript III (Invitrogen) | 0.8 | 0.8 | 0.8 [RT+] | 10 | | 5x Forward Strand Synthesis buffer | 1.6 | 4 | 9.2 | | S1330S random primer mix (NEB) | 0.4 | 0.4 | | M0300S T4 gene 32 protein (NEB) | 0.1 | 0.1 | | 25mM dNTP mix | 0.4 | 0.4 | | 100mM DTT | 0.4 | 0.4 | | Ribolock | 0 | 1 | | H2O | 0.3 | 2.9 | | **Total** | **8** | **20** |  |  |   Setup following program on a PCR block  25°C 10’  42°C 60’  85°C 5’ (terminate the reaction)  Now you have 20µl first-stand cDNA-RNA duplex. |