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| Step 4  Second Strand synthesis  Time:  3h (2h waiting time) | **Second Strand synthesis**  Do second strand synthesis on both RT+ and RT- samples:   |  |  |  |  | | --- | --- | --- | --- | | Reagents | Vol. (µl) |  |  | |  | Single cell | 1000 cells |  | | First strand reaction | 8 | 20 |  | | NEB buffer 2 | 4 | 4 | 20 | | Klenow M0210L (NEB) | 0.5 | 0.5 | | RNase H M02975 (NEB) | 0.2 | 0.2 | | 10mM dNTP | 1 | 1 | | H2O | 26.3 | 14.3 | | **Total** | **40** | **40** |  |   15°C 2 h or 16°C 2h30min.  75°C 20 min to inactive enzyme.  Clean RT+ and RT- cDNA with Ampure Beads, resuspend RT+ cDNA in 40µL and RT- with 20µL.  Take out 4µL RT+ for qPCR validation, and 16µL H2O to make 20µL solution. Use 2µL per reaction, this is enough for three amplicon with triplicates.   |  |  |  |  |  | | --- | --- | --- | --- | --- | |  | Vol.  [RT] | Vol.  [Final] | Dilution | Vol. [qPCR] | | RT(–) | 1 | 20 | 1:20 (1µL🡪20µL) | 20 | | RT(+) | 10 | 40 | 1:4x5  (10µL🡪40µL) (4µL🡪20µL) | 20 |   Add 14µL H2O to the rest 36µL solution, proceed with sonication followed by Ampure Beads clean up for library construction. |