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| Step 1  RNA isolation and DNase I treatment  Time:  2h  Vol: | RNA isolation and DNase I treatment  1. RNA isolation using TRIzol method  1) Spin down the cells at 2500 rpm at 4°C for 5 minutes.  2) Remove most of the liquid, leave ~10 µl behind.  3) Add 500 ul of Trizol to lyse the cells completely, mix well by pipetting.  4) Add 100 ul of chloroform and mix thoroughly by vertexing.  5) Spin at 12,000g at 4°C for 15 minutes.  6) Take out aqueous supernatant, leave some behind to avoid contamination of DNA resides in the interphase.  7) Precipitate using 1 µl LPA and an equal volume (~500 µl) of isopropanol. Precipitate at -20°C for 30min or overnight.  8) Centrifuge the tube for 15~30min at >=12,000 xg at 4°C. Carefully discard the supernatant without disturbing the pellet.  9) Add 500µL 70% EtOH.  10) Centrifuge the tube for ~5min at >=12,000 xg at 4°C. Carefully discard the supernatant without disturbing the pellet.  11) Air dry the pellet for ~5min. Re-suspend the pellet in 7µl RNAse-free water.  It can be stored at -80°C or proceed to DNase I treatment to remove residual DNA contamination.  2. DNase I treatment  1) Prepare DNase I mix   |  |  |  | | --- | --- | --- | | RNA | 7µl | 9x | | RQ1 DNAse (RNase free) | 1 | 9 | | RQ1 DNAse buffer | 1 | 9 | | Ribolock | 1 | 9 | | **Total** | 10 | 27 |   2) Incubate at 37 °C for 30 min.  3) Add 2 µl of RQ1 Stop buffer to the mix, vertex to mix well.  4) Incubate at 65 °C for 10 min to stop the reaction.  Now you have 12µl DNA free RNA. |