Total RNA preparation from intraerythrocytic Plasmodium falciparum for transcriptome analysis and quantitative PCR

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Abstract

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Protocol

Step 1.

step 1

Isolation of *Plasmodium falciparum* from infected RBCs (PfRBCs):

1. Add 12 ml of ultrapure water* at 4 °C to 0.8 ml of packed PfRBCs.
2. Keep standing it on ice for 10 min for lysis of PfRBCs.
3. Centrifuge the sample for 10 min at 1750g at 4 °C.
4. Transfer the sediment containing free parasites to a new 1.5 ml micro tube.
5. Centrifuge the sample in a standard micro centrifuge for 5 min at 3500g at 4 °C.
6. Completely aspirate the supernatant.
7. Add 100 µl RNAprotect Cell Reagent (Qiagen GmbH, Hilden, Germany) to the sediment, and suspend completely by vortexing to protect the nucleic acids of the parasites from degradation.
8. Store the mixture at -20 °C or -80 °C.

* Wako Pure Chemical Industries, Osaka, Japan, catalogue no. 210-01303

Step 2.

step 2

Harvesting total RNA from the parasites using the RNase plus Mini kit*.

1. Pellet the appropriate number of parasites (less than 1x 10^7 parasites) by centrifugation for 5 min at 3500g.
2. Remove the supernatant.

3. Add 600 µl Buffer RLT Plus enriched with 1% ß-mercaptethanol and vortex the tube to make lysate of parasites.
4. Homogenize the lysate with a QIAshreddar spin column (Qiagen) placed in a 2 ml collection tube, and centrifuge for 2 min at maximum speed (14000g).
5. Transfer the homogenized lysate to a gDNA Eliminator spin column placed in a 2 ml collection tube. Centrifuge for 30 s at 8000g and save the flow-through.
6. Add equal volume of 70% ethanol (600 µl) to the flow-through, and mix well.
7. Transfer the sample to a RNeasy spin column placed in a 2 ml collection tube.
8. Centrifuge it for 15 s at 8000g. Discard the flow-through.
9. Add 700 µl Buffer RW1 to the RNeasy spin column, and centrifuge for 15 s at 8000g to wash the column membrane. Discard the flow-through.
10. Add 500 µl Buffer RPE to the RNeasy spin column, and centrifuge for 15 s at 8000g to wash the column membrane. Discard the flow-through.
11. Add 500 µl Buffer RPE to the RNeasy spin column, and centrifuge for 15 s at 8000g to wash (the second) the column membrane. Discard the flow-through.
12. Place the RNeasy spin column in a new 2 ml collection tube, and centrifuge at full speed (14000g) for 1 min.
13. Place the RNeasy spin column in a new 1.5 ml collection tube. Add 30 µl RNase-free water directly to the spin column membrane, and centrifuge for 1 min at 8000g to elute the RNA.

*Qiagen, catalogue no. 74134

Step 3.

Step 3

Confirmation of the concentration and purity of harvested RNA:

1. Estimate the concentration of harvested RNA by NanoDrop ND-100 (Thermo Fisher Scientific Inc., Tokyo, Japan).
2. Confirm the purity of harvested RNA using an Agilent 2100 Bioanalyzer (Agilent Technologies Japan, Ltd., Tokyo, Japan). The suitable quality of the harvested RNA can be confirmed with clear two peaks (18s rRNA and 28s rRNA) in electrophoregram.