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Procedure

RT-qPCR protocol for Samples Enriched with IsoFlux

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1. Upon completion of CTC enrichment with IsoFlux, transfer the cells/beads sample into a 1.5mL microfuge tube (RNAse/DNA-free). Use additional Binding Buffer to rinse if necessary.
2. Place the tube on the magnet for 30 seconds. While keeping the tube on the magnet, remove and discard the supernatant.
3. Add 350µL of RLT buffer containing 2-mercaptoethanol (see Qiagen RNeasy Micro Kit protocol).
4. Pulse vortex to lyse the cells for 30 seconds. Sample may be processed for RNA isolation immediately or frozen quickly on dry ice and stored at -80C.
5. When ready for RNA isolation thaw sample on bench, add RNA carrier (refer to Qiagen RNeasy Micro Kit protocol for RNA carrier preparation).
6. Follow Qiagen RNeasy Micro Kit protocol for RNA isolation.
7. When ready to elute RNA, add 1µL of RNase inhibitor (RNAse Out, Life Technogies) to the 1.5mL microfuge tube.
8. Place the RNA column on this tube. Elute with 20µL of RNase-free water. (Tip: elute with 14µL RNase-free water first, then again with 6µL to maximize the recovery of RNA)
9. Use 10µL of the eluted RNA for reverse transcription into cDNA in a 30µL reaction volume. Store the remaining RNA at -80C.
   We use SuperScript Villo cDNA kit from Life Technologies (see protocol).
10. After a successful RT, the cDNA concentration is typically around 2µg/µL (based on Nanodrop measurement).
11. Dilute the cDNA with nuclease-free water to 50µL. Use 2 to 5µL of the cDNA for each 20µL qPCR reaction.

Tip: If multiple probes are to be used, pre-amplification is recommended.