RNA isolation and cDNA synthesis from BJAB, BC-3, and JSC-1 cells

Goal: Determine basal levels of all latent transcripts synthesized by KSHV in BC-3 and JSC-1.

Strategy: Isolate total RNA from frozen 1E7 BJAB, BC-3, and JSC-1 cells. Generate cDNA (first-strand only) and use in real-time PCR reaction with probes to full-length LANA2, spliced LANA2, LANA1, spliced LANA1, and v-cyclin/v-FLIP transcripts. Additional control probes include Orf50 (a lytic gene whose expression is predicted to be lower except for those cells undergoing the lytic phase of infection) and GAPDH (used a cell number control for normalization).

RNA isolation (adapted from Invitrogen's protocol for TRIzol)

- Pellet cells by centrifugation and add 1 ml TRIzol (1ml/1E7 cells). Pipet up and down to lyse cells. Use 800 µl for less than 1E7 cells.
- Incubate samples for 5 min. at room temperature to allow dissociation of nucleoprotein complexes. Add 200 µl chloroform (per 1 ml TRIzol). (Use 160 µl for less than 1E7 cells). Shake vigorously by hand for 15 seconds and incubate at room temperature for 2 – 3 min.
- Centrifuge no more than 11,500 rpm for 15 min. at 4C. The RNA will be found exclusively in the aqueous phase which will be about 60% of the volume of TRIzol used.
- Transfer to fresh tube and precipitate with 1 µl glycogen and 500 µl isopropanol (per 1 ml TRIzol). Incubate at room temperature for 10 min and centrifuge at 12k g for 10 min at 4C.
- Decant supernatant and wash with 1 ml 75% EtOH. Vortex and centrifuge at 9,000 rpm for 5 min at 4C.
- Decant and air-dry pellet.
- Dissolve in RNase-free H2O (40 µl for 1E7 cells or more and 20 µl for less)
- If there is less than 5 µg of RNA generated, then use the speed-vacuum to remove most of the H2O to accommodate the cDNA reaction.

cDNA synthesis (adapted from Invitrogen's SuperScript Double-stranded cDNA kit)

- Use 5 µg total RNA/reaction, 1 µl oligo dT primer (100 pmol/µl), 10 µl RNase-free H2O. Total volume = 11 µl
- Heat reaction at 70C for 10 minutes and quick chill on ice. Centrifuge briefly and add: 4 µl 5x First-strand reaction buffer, 2 µl 0.1 M DTT, 1 µl 10 mM dNTP mix. Vortex and centrifuge briefly. Place tube at 45C for 2 min to equilibrate before adding reverse trancriptase.
- Add 1 µl SuperScript II RT (total vol. ~ 20 µl) and incubate aqt 45C for 1 hours.
- Place tube on ice to terminate reaction or store in freezer (-20C).
- Use 1 µl per reaction.