**TRIzol/RNeasy hybrid RNA Extraction**

- Adapted from [Pilar Quintana](https://www.researchgate.net/post/Could_anyone_tell_me_which_is_the_best_method_to_extract_total_RNA_from_in_vitro_cultured_Plasmodium_falciparum_strains), [Mauricio Rodriguez-Lanetty](http://people.oregonstate.edu/~weisv/assets/trizol_rneasyhybrid.pdf), and Rebecca W Beerman’s RNA Extraction from Tissue for Q-PCR protocol by JWS

-Combines TRIzol and Qiagen RNeasy kit

1. Isolate cells and resuspend in TRIzol
   1. Use 1mL TRIzol/50-100 mg cells
2. Homogenize tissue in TRIzol by passing it through a 0.6 mm blunt needle coupled to a 1ml syringe 5 times
   1. Let sit at room temperature for 5 minutes
3. Spin tubes 10 min 12,000 x g at 4C and transfer supernatant to new tube
   1. Can store at -80C or continue to Step 4
4. Add 200µl chloroform per 1mL TRIzol
   1. Shake vigorously by hand for 20 seconds
   2. Incubate at room temperature for 3 minutes
5. Spin 15 minutes at 12,000xg 4C
   1. Carefully transfer upper aqueous phase to new tube
      1. **IMPORTANT**: Stay away from the aqueous/organic interphase. This is where the DNases and RNases are. It is suggested to sacrifice aqueous material rather than risk taking this precipitate
   2. Mix immediately with 1 volume RNase-free 70% EtOH
6. Add up to 700µl of this mixture to RNeasy mini column
   1. Spin 15 sec ≥ 8,000 g at room temperature, discard flowthrough. Repeat until entire sample has passed through column

**DNase treatment and clean up**

1. Add 350µl Buffer RW1 and spin 15 sec ≥ 8,000g at room temperature, discard flowthrough
2. Add 80µl (10µl DNase stock solution + 70µl Buffer RDD) per column and let stand for 15 minutes at room temperature
3. Add 350µl RW1 to column and spin 15 sec ≥ 8,000g at room temperature, discard flowthrough
4. Add 500µl RPE to column and spin 2 minutes ≥ 8,000g at room temperature, discard flowthrough
   1. Spin again 1 minute ≥ 8,000g before placing into clean tube and adding water (gets off last bit of EtOH)
5. Transfer column to new 1.5µl collection tube and add 30µl RNase free water directly onto the column membrane
   1. Allow sample to sit at room temperature for 1-2 minutes
   2. Spin 1 minute at full speed at room temperature to elute RNA
6. Measure RNA concentration with Qubit RNA HS Assay
7. Store RNA at -80C until use