title: “Hahn lab protocols” output: prettydoc::html\_pretty: theme: architect toc: yes pdf\_document: number\_sections: yes toc: yes fontsize: 18pt =======

List of protocols:

* [Ecdysteroid Extraction](2018-01-08_ecdysteroid_extraction.html)

Dan hahn is cool.

Welcome to the Hahn lab protocols page. Here is a master list of lab procedures we use for gene and protein expression. Please visit [our lab page](http://entomology.ifas.ufl.edu/hahn/lab/) to find out more about our work. Click [here](https://github.com/adnguyen/Hahn_lab_protocols) for the github repository for this website.

updated: 2017-12-22

# Gene expression workflow

## RNA isolation

### Reagents:

* [TRI Reagent](https://www.thermofisher.com/order/catalog/product/15596026) (cat# 15596026)
* [BCP(1-bromo-3-chloropropane)](https://www.fishersci.com/shop/products/bcp-47/nc9551474#?keyword=BCP)(cat# NC9551474)
* [THE RNA storage solution](https://www.thermofisher.com/order/catalog/product/AM7001) (cat# AM7001)
* [DNA-free™ DNA Removal Kit](https://www.thermofisher.com/order/catalog/product/AM1906) (cat# AM1906)
* [Qubit™ dsDNA BR Assay Kit](https://www.thermofisher.com/order/catalog/product/Q32850) (cat# Q32850)
* [Qubit™ RNA IQ Assay Kit](https://www.thermofisher.com/order/catalog/product/Q33221?SID=srch-srp-Q33221) (cat# Q33221)

### Protocol:

[RNA isolation protocol (originated from Applied Biosystems and adpted to our lab condition)](https://assets.thermofisher.com/TFS-Assets/LSG/manuals/9738M_D.pdf)

**Homogenize**

1. Homogenize in 1ml TRI Reagent and 5 beads by the Bead Mill Homogenizer (Precellys® Evolution), use the soft tissue program which will homogenize 15s, break for 30s and then homogenize for an additional 15s.
2. Icubate the homogenates for 5 min at room temperature.

RNA extraction 3. Add 100 μL BCP per 1 mL of TRI Reagent solution,mix well, and incubate at room temp for 5–15 min. 4. Centrifuge at 12,000 x g for 10–15 min at 4°C, then transfer the aqueous phase to a fresh tube.

RNA Precipitation and Wash 5. Add 500 μL of isopropanol per 1 mL of TRI Reagent solution, vortex for 5–10 sec, and incubate at room temp for 5–10 min. 6. Centrifuge at 12,000 x g for 8 min at 4–25°C, and discard the supernatant. 7. Add 1 mL of 75% ethanol per 1 mL of

TRI Reagent solution. 8. Centrifuge at 7,500 x g for 5 min, remove the ethanol, and vacum dry the RNA pellet for 5 min.

RNA Solubilization 9. Dissolve RNA in the THE RNA storage soluStep 2: Remove contaminating DNA from RNA

## Remove contaminating DNA from RNA:

### Reagents:

[*Dnase1 Treatment with DNA-free kit*](https://www.thermofisher.com/order/catalog/product/AM1906)  
[HyClone™ Water, Molecular Biology Grade](https://www.fishersci.com/shop/products/hyclone-water-molecular-biology-grade-6/p-7058038#?keyword=molecular+grade+water)  
0.5ml centrifuge tubes

### Protocol:

This protocol is for a 50ul reaction volume with up to 10ug RNA.

1. Prepare the master mix as follows (prepare 5 tubes extra) Dnase1 10× buffer = 5.0ul (0.1× reaction volume) × (number of tubes+5) Dnase1 = 1.0ul × (number of tubes+5) Molecular grade water = 4ul × (number of tubes+5)
2. Pipette out 10ug RNA samples to new 0.5ml tubes (volume depends on the concentration of RNA samples), then add molecular grade water (volume=40ul-volume of RNA) to make the final volume to 40ul.
3. Add 10ul master kit to each of the above reaction tube (now the total volume is 50ul)
4. Incubate at 37˚C for 30min.
5. Add 5ul (0.1× reaction volume) suspended DNase Inactivation Reagent and mix well.
6. Incubate 2 min at room temperature. Mix every 30s to 60s (This step is very important in order to redisperse the DNase inactivation reagent.)
7. Centrifuge at 10,000 × g for 1.5 min and transfer the RNA to a fresh tube. Note: This centrifugation step pellets the DNase Inactivation Reagent. After centrifuging, carefully transfer the supernatant, which contains the RNA, into a fresh tube. Avoid introducing the DNase Inactivation Reagent into solutions that may be used for downstream enzymatic reactions, because it can sequester divalent cations and change the buffer conditions. ion.

## dsDNA contamination and RNA quality control

Reagents:

*Agarose*  
10× TAE Buffer  
*Commercial Bleach, 6% Sodium Hypochlorite*  
Ethidium Bromide (10 mg/ml)  
\*10× DNA Loading Buffer (1.9 mM xylene cyanol, 1.5 mM bromophenol blue, 25% glycerol in sterile dH20)

## Protocol

## Prepare the gel

1. Add 1.0% w/v agarose to 1× TAE buffer. e.g. 0.5 g in 50 mL.
2. Add 1.0% v/v Clorox® bleach, e.g. 500 μL in 50 mL, and incubate at room temperature for 5 minutes, with occasional swirling
3. Heat the suspension to melt the agarose (10 sec intervals with swirling) until all the agarose dissolves).
4. Allow solution to cool to about 50C (i.e. you can touch it for a couple seconds without discomfort)
5. Adding ethidium bromide to a final concentration of 0.5 μg/mL. e.g. 25ug in 50ml. Swirl thoroughly.
6. Pour solution into gel mold and allow the ‘bleach gel’ to solidify (about 30min in room temperature)
7. Position the gel into the gel electrophoresis tank. Avoid bubbles.
8. Add enough 1× TAE buffer to cover the gel to a depth of about 5mm.  
   ## Load and Run RNA Samples
9. Mix the samples of RNA with DNA loading buffer  
   1ug RNA+1ul\_10× DNA loading buffer + molecular grade water to make final volume of 10ul.
10. Load the mixtures and RNA ladder slowly into the slots. Avoid making bubbles!
11. Attach the electrical leads so that RNA can move toward the anode.
12. Apply a voltage of 100 V for approximately 35 minutes and then visualizing by UV trans illumination  
    ## Visualizing by UV trans-illumination
13. sdd

## RNA quantification and quality control

RNA concertration 1. the concentration of an RNA solution is determined by NanoDrop.

Dnase1 Treatment with DNA-free kit(<https://www.thermofisher.com/order/catalog/product/AM1906>)

dsDNA contamination and RNA quality check

## cDNA synthesis: reverse transcription

## Quantitative Real Time PCR

## Data processing and handling

# Protein expression workflow

## Protein Isolation

### Reagents

### Protocol

## Quantifying protein and quality control

## Polyacrylamide Gel Electrophoresis

## Western Blotting

## Data processing and handling