Preparation of whole insect specimens

* Take weight of larvae
* Add [150]μl of PBS buffer to tube and bead beat for 30secs
* Centrifuge the homogenate at 13000rpm for 30min at 4°C
* Transfer supernatant to a new tube – try to leave the fat behind
* Discard beads and bug mush. Store supernatant overnight at -80°C

Extract the hemolymph

* <https://youtu.be/im78OIBKlPA>
* Fold larvae over and clip one foreleg to release hemolymph
* Using a capillary tube draw up the hemolymph into the tube and blow it into a collection tube with antioxidant

Protein extraction and purification

* Denature protein in sample using Laemmli buffer with β-mercaptoethanol
  + <http://www.bio-rad.com/en-us/faq/268440261/technical-support-faq>
  + <https://intranet.pasteur.edu.uy/publico/bonilla/Protocolos/Acrilamida/Laemmli%20buffer%20Background.pdf>
  + Laemmli buffer includes glycerol, SDS, TRIS, BME, bromophenol blue
    - BME is a biological antioxidant that scavenges –OH groups
    - BME is very volatile out of solution.
    - BME is used in large excess to drive the equilibrium reaction toward completion and keeps the proteins from reoxidation.
    - If they reoxidize, they can electrophorese as fuzzy bands, or spurious artifactual bands may appear.
* Load and run SDS-PAGE gel to find target protein pool verified via mole weight against marker standard

Western Blot

* Transfer to Incubate transferring membranes with SP, incubate total protein spread with SP-specific Abs

Protein Analysis

PBS: phosphate buffer solution, maintains physiological pH and osomolarity

BCA: bicinchoninic acid, for the colorimetric detection and quant of total protein. It uses Cu