**DNA extraction for Psyllid (Insects)**

*Entomology Lab, Aberdeen Research Extension. Updated August 2017*

Reference: Marzachi, C., Veratti, F., and Bosco, D., 1998. Direct PCR detection of phytoplasmas in experimentally infected insects. *Annals of Applied Biology* 133:45-54.

1. Grinding by pestle: Put one or two insects in tube and 300ul of CTAB 2% and grind it with pestle (use tube of 1.5ml). Then add 200ul more of CTAB (final volume is 500ul).

**Grinding by homogenizer: Add 500ul of CTAB 2% and grind 1 min in high speed and 1 min in medium speed.**

* Grinding procedure may vary according to the tissue hardness.

1. Incubate at 60 ͦ C for 30min. Mix gently by inversion every 10 minutes.
2. Centrifuge at 14000 rpm for 5min, then transfer supernatant to new tube of 2ml.
3. Add the same volume (500ul) of chloroform: isoamyl alcohol (24:1) to the supernatant, mix by vortex about 20 seconds. Centrifuge at 14000 rpm for 5-**10** min.
4. Transfer clean supernatant (upper phase) to new tube and add 2/3 volumes\* of cold isopropanol. Put in -20C for 20-30min.
5. DNA precipitation: Centrifuge for 20min at 14000 rpm. Pour gently the isopropanol and keep pellet (DNA). Be careful since pellet is the bottom of the tube, it is hardly visible.

\*For example: if the volume of supernatant is 400ul, add 267ul of cold isopropanol.

400 x 2/3 = 266.66 = 267ul

1. Wash the pellet: Add 300ul ethanol 70% and centrifuge 5min at 10000 rpm.
2. Remove by pouring/using pipette the ethanol and dry pellet on fume hood (usually overnight). When pellet is dried, add 30-50ul (pellet from 1 psyllid: 30ul, pellet from 3 psyllids: 50ul) of nuclease-free water and keep in -20C.