**Purification of total DNA from insects using the DNeasy® Blood &**

**Tissue Kit**

**Things to do before start**

Buffer AL may form precipitates upon storage. If necessary, warm to 56°C until the precipitates have fully dissolved.

Make sure that ethanol is added to buffer AW1 and AW2.

Sterilize metal beads.

## 1.

Dry the insect material if stored in ethanol (20-30 min).

Add **180 μl PBS** in **2 ml** screw cap tubes

Add **metal beds**

Place **up to 50 mg** dried insect material in the tubes

## 2.

**Homogenize** the sample using the TissueLyser

(4 min on 30 Hz)

Quickly spin down samples in centrifuge

## 3.

For each sample, do the following procedure before continuing with the next sample:

Add **20 μl proteinase K**

Add **200 μl Buffer AL using filter tip** followed by mixing with the pipette (it is important that the sample is mixed with buffer AL immediately)

Mix by inverting/shaking the tube

When done for all samples:

Mix **thoroughly** by **vortexing** for approximately 30 sek

4.   
**Incubate** at 56°C for at least 3 h

5.   
Quickly spin down samples in centrifuge.

Add **120 μl Buffer P3** and mix by inverting the tube ~3 times immediately for each sample.

**Incubate on ice for 5 min**.

6.  
**Centrifuge** for **5 min** at **14´000 rpm**.

7.  
**Pipet** the **lysate** into the **QIAshredder Mini spin column (lilac)** placed in a 2 ml collection tube, and centrifuge for **2 min** at 20,000 x g **(14,000 rpm)**.

8.   
**Pipet** the **lysate** without disturbing the pellet into a 1.5 ml tube

## 9.

Add **4** **μl RNAse** and mix by pipetting + inverting the tube 1 time.

**Incubate** in room temperature for **2 min**

10.  
Add **200 μl ethanol** (96–100%) to the sample and mix immediately by pipetting until the liquid looks homogenized + inverting the tube 1 time.

## 11.

**Pipet** the mixture (not more than 660 μl) into the DNeasy Mini spin column placed in a **2 ml collection tube** (provided).

Centrifuge at **10 000 rpm** for **1 min**.

Discard flow-through and collection tube.

12.

**Place** the Mini spin column in a new 2 ml collection tube (provided).

Add **500 μl Buffer AW1**

Centrifuge for **10 000 rpm** for **1 min**.

Discard **flow-through** and collection tube.

13.

Make sure membrane is dry after centrifugation in this step!

Place the Mini spin column in a new 2 ml collection tube (provided)

Add **500 μl Buffer AW2**

Centrifuge for **3 min** at 20,000 x *g* (**14,000 rpm**) to dry the membrane.

Discard **flow-through** and **collection tube**. Do this carefully so that no Buffer AW2 comes in contact with the spin column!

14.

Place the Mini spin column in a clean **1.5 ml or 2 ml microcentrifuge tube** (not provided).

Pipet **60 μl Buffer EB** **directly** onto the DNeasy membrane.

**Incubate** at room temperature for **at least 6 min.**

Centrifuge for **1 min** at **8500 rpm** to elute.

(Optional: repeat one more time with the same EB buffer and the same collection tube).

**Gel**

Mix 30 - 35 ml of 1 % agarose gel

(% agarose depends on the length of the DNA, 1% is for longer fragments. We used 1% 14/11)

Add 5 μl Gel-red

Prepare 2 μl from each sample in the DNA-lab (i små PCR-provrör)

Bring into post PCR lab

Add 4 μl Stop-Mix to the samples

Use 3 μl ladder

80 V for 30 min (man kan också öka på tiden lite)

**Regent order**

PBS

Proteinase K

Buffer AL

RNAse

96 – 100 % Ethanol

AW1

AW2

Buffer EB

**Qbit**

Skala: ng/μl

Standard håller 3 dygn

Standard: 190 μl buffer + 10 μl standard

Sample: 199 μl buffer + 1 μl sample

Vortexa minst 3 min

Spinna ner snabbt

Inkubera i rumstemp minst 2 min