DNA Extraction from BLOOD, Using Qiagen DNeasy Blood & Tissue Kit (UPrep columns)

updated Feb 2016 Bronwyn Butcher

IMPORTANT BASICS

- **FOLLOW THIS STANDARD OPERATING PROCEDURE WHENEVER HANDLING BLOOD IN LYSIS BUFFER FOR DNA EXTRACTION.**
- **Wear gloves throughout.**

Day 1:

Before you start:

1. Make a **data sheet for your samples:** ID#, date, initials
2. Retrieve **Proteinase K** from -20 freezer (20 ul per sample). Thaw, vortex, centrifuge, place on ice.
3. Make sure **Incubator** (by the door) is set to 64C.

Extraction Procedure:

1. Centrifuge blood tube at full speed for 1 minute.

2. Prepare one 1.5 ml microcentrifuge tube for each blood sample. Place in rack and number numerically (1 to x) – label top and side of each tube.

3. To each labeled, empty microcentrifuge tube, add **20uL Proteinase K**.
   RED P20, set to 20, filter tip
   Reuse one tip – watch bubble bead on side. Change tips as needed to avoid bubbles.

1. Add **150 uL PBS .**
   RED P200 set to 150, nonfilter

2. Add **50 uL blood in lysis buffer.**
   YELLOW P1000, set to ~600 (whatever gives enough suction), **filter** tip, new tip per sample

3. Add **200uL Buffer AL. Vortex well immediately,** for ~5 seconds. (Do not mix AL with any bleach).
   RED P1000 set to 200, nonfilter, new tip per sample
   CAUTION: **BUFFER AL is Highly reactive when combined with bleach!**
   Dangerous components**: guanidinium choloride** (25-50%), which CANNOT go down drain.

4. **Incubate** overnight (up to 24 hours) at 64C in incubator on shaking platform set at 164rpm

5. Clean counters with **water first**, then 10% bleach; empty and rinse used tip container.

Day 2:

Prep:

1. Turn on water bath and set temperature to 64C (for blood samples) or 56C (for tissue samples)
2. Centrifuge the digests at full speed for 0.5 minutes. Add **200uL Ethanol** (96-100%) to the digests, working with up to 12 tubes at a time. VORTEX well.
3. RED P1000, set to 200, non-filter tip, 1 tip per~10 samples
4. **Prepare one spin column in its tube for each blood sample by placing in a rack and labeling each column on it's lip. Label numerically, 1 to x according to the number of samples (x) you will be extracting.**
5. Get out **Pipetters:** RED P1000 set to 200 – non-filter tips (Ethanol); YELLOW P1000 set to 650 – filter tips (sample) RED P1000 set to 500 – non-filter tips (Buffer); RED P200 – non-filter tips (Elution buffer)
6. Get a 250 ml glass beaker for buffer disposal

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Extraction Procedure :

1. **Using a new tip for each sample, Add ALL of liquid into the spin column including any undigested material.** YELLOW P1000 set to 650 – filter tips, new tip per sample

2. Centrifuge spin tubes at full speed ≥ 8000 rpm for 1 minute. **NOTE: BE SURE TO CHECK THAT ALL OF THE LIQUID HAS PASSED THROUGH THE COLUMN. CENTRIFUGE AGAIN FOR ANOTHER 2-3 MINUTES IF IT HAS NOT PASSED THROUGH.**

WASHING (Do not mix any wash buffers with bleach).

1. Collect the liquid that passes through column into glass beaker for later disposal, blot tube dry; replace spin column to tube.
   To blot: put plastic wrap on counter + 2 layers of KimWipes; Collect the liquid that passes through column into glass beaker for later disposal; tap/blot on KimWipe.

2. Add 500uL Buffer AW1 to each column. - RED P1000 set to 500, non-filter tips, new tip per sample if needed.
   CAUTION: **BUFFER AW1 is Highly reactive when combined with bleach!**
   Dangerous components**: guanidinium choloride** (25-50%), which CANNOT go down drain.

3. Centrifuge spin tubes at full speed ≥ 8000 rpm for 1 minute. Collect the liquid that passes through column into a glass beaker for later disposal as in step 4; replace column to tube.

4. Add 500uL Buffer AW2 to each column. - RED P1000 set to 500, non-filter tips, new tip per sample.

5. Centrifuge spin tubes at full speed for **3 minutes**. Column must be dry. If not, centrifuge 1 more minute. This pass through liquid can go into the normal trash, with the collection tube.

ELUTION OF DNA FROM COLUMN

1. Place a new set of **1.5** ml labeled tubes into a rack for collection of the DNA from the column and add spin column.

2. First elution - Add 50uL warmed, nuclease free H20 directly onto the spin column membrane.- RED P200 – non-filter tip, new tip per sample.

3. Incubate at room temperature for 1 minute., then Centrifuge spin tubes at full speed rpm for 1 minute. (line the caps of the tubes up in the opposite direction of the rotation of the rotor)
   THE **LIQUID COLLECTED IN THE TUBE IS YOUR DNA!!**

4. Second elution – add spin column to new 1.5mL labelled tube. Add 100uL warmed, nuclease free H20 directly onto the spin column membrane.- RED P200 – non-filter tip, new tip per sample.

5. Incubate at room temperature for 1 minute., then Centrifuge spin tubes at full speed rpm for 1 minute. (line the caps of the tubes up in the opposite direction of the rotation of the rotor)
   THE **LIQUID COLLECTED IN THE TUBE IS YOUR DNA!!**

6. For maximum DNA yield: repeat elution, (usually not needed, and it dilutes the DNA).

7. LABEL tubes as you throw away column. Include DATE, "DNA" and identification info (e.g. USFW# for birds) on TOP and SIDE of tubes. (ALTERNATIVELY, PRELABEL THESE TUBES THE DAY BEFORE AND STORE CLOSED UNTIL NEEDED).

CLEAN UP

1. Dispose of AL and AW1 buffers in appropriate collection jug, underneath Fume Hood. Clean used glass beaker with hot tap water, rinsing very well (no bleach!!).

2. Clean counters with **WATER FIRST**, then 10% bleach; empty and rinse and dry used tip container.