Step 2: Protocol for extraction and purification of DNA

Protocol

The procedure for purification of DNA is carried out with the QIAamp DNA Mini Kit (QIAGEN). The kit can be stored at room temperature (15-25°C) for up to 12 months. QIAamp DNA Mini Kit contains ready-to-use Proteinase K solution, which is stored at 4°C (fridge).

Equipment and Reagents to Be Supplied by User:

- QIAamp DNA Mini Kit (Qiagen), catalog Nos. 51304 (50 rns). & 51306 (250 rns.)
- Absolute ethanol (96-100%) (stored at 4°C)
- Syringe 2.5 ml with needle 21 Gauge (Terumo)
- Micropipets and pipet tips with aerosol barrier
- Microcentrifuge (with rotor for 1.5-2 ml tubes)
- Vortexer
- Water bath or heating block at 56°C
- Phosphate-buffered saline (PBS 1X) (stored at 4°C)

Buffer Preparation:

Preparation of Wash Buffer- AW1 and **AW2** buffers are supplied as a concentrate. Before using for the first time, add the appropriate amount of ethanol (96–100%) as indicated on the bottle. AW1 and AW2 buffers are stable for 1 year when stored closed at room temperature.

Preparation of Cell Lysis Buffer

1. Introduce 220 µl **Buffer PBS 1X** into a 1.5 ml microcentrifuge tube. Add 22 µl QIAGEN **Proteinase K** to the microcentrifuge tube.

2. Mix by pulse-vortexing for 1 second and briefly centrifuge the tube for 2 seconds.

3. Add 220 µl **Buffer AL**. Mix by pulse-vortexing for 2-5 seconds and briefly centrifuge the tube for 2 seconds.

   Note: *Mix Buffer AL thoroughly by shaking before use. Do not add Proteinase K directly to Buffer AL.*

1. Disconnect the needle and the piston from the syringe.

2. Connect the **Filter** to the syringe, then to the needle, and placed the system in 2 ml microcentrifuge tube.

   Note: *If the Filter has been stored at -20°C, leave it on the bench 5 min at room temperature before the lysis step*

3. Introduce 462 µl of **Cell Lysis Buffer** into the syringe, then reinsert the piston.

4. Pass the lysate 3 times through the filter by aspiration/discharge operation and pushing/pulling the piston slowly.

Note: *If foaming occurs, we recommend to briefly centrifuge of the tube for 2 seconds.*

5. Incubate the sample at 56°C for 15 minutes (water bath or heating block).

6. Briefly centrifuge the 2 ml microcentrifuge for 2 seconds to remove drops from the lid or sides.

7. Add 220 µl of **Ethanol** to the sample and mix by pulse-vortexing for 10-15 seconds. After mixing, briefly centrifuge the 2 ml microcentrifuge for 2 seconds to remove drops from the lid or sides.

8. Introduce the mixture (682 µl) onto the **QIAamp Mini spin column** (in a 2 ml collection tube). Close the cap, and centrifuge at full speed (14,000 rpm, 16,800 rcf) for 1 min. Discard the flow-through and collection tube.

9. Place the QIAamp Mini spin column in a new 2 ml collection tube and add 500 µl **Buffer AW1.** Close the cap, and centrifuge at full speed (14,000 rpm, 16,800 rcf) for 1 min. Discard the flow-through and collection tube.

10. Place the QIAamp Mini spin column in a new 2 ml collection tube and add 500 µl **Buffer AW2.** Close the cap, and centrifuge at full speed (14,000 rpm, 16,800 rcf) for 3 min. Discard the flow-through and collection tube.

Note: Alternative protocol. *For steps 8, 9 & 10 using the* ***QIAvac 24 Plus*** *(Qiagen). Ensure that the main vacuum valve is closed. Switch on the vacuum pump by pressing the power switch. Adjust the needle of the vacuum approximately at* ***-600 mbar****. Insert the VacConnector into the luer slot, then the QIAamp Mini spin column of the QIAvac 24 Plus. Introduce the mixture from the step 7 (682 µl) to the QIAamp Mini spin column and leave the lid open. Open the main vacuum valve and ensure that the needle is stablilized at -600 mbar. After all lysate has been drawn through the spin column, switch off the vacuum pump. Apply 750 µl* ***Buffer AW1*** *to the QIAamp Mini spin column and switch on the vacuum pump. After all of Buffer AW1 has been drawn through the spin column, switch off the vacuum pump. Apply 750 µl* ***Buffer AW2*** *to the QIAamp Mini spin column and switch on the vacuum pump. After all of Buffer AW1 has been drawn through the spin column, switch off the vacuum pump. Close the lid of the spin column, remove it from the vacuum manifold, and discard the VacConnector.*

11. Place the spin column into a 1.5 ml microcentrifuge tube and centrifuge at full speed for 1 min to eliminate possible traces of Buffer AW2, which will be removed by pipetting.

12. Add 50 µl of **Buffer AE** directly to the column matrix, and incubate at room temperature for 5 minutes. Centrifuge at full speed for 1 min to elute the DNA.

13. Determine the concentration of DNA by fluorometry (Qubit® fluorometer recommended) or UV spectrometry (e.g., NanoDrop).

The DNA solution is ready for immediately analysis or can be stored at --20°C for later use.