DNA ISOLATION FROM LASER CAPTURE MICRODISSECTION BIOLOGICAL SAMPLES USING QIAamp® DNA FFPE TISSUE KIT

Purpose: Isolate DNA from laser capture microdissection (LCM) samples
Reference: QIAGEN QIAamp DNA FFPE Tissue Handbook

Approach: Use QIAamp® DNA FFPE Tissue kit

Background Information

1. If you are expecting **50- 100 ng of DNA**, **3 times elution** with 30 µl buffer ATE should be enough to recover the maximum amount of input DNA from the column. The efficiency of DNA recovery was tested by using 25, 50 and 100 ng of soybean cotyledon-stage seed genomic DNA and most of DNA was recovered by 2 times elution, but 7-10% more DNA could be recovered by a third elution.
2. If you are expecting **more than 100 ng of DNA**, **5 times elution** should be enough to recover the maximum amount of input DNA from the column.
3. If there are **multiple tubes containing samples** and the amount of expected DNA is **less than 2 µg** (the capacity of DNA column), you can add the lysate from each tube (treated as step 2 to 6) to one QIAamp MinElute column by repeating step 7 and then process the column for DNA isolation. We tested by adding 50ng of soybean cotyledon-stage seed genomic DNA onto the column five times and could recover 93-100% (233-250 ng) of input DNA.
4. Include DNA sample with known concentration as experimental control
5. This Protocol is written based on QIAamp® DNA FFPE Tissue Handbook (Catalogue number 56404, QIAGEN)

Important points before starting

1. QIAamp MinElute columns should be stored at 2–8°C upon arrival
2. Test RNase before use to make sure that RNase is not contaminated
3. With a thickness of 10 µm and a surface area of up to 100 mm^2, can be combined in one preparation (in 180ml Buffer ATL).
4. Equilibrate all the buffers at room temperature and check whether precipitate has formed in Buffer ATL and AL before starting the procedure

Procedure

1. Combined LCM capture tissues up to 100 mm^2.

2. Add Buffer ATL up to 180 µl. Then, add 20 µl proteinase K, and mix by vortexing.
3. Incubate at 56°C for 1 h (or until the sample has been completely lysed).
4. Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the lid.
5. Add 2 µl RNase A (100 mg/ml) (10 µl Invitrogen RNaseA (20 mg/ml)) and incubate for 2 min at room temperature.
5. Add 200 µl Buffer AL to the sample, and mix thoroughly by vortexing. Then add 200 µl ethanol (96–100%), and mix again thoroughly by vortexing.
* A white precipitate may form on addition of Buffer AL and ethanol. Based on QIAamp DNA FFPE Tissue Handbook, this precipitate does not interfere with the QIAamp procedure.
6. Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the lid.
7. Transfer the lysate to the QIAamp MinElute column (in a 2 ml collection tube) and centrifuge at 6000 xg (8000 rpm) for 1 min. Place the QIAamp MinElute column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.
8. Add 500 µl Buffer AW1 to the column and centrifuge at 6000 xg (8000 rpm) for 1 min. Place the column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.
9. Add 500 µl Buffer AW2 to the column and centrifuge at 6000 xg (8000 rpm) for 1 min. Place the QIAamp MinElute column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.
10. Centrifuge at full speed (20,000 xg; 14,000 rpm) for 3 min to dry the membrane completely.
11. Place the column in a 1.5 ml microcentrifuge tube and apply 30 µl Buffer ATE to the center of the membrane.
12. Incubate at room temperature for 5 min. Centrifuge at full speed (20,000 xg; 14,000 rpm) for 1 min.
13. Repeat Step 11 and 12. Use same tube to collect DNA from additional elution.