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DNA Extraction from Filters using QIAgen DNeasy and QIAshredder

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1 Works for me dx.doi.org/10.17504/protocols.io.jx5cpq6

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ABSTRACT

From the Qiagen DNeasy Handbook (07/2006)
http://www.qiagen.com/products/catalog/sample-technologies/dna-sample-technologies/genomic-dna/dneasy-blood-and-tissue-kit

Reagent Preparation

1

Do the following once per kit:
1. Add 25 mL ethanol to Buffer AW1; check box on bottle (✔).
2. Add 30 mL ethanol to Buffer AW2; check box on bottle (✔).

Do the following in preparation for each extraction:
1. Confirm Buffers ATL and AL are completely in solution; warm to 56° C if necessary.
2. Prepare a solution of 100 µL Buffer ATL+ 30 µL Proteinase K X number of samples.

If Proteinase K is in powder form, create a 10 mg/ml solution in nuclease free water. Prepare only the amount you need for the set of extractions that day.

Bench Preparation

2
1. Turn on water bath or incubator and set to 56°C.
2. Wipe down bench surface with 70% EtOH.
3. Fill beaker with 70% EtOH and rest forceps in beaker for flame sterilization.
4. Position a Bunsen burner nearby.
5. Retrieve samples from freezer and allow to thaw on ice. No more than 6 filters or 16 plastic pieces at one time.

Sample Record

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Reference Number (Barcode Number):
Liters of Water Represented:
Filter Pore Size:
Portion of Filter Used:
Number of Portions Processed:
Plastic Description (type, size, thickness, color, opacity):
DNA Elution Volume at the End of this Procedure:
Other Notes/Details:

Filter Preparation and Cell Disruption

4
1. Label a 1.5 mL tube for each sample. Add 130 µL of ATL & Proteinase K solution to each tube.
2. Flame-sterilize forceps and blade over Bunsen burner and allow to cool on a Kimwipe.
3. Using two sterilized forceps, pull filter out of vial and lay on the inside of the plate top. Cut with sterilized blade to desired size and return unwanted portion of filter to the vial. Fold the desired portion of filter in half with the sample folded inside.
4. Unfold filter and transfer into labeled 1.5 mL tube.
5. Discard empty used vials and wipe down bench top between each sample.

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Once all filters or plastics are in the ATL solution, mix by vortexing (10 sec).
Add 300 ul Buffer AL, and mix by vortexing (10 sec).
Incubate for 60 minutes at 56°C, mixing by vortexing every 15min (or carry out in incubation oven with rotation).

01:00:00 Incubation time, vortex every 15 mins

THIS IS A GOOD TIME TO LABEL THE TOP AND SIDES OF THE QIASHREDDER AND DNEASY COLUMNS FOR FUTURE USE. ALSO LABEL ONE 1.5 ML LO-BIND TUBE WITH EXTRACTION NUMBER AND ONE 1.5 ML LO-BIND TUBE WITH STICKERS WITH SAMPLE ID.
Vortex at max speed for 10 minutes.

00:10:00 vortex at max speed using multi-tube adapter on vortex

Centrifuge for 30 seconds at 20K x g.

Cell Homogenization using QIAshredder kit

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1. Flame-sterilize forceps for filter removal.
2. Pipette as much lysate as possible into QIAshredder (purple) column. Place plastic or filter in column along with this lysate.
3. Centrifuge for 15 seconds at 20K x g.

DNA Capture and Purification

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1. Add 300 µL of 100% Ethanol to flow through. Mix by vortexing. You can clip the purple cap off the column and use that to cover the tube while vortexing.
2. Transfer 700 µL lysate+ethanol mixture onto DNA Mini Spin (clear) column.
3. Centrifuge for 15 seconds at 20K x g.
4. Discard flow-through (not compatible w/bleach).
5. Repeat from step 2 until all lysate+ethanol mixture has passed through column. Discard flow through and collection tube when it has all passed through the column.
6. Place column in new, labeled, 2 mL collection tube.
7. Add 500 µL Buffer AW1 to DNA Mini Spin column.
8. Centrifuge for 15 seconds @ 20K x g.
9. Discard flow-though (not compatible w/ bleach).
10. Add 500 µL Buffer AW2 to DNA spin column.
11. Centrifuge for 2 min @ 20K x g.
12. Empty flow-through and spin again for 1 minute.

DNA Elution

8
1. Place DNA Mini Spin column in clean 1.5 mL tube labeled with extraction number in sharpie.
2. Add 50 µL Buffer AE directly onto column membrane.
3. Incubate @ room temperature for 3 minutes. **00:03:00** **incubate at room temperature**
4. Centrifuge for 1 minute @ ≥8000 x g. This is "Elution #1."
5. Transfer column to new 1.5 mL tube labeled with same name and "Elution #2."
6. Add 50 µL Buffer AE directly onto column membrane and centrifuge for 1 minute @ ≥8000 x g into new tube. This is Elution #2.
7. Store samples (Elution #1 and Elution #2) at 4°C. If DNA will not be used for several months, make aliquots for the fridge and keep the rest in the -20°C or -80°C freezer.

Overview

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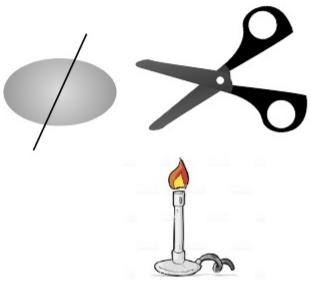
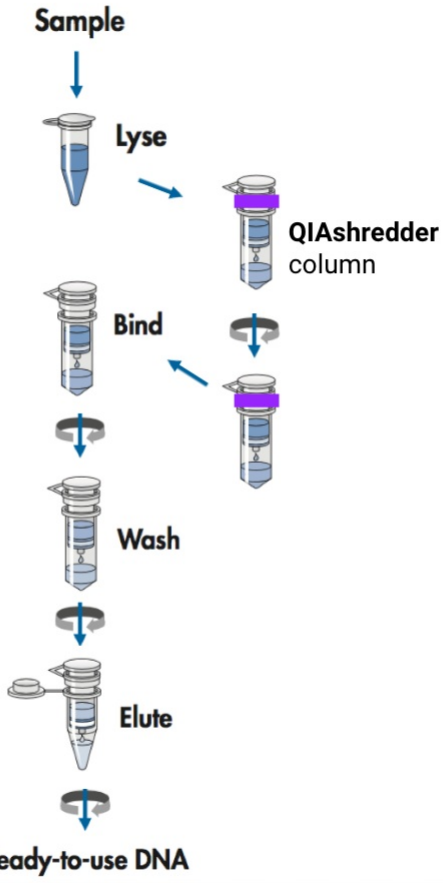
Prepare filters.
Cut in half with sterilized scissors.
Return ½ filter to freezer.





Filter with **buffer ATL** **proteinase K**. Vortex

Buffer AL. Vortex. Incu

Ethanol to flow-throug

Transfer all material ba DNeasy column.

Buffer AW1, centrifuge **Buffer AW2**, centrifuge

Buffer AE, centrifuge i clean tube (Elution 1). **Buffer AE**, centrifuge i clean tube (Elution 2).