AquaGenomic™ Instruction Manual

General Information

Description

AquaGenomic™ is an aqueous genomic DNA extraction reagent enabling rapid, scalable, non-toxic DNA isolation from cells, tissues, microbes, saliva, stool, soil, and blood. Diluted lysates may be used directly for PCR.

Specification

Product Name	AquaGenomic™ Solution
Product #	AG2030M
Size	30 ml AquaGenomic™ for 300 minipreps from cultured cells
MSDS	Available at www.boltii.com
Storage	Store tightly capped at room temperature (~22°C).
Note	Order these ancillary reagents with AquaGenomic™ for specific applications: • AquaPrecipi™ (AP3015M) for stool and soil DNA • AquaRemove™ (AR1208M) for avian blood DNA

Terms & Condition

Product Usage: For *In Vitro* Laboratory Research Use Only. NOT to be administered to humans or used for medical diagnosis.

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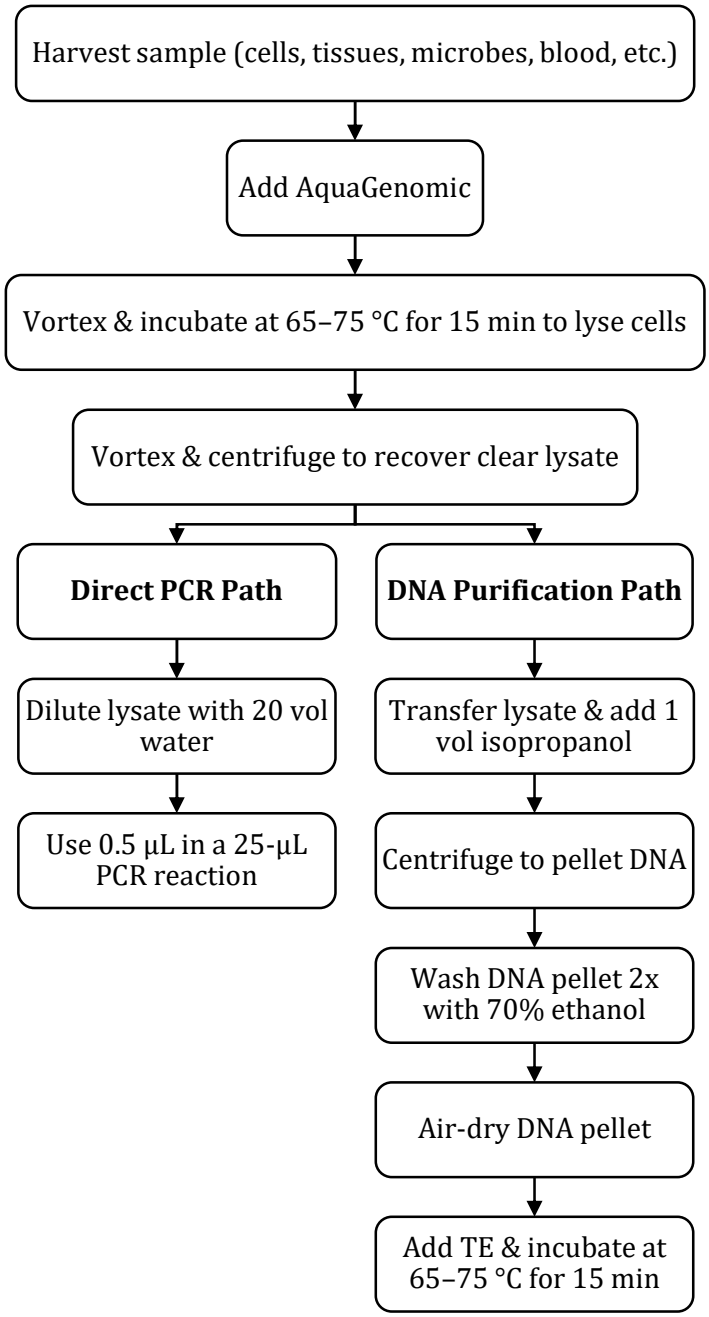

AquaGenomic™ Protocol Flowchart

Cultured Cell DNA Protocol

Extract genomic DNA from cultured cells (~1–2 million cells).

1. Harvest Cells

- Pellet 1–2 × 10^6 cells at 3,000×g for 5 min.
- Carefully aspirate all medium.

2. Cell Lysis

- Add **100 µL AquaGenomic™** to the cell pellet.
- Vortex vigorously for 1 min until no visible clumps remain.
- Incubate at **65–75 °C for 15 min** to lyse cells.

3. Pellet Debris

- Centrifuge at 12,000×g for 5 min.
- Transfer the clear supernatant to a new tube without disturbing the pellet.

Note: For direct PCR, dilute an aliquot of the lysate with **20 volumes of water** and use **0.5 µL** of the diluted lysate in a **25-µL PCR reaction**.

4. DNA Precipitation

- Add an **equal volume** of isopropanol.
- Vortex 30 s (solution will appear cloudy).
- Centrifuge 12,000×g for 5 min to pellet DNA.
- Flip tube to discard supernatant.

5. Ethanol Wash

- Rinse the pellet with **70% ethanol** (fill the tube, including cap).
- Discard ethanol and repeat once.
- Tap tube on paper towel to remove residual ethanol.
- Air-dry pellet for 15 min.

6. DNA Solubilization

- Add **100 µL TE buffer**; pipette and vortex to disperse.
- Incubate at **65–75 °C for 15 min** to solubilize DNA and inactivate DNases.
- *Optional:* For maximum yield, incubate at **4–22 °C overnight** before spinning.
- Spin 5 min and transfer the DNA-containing supernatant to a new tube.

Tissue DNA Protocol

Extract genomic DNA from animal or plant tissues (~10 mg).

1. Prepare Sample

- Cut ~2 mm tissue cubes (~10 mg).

2a. Lysis Method A: Homogenization

- Add **200 µL AquaGenomic™**.
- Homogenize thoroughly until no fragments remain.
- Incubate at **65–75 °C for 15 min**.

2b. Lysis Method B: Proteinase K Digestion for Tough Tissues

- Add **200 µL AquaGenomic™ + 10 µg Proteinase K**.
- Incubate at **55 °C for ≥90 min**.
- Heat at **95 °C for 10 min** to inactivate Proteinase K.
- Pipette to break remaining tissue.

Proceed through Steps 3–6 as described in the Cultured Cell DNA Protocol.

Microbial DNA Protocol

Extract genomic DNA from Gram-negative bacteria, Gram-positive bacteria, or yeast.

1. Harvest Cells

- Pellet 1 mL overnight culture at 12,000×g for 1 min.
- Aspirate supernatant.

2. Cell Lysis

- Pre-treat Gram-positive bacteria with **lysozyme**, or yeast with **lyticase**.
- Pellet cells and remove supernatant.
- Add **20 mg sterile sand + 200 µL AquaGenomic™ + 10 µg Proteinase K**.
- Incubate **55 °C for 60 min → 95 °C for 15 min**.
- Vortex vigorously for 1 min.

Proceed through Steps 3–6 as described in the Cultured Cell DNA Protocol.

Stool and Soil DNA Protocol

Extract DNA from stool or soil; removes PCR inhibitors using AquaPrecipi™.

1. Sample Preparation

- Feces: ~15 mg (or 10 mg dry rodent pellet).
- Soil: ~30 mg.

2. Lysis

- Add **200 µL AquaGenomic™**.
- Homogenize 1–2 min.
- For mitochondrial DNA:
    - Add Proteinase K to **100 µg/mL**
    - Incubate **55 °C 60 min → 95 °C 15 min**

3. Pellet Debris

- Centrifuge 12,000×g for 5 min; transfer clear lysate.

4. DNA Precipitation

- Add **0.5 vol AquaPrecipi™ + 0.5 vol ethanol**.
- Vortex 30 s.
- Centrifuge 12,000×g for 5 min; discard supernatant.

Proceed through Steps 5–6 as described in the Cultured Cell DNA Protocol.

Saliva DNA Protocol

Extract DNA from human saliva or mouthwash.

1. Collection

- Saliva: Collect **250 µL** after cheek rubbing.
- Mouthwash: Rinse with 10–20 mL Scope®; pellet **250 µL**.

2. Lysis

- Mix **250 µL sample + 250 µL AquaGenomic™**.
- Incubate **15 min at 65–75 °C**.

Proceed through Steps 3–6 as described in the Cultured Cell DNA Protocol.

Whole Blood DNA Protocol

Extract genomic and cell-free DNA from whole blood.

1. Collection

- Collect anticoagulated blood using standard procedures.

2. Lysis

- Add **250 µL blood to 250 µL AquaStool™**.
- Vortex and incubate **15 min at 75 °C**.

Proceed through Steps 3–6 as described in the Cultured Cell DNA Protocol.

Avian Blood DNA Protocol

Extract 50–100 µg DNA from avian blood stored in Queen's lysis buffer.

1. Collection

- Collect **10 µL avian blood** into **90 µL Queen's lysis buffer**.

2. Lysis

- Add **200 µL AquaGenomic™**; vortex and incubate **15 min at 75 °C**.

3. Debris Removal

- Add **100 µL AquaRemove™** (1:1 diluted with isopropanol).
- Centrifuge 12,000×g for 5 min.
- Transfer clear supernatant to new tube.

Proceed through Steps 4–6 as described in the Cultured Cell DNA Protocol.

Frequently Asked Questions

Please read through these questions carefully. The answers provide additional helpful tips and useful information for the successful use of AquaGenomic.

1. Do I need to keep AquaGenomic in the freezer?

No, AquaGenomic Solution is stable at room temperature (~22 °C) for >1 year.

2. Does AquaGenomic Solution contain Proteinase K?

No. AquaGenomic can be used to extract DNA from most cells and tissues without needing protease digestion. However, adding Proteinase K (50 µg/mL) to AquaGenomic solution can increase DNA yield and is required for mitochondrial DNA extraction. You may homogenize the sample in AquaGenomic containing Proteinase K, incubate it at 55 °C for 1-2 hrs and then at 95 °C for 10-15 min to inactivate the Proteinase K.

3. I am worried about cross-contamination using homogenizers, any tips?

Between uses, you may wash the homogenizer with soap and running water, soak it in 10% bleach for ~5 min, and then rinse it with running deionized water. Alternatively, you may use Proteinase K digestion for tissue lysis without using a homogenizer, or use a multichannel bead beater for homogenization in disposable screw-capped tubes.

4. How may I maximize the DNA yield?

Incubating the crude lysate at 65-75 °C for better cell lysis and/or incubating the DNA pellet in TE buffer at 4-22 °C overnight before centrifuging to remove any insoluble in DNA pellet may maximize DNA recovery.

5. Do I have to use the lysate immediately for PCR?

No, you may store the lysate at 4 °C until analysis. If the lysate has been incubated at 75-85 °C for 15 min, it may even be left at room temperature until analysis.

6. I got a weak PCR amplification using the lysate directly, how may I improve it?

You may try a few things to optimize the amplification: a) try different amount (0.25–2.5 µL) of 10-20x diluted lysate per 25-µL PCR reaction, b) add 0.1 mg/mL BSA to the PCR reaction, c) add 1 mg/mL DTT to the PCR reaction, and d) increase the PCR cycle number to 45 cycles.