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EPMotion - DNA Extraction

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CGORD Palmer Wet-Lab Protocols

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ABSTRACT

This protocol is designed for Agencourt's DNAdvance Extraction kit on the EPmotion 5075. Samples are extracted in a 96 well plate. This is a continuation of the "Sample Cutting/Processing" Protocol

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PROTOCOL CITATION

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59577

GUIDELINES

This protocol is a continuation to the *Sample Cutting/Processing Protocol* . If you do not have an EPmotion, follow the Agencourt DNAdvace protocol (download the handbook from Step 1 of this protocol).

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MATERIALS TEXT

EPmotion tips are very limited during this pandemic. The tips listed below are tip reloads. You will need to request eppendorf to send a minimum of 8 x 300ul tip trays and 1 x 50ul tip tray.

Equipment

- Eppendorf ThermoMixer C **Catalog No. 5382000023**
- Eppendorf SmartBlock plates attachment **Catalog No. 5363000039**
- Eppendorf Lid for Thermomixer **Catalog No. 5363000233**
- epMotion 5075
- 8 Channel Dispensing Tool (300uL) **Catalog No. 960001052**
- 8 Channel Dispensing Tool (50uL) **Catalog No. 960001044**
- Gripper Tower
- Eppendorf Magnum FLX Magnet Adapter **Catalog No. 960001044**
- Thermoadaptor for PCR **Catalog No. 960002199**
- Eppendorf Reservoir Rack (up to 7 reservoirs) **Catalog No. 960002148**
- Centrifuge that can spin down plates
- DynaMag™-96 Side Magnet **Catalog No. 12331D**

Reagents Supplied by User

- 100% Ethanol 200 Proof

  1M DTT **Sigma**
- **Aldrich Catalog #43816** Step 2 or equivalent
- Ultrapure Distilled, Nuclease Free Water **Contributed by users** Step 22

Consumables

- 50mL Falcon Tube
- 25mL Reagent Reservoir
- 200uL tips
- 200uL Multichannel Pipette
- 1-50uL epT.I.P.S. Motion **Catalog No. 0030014413**
- 20-300uL epT.I.P.S. Motion **Catalog No. 0030015231**
- epMotion reservoir 30mL **Catalog No. 960051009**
- Thermo Scientific™ 96-well Sealing Mats, Square **Cat. AB0675**
- MicroAmp™ Optical 96-Well Reaction Plate with Barcode **Catalog No. 4306737**
- Adhesive PCR Plate Seals **Catalog AB0558**

SAFETY WARNINGS

You will be working with animal tissues.

BEFORE STARTING

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This protocol assumes that you have the EPmotion set up and epBlue software downloaded. Complete the "Sample Cutting/Processing Protocol" before you start on this protocol.

Agencourt DNAdvance Reagent Preparation 30m

1 Download the DNAdvance Handbook from Beckman Coulter Genomics. Google this - (PN B66866AC) or request from Beckman Coulter Group.

Prepare Proteinase K Solution following instructions on page 9 of the Handbook.

- Create **785 µL** aliquots of proteinase K solution in 1.5mL tubes.
- Store Proteinase K solution at **-20 °C**
- Note: 1 Aliquot will provide enough reagent for 1 plate.

2 1M DTT **Sigma**

Create **565 µL** aliquots of **Aldrich Catalog #43816** into 1.5mL tubes

- Store DTT at **-20 °C**
- Note: Note: 1 Aliquot will provide enough reagent for 1 plate.

3 Aliquot Bind BBE (magnetic beads) **Beckman Coulter Genomics** into 50mL falcon tubes for ease of use.

- Ensure you fully vortex and shake the beads (there should be no beads collected at the bottom of the bottle)

Lysis Master Mix 5m 30s

4 Note: The following amounts will make a master mix for 96 samples.

Lysis Buffer **Beckman Coulter**

Add **15.18 mL** of **Genomics Catalog #C42203** to a new 50mL falcon tube.

5 Add **770 µL** of Proteinase K to previous 50mL falcon tube

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6 Add **550 µL** of DTT to previous 50mL falcon tube

3m 30s

7 Shake vigorously and vortex for **00:00:30**

- Place **On ice** for **00:03:00** to allow bubbles to settle

Overnight Lysis

8 Spin down Deepwell plate with processed samples from the Sample Cutting/Processing Protocol.

- Defrost Deepwell plate with processed samples if stored in **-80 °C** on ice.
- Spin at 2500rpm for 5 sec

9 Pour Master Mix into a reagent reservoir and Pipette **150 µL** of Master Mix into each well of the deepwell plate.

- Use Multichannel pipette for efficiency

10 Seal deepwell plate with a Square sealing mat (Cat. AB0675) and quickly spin down on centrifuge

- Ensure all wells are tightly covered.
- Spin at 2500rpm for 5 sec

11 Place deepwell plate on Thermomixer C and incubate overnight at **900 rpm, 55°C**

- Use plates attachment and lid
- Incubate between **12-20 hours** **Overnight**

Setting up the EPmotion 30m

30m

12 Next Day,
Equilibrate Bind BBE (magnetic beads) **Beckman Coulter Genomics** to room temperature for **00:30:00**

- Ensure there is at least **25 mL** of beads in the 50mL falcon tube that you had aliquoted in step 3.

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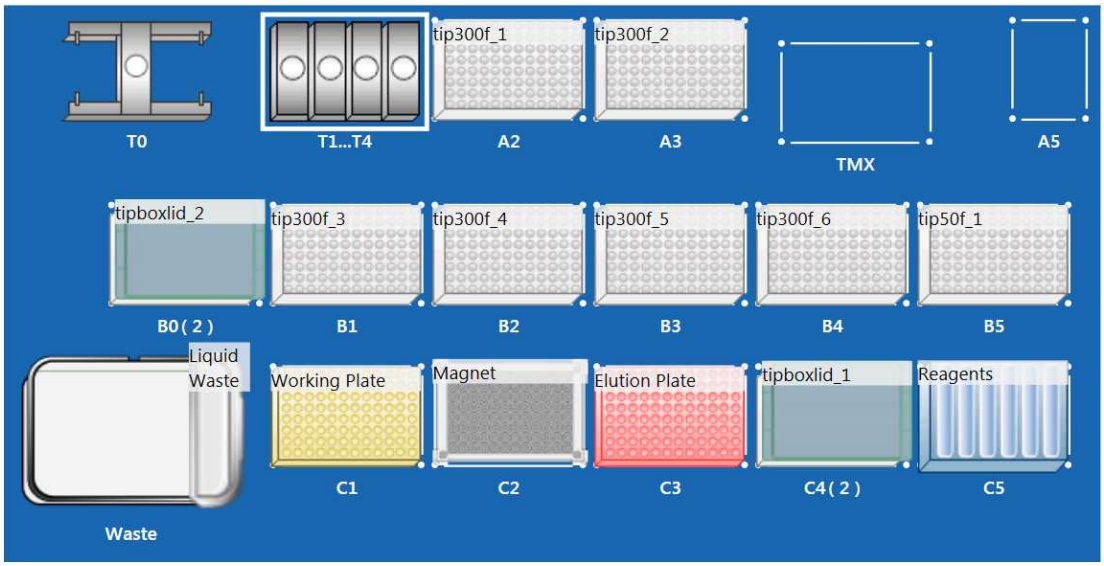
13 Open epBlue Application.

14 In the epBlue Studio, open the Application Editor.

15 Import the epblue protocol **Agencourt DNAdvanced Extraction.export7**

16 Open the imported protocol.
- Named as "Agencourt DNAdvanced Extraction"

17



DNA Extraction Worktable Setup

Set up EPmotion following the worktable figure above.
- Place 300ul EPmotion tip rack on positions A2, A3, B1-B4 without tip rack lid.
- Place 300ul EPmotion tip rack on positions B0 and C4 with tip rack lid.
- Place 50ul EPmotion tip rack on position B5 without tip rack lid.
- Place eppendorf Thermoadapter PCR 96 on position C3
- Place eppendorf magnum FLX magnet on position C2
- Ensure Tip waste basket is empty and liquid waste reservoir is empty.

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18 Labelling epMotion Reservoirs
- Grab 7 epMotion 30mL Reservoir Racks
- Label 1 epMotion Reservoir as "Bind 1"
- Label 1 epMotion Reservoir as "Beads"
- Label 3 epMotion Reservoirs as "70% EtOH"
- Label 1 epMotion Reservoir as "EB"
- Label 1 epMotion Reservoir as "Empty"

19 Place reservoir labelled as "Empty" onto position 7 of the epMotion Reagent Reservoir Rack

20 Add **25 mL** of Pre-Bind PBBA **Beckman Coulter Genomics** to reservoir labelled as "Bind 1".
- Place "Bind 1" onto position 1 of the epMotion Reagent Reservoir Rack

21 Add **25 mL** of Elution EBA **Beckman Coulter Genomics** to reservoir labelled as "EB".
- Place "EB" onto position 6 of the epMotion Reagent Reservoir Rack.

22 Take two clean 50mL falcon tubes and make **100 mL** of 70% EtOH.
- Add **35 mL** of 100% EtOH **Contributed by users** to each 50mL falcon tube
- Add **15 mL** of Ultrapure Distilled, Nuclease Free Water **Contributed by users** to each 50ml falcon tube

23 Add **30 mL** of 70% EtOH made from the previous step to the 3 resevoirs labelled as "70% EtOH"
- Place the three "70% EtOH" reservoirs onto positions 3, 4, and 5 of the epMotion Reagent Reservoir Rack.

24 Shake and Vortex the previously aliquoted 50mL Bind BBE (magnetic beads) **Beckman Coulter Genomics** for **00:05:00** . 5m
- Ensure there is no bead pellet collected at the bottom of the tube

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25 Add **25 mL** of Bind BBE (magnetic beads) **Beckman Coulter Genomics** to reservoir labelled as "Beads"
- Place "Beads" onto position 2 of the epMotion Reagent Reservoir Rack

26 Remove all lids off reagent reservoirs (including "Empty" reservoir) and place reservoir rack onto position C5 of the epMotion worktable.

27 Remove deepwell plate containing all lysed samples from Thermomixer and spin down.
- Remove mat seal from deepwell plate and place on position C1 of the epMotion worktable.
- Ensure that the plate is placed in the correct orientation. Letters and Numbers should be right side up on the plate.

28 Take new a MicroAmp™ Optical 96-Well Reaction Plate with Barcode **(Catalog No. 4306737)** and label with same plate name as the deepwell plate, your initials and today's date.
- Place labelled plate on the thermoadapter on position C3
- Ensure that the plate is placed in the correct orientation. Letters and Numbers should be right side up on the plate.

29 Close epMotion hood when all tips/plates/reagents are in position

Running the Protocol

30 Press the play button

31 Select your EPmotion ID and press "Next"

32 Select the "Input volumes manually" option under the Volume Settings section and press "Next"

33 Press "Next" again until you see this page below.

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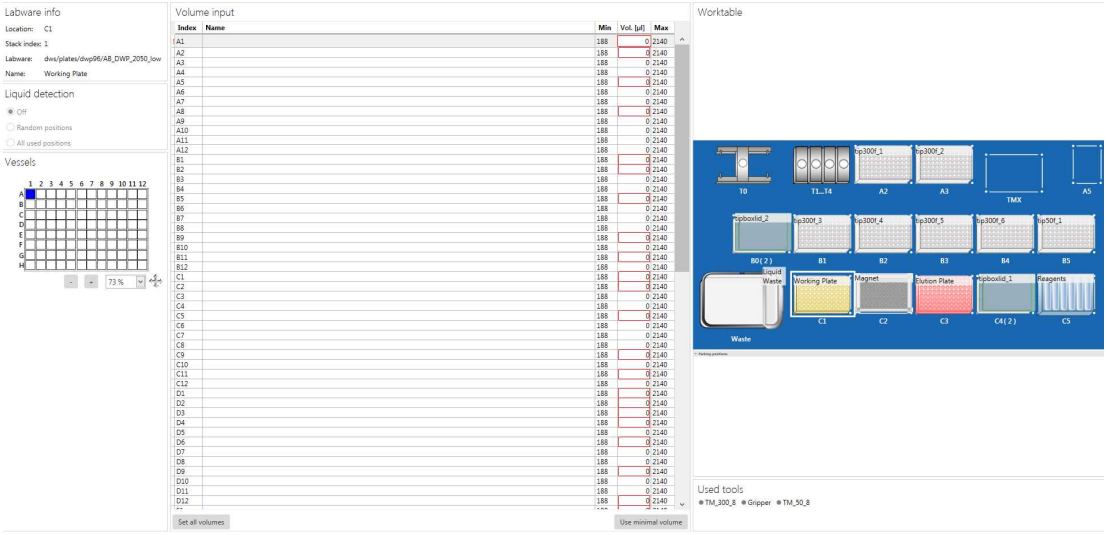

When on the page above, select the "Set all volumes" option and enter "200".

- This will enter the volume of lysate in each well of the deepwell plate to 200ul.

'

Press "Next" when all the fields are set to 200ul.

34 Ensure that the volumes of the reagents are set to:

- Bind 1 - 25000ul
- Bind 2 - 25000ul
- EtOH 1 - 30000ul
- EtOH 2 - 30000ul
- EtOH 3 - 30000ul
- Elution Buffer - 25000ul

35 Press "Run"

36 About halfway through the protocol, there will be a "user intervention" step. The robot will stop and ask you to spin down the deepwell plate and return to magnet.

- Take deepwell plate out and pulse-fuge. Return to magnet and Press "OK".

37 Once the robot is done, open the hood and remove the Elution plate from position C3.

- **NOTE: There will be bead carryover to the elution plate.**
- Seal elution plate with a PCR adhesive seal
- Place elution plate on DynaMag - 96 Side Magnet
- Place magnet and plate at 4C and leave **Overnight**

Day After Extraction

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38 Transfer **180 µL** of supernatant to a new MicroAmp™ Optical 96-Well Reaction Plate with Barcode (**Catalog No. 4306737)**

- Label the new plate the same as the old plate
- If magnetic beads are not separated from the supernatant, wait for a few more hours
- Try not to carry over beads to the new plate

39 Seal, shake and spin down the newly transferred plate.

40 Nanodrop the plate.

- Use the Elution EBA **Beckman Coulter Genomics** to blank the Nanodrop.
- Save the nanodrop table only values and assign the file name as the DNA plate code and date.

Database Entry

41 Open the Extraction Database that you created in the "Sample Cutting/Processing Protocol".

42 Copy and paste the nanodrop concentrations, 260/280, and 260/230 values into the google sheets Extraction Database. (Columns H-J)

43 Enter the date in which you performed the extraction in Column G

44 Enter the plate barcode (as shown below) into Column B of the Extraction Database.

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