Sep 12, 2022

EPMotion - Normalization and Randomization

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dx.doi.org/10.17504/protocols.io.261genw5dg47/v1

CGORD Palmer Wet-Lab Protocols

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ABSTRACT

This protocol normalizes and randomizes samples on the EPmotion for the Twist 96-Plex (Formerly riptide) library prep protocol. The procedure outlines how to complete normalization calculations and the randomization process for up to 96 samples, as well as how to set up the EPMotion robot to complete normalization on a 96-well plate.

DOI

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PROTOCOL CITATION

Katarina A Cohen, Khai-Minh H Nguyen, Oksana Polesskaya, Abraham Palmer 2022. EPMotion - Normalization and Randomization . **protocols.io** https://protocols.io/view/epmotion-normalization-and-randomization-b72nrqde

CREATED

Apr 22, 2022

LAST MODIFIED

Sep 12, 2022

PROTOCOL INTEGER ID

61230

MATERIALS TEXT

Equipment

- PC Running Excel
- EPmotion 5075
- Thermoadaptor for PCR **Catalog No. 960002199**
- Eppendorf Reservoir Rack (up to 7 reservoirs) **Catalog No. 960002148**
- Eppendorf TS 50 single-channel dispensing tool, 1-channel, 1.0 – 50 µL **Catalog No. 960001010**
- Eppendorf 8 Channel Dispensing Tool (50uL) **Catalog No. 960001044**
- TS 300 single-channel dispensing tool, 1-channel, 20 – 300 µL **Catalog No. 960001028**

Reagents

- UltraPure Distilled Water **Invitrogen - Thermo Fisher Catalog #10977-015** Step 13

Consumables

- 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**
- Adhesive PCR Plate Seals **Catalog AB0558**
- PCR Plate, 96-well, semi-skirted, flat deck, black lettering **Catalog AB1400L**

SAFETY WARNINGS

Follow all safety precautions per EPmotion guidelines.

BEFORE STARTING

This protocol assumes you have set up the EPmotion already. All required equipment and reagents are listed in the Materials section.

Excel Calculations

1

Enter sample information and values into the "Riptide Library Template" Excel worksheet:

Riptide Library Template for Low_Concentration Samples.xlsx

Riptide Library Template for High-Concentration Samples.xlsx

- Only use Low-Concentration Template when average concentration is lower than 90ng/ul.
- Enter all information under the "Sample_Information" tab (all information should be filled in from the Google Sheets Extraction Database you created in the "Sample Cutting/Processing Protocol."
- If missing a transponder ID, enter the sample's barcode ID.

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- Note: This protocol is designed to input 100ng of DNA per sample.

2 The "Sample_Randomization" tab will be prepopulated with the values entered under "Sample_Information."
- For samples with concentrations less than 35 ng/uL:
  Low-Concentration Samples -- set the sample volume to 4 uL and the water volume to 0 uL.
  High-Concentration Samples -- set the sample volume to 30 uL and the water volume to 0 uL.
- "Sample (uL)" and "Water (uL)" cells will be highlighted in red if the volume is less than 1 uL. To rectify this, if the sample volume is below 1 uL, round up to 1 uL.
- If the water volume is below 1 uL, adjust the sample volume so that the final water volume is either 0 uL or 1 uL.

3 Randomize the samples in the Sample_Randomization Tab.
- Drag and fill the "Rand" column to replace the random numbers.
- Select columns B-K
- Select "Sort & Filter" and "Custom Sort" the samples by "Rand"

4 Save and Rename the Library file.
- Our lab names the file as follows: Riptide## Library (DNA plate Code)
- EX**. Riptide05 Library (John03)**

5 Open the previously created Extraction Database on google sheets and enter the Riptide## you assigned to this library into column Q of the Extraction Database.

6 Create water and sample .csv file for the EPMotion robot using the following template:
ep_normalization template.csv

6.1 For **water**, enter the following values in their respective columns:
1. Rack: 1
2. Source: 1
3. Rack: 1
4. Destination: A1-A12, B1-B12, etc.
5. Volume: Copy these values from Excel randomization, rounded to two decimal points
   - If the water volume for a well is 0 uL, delete the entire corresponding row.
6. Tool: '1' if volume is under 50 uL, '2' if it is over 50 uL

6.2 For **samples**, enter the following values in their respective columns:
1. Rack: 1
2. Source: Randomized list of the gDNA plate's well IDs from Excel

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3. Rack: 1
4. Destination: A1-A12, B1-B12, etc.
5. Volume: Copy these values from Excel randomization, rounded to two decimal points
6. Tool: 1

Setting up the EPMotion Robot

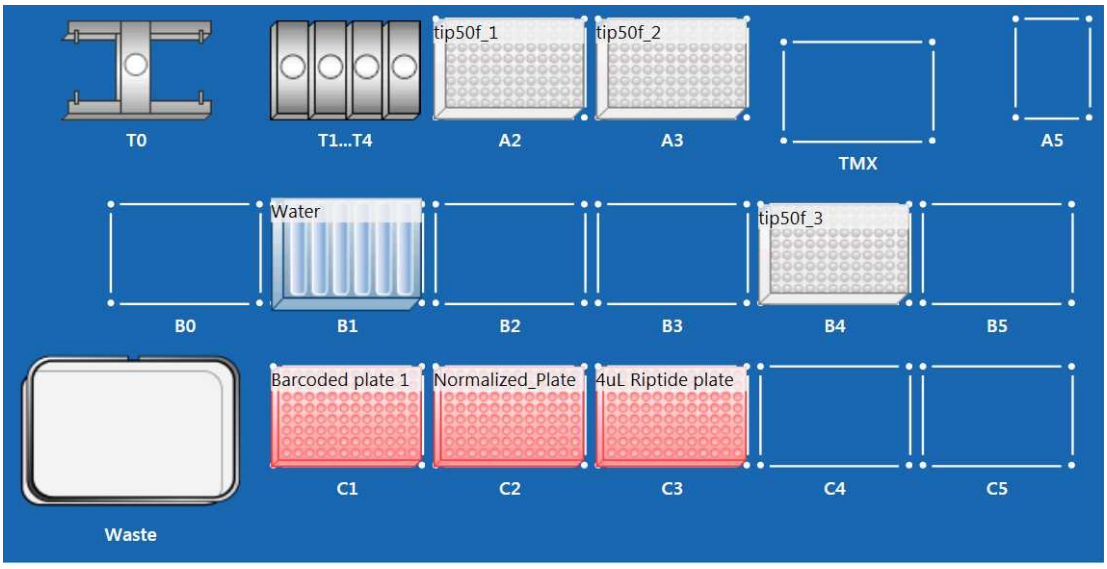
7 Open epBlue Application.

8 In the epBlue Studio, open Application Editor.

9 Import the epBlue normalization protocol.

- High-concentration samples: **Sample_Normalization.export7**
- Low-concentration samples: **Low_Concentration_Sample_Normalization.export7**

10



High-Concentration Sample Normalization Worktable Setup.

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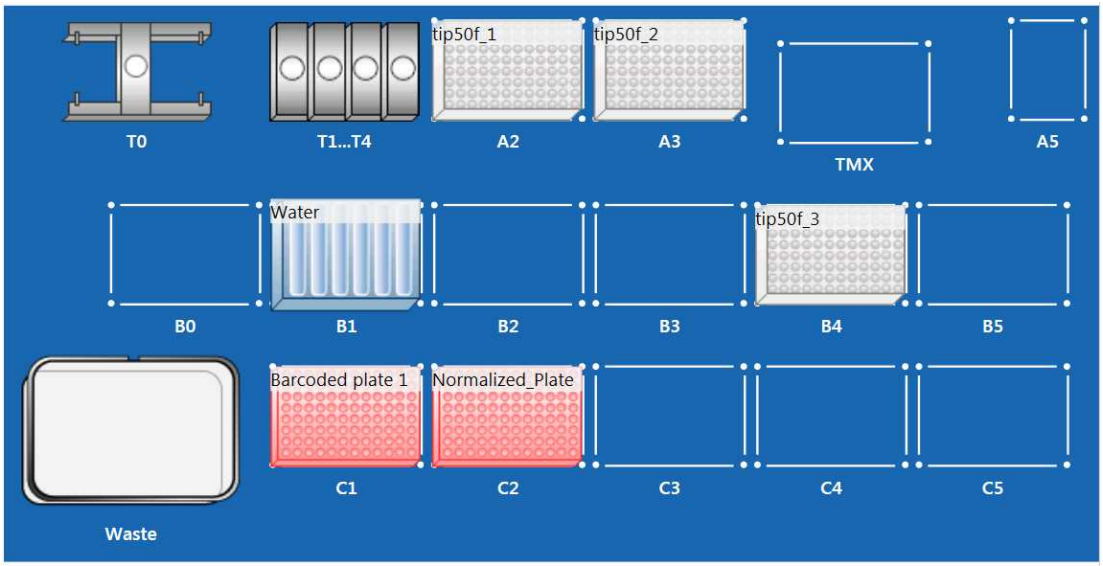

Low-Concentration Sample Normalization Worktable Setup.

Open the imported protocol, and set up the EPMotion following the above worktable figure.

- Place 50 uL EPMotion tip racks in position A2, A3, and B4 without tip rack lid.
- Place EPMotion Reagent Reservoir Rack in position B1
- Place Eppendorf Thermoadapter PCR 96 in position C1 and C2
- Ensure tip waste basket is empty and liquid waste reservoir is empty

11 Label EPMotion 30 mL reservoirs.

- Label one reservoir "Water"
- Label one reservoir "Empty"

12 Place reservoir labelled "Empty" in position 7 of the EPMotion Reagent Reservoir Rack with its lid removed.

13 Add **30 mL** of

UltraPure Distilled Water **Invitrogen - Thermo Fisher Catalog #10977-015** to reservoir labelled "Water."

- Place "Water" in position 1 of the EPMotion Reagent Reservoir Rack with its lid removed.

14 Label a new ThermoScientific Thermo-fast 96 Detection Plate with Black Lettering **(Catalog No. AB-1400-L)** with the number of the Riptide library, user's initials, date of normalization, and sample volume.

Ex. "Riptide 42 Normalized Plate K.C. 4/3/2021 30 uL"

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- Place labelled plate on the thermoadapter in position C2.
- Ensure that the plate is placed in the correct orientation. Letters and numbers should be right side up on the plate.

14.1 **NOTE:** For low-concentration plates, the samples are normalized to 4 uL. Because of this, only the "4-uL Plate" is required and is placed in position C2 (not a separate "Normalized Plate"). Skip Step 12, and proceed to Step 13.

15 Label a new ThermoScientific Thermo-fast 96 Detection Plate with Black Lettering **(Catalog No. AB-1400-L)** with the number of the Riptide library, user's initials, date of normalization, and sample volume.

Ex. "Riptide 42 4-uL Plate K.C. 4/3/2021 4 uL"

- Place labelled plate in position C3.
- Ensure that the plate is placed in the correct orientation. Letters and numbers should be right side up on the plate.

16 After thawing the gDNA plate, ensure the plate is properly sealed with a PCR adhesive seal. Briefly vortex and spin down the plate.

17 Remove seal from gDNA plate, and place it on the thermoadapter in position C1.
- NOTE: gDNA plate must be in MicroAmp™ Optical 96-Well Reaction Plate with Barcode **Catalog No. 4306737**
- The imported protocol is programmed for these specific plates.

18 Close EPMotion hood when all tips, plates, and reagents are in position.

Running the Normalization Protocol

19 Press the play button.

20 Select your EPMotion ID, and press "Next."

21 Select the "Input Volumes Manually" option under "Volume Settings," and press "Next."

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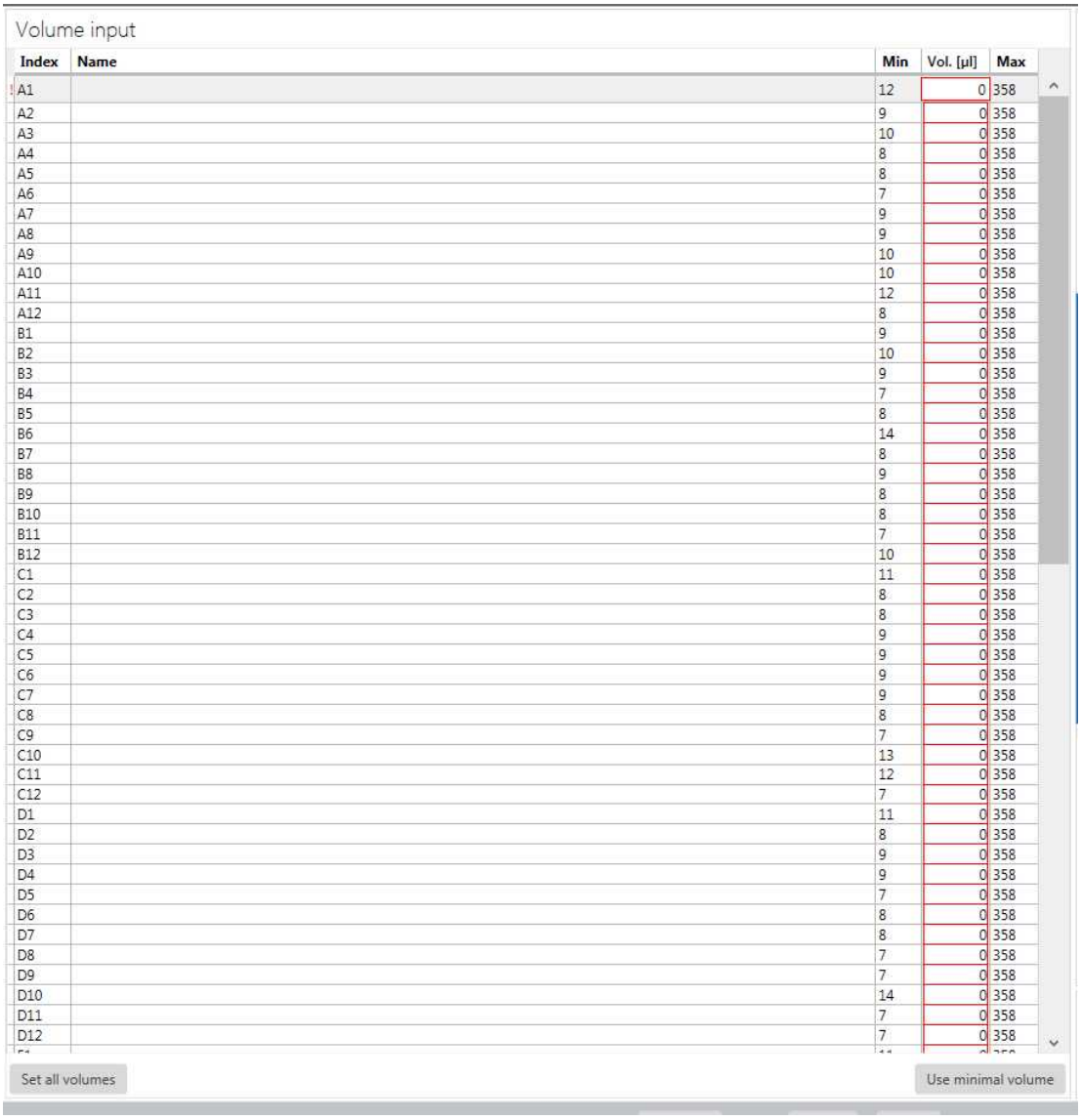
22 Set volume for water at 30000 uL (30 mL), and press "Next."

23 Set volume for samples in the barcoded plate. This is usually ~190 uL for all samples. Press "Set all volumes" in the bottom-left corner, and enter the desired volume. Else, select "Use minimal volume" in the bottom-right corner.



Index	Name	Min	Vol. [µl]	Max
A1		12	0	358
A2		9	0	358
A3		10	0	358
A4		8	0	358
A5		8	0	358
A6		7	0	358
A7		9	0	358
A8		9	0	358
A9		10	0	358
A10		10	0	358
A11		12	0	358
A12		8	0	358
B1		9	0	358
B2		10	0	358
B3		9	0	358
B4		7	0	358
B5		8	0	358
B6		14	0	358
B7		8	0	358
B8		9	0	358
B9		8	0	358
B10		8	0	358
B11		7	0	358
B12		10	0	358
C1		11	0	358
C2		8	0	358
C3		8	0	358
C4		9	0	358
C5		9	0	358
C6		9	0	358
C7		9	0	358
C8		8	0	358
C9		7	0	358
C10		13	0	358
C11		12	0	358
C12		7	0	358
D1		11	0	358
D2		8	0	358
D3		9	0	358
D4		9	0	358
D5		7	0	358
D6		8	0	358
D7		8	0	358
D8		7	0	358
D9		7	0	358
D10		14	0	358
D11		7	0	358
D12		7	0	358

Volume Input Screen for Barcoded gDNA Plate.

24 Press "Run."

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24.1 **EQUIPMENT CHECK:** The EPMotion report will show a warning that there is nothing located in space C3. Press "Ignore" to allow the protocol to continue

24.2 **USER INTERVENTION:** For high-concentration plates, after normalizing the first plate, the protocol will pause. Seal, shake, and spin down the normalized plate, and return it to C2. Remove the barcoded gDNA plate, and store it in a -20°C freezer. Move the Eppendorf Thermoadapter PCR 96 from position C1 to C3, and place the 4 uL plate in C3. Continue the protocol.

25 When the protocol ends, seal the plates. Store normalized plates and original gDNA plates in cardboard box.

- **Box Label EX.** Riptide 140/141, MM/DD/YYYY, initials, extracted gDNA
- 50mL falcon tube storage boxes with the dividers taken out can be used as storage. This will fit more than 4 plates. 2 libraries worth of plates can be stored in a single box

Store at plates **-20 °C**

26 Enter the name of the storage box of the gDNA plates into the google sheets Extraction Database in column S.

27 Store 4uL plate at **-20 °C** or continue with Twist 96-Plex (Riptide) protocol.

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