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Twist 96-Plex (Riptide) Library Prep

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CGORD Palmer Wet-Lab Protocols

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ABSTRACT

This protocol is designed for Twist 96-PLex Library Prep. We use the EPmotion 5075 to add sample barcodes (can also easily be done manually with a multichannel pipette). This is a continuation of the "EPMotion - Normalization and Randomization" protocol.

DOI

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PROTOCOL CITATION

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CREATED

May 02, 2022

LAST MODIFIED

Sep 12, 2022

PROTOCOL INTEGER ID

61801

GUIDELINES

It is optional to use the EPmotion in this protocol. Visit Twist Bioscience site for updated protocols.

MATERIALS TEXT

Please see Twist 96 Plex Protocol on Twist Bioscience Site for full list of materials needed

Equipment

- epMotion 5075
- 8 Channel Dispensing Tool (50uL) **Catalog No. 960001044**
- Qubit Flourometer
- Bioanalyzer
- Nanodrop
- Pipettes
- Multichannel pipette (10ul) if EPmotion is not available.

Consumables

- 1-50uL epT.I.P.S. Motion **Catalog No. 0030014413**
- Qubit HS Assay
- Tapestation D1000
- Reagent Wells
- Ultrapure DI Water
- Pipette Tips: 10ul, 200ul, 1000ul

Adding Primer A with EPmotion

1 Review the Twist 96-Plex Protocol
Note: Protocol was created for Document Version (DOC-001284 REV 2.0)
Twist Bioscience Protocol

2 Fill ice pan with ice chips.

3 Defrost 4uL randomized/normalized sample plate from the "EPMotion - Normalization and Randomization" protocol **On ice**

4 Open epBlue application

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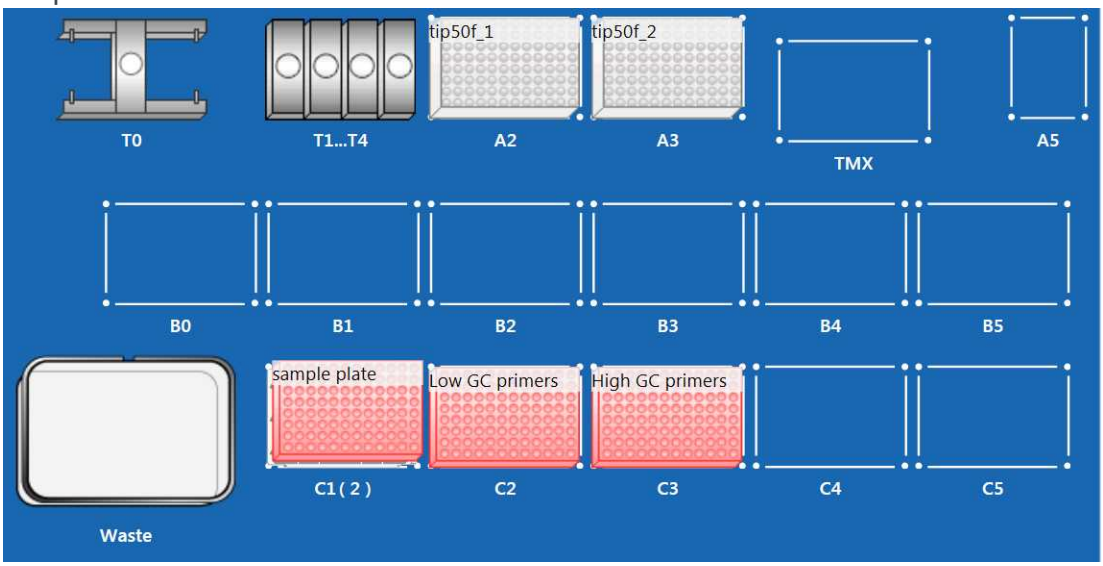
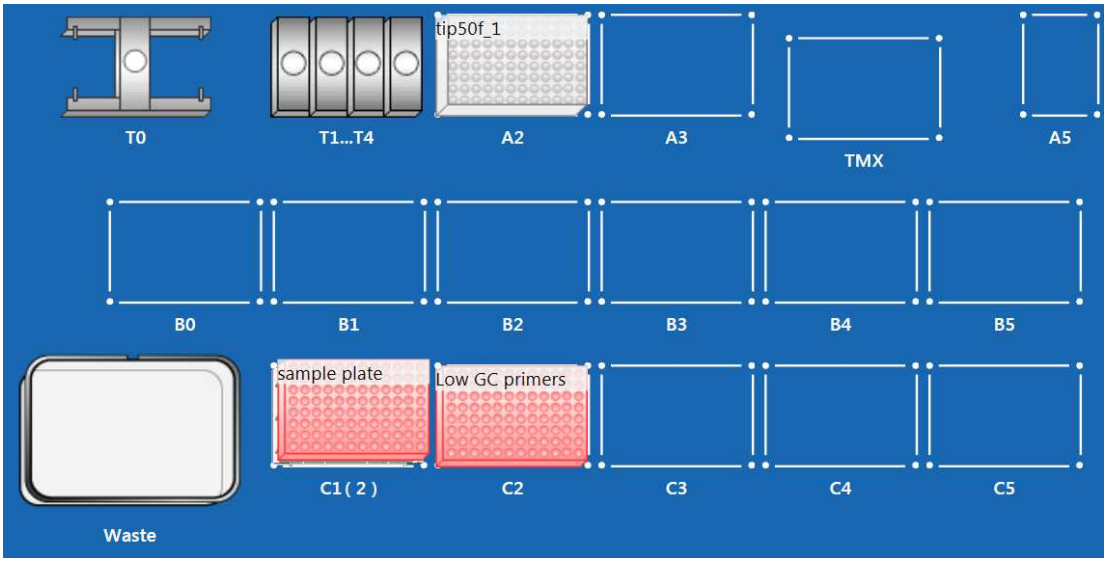
5 Download and import **50% GC Content Adapters.export7** and **Low GC Content Adapters.export7**

- Depending on the GC content of the species you are working with, choose the protocol accordingly.
- Use 50% GC Content Adapters if samples have GC content between 40% - 60%
- Use Low GC Primers if samples have GC content below 40%
- Use High GC Primers if samples have GC content above 60%

6 Set up the EPmotion as shown on the worktable below



Worktable for High and Low GC Content Primers - 50%



Worktable for Only Low GC Content Primers

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Library Prep

7 Follow the Twist 96-Plex Protocol
Note: Protocol was created for Document Version (DOC-001284 REV 2.0)

- **IMPORTANT:** Size selection Bead Volumes used: **50ul:20ul (Option 3)** worked best for us.
- Make sure to record which pool barcode index for each library
- PRO TIP: We try to match the last digit of the library number (Ex. Riptide 53) with the pool barcode index used.
- In the case of Riptide 5**3.** We would use the pool barcode #3 for this particular library.

QC

8 Perform on each library and record keep all QC for pooling steps

- Nanodrop
- Qubit (HS Assay)
- Tapestation (D1000)

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- Libraries should have an average fragment size between 420bp - 650bp.
- 260/280 should be around 1.80 - 2
- 260/230 should be around 2-2.2.
- **We have been able to get good data from libraries with relatively poor nanodrop purities**.
- Qubit concentrations can widely range. We get a range from 10ng/ul - 60ng/ul

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