

Supplementary Laboratory User guide for Twist Target enrichment

For use with biomodal duet multiomics solution duet evoC & Twist Human Methylome/ Twist Alliance Pan-Cancer Methylation Panels

This addendum is for the use of the duet evoC protocol in combination with a targeted panel.
For targeted purposes, below are the instructions to use the duet evoC kit in conjunction with:

- The **Twist Human Methylome Panel 123 Mb.**
- The **Twist Alliance Pan-Cancer Methylation Panel-1.5 Mb.**

 This user guide is specifically compatible with **Product Numbers: 6205-1 / 6205-2 (8 reactions)** and **6206-1 / 6206-2 (24 reactions)**. If you have a different product number, please refer to the appropriate guide or contact biomodal support for assistance.

Contents

Supplementary Laboratory User guide for Twist Target enrichment.....	1
For use with biomodal duet multiomics solution duet evoC & Twist Human Methylome	3
I. Reagents.....	3
II. For Target Enrichment with Twist Human Methylome Panel	3
III. Software guide for Target Enrichment.....	4
For use with biomodal duet multiomics solution duet evoC & Twist Alliance Pan-Cancer Methylation Panel.....	5
I. Reagents.....	5
II. For use of duet evoC with the Twist Alliance Pan-Cancer Methylation Panel	5
III. For Target Enrichment with Twist Alliance Pan-Cancer Methylation Panel	6
IV. Software guide for Target Enrichment	7
V. Revision History	7

For use with biomodal duet multiomics solution duet evoC & Twist Human Methylome

I. Reagents

If using a targeted panel, we recommend Twist Human Methylome Panel.

II. For Target Enrichment with Twist Human Methylome Panel

For running a targeted assay, below are the instructions to use the duet evoC kit in conjunction with the Twist Human Methylome Panel.

These are the changes that you need to implement in the Twist user guide (Twist Targeted Methylation Sequencing Protocol [DOC-001222 REV 4.0](#)) and the Appendix B when used with duet evoC:

Follow step 1.1 of the Twist protocol.

- 1.2** Transfer the calculated volumes from each amplified indexed library to a 0.2 ml PCR tube.

Follow the Twist protocol from step 1.3 to step 2.1 (step 1.3 is in Appendix B of the Twist protocol for use with the Twist Human Methylome Panel).

- 2.2** Resuspend the dried methylation pre-hybridization material from Step 1.5 in 20 µl Fast Hybridization Mix using a P20 pipette*. Mix by pipetting up and down 15 times slowly with a P20 pipette.

****We recommend a P20 pipette, as the Fast Hybridisation mix is extremely viscous and this enables better liquid handling.***

Follow the Twist protocol from step 2.3 to Step 3 (step 2.6 is in Appendix B of the Twist protocol for use with the Twist Human Methylome Panel).

- 3** Set the temperature of the thermomixer for Fast Wash Buffer 1 to 63°C*. Use an immersion glass thermometer to monitor the temperature.

Preheat 450 µl Fast Wash Buffer 1 to 63°C.

**** It is critical that the Fast Wash Buffer 1 washes are performed at 63° C. The temperature of the preheated Fast Wash Buffer 1 influences the off-target rate and uniformity.***

Follow the Twist protocol from step 3.1 to 3.26 (steps 3.12, 3.13, 3.16, and 3.17 are in Appendix B of the Twist protocol for use with the Twist Human Methylome Panel. Fast Wash Buffer 1 temperature is 63°C).

3.27 Resuspend the pellet in 22.5 µl of water and use the entire slurry for the next step.

Follow the Twist protocol from step 3.28 and onward.

III. Software guide for Target Enrichment

To run the biomodal duet multiomics solution duet evoC pipeline in targeted mode, please refer to the section 'target enrichment' of the [duet software installation and running guide](#).

It is assumed that you have successfully installed and tested the Command Line Interface (CLI) for the biomodal duet multiomics solution on your chosen platform and that you now want to run the pipeline on data generated using a targeted panel.

Therefore, this document does not discuss the installation, configuration, or basic usage of the CLI, nor how to process a test dataset through the pipeline. It is assumed that the installation of dependencies and configuration of your environment have been addressed already and that you have been able to successfully use the CLI to process a non-targeted test dataset.

The scripts, configuration files, and reference files needed to run the pipeline in targeted mode are included as part of the standard CLI installation and configuration and should all be available already as part of the resources downloaded when configuring the CLI.

For use with biomodal duet multiomics solution duet evoC & Twist Alliance Pan-Cancer Methylation Panel

I. Reagents

If using a targeted panel, we recommend Twist Alliance Pan-Cancer Methylation Panel-1.5 Mb.

II. For use of duet evoC with the Twist Alliance Pan-Cancer Methylation Panel

Please follow the duet evoC user guide until you reach Section 17.4.

17.4 Amplify in a thermal cycler programmed as below with a heated lid set to **105°C**:



Important: for target enrichment, check PCR cycle guidance below.

Step	Temp (°C)	Time	Cycles	Heated Lid	Volume (μl)
Initial Denaturation	98	30 secs	1	105°C	50.0
Denaturation	98	10 secs	8-11 cycles*		
Annealing	62	30 secs			
Extension	65	60 secs			
Final extension	65	5 mins	1		
Hold	4	Hold	1		

***If performing target enrichment, the number of PCR cycles needs to be adjusted. For 375 ng input, we recommend the following number of PCR cycles. These are recommendations only, some optimization may be required:**

5 to <10 ng = **11** cycles

10 to <20 ng = **10** cycles

20ng to ≤30 ng = **9** cycles

>30ng to ≤80 ng = **8** cycles.



IMPORTANT: recommended input range for gDNA is 10-80ng. Recommended input range for cfDNA is 5-30ng.

Follow the duet evoC protocol to section E.

III. For Target Enrichment with Twist Alliance Pan–Cancer Methylation Panel

For running a targeted assay, below are the instructions to use the duet evoC kit in conjunction with the Twist Alliance Pan–Cancer Methylation Panel–1.5 Mb.

Please follow the steps outlined in the duet evoC user guide, implementing the modifications above in Section II: For use of duet evoC with the Pan–Cancer Methylation Panel.

These are the changes that you need to implement in the Twist user guide (Twist Targeted Methylation Sequencing Protocol [DOC-001222 REV 4.0](#)) when used with duet evoC:

- 1.1 If multiplexing 8 libraries per hybridisation reaction, the amount of each library will be 375 ng and the total mass of the pool will be 3000 ng. We recommend 3000 ng as standard pool input.
- 1.2 Transfer the calculated volumes from each amplified indexed library to a 0.2ml PCR tube.
Follow the Twist protocol from step 1.3 to 2.1.
- 2.2 Resuspend the dried methylation pre-hybridization material from Step 1.5 in 20 µl Fast Hybridization Mix using a P20 pipette*. Mix by pipetting up and down 15 times slowly with a P20 pipette.

*** We recommend a P20 pipette, as the Fast Hybridisation mix is extremely viscous and this enables better liquid handling.**

Follow the Twist protocol from step 2.3 to Step 3.

3. Set the temperature of the thermomixer for Fast Wash Buffer 1 to 66°C*. Use an immersion glass thermometer to monitor the temperature.

Preheat 450 µl Fast Wash Buffer 1 to 66°C.

***It is crucial that the Fast Wash Buffer 1 washes are performed at 66° C. The temperature of the preheated Fast Wash Buffer 1 influences the off-target rate and uniformity.**

Follow the Twist protocol from step 3.1 to 3.11.

- 3.12 Add 200 µl preheated (66°C) Fast Wash Buffer 1. Mix by pipetting up and down 15 times.

- 3.13 Incubate the tube(s) for 5 minutes at 66°C.

Follow the Twist protocol for steps 3.14 and 3.15.

- 3.16 Add 200 µl preheated (66°C) Fast Wash Buffer 1. Mix by pipetting up and down 15 times.

3.17 Incubate the tube(s) for 5 minutes at 66°C.

Follow the Twist protocol from steps 3.18 to 3.26.

3.27 Resuspend the pellet in 22.5 µL of water and use the entire slurry for the next step.

Follow the Twist protocol from step 3.28 and onward.

IV. Software guide for Target Enrichment

To run the biomodal duet multiomics solution duet evoC pipeline in targeted mode, please refer to the section 'target enrichment' of the [duet software installation and running guide](#).

It is assumed that you have successfully installed and tested the Command Line Interface (CLI) for the biomodal duet multiomics solution on your chosen platform and that you now want to run the pipeline on data generated using a targeted panel.

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V. Revision History

Version	Date	Description
5.0	February 2025	Protocol release