Dovetail® Micro-C Stage 1 Protocol for Drosophila Imaginal Discs

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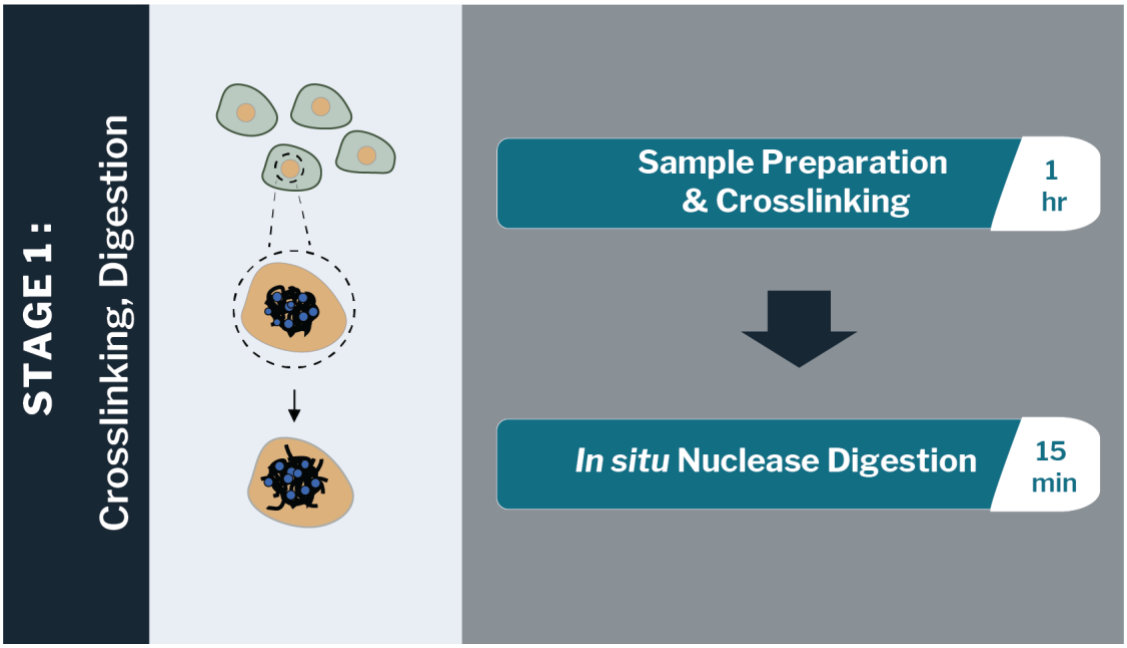
Stage 1: Crosslinking and Digestion

As you prepare for Stage 1, keep the following in mind:

- Sample preparation takes ~ 1.5 hours.

Figure 1. Stage 1: Crosslinking and Digestion



Before You Begin

- The 10X Wash Buffer might have precipitated in storage. Incubate at 50°C for 15 minutes or until the precipitate is no longer visible. Vortex to mix prior to use.
- Dilute 10X Wash Buffer to 1X with UltraPure™ Water. Store at room temperature. 1X Wash Buffer is stable at room temperature for 2 months. You need ~4 mL of 1X Wash Buffer per sample for the entire protocol.
- Prepare 0.3 M DSG in DMSO (anhydrous) by dissolving 1 mg of DSG in 10.22 µL DMSO. DSG is water-insoluble and moisture-sensitive. Prepare **immediately** before use. Do not store DSG in solution.

- Prepare fresh 1X Nuclease Digest Buffer and store at room temperature. 1X Nuclease Digest Buffer is stable for 1 day at room temperature. You need 50 µL of 1X Nuclease Digest Buffer per sample. To prepare 1X Nuclease Digest Buffer (50 µL), mix the following components:

Reagent	Volume Per Reaction	10% Extra		# Reactions		Final
UltraPure Water	40 µL	44 µL	x	8	=	352 µL
10X Nuclease Digest Buffer	5 µL	5.5 µL	x	8	=	44 µL
100 mM $MgCl_2$	5 µL	5.5 µL	x	8	=	44 µL
Total	50 µL					

- Set the thermal mixer at 22°C, shaking at 1,250 rpm.
- Thaw 0.5 M EGTA at room temperature. Vortex to mix prior to use.

Follow the steps below for Crosslinking and Digestion:

1. Dissect 100 of third instar imaginal leg discs (or 50 of third instar imaginal eye discs or wing discs) in Drosophila Schneider S2 Medium.
2. Transfer the discs into BioMasher II tube (PeloBiotech, PN: 320103)
3. Spin down the tube at 2,000 x g for 3 minutes. Discard the supernatant.
4. Flash freeze the discs in liquid nitrogen then place at -80°C for a minimum of 10 minutes.
5. Prepare and place on the bench a solution containing:
   - 1 mL of 1X PBS
   - 10 µL of 0.3 M DSG (freshly prepared)
6. Thaw the discs at room temperature for 5 minutes.
7. Resuspend the thawed discs in 150 µL of the PBS/DSG solution.
8. Homogenize the discs with the plastic pestle that is provided with the BioMasher II tubes.
9. Add the remaining 850 µL of the PBS/DSG solution.
10. Rotate the tube for 10 minutes at room temperature. Cells should not settle.
11. Add 27 µL of 37% formaldehyde.
12. Rotate the tube for 10 minutes at room temperature. Cells should not settle.
13. Spin the tube at 3,000x g for 5 minutes. Carefully remove and discard the supernatant.
14. Wash the pellet with a total of 1 mL of 1X Wash Buffer: first add 200 µL of Wash Buffer and pipet to break up clumps, then add the remaining 800 µL. Pipet up and down to fully resuspend the pellet.
15. Spin the tube at 3,000 x g for 5 minutes. Carefully remove and discard the supernatant.
16. Repeat steps 14 and 15 once.
17. After removing the second wash, resuspend the pellet in 1 mL of 1X Wash Buffer. Pipet up and down to fully resuspend.
18. Using a 1 mL syringe, gently push the 1 mL of resuspended sample through a 50 µm filter into a new 1.5 mL tube.
19. Gently pass an additional 500 µL of 1X Wash Buffer through the same 50 µm filter into the 1.5 mL tube. Your tube should now contain a total volume of ~1.5 mL.
20. Spin the tube at 3,000 x g for 5 minutes. Carefully remove the supernatant.
21. Resuspend the cell pellet in 50 µL 1X Nuclease Digest Buffer (freshly prepared, see Before You Begin).

22. Add 0.5 µL of **1:10 diluted** MNase Enzyme Mix. Pipet up and down to fully mix. You can prepare the 1:10 dilution, by mixing 1 µL of kit supplied MNase Enzyme Mix with 9 µL of 1X Nuclease Digest Buffer (freshly prepared).
23. Incubate the tube at 22°C for exactly 15 minutes in an agitating thermal mixer set at 1,250 rpm.
24. Stop the reaction by adding 5 µL of 0.5 M EGTA. Pipet up and down to fully mix.
25. Continue to Stage 2: Sample Preparation QC.