



# Dovetail<sup>®</sup> Micro-C Stage 1 Protocol for Rice

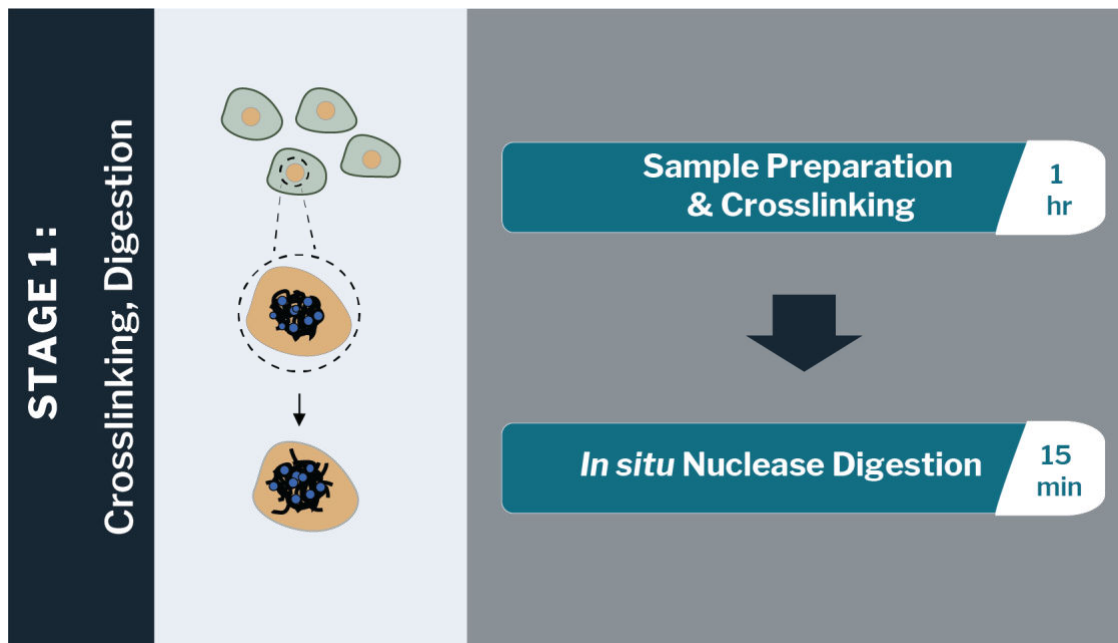
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## Stage 1: Crosslinking and Digestion

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  $\mu$ L DMSO. DSG is water-insoluble and moisture-sensitive. Prepare **immediately** before use. Do not store DSG in solution.
- 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.

- 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  $\mu\text{L}$  of 1X Nuclease Digest Buffer per sample. To prepare 1X Nuclease Digest Buffer (50  $\mu\text{L}$ ), mix the following components:

Reagent	Volume Per Reaction	10% Extra		# Reactions		Final
UltraPure Water	40 $\mu\text{L}$	44 $\mu\text{L}$	x	8	=	352 $\mu\text{L}$
10X Nuclease Digest Buffer	5 $\mu\text{L}$	5.5 $\mu\text{L}$	x	8	=	44 $\mu\text{L}$
100 mM $\text{MgCl}_2$	5 $\mu\text{L}$	5.5 $\mu\text{L}$	x	8	=	44 $\mu\text{L}$
Total	50 $\mu\text{L}$					

### Follow the steps below for Crosslinking and Digestion:

1. Weigh out 50 mg of frozen leaf tissue.
2. Grind the tissue to a **fine powder** with a mortar and a pestle in a liquid nitrogen bath.
3. Transfer the ground tissue to a 5 mL Tube.
4. Resuspend the tissue in 1.5 mL of 1X PBS and pipet up and down to ensure no clumps are present.
5. Add 15  $\mu\text{L}$  of 0.3M DSG (freshly prepared).
6. Rotate the tube for 10 minutes at room temperature. Cells should not settle.
7. Add 40.5  $\mu\text{L}$  of 37% formaldehyde.
8. Rotate the tube for 10 minutes at room temperature. Cells should not settle.
9. Spin the tube at 5,000 x g for 6 minutes. Carefully remove and discard the supernatant.
10. Wash the pellet with a total of 1 mL of 1X Wash Buffer: first add 500  $\mu\text{L}$  of Wash Buffer and pipet to break up clumps, then add the remaining 500  $\mu\text{L}$ . Vortex to fully resuspend the pellet.
11. Spin the tube at 5,000 x g for 6 minutes. Carefully remove and discard the supernatant.
12. Repeat steps 10 and 11 once.
13. After removing the second wash, resuspend the pellet in 500  $\mu\text{L}$  of 1X Wash Buffer. Vortex to fully resuspend.
14. Using a 1 mL syringe, gently push the 500  $\mu\text{L}$  of resuspended sample through a 200  $\mu\text{m}$  filter into a new 5 mL tube.
15. Gently pass an additional 500  $\mu\text{L}$  of 1X Wash Buffer through the same 200  $\mu\text{m}$  filter into the 5 mL tube. Your tube should now contain a total volume of ~1 mL.
16. Using the same syringe but changing to a 50  $\mu\text{m}$  filter, re-filter the 1 mL sample into a new 5 mL tube.
17. Gently pass an additional 500  $\mu\text{L}$  of 1X Wash Buffer through the 50  $\mu\text{m}$  filter into the 5 mL tube. Your tube should now contain a total volume of ~1.5 mL.
18. Spin the tube at 2,000 x g for 5 minutes. Carefully remove the supernatant.
19. Resuspend the cell pellet in 50  $\mu\text{L}$  1X Nuclease Digest Buffer (freshly prepared, see Before You Begin).
20. Add 1  $\mu\text{L}$  of MNase Enzyme Mix. Pipet up and down to fully mix.
21. Incubate the tube at 22°C for exactly 15 minutes in an agitating thermal mixer set at 1,250 rpm.
22. Stop the reaction by adding 5  $\mu\text{L}$  of 0.5 M EGTA. Pipet up and down to fully mix.
23. Continue to Stage 2: Sample Preparation QC following the Dovetail® Micro-C Kit User Guide v2.0 for tissue and blood.