Solid tissues

Chemical cross-linking protocol

Required materials:

- PBS
- Cross-linking agent. Use either:
  - ~37% formaldehyde stock solution in ~10-15% methanol (preferably opened not more than 3 months before usage)
  - ~50% or ~25% glutaraldehyde stock solution in H_2O (preferably opened freshly)
- Glycine solution (typically 2.5 M) in PBS
- A desiccator (excicator) with a pump or similar setup to create a vacuum

Before you start:

Make sure that PBS, Glycine solution, and formaldehyde/glutaraldehyde stock solution are all free of contaminations and readily available in sufficient amounts for the planned procedure. Equilibrate them to room temperature if necessary.

Prepare 1% solution of formaldehyde (<u>be mindful that the stock solution is typically **~37% concentrated**</u>) or 1% solution of glutaraldehyde (<u>be mindful that the stock solution is typically **~25% or ~50% concentrated**</u>) solution in PBS shortly in advance (preferably as shortly as possible, not longer than 1h before use) to make sure that it is ready to use immediately after the tissues are prepared.

Cross-linking

If possible, freshly harvested tissues should be used for cross-linking, however, non-homogenized tissues flash-freezing in liquid nitrogen and stored at -80°C can also be used if necessary.

> **<u>Important:</u>** Frozen tissues have to be thawed before cross-linking. If using frozen tissues transfer the container with the tissue from the freezer into ice and let the content thaw completely before proceeding to the next step (the required incubation time on ice might be variable depending on the tissue. Typically, ~30 min is sufficient).

1. Add 5-10 ml of room temperature 1% formaldehyde or glutaraldehyde solution in PBS directly to the tube containing the tissue. Make sure that all of the tissue fragments are submerged (for high amounts of tissues in the sample the volume of formaldehyde solution may have to be adjusted to ensure the full submerging).
2. Minimize the air accumulation from the submerged tissues by flicking the tube several times (<u>**be careful, formaldehyde and glutaraldehyde are very toxic**</u>).
3. Place the tube with a loosely fitted cap (<u>**the cap must be placed in a way that allows easy air exchange between the tube content and the surroundings, but tight enough to prevent formaldehyde splattering**</u>) into the desiccator.
4. Close the desiccator and turn on the pump. Incubate the samples at room temperature in the vacuum for 30 min (the optimal incubation time may be varied and may have to be adjusted. The recommended 30 min, however, should be optimal in most cases).
5. Turn off the pump, take the sample out, and add to it Glycine solution in PBS to a final Glycine concentration of 125 mM, mix the sample well, but gently (be careful not to introduce air bubbles around the submerged tissue).
6. Put the sample with a loosely fitted cap back into the excicator, start the pump, and incubate the sample at room temperature in the vacuum for 10 min.
7. Remove as much liquid from the tissue as possible.
8. Add 12 ml of PBS to the tube containing the tissue, close it, and turn it upside down a few times.
9. Remove as much PBS from the tissue as possible (the majority of PBS can be simply decanted, the leftover liquid might be carefully removed with a pipette. Alternatively, the tissue can be removed from the tube and the excess liquid can be gently removed with a paper towel).

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10. Flash freeze the tissues in liquid nitrogen.
11. Grind the tissues in liquid nitrogen to a very fine powder (it is paramount for the effectiveness of the downstream tissue lysis for the material to be ground into as fine powder as possible).
12. Weight the powder and store it at -80°C or use it immediately for downstream experimental procedure.