Method to Reduce the Recovery Volume (increase the concentration) of the Sample when MesoDissecting

(Research Use Only)

Overview:
Utilizing the AvanSci Bio's MeSectr Instrument and xScisor consumable, the method below will help reduce the recovery volume of the sample while maintaining recovery yield. The method involves generating either an air or liquid bolus above the liquid column such that the sample can be completely ejected leaving only air or clean dissection solution in the tip of the xScisor (the tip of the xScisor has a 3 ul dead volume). The disadvantage is the bolus will reduce the total volume available for dissection by a few microliters and it takes a bit longer to prepare each xScisor.

Volume Reduction Method:

1. Fill xScisor with dissection solution in usual manner.
2. Generate an air bolus as follows:
    a. Load xScisor into collet; leave mill head in up position. Go to Dissect page in 2iD software.
    b. Hold a clean paper towel against the tip of the xScisor. Simultaneously hold both the pulse button and either of the engage buttons to start withdrawing the plunger rod (Same process as done for aspirator calibration. If plunger rod does not move, move joystick side to side until it does).
    c. Release both buttons after the plunger in the xScisor has moved up a few millimeters. A few microliters of dissection solution should be absorbed by the paper towel.
3. Lower Head Assembly into Ready Position and dissect in the usual manner.
4. Recover sample and eject xScisor in the usual manner but do not plunge xScisor or unused dissection solution will dilute the sample.

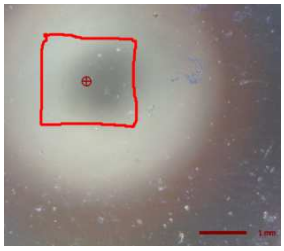
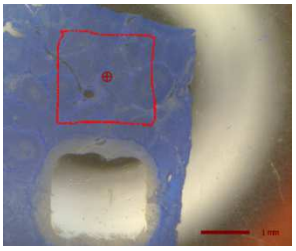
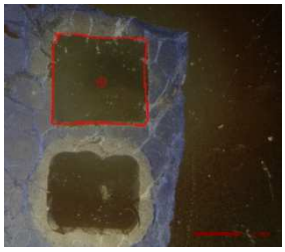
NOTE: To generate a liquid bolus, do not use the paper towel. It is important to generate a sufficiently large bolus that an air gap will separate the liquid bolus from the sample solution.

Method Validation:
The following variables were examined:

- 400 micron and 800 micron xScisor blade.
- Slowest aspiration rate setting and fastest aspiration rate setting.
- Air and liquid bolus.
- Partial and fully deparaffinized tissue.

Method used:

1. Weighed empty recovery tube.
2. Filled xScisor with aqueous milling solution.
3. Generated template to serve as dissection guide.

4. Aligned template to area to be dissected.
5. Used digital template to dissect same size (4.1 mm^2) and shape area for each sample.
6. Recover liquid using a single depression of the plunger.
7. Weighed recovery tube to determine recovered volume.
8. Adjusted volumes of all recovered samples to be equal to largest sample volume recovered.
9. Add proteinase K
10. Ran on THOR 65°C for 20 min, 95°C for 10 min, 25°C for 1 min.
11. Centrifuged recovery tubes, determine DNA yield of 5 ul of supernatant using PicoGreen.

Results:

		400 micron blade		800 micron blade	
Aspiration rate and use of bolus	tissue deparaffinization	Total recovered vol-ul	Total recovered DNA-ng	Total recovered vol-ul	Total recovered DNA-ng
Slow-air bolus	partial	17	11	8	13
	full	17	17	9	16
Slow-liquid bolus	partial	30	18	21	17
	full	26	13	27	11
Fast-no bolus	partial	64	13	48	15
	full	66	12	55	14

The following allow recovery in smaller volumes:

- Reducing aspiration rate
- Air bolus
- Larger blade

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