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Sample preparation protocol for hair fiber curvature analysis

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1 *Works for me* dx.doi.org/10.17504/protocols.io.bbweipbe

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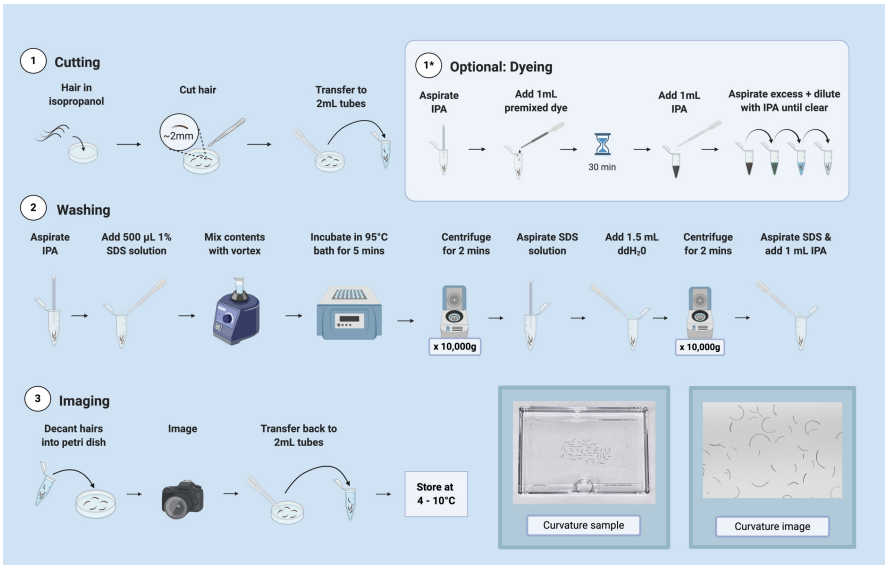
ABSTRACT

This protocol prepares hair fibers per sample for curvature analysis.

Diagrams were created with BioRender.com



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EXTERNAL LINK

https://tinalasisi.github.io/2020_HairPheno_manuscript/index.html

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PROTOCOL CITATION

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KEYWORDS

hair morphology, hair fiber curvature, hair curl, imaging, hair form, hair texture, hair

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MATERIALS TEXT

Materials:
1. Disposable petri dishes (for prep)
2. Square petri dishes (for imaging)
3. 2 mL microcentrifuge tubes
4. 50 mL Falcon tubes
5. #11 scalpel blades
6. Disposable transfer pipettes
7. Aspirating pipettes/ Pasteur pipettes
8. Parafilm

Reagents
1. SDS
2. Double Distilled H20 (ddH2O)
3. 100% isopropanol (ISA)

Equipment:
1. 5 mm size guide
2. Dryblock water bathb
3. Thermometer
4. Small, straight scissors
5. Vortex
6. Stepper pipette
7. Microcentrifuge
8. Tweezers
9. Aspirator
10. Camera
11. Copy Stand to hold camera

SDS	20 g
ddH2O	80 mL

Proportions for 20% SDS stock solution

SDS 20% stock solution	1 mL
ddH2O	19 mL

Proportions for 1% SDS master mix

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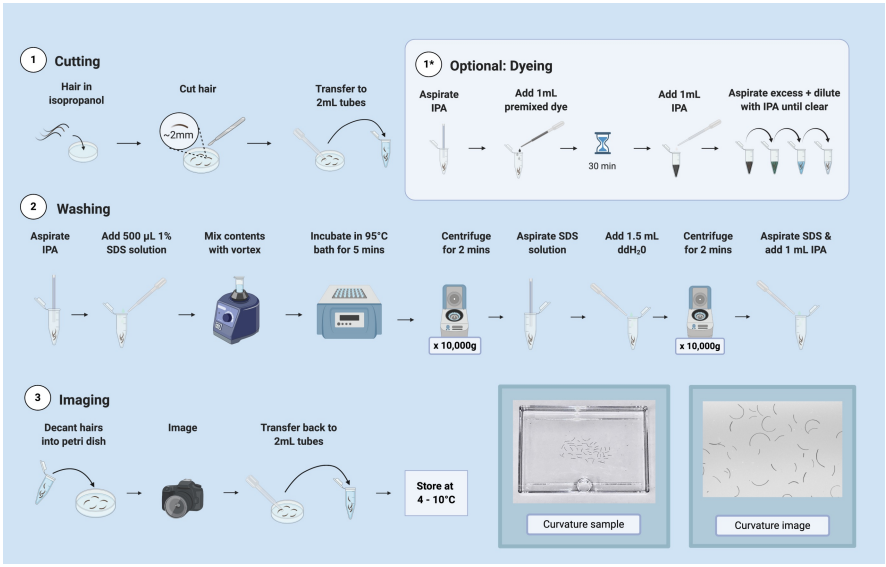

Preparing hair fragments 5m

1 Prepare a batch of 6 hairs for the protocol.

Preparing the hair fragments for a single sample will require:

- 5 hairs of (around 30mm) per sample minimum
- double distilled water (ddH2O)
- 1% sodium dodecyl sulfate (SDS)
- isopropanol (IPA)

1.1 Place hairs onto Petri dish filled with 5mL IPA

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1.2 Cut hairs into <3mm fragments with a #10 scalpel blade.

1.3 Transfer the cut hairs into 2mL tubes using a new transfer pipette for each sample.

In between pipetting, allow the hairs to sink to the bottom of the tube.

Then pipette excess IPA from the 2mL tube and use it to rinse the Petri dish and draw in more samples.

Repeat this process until all (or most) of the samples are in the 2mL tube.

At this point, the samples can be stored, or you may continue on to the next section.

2 Optional: dyeing

Are your hair samples lightly pigmented? Dye them for better contrast.

Before washing the hairs, check each sample and take note of whether the hair sample is darkly pigmented or not.

If the hairs are readily visible in the 2mL tube, even from a distance, you may consider them darkly pigmented for the purposes of this protocol.

If you cannot see the hairs well, follow the steps for lightly pigmented hairs below to dye them before the washing and imaging steps.

Dyeing them before washing ensures that fewer hairs will be lost in the process due to a researcher's inability to see readily see them during transfers between tubes.

Caution: Permanent hair dyes are slightly basic to open the cuticle of the hair fiber and allow for the dye pigments to enter the hair shaft.

Relaxers and perms similarly use basic solutions, however, they are designed for the purpose of breaking the disulfide bonds in hair and altering hair texture.

As hair dyes tend to be around pH7 or pH8 , while texture-altering solutions are much more basic (pH9 to pH14), this should not be a concern for relatively short periods of time. However, the effects of hair dyes on curvature are yet to be studied, so these dyeing steps must be done with caution.
Keep dye and developer from kit well sealed between uses.

Also check for the expiration date as the dye and developer may not have the desired effect past this date.

2.1 Prepare **10 mL** dye from a commercial dye kit (according to instructions on kit) in 50mL Falcon tube and set aside.

2.2 Use an aspirator or transfer pipette to remove most of the IPA from the 2mL tubes without disturbing

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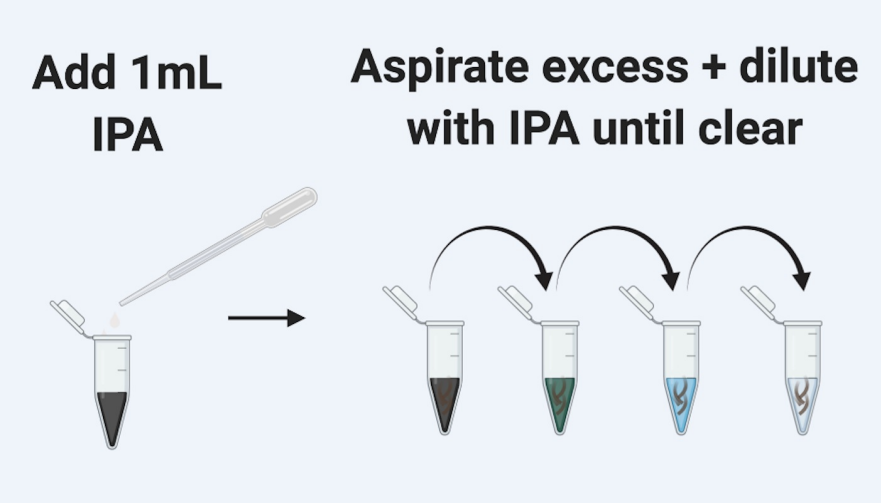
the hairs.

2.3 Use a disposable transfer pipette to add **1 mL** of the mixed dye to each 2mL tube being processed. Then let the dye develop for a minimum of **00:30:00**

2.4 Rinse each 2mL tube of dyed hair with IPA.



This may require several rounds of pipetting fresh IPA into the sample until the liquid appears clear.

Washing the hairs 20m

3

The washing steps are taken to remove any products from the surface of the hair that may influence its longitudinal shape (aka. curl or curvature). The samples are set in a hot water bath to "revert" any changes to the hair due to extrinsic factors such as brushing, flat-ironing, braiding etc.

Various tools and products used to alter hair texture/form work by breaking and reforming the (relatively weak) hydrogren bonds in the hair. Heat and moisture both break these bonds causing any artificially formed shape to the hair to be lost.

There are limitations to this. Repeated styling over long periods of time may start influencing the structural integrity of the fiber beyond the flexible hydrogen bonds. Aditionally, any treatment affecting the disulfide bonds in the hair (e.g. perms) will not be undone by washing in hot water.

As such, we recommend that researchers ask extensive questions about hair treatment history during hair sample collection, if they wish to study the intrinsic factors influencing hair morphology.

Set aside materials for washing hairs

The washing steps will require

- **500 µl** of 1% SDS per hair in the sample batch (see comment and materials).
- **1.5 mL** ddH2O per sample
- **1 mL** IPA per sample
- An aspirator with replacement for each sample *or* a transfer pipette for each sample

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- A (Vortex) mixer
- A pair of forceps
- A (Dryblock) water bath (with a thermometer) - this will need to be heated to **95 °C** for use.

3.1 Using an aspirator or pipette, remove and discard as much IPA from the sample without disturbing the hairs.

3.2 Add **500 µl** 1% SDS solution and mix the contents of the 2mL tube using a Vortex mixer.

Avoid turning the tube upside down as hairs may get stuck to the lid

3.3 Place the samples in a bath where the surrounding water has been heated to **95 °C** . And leave the samples in the bath for **00:05:00** . Use forceps to remove the hairs from the bath and place in trays to cool for at least **00:10:00** 15m

Heating the tubes may cause vapor pressure to build within them, resulting in caps popping open. Watch your samples and use cap locks where possible.

3.4 Once the hairs have cooled, centrifuge the hairs **10000 x g, 00:02:00**

This step is critical as the hairs will not always (uniformly) sink to the bottom. The more hairs are collected at the bottom of the tube, the fewer will be lost during the subsequent rinses.

3.5 Using an aspirator or pipette, remove as much of the SDS solution as possible without disturbing the hairs.

3.6 Fill the 2mL tube with **1.5 mL** of ddH2O, mix using Vortex and spin in microcentrifuge at **10000 x g, 00:02:00** .

3.7 Again, using an aspirator or pipette, remove as much of the ddH2O as possible.

3.8 Add **1 mL** of IPA to each sample and mix again.
You can now store the hairs until imaging.

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Imaging hair samples 4m

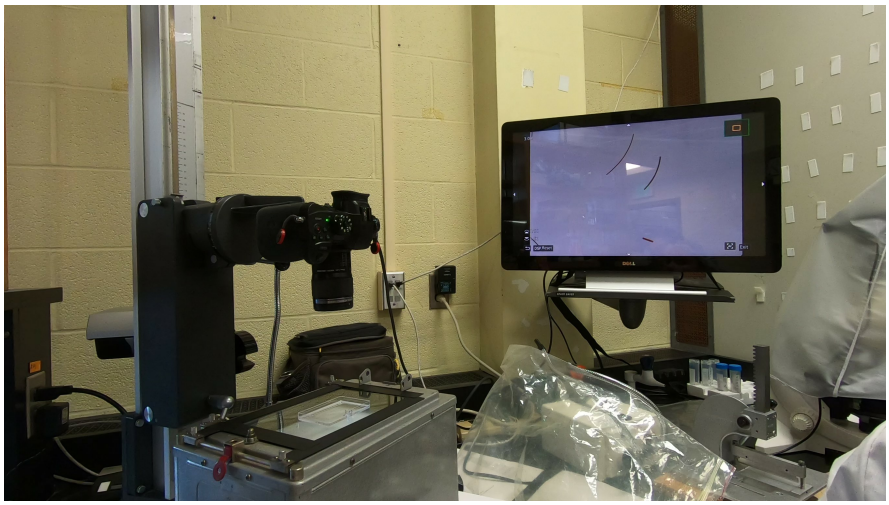
4 Set up imaging station.

For imaging at this macroscopic scale, we recommend setting up a camera to point down at an elevated stage with a white platform (a white sheet of paper can be used).

For each sample, you will need:

- a Petri dish
- **5 mL** of IPA

We recommend you go through these imaging steps for a single sample at a time as the IPA will begin to evaporate when placed in the Petri dish.



4.1 Mix the contents of the 2mL tube with your sample and decant immediately into the Petri dish on the stage. You can now take an image of the hairs.

Decanting will require some care not to stain the stage. if there are residual hairs in the 2mL tube after decanting, use a pipette to remove some IPA from the Petri dish and rinse hairs into the dish.

Always use a new transfer pipette for a new sample to avoid contamination. If the majority of the hairs are in the dish, this may be sufficient for imaging.

4.2 Dispose of hairs or retransfer to 2mL tubes with **1 mL** IPA for storage.

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