Silk Fibroin Extraction from B. Mori Cocoons

http://www.nature.com/nprot/journal/v6/n10/pdf/nprot.2011.379.pdf%3FWT.ec_id%3DNPROT-201110

Materials (yellow = probably don't have and need to buy, green = probably don't have but should be easy to scavenge)

REAGENTS	EQUIPMENT
Extraction 1) Silk cocoons (Tajima Shoji or equivalent) 2) Sodium carbonate (Sigma-Aldrich, cat. no. 451614, http://www.sigmaaldrich.com/) 3) Ultrapure water 4) Lithium bromide (LiBr, Sigma-Aldrich, cat. no. 213225, http://www.sigmaaldrich.com/)	**Extraction** 1) Titanium scissors 2) Small and Large stir bars 3) Spatula 4) Glass beakers (50 ml, 1L, and 2L) 5) Plastic beaker (2 L) 6) Aluminum foil 7) Hot plate 8) Small, Medium, and Large weigh boats 9) Analytical balance 10) Hot hand protectors 11) Graduated cylinder (50 ml) 12) Slide-A-Lyzer dialysis cassette 3500 MWCO, 3–12 ml capacity (Thermo Scientific, cat. no. 66110, http://www.fishersci.com/) 13) Dialysis cassette buoy (Thermo Scientific, cat. no. 66432, http://www.fishersci.com/) 14) Syringe (20 ml; BD Medical, cat. no. 309661, http://www.fishersci.com/) 15) Needles (18G) 16) Conical tubes (50 ml) 17) Centrifuge 18) Fixed-angle rotor (Eppendorf, cat. no. 022637207, http://www.fishersci.com/) <-centrifuge? 19) Lyophilizer (optional: use only if lyophilized silk is required, Labconco, cat. no. 7751030, http://www.fishersci.com/) 20) Freezer (− 80 °C; optional: use only if lyophilized silk is required) 21) Kimwipes
Concentrating silk fibroin 1) Fibroin solution (7–8%, wt/vol); PROCEDURE Step 22) 2) PEG (10,000 MW, Sigma-Aldrich, cat. no. P6667, http://www.sigmaaldrich.com/)	**Concentrating silk fibroin** 1) Syringes (3ml and 10 ml) 2) Needles (18G) 3) Slide-A-Lyzer dialysis cassette (0.5–3.0 ml; Thermo

3) Ultrapure water	Scientific, cat. no. 66330, http://www.fishersci.com/) 4) Beakers (100 ml and 1L) 5) Large stir bar 6) Small dialysis cassette buoy (Thermo Scientific, cat. no. 66430, http://www.fishersci.com/) 7) Aluminum foil 8) Microcentrifuge tubes
Silk films 1) Aqueous silk solution, 8% (wt/vol)	**Silk Films** 1) Petri dish, non–tissue culture treated (100 mm) 2) Tweezers 3) Vacuum desiccator
Electrospinning 1) Aqueous silk solution, 8% (wt/vol) 2) Polyethylene oxide (PEO, 900 kDa; Sigma-Aldrich, cat. no. 189456, http://www.sigmaaldrich.com/) 3) Ultrapure water 4) Methanol	**Electrospinning** 1) Glass scintillation vials (20 ml) 2) Small stir bar 3) Hot plate 4) Syringe (10 ml) 5) Blunt-tip needle (16 gauge), McMaster-Carr, cat. no. 75165A552, http://www.mcmaster.com/) 6) Syringe pump (Fisher Scientific, cat. no. 14-831-200, http://www.fishersci.com/) 7) High voltage supply (Glassman Series EH, cat. no. EH30P3, http://www.glassmanhv.com/) 8) Nonstick aluminum foil (Reynolds Wrap) 9) Reciprocating shaker (MaxQ, Thermo Scientific, cat. no. 11-675-152, http://www.fishersci.com/) 10) Insulated electrical wire 11) Alligator clips (McMaster-Carr, cat. no. 7236K252, http://www.mcmaster.com/)

Protocols

Fibroin extraction ● TIMING 2.5 h active, overnight drying (day 1)

1| Prepare a 2-liter glass beaker filled with 2 liters of ultrapure water, cover it with aluminum foil and heat until boiling.

! **CAUTION** Do not leave the beaker unattended while heating and boiling. Because of high temperatures, plastic beakers should not be used.

2| Meanwhile, cut cocoons with titanium scissors into dime-sized pieces and dispose of silkworms. Measure out 5 g of cocoon pieces into a large weigh boat.

3| Measure 4.24 g of sodium carbonate in a medium weigh boat.

4| Add the measured sodium carbonate to the water and let it completely dissolve (to prepare a 0.02 M solution of Na_2SO_3).

! **CAUTION** If water is boiling, add sodium carbonate slowly to avoid boiling over.

5| Add the cocoon pieces once the water starts to boil and continue boiling for 30 min. Occasionally, stir with a spatula to promote good dispersion of fibroin.

☐**CRITICAL STEP** To increase reproducibility, boil for exactly 30 min every time. If boiling for longer or shorter times, indicate this on the batch label. Increasing the boiling time will degrade the fibroin.

6| Remove the silk fibroin with a spatula and cool it by rinsing in ultrapure cold water. Squeeze excess water out of the silk. Discard the sodium carbonate solution in the sink.

! **CAUTION** Silk fibroin and solution will be hot; use hand protectors.

7| Place fibroin in a 1-liter beaker filled with 1 liter of ultrapure water and a stir bar.

! **CAUTION** If you are using a plastic beaker, ensure that the hot plate has cooled.

8| Rinse the fibroin in water for 20 min while gently stirring on a stir plate.

9| Repeat Steps 7 and 8 twice for a total of three rinses.

10| After the third wash, remove the silk, squeeze it well and then spread it out on a clean piece of aluminum foil.

11| Allow the silk fibroin to dry in a fume hood overnight.

☐**PAUSE POINT** Degummed silk fibroin, in which the sericin has been removed, can be stored indefinitely at room temperature. For long-term storage, place it in a clean plastic bag or wrap it in aluminum foil. Be sure to indicate the length of the boiling step on the label.

Dissolve silk fibroin in LiBr ● TIMING 4.5 h (day 2)

12| Calculate the amount of 9.3 M lithium bromide needed to prepare a 20% (wt/vol) solution based on the amount of dried fibroin available. As 20% of the solution will be silk, 80% will be LiBr. That is, a ratio of 1:4 (1 g to 4 ml) silk to LiBr. Therefore, multiply the amount of the dried silk fibroin by 4 to obtain the total volume of 9.3 M LiBr needed (X).

13| Prepare a 9.3 M LiBr solution.

! **CAUTION** Adding LiBr to water results in an exothermic reaction; be mindful of the heat generated. When preparing large volumes, we recommend carrying this out on ice.

□**CRITICAL STEP** LiBr has a low density and its volume should be taken into account while preparing the solution. We suggest adding only 60% of the calculated volume of water and then bringing the solution up to the final volume. Stir with a small stir bar.

14| Pack silk fibroin tightly into a 50-ml glass beaker and add the required amount of LiBr solution on top.

□**CRITICAL STEP** The LiBr must be added to the silk rather than adding silk to the LiBr so that the silk will eventually be covered and dissolved by the LiBr. It is also helpful to use the smallest glass container that will still hold the silk and LiBr solution.

15| Let fibroin dissolve in an oven at 60°C for 4 h. Once the silk fibroin is completely dissolved, it will appear amber in color and will be transparent. Black bits from the silkworm may be visible but will be removed later. This solution will be highly viscous but should not contain any intact fibers, as determined by visual assessment.

? **TROUBLESHOOTING**

Dialysis and centrifugation ● TIMING 49 h (days 2–4)

16| Hydrate dialysis cassettes in water for a few minutes.

17| With a 20-ml syringe and an 18-gauge needle, insert 12 ml of the silk-LiBr solution into a 3–12-ml dialysis cassette.

□**CRITICAL STEP** Be careful not to puncture or touch the dialysis membrane. The solution will be very viscous, and this step will be easier if the solution is kept warm before adding to the cassette. It is important to avoid shearing the solution whenever possible to avoid the induction of B-sheet within the silk. Therefore, only use the needle when injecting into the cassette. Moreover, have an additional needle and insert it into another top port of the cassette to allow air to escape. Remove the extra needle once all the air has been purged.

18| Dialyze against 1 liter of ultrapure water per 12 ml cassette. To ensure mixing, use a large stir bar and place on a magnetic stir plate. Change the water after 1 h, 4 h, that evening, the next morning and night, as well as in the morning on the following day (i.e., six changes within 48 h).

19| Remove silk from the cassettes with another 20-ml syringe and an 18-gauge needle. Place silk in a 50-ml conical tube. Depending on the volume, either split it between two tubes (if more than 40 ml) or fill one tube and use a counterbalance of water.

20| Centrifuge to remove impurities. Place in a centrifuge and spin at 9,000 r.p.m. (~12,700*g*) at 4°C for 20 min.

21| Carefully remove tubes from the centrifuge and either pour or transfer the silk solution with a 25 ml pipette into another centrifuge tube. Be sure to leave any white flocculent or brown matter behind.

22| Repeat Steps 20 and 21 again.

23| To determine the concentration of the silk in solution, measure the weight of a small weigh boat. Thereafter, add 0.5 ml of the silk solution to the boat and allow it to dry at 60°C. Once the silk is dry, determine the weight of the silk and divide it by 0.5 ml. This will yield the weight per volume percentage.

□**CRITICAL STEP** A batch of 5 g of silk cocoons generally yields 25 ml of 7–8% (wt/vol) silk solution. The solution will be tinted yellow but should be relatively clear and slightly more viscous

than water. If there are impurities such as white flocculents or dark particulates, it is best to re-centrifuge to remove them.

❑**PAUSE POINT** The silk solution can be stored at 4 °C for at least a month. Depending on the purity, stored silk will eventually gel but gelation times will vary. Once the silk has gelled, it cannot be used for protocols that require solution and therefore another batch will need to be extracted.

Lyophilization and Concentration (optional)

24| The fibroin solution (25 ml at concentration 7–8% (wt/vol)) can either be used as is or it can be *lyophilized (option A)* or *concentrated (option B)*. For storage for longer than 1 month, the silk solution should be lyophilized. In this form, the silk will be stable for years at room temperature and can be reconstituted in HFIP. The concentrated solution (20–30%, wt/vol) can be used directly for preparing silk tubes.

(A) Lyophilization (optional) ● **TIMING 3 d**

(i) Divide the aqueous silk solution into 50-ml conical tubes with no more than 20 ml per tube.

(ii) Place vertically in a − 80 °C freezer for several hours until the solution is completely frozen. If a −80 °C freezer is not available, silk can also be frozen at − 20 °C overnight or placed in liquid nitrogen until the solution is frozen.

(iii) Fold a Kimwipe over the top of the tube and attach with either a rubber band or tape. Keep caps for later use.

(iv) Place frozen samples on a lyophilizer for 2–3 d until all of the water is removed from the solution. The tube will no longer feel cold.

(v) Remove from lyophilizer, cap and store at room temperature (20–25 °C).

❑**PAUSE POINT** The lyophilized material can be stored at room temperature indefinitely.

(B) Concentrating silk fibroin (optional) ● **TIMING 21–25 h**

Not Included. I doubt we would need this.

25| The silk fibroin can now be used to prepare a number of different materials (**Fig. 1**). Using the concentrated solution (prepared in Step 24B) you can prepare silk tubes by either a simple dip method to create thin-walled tubes (option A, **Fig. 3**) or by gel spinning, wherein the fibroin is extruded onto a rotating mandrel (option B, **Fig. 4**). Alternatively, silk solution may be used to prepare hydrogels via vortexing, sonication, electrical current or pH change (options C–F, **Fig. 5**); nonpatterned or patterned silk films (options G and H, **Fig. 6**); silk microspheres using the DOPC (option I, **Fig. 7**) or PVA (option J, **Fig. 8**) method; electrospun silk fibers (option K, **Fig. 9**); or silk sponges that are either aqueous-based (option L, **Fig. 10**) or HFIP-based (option M, **Fig. 11**).