Tip Pulling

Nanoelectrospray Capillary Preparation for Native MS

ERIN PANCZYK
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Electrospray ionization (ESI) is an ambient pressure ionization method in which macromolecular complexes can be transferred from solution-phase to gas-phase ions for mass spectrometry analysis. ESI offers several advantages compared to other ionization methods; most importantly, the non-covalent interactions that maintain protein complex native structure are preserved. Additionally, nanoESI is commonly used to ionize large proteins and protein complexes because only nanomolar to micromolar protein concentrations are required which are typical of samples that may be expensive or difficult to purify.

Borosilicate nanoESI capillaries (http://www.kimble-chase.com/advancedwebpage.aspx?cg=930&cd=3&SKUTYPE=202&SKUFLD=SKU&DM=1250&WEBID=7911) are prepared in-house using a Sutter Instrument P-97 Flaming/Brown Micropipette Puller (https://www.sutter.com/MICROPIPETTE/p-97.html). Additional sizes of capillaries are available for purchase from the Sutter website, however, pulling parameters may need optimization. The P-97 Micropipette Puller is programmable and allows researchers to control the capillary shape and size. A full description of the features is available in the *Pipette Cookbook* and product description found on the Sutter Instrument website listed above.

A brief description of the parameters is given below:

- *Heat:* controls the electrical current supplied to the filament. This setting will affect the length and size of the tip. Generally, higher heat will lead to longer and finer tips.
- *Pull:* controls the force of the hard pull. Lower pull values will give larger tips with a shorter taper, while values between 120-250 will yield smaller tips.
- *Velocity:* controls the speed at which the glass softens and beings to pull apart. The lower the velocity value, the larger the tip and shorter the taper.
- *Time:* controls the length of time the cooling air is active. Decreasing the time value will lead to a longer taper and smaller tip.
- *Pressure:* controls the pressure of the cooling air delivered to the filament. The higher the pressure, the shorter the pipette taper will be. For thin-walled capillaries, values less than 300 for pressure are recommended. For thick-walled capillaries, 500 is recommended.

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Important: Pulling conditions can vary over time and laboratories, so additional tuning may be required to yield the desired capillary size and shape. Typical settings for borosilicate glass capillaries commonly used in the National Resource are listed below:

Heat	585
Pull	20
Velocity	19
Time	200
Pressure	500

After capillaries are pulled, the opening is examined under a dissecting microscope to evaluate whether the size and shape are suitable for analysis.

- Capillary openings should ideally be less than 20 µm; larger openings will be difficult to yield protein signal due to large droplet size and may require higher spraying voltages which can disrupt native structure.
- Typical nanoESI spray voltages range from 0.5-1.2 kV.
- A long taper should also be avoided because the capillaries will become difficult to fill without any bubbles present and bubbles can disrupt spray stability.
- It is possible for the tip of the capillary to be closed based on the pulling program entered. In this case, the tip is gently clipped, generally with the flat end of forceps to create a small opening. Care must be taken not to over-break the tip or cause glass to clog the opening.

Once a suitable capillary is obtained, a 10 µL Hamilton syringe is used to introduce the sample solution into the capillary. To prevent the formation of bubbles, the solution is often deposited into the middle of the capillary and shaken down into the tip. The capillary is then re-examined under a microscope to ensure no bubbles are present and that the solution has fully migrated to the end of the tip.

For most reproducible results, maintaining similar tip size and shape is highly encouraged for analysis. **Please NOTE:** for reproducible tips and filament longevity, it is required to allow the wire filament to cool down to room temperature (approximately 5 minutes) in between pulls.

Several other micropipette pullers are commercially available. Another common model is the Sutter Instrument P-2000 Laser-Based Micropipette Puller (https://www.sutter.com/MICROPIPETTE/p-2000.html), which is capable of pulling quartz glass capillaries.