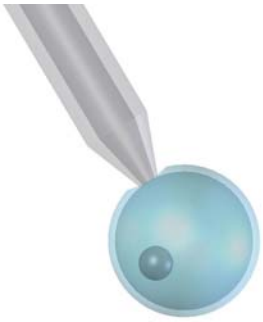
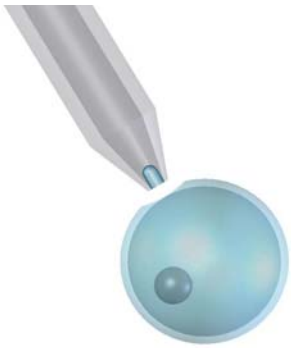
Patch Pipettes

The patch clamp technique is used in electrophysiological research to study the electrical activity of neurons at the cellular level. The technique requires using a blunt pipette with a 3-4mm short taper and a 1-3μm tip to isolate a patch of membrane. In general, patch pipettes are used to electrically isolate and study the movement of charges (ions) through the pores (ion channels) of the neuronal surface membrane. There are basically four different approaches to the patch technique: cell-attached patch, whole cell recording, and excised patch (outside-out patch and inside-out patch).

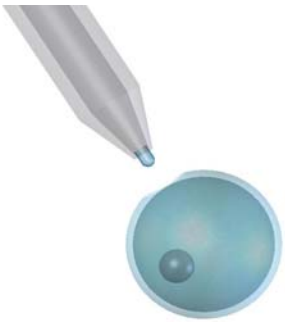
Cell-Attached Patch



Whole Cell Patch



Inside-Out Patch



Outside-Out Patch

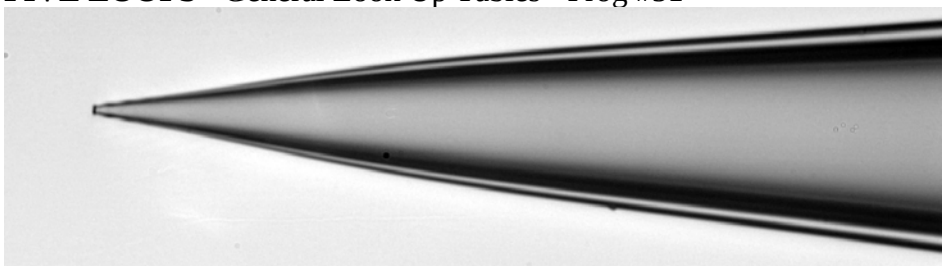
The patch technique is based on the electrical isolation of a small patch of membrane from the rest of the cell. To achieve this isolation, the patch pipette is placed against the cell membrane, and a slight suction or negative pressure is generated within the pipette. A tight seal is created between the pipette and the lipids of the cell membrane which is referred to as a "giga-seal" due to the high resistances (in the GΩ range) created between the outside of the patch pipette and the surrounding bath solution. The cell-attached patch configuration is a non-invasive approach which is used to measure the currents (current clamp) of single ion channels of the intact cell. The whole cell patch configuration is achieved when additional negative pressure is applied to the cell membrane through the pipette as it is in the cell-attached configuration. The suction through the pipette causes the cell membrane to rupture and create the whole cell patch where the cell is perfused by the solution in the pipette. In this case, the interior of the cell and the solution of the pipette become contiguous and the currents passing through the entire cell membrane are recorded. This whole cell recording configuration is equivalent to intracellular recording with sharp microelectrodes and has the advantage that it can be applied to very tiny or flat cells that in most other situations would be impossible to impale.

Patch Pipette Images

Whole Cell Recording, Cultured and Dissociated Cells

The ideal morphology for a patch pipette intended for dissociated/cultured cells is a short stubby taper, a high cone angle, and a 2-3 micron tip. This is best generated by using **Thick Walled Glass** and a 2.5mm or 3.0mm box filament.

FIVE LOOPS - General Look Up Tables - Prog #51

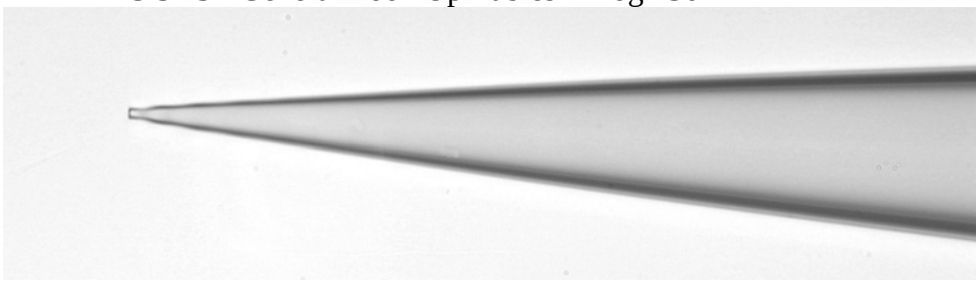


1.5mm x 0.86mm thick walled glass, ~2µm Tip, 3-4mm taper (400X mag)

Tissue Slice Recording

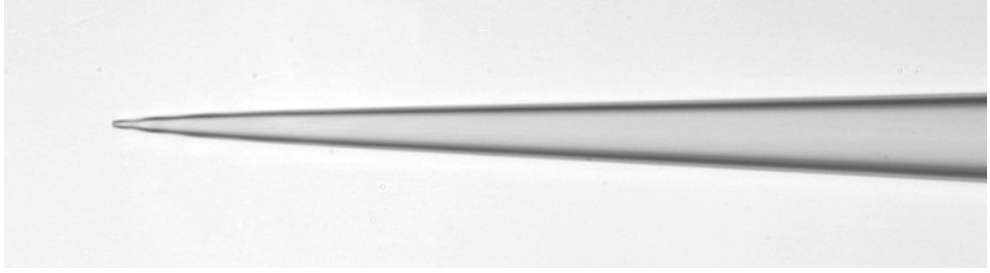
The ideal morphology for slice recording is a gradual slender taper, a low cone angle, and a 1-2 micron tip. This is best generated by using **Thin Walled Glass**, a 2.5mm or 3.0mm box filament and a program that allows the puller to loop 2-3 times. If you are going deep into the slice and find you are losing your seal or "chasing your cells," often due to a final taper that is too short and wide, select a velocity setting that allows two loops instead of three loops. This will reduce the cone angle and allow for a more slender taper.

THREE LOOPS - General Look Up Tables - Prog #56



1.5mm x 1.1mm thin walled glass, 2-3µm Tip, 4mm taper (400X mag)

TWO LOOPS - General Look Up Tables - Prog #56 with a higher velocity



1.5mm x 1.1mm thin walled glass, 1-2µm Tip, 5mm taper (400X mag)

Patch Pipettes - Recommended Programs

Whole cell patch applications often require a tip between 2-3µm, a short 33-4mm taper, and a resistance between 1-5 MΩ. To achieve this morphology, it is best to use thick walled 1.5 x 0.86 glass with a 2.5mm x 2.5mm box filament (FB255B) or a 3mm x 3mm box filament (FB330B). For those requiring higher resistances between 5-10MΩ, using a higher velocity to allow 4 loops instead of 5 loops will help achieve this goal.

Those working within a slice preparation often require a slightly smaller tip of 1-2µm and more gradual and long taper. In this case it is best to use thin walled glass (1.5mm x 1.1mm, 1.2 x 0.96mm, and 1.0mm x 0.78mm) and a trough or box filament.

If you are aiming for pipette resistance between 1-10 MΩ. and a tip size between 1-3 µm, any of the below programs should work well.

If you have a **2.5 x 2.5 box filament (FB255B)** installed in your puller, please choose the program below intended for 1.5 OD thin walled or thick-walled glass.

Glass OD/ID	Glass Item #	Heat	Pull	Vel	Time/Del	Pressure	Loops
1.5mm x .86mm	BF150-86-10	Ramp	0	21	1 (delay)	500	4-5

Glass OD/ID	Glass Item #	Heat	Pull	Vel	Time/Del	Pressure	Loops
1.5mm x 1.1mm	BF150-110-10	Ramp	0	65	250 t	500	2-3

If you have a **3.0 x 3.0 box filament (FB330B)** installed in your puller, please choose the program below intended for 1.5 OD thin walled or thick walled glass.

Glass OD/ID	Glass Item #	Heat	Pull	Vel	Time/Del	Pressure	Loops
1.5mm x .86mm	BF150-86-10	Ramp	0	19	1 (delay)	500	4-5

Glass OD/ID	Glass Item #	Heat	Pull	Vel	Time/Del	Pressure	Loops
1.5mm x 1.1mm	BF150-110-10	Ramp	0	65	250 t	500	2-3

If you have a **3mm trough filament (FT330B)** installed in your puller, please choose the program below intended for 1.5 OD thin walled or thick-walled glass.

Glass OD/ID	Glass Item #	Heat	Pull	Vel	Time/Del	Pressure	Loops
1.5mm x 0.86mm	BF150-86-10	Ramp+10	0	55	150 t	500	5-6

Glass OD/ID	Glass Item #	Heat	Pull	Vel	Time/Del	Pressure	Loops
1.5mm x 1.10mm	BF150-110-10	Ramp+15	0	90	150 t	500	3

You have a choice of using “filamented” or “non-filamented” capillary glass. Filamented capillaries have a small thin rod of glass adhered to the inner wall of the capillary to facilitate the capillary action of drawing solution to the tip of your pipette. This is absolutely required for microelectrodes which have a tip size less than 1 micron, but most researchers choose to use filamented glass even for patch pipettes with 1-3µm tips. The filament will not interfere with establishing a gigolm seal and might help reduce the development of air bubbles in the pipette when it is being filled.

* *If your filament and glass combinations are not found here, please refer to the “Patch Pipette Look Up Table” on Page 32 or refer to the “General Look Up Tables” and install a* ***Type A*** *program designated for your filament and glass.*

Writing a Stable Patch Program….the **Mid-Point Velocity** is the **Stable Velocity!!!**

To make a program with consistent results, you must find the mid-point velocity which will be your stable velocity setting. To do this you must find the entire range of velocity settings that loop the number of times recommended on the table. By using the absolute mid-point of the velocity range, you will create the most stable and reproducible program.

THICK WALLED GLASS – WHOLE CELL RECORDING
Using 1.5 x .86 glass and a 2.5 x 2.5 box filament install the following setting:

Heat	Pull	Velocity	Delay	Pressure	Ideal # of Loops
Ramp	0	22	1	500	5 times

Using the above one line program, pull a series of pipettes using all the same settings and only change the velocity **(**up and down in ONE unit increments) to find the entire range which allows for **five loops**. To find the entire range, gradually increase the velocity in 1 unit increments until the puller loops four times and then gradually decrease the velocity until the puller loops six times. See example below:

Velocity	17	18	19	20	**21**	22	23	24	25
Loops	7	6	6	5	**5**	5	4	4	4

(Mid-Point Velocity)

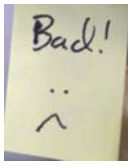
THIN WALLED GLASS – SLICE RECORDING
Using 1.5 x 1.1 glass and a 2.5 x 2.5 box filament install the following setting:

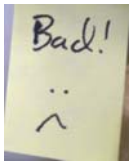
Heat	Pull	Velocity	Time	Pressure	Ideal # of Loops
Ramp	0	65	250	500	2 times

Pull a series of pipettes using all the same settings and only change the velocity (up and down in THREE unit increments) to find the entire range which allows for **two loops**. To find the entire range, gradually increase the velocity in 3 unit increments until the puller loops one time and gradually decrease the velocity until the puller loops three times. See example below:

Velocity	53	56	**59**	**62**	**65**	**68**	**71**	74	77
Loops	3	3	**2**	**2**	**2**	**2**	**2**	1	1

Threshold **(Mid-Point Velocity)** **Threshold**





Pre-Heat Mode: When using the P-1000 Puller, you can also use the **THERMOLOCK**™ feature which pre-heats and maintains the jaw temperature at 70° C (Please see P-1000 manual for instructions). The variation of heat retained in the brass jaws (which hold the filament) can introduce variability if you have an "unstable" velocity setting. By choosing the **stable mid-point velocity** value, you will buffer these slight changes in heat, and your puller will loop the number of times you have intended. Using a **threshold velocity will result in variability** in cone angle and number of loops. You will find that there is not much leeway on each side of the mid-point velocity, so it is important to identify the exact range of velocities. If you have a P-1000 puller, try the Pre-Heat Mode (activated in the Menu screen) to help stabilize your program.

If you are using 1.5mm x 0.86mm glass, it is best to use a 2.5mm x 2.5mm box filament or a 3mm x 3mm box filament and aim for **4 to 5 loops**. Using a 2.5mm or 3mm box filament in combination with the delay mode for cooling will produce the shortest, most stubby taper and highest cone angle.

If you are using 1.5mm x 1.1mm glass, it is best to use a 2.5mm x 2.5mm box filament or a 3mm trough filament and aim for **2 to 3 loops**. Using a trough filament and the time mode for cooling will produce the most stable results, while using a 2.5mm or 3mm box and the delay mode for cooling will produce the shortest, most stubby taper and highest cone angle. When more than 3 loops or 3 lines are used to pull thin walled glass, it can often lead to variability in tip size, and the tips will more likely have an uneven, flared tip, splintered or cracked tip.

A. To make a pipette with a **SMALLER TIP & HIGHER RESISTANCE . . .**
Increase the velocity to allow the puller to loop one less time. Aim for 2 loops instead of 3 loops for thin walled glass and aim for 4 loops instead of 5 loops for thick walled glass.

Find the range of velocities that loops 4 times (instead of 5) by gradually increasing the velocity and choosing the mid-point value. For example, using a 2.5mm box filament, 1.5 x .86 glass and the following settings:

Find the range of velocities that allows 4 loops and install the mid-point value…

Heat	**Pull**	**Velocity**	**Delay**	**Pressure**	**Loops**
525	0	**26**	1	500	**4 (**instead of 5)

If you would like a **SMALLER TIP but NOT A LONGER TAPER**, write out your program into five identical lines based on your one-line five-looping program you previously established. Then reduce the velocity on the fourth line by three units (which "saves" a little more glass for line five when the tip is forming. You can also add a slight amount of pull on the last line and/or increase the heat on the last line by 5-20 units. So as not to get "lost and confused" make only one modification at a time.

Line	**Heat**	**Pull**	**Velocity**	**Delay**	**Pressure**
1	525	0	21	1	500
2	525	0	21	1	500
3	525	0	21	1	500
4	525	0	**18**	1	500
5	**525+**	**10-40**	**21-25**	1	500

If you are using *thin walled glass and require a smaller tip* but do not want to increase the taper length, write the program out into multiple lines and *reduce the heat on the last line* instead of increasing it. Using a lower heat to produce a smaller tip might appear counter-intuitive but, if there is too much heat or too little cooling supplied to thin walled glass when the tip is forming, on the last line, one will produce a blunt tube-like tip instead of a fine tip.

B. To make a pipette with a **LARGER TIP & LOWER RESISTANCE . . .**
Lower the velocity to allow the puller to loop one additional time. Aim for 3 loops instead of 2 loops for thin walled glass and aim for 5 loops instead of 4 loops for thick walled glass.

Heat	Pull	Velocity	Delay	Pressure	Loops
525	0	24	1	500	4
		(change to 19-21) →	→	→	→ **(now looping 5 times)**

LOOK UP TABLE for Patch Pipette Programs, 1-10MΩ, 1-3µm Tip, with a 3-4mm short taper

Filament	Glass OD/ID	Glass Item #	Heat	Pull	Vel	Time/Del	Pressure	Loops
3mm Trough	1.5 x .86	BF150-86-10	Ramp+10	0	55	150 t	500	4-5
3mm Trough	1.5 x 1.1	BF150-110-10	Ramp+15	0	90	150 t	300	3
3mm Trough	1.2 x .69	BF120-69-10	Ramp+15	0	45	150 t	500	4
3mm Trough	1.2 x .94	BF120-94-10	Ramp	0	65	150 t	500	3
3mm Trough	1 x .50	BF100-50-10	Ramp+15	0	50	150 t	500	4
3mm Trough	1 x .78	BF100-75-10	Ramp+15	0	90	150 t	500	3

Filament	Glass OD/ID	Glass Item #	Heat	Pull	Vel	Time/Del	Pressure	Loops
2.5 x 2.5 box	1.5 x .86	BF150-86-10	Ramp	0	21	1 (delay)	500	4-5
2.5 x 2.5 box	1.5 x 1.1	BF150-110-10	Ramp	0	65	250 t	500	3
2.5 x 2.5 box	1.2 x .69	BF120-69-10	Ramp	0	20	250 t	500	4
2.5 x 2.5 box	1.2 x .94	BF120-94-10	Ramp	0	45	200 t	500	3
2.5 x 2.5 box	1 x .50	BF100-50-10	Ramp	0	30	250 t	500	4
2.5 x 2.5 box	1 x .78	BF100-78-10	Ramp	0	40	200 t	500	3

Filament	Glass OD/ID	Glass Item #	Heat	Pull	Vel	Time/Del	Pressure	Loops
3 x 3 box	1.5 x .86	BF150-86-10	Ramp+ 5	0	22	1 (delay)	500	4-5
3 x 3 box	1.5 x 1.1	BF150-110-10	Ramp	0	65	250 t	500	3
3 x 3 box	1.2 x .69	BF120-69-10	Ramp+ 5	0	25	250 t	500	4
3 x 3 box	1.2 x .94	BF120-94-10	Ramp+ 5	0	45	200 t	500	3
3 x 3 box	1 x .50	BF100-50-10	Ramp+ 5	0	30	250 t	500	4
3 x 3 box	1 x .78	BF100-78-10	Ramp+ 5	0	40	200 t	500	3

*The 2.5 x 4.5 box filament programs listed below are **not ideal for making short tapered patch pipettes** and are only recommended if you need a <u>much</u> longer taper.

Filament	Glass OD/ID	Glass Item #	Heat	Pull	Vel	Time/Del	Pressure	Loops
2.5 x 4.5 box	1.5 x .86	BF150-86-10	Ramp+ 5	0	23	1 (delay)	500	5-6
2.5 x 4.5 box	1.5 x 1.1	BF150-110-10	Ramp+ 5	0	65	250 t	500	3
2.5 x 4.5 box	1.2 x .69	BF120-69-10	Ramp	0	20	250 t	500	4-5
2.5 x 4.5 box	1.2 x .94	BF120-94-10	Ramp	0	40	200 t	500	3
2.5 x 4.5 box	1 x .50	BF100-50-10	Ramp	0	25	250 t	500	4
2.5 x 4.5 box	1 x .78	BF100-78-10	Ramp	0	25	250 t	500	3

References for Electrophysiology - For additional information about electrophysiology, its history, and the various approaches, please refer to the following sources:

- Advanced Micropipette Techniques for Cell Physiology, K.T Brown, D.G. Flaming
- The Axon Guide – For Electrophysiology & Biophysics: Laboratory Techniques http://www.moleculardevices.com/pdfs/Axon_Guide.pdf
- Patch Clamping An Introductory Guide to Patch Clamp Recording, Areles Molleman
- The American College of Neuropsychopharmacology web site http://www.acnp.org/g4/GN401000005/CH005.html
- Curtis, H.J. & Cole, K. S. Membrane action potentials from the squid giant axon. J. Cell. & Comp. Physiol. 15: 147-157, 1940
- Huxley AL and Hodgkin AF**.** Measurement of Current-Voltage Relations in the Membrane of the Giant Axon of Loligo. *Journal of Physiology* 1: 424-448, 1952(a).
- Neher E and Sakmann B. Noise analysis of drug induced voltage clamp currents in denervated frog muscle fibers. *J Physiol* 258: 705–729, 1976
- General descriptions about electrophysiology and various approaches: http://www.answers.com/topic/electrophysiology
- "Single Channel Recording" 2nd edition 1995 by Sakmann & Neher

Where to take a concentrated short course to learn electrophysiology techniques

Marine Biological Lab, Woods Hole, MA
http://www.mbl.edu//education/courses/summer/

Cold Spring Harbor Lab, Cold Spring Harbor, Long Island, NY
http://meetings.cshl.edu/courses.html

MBL - Marine Biological Labs, Eel Pond, Woods Hole, MA Photo courtesy of David McFarland