Supplemental File 1: Assembly and transfection instructions

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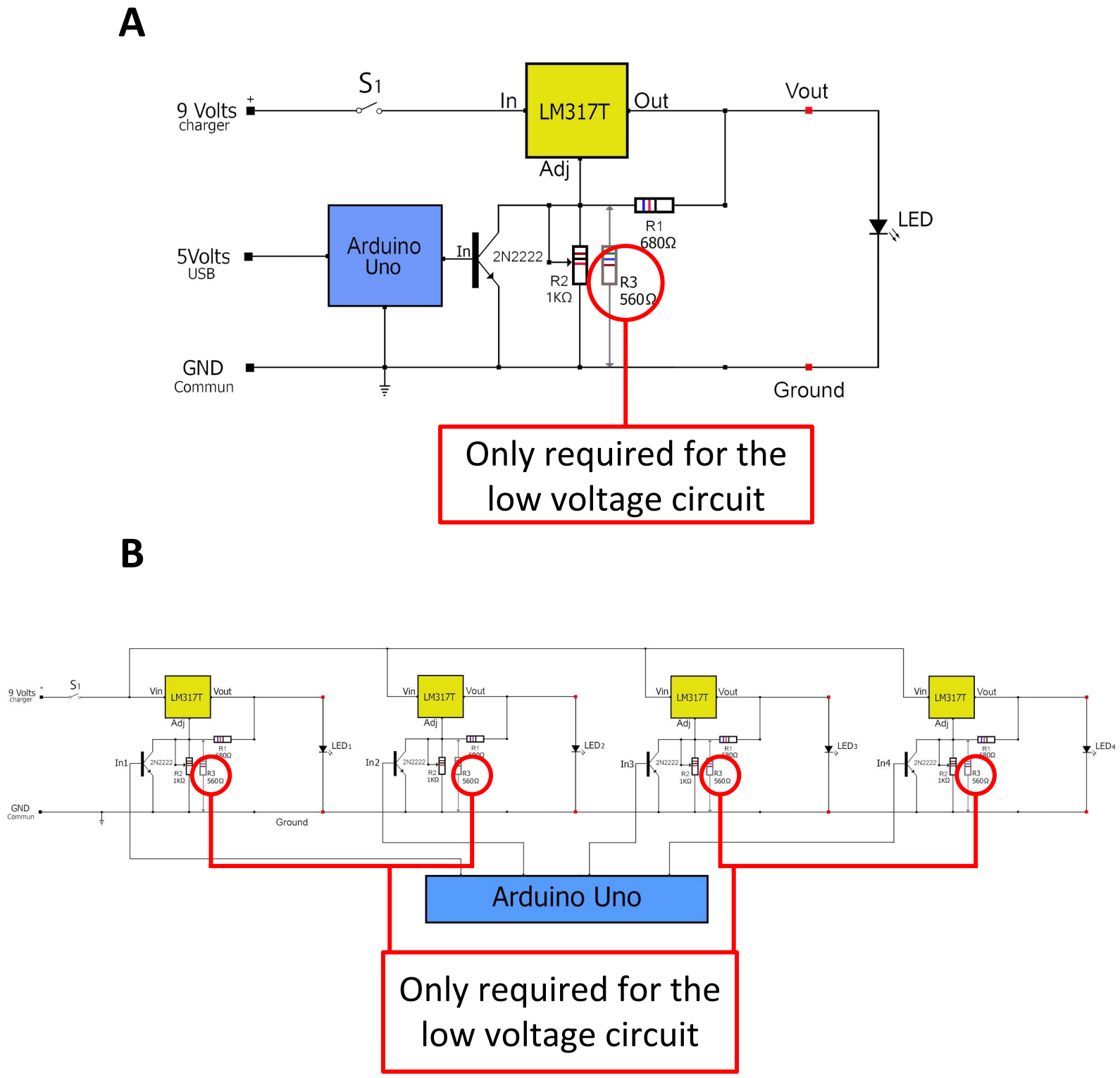


Figure S1: Electronic Driver Circuit for multiple LEDs. (A) The circuit diagram for a single LED system. (B) The circuit diagram for a four LED system.

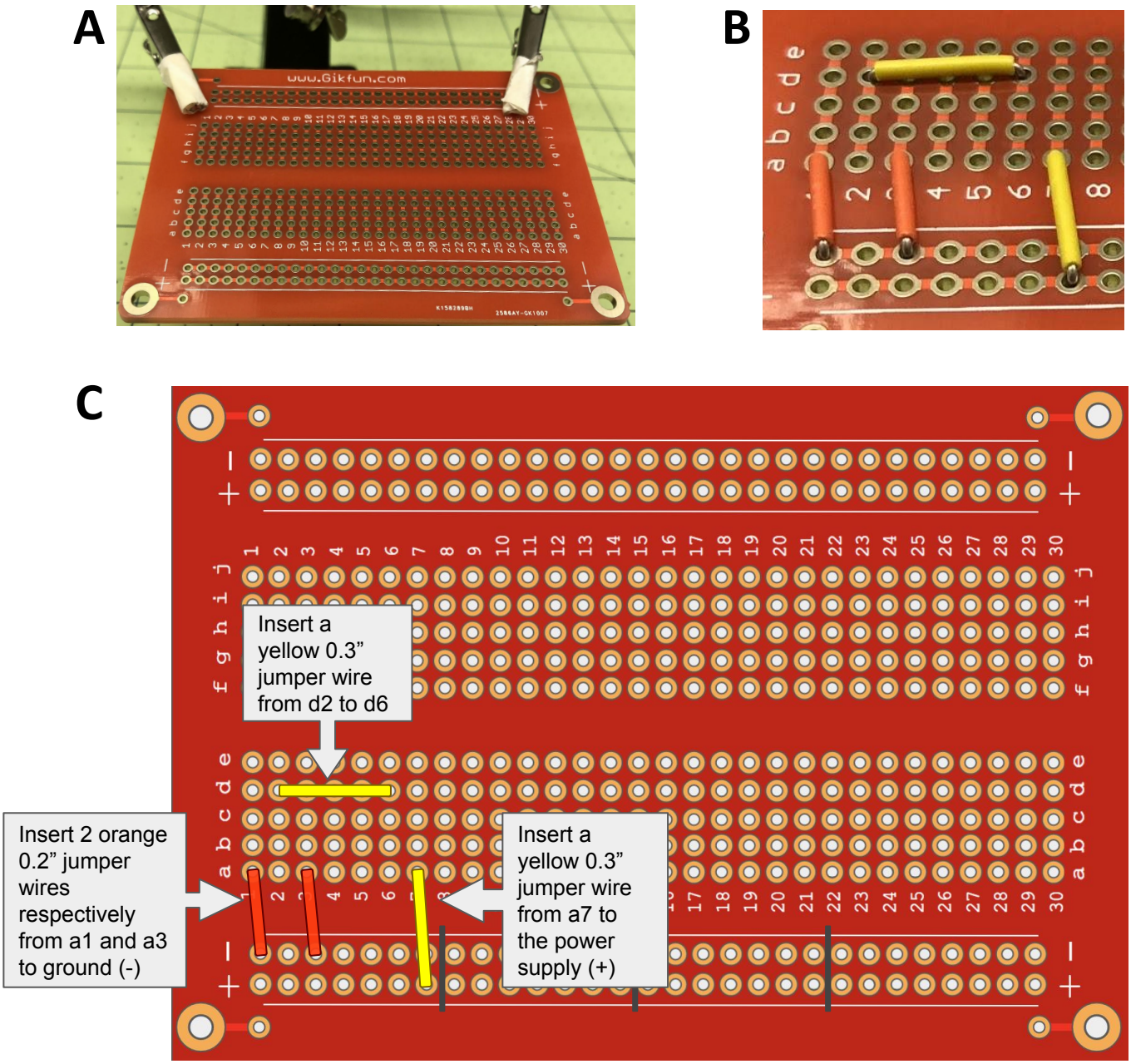


Figure S2: Placing the circuit Interconnections. (A) Clip your PCB board onto your helping hands. (B) Position of main circuit jumpers into the through holes in the picture. (C) Diagram of wire connectors mapping the coordinates. For the four LED system, draw lines dividing each circuit as shown (black vertical lines). Figures S31-S38 describe the assembly of four circuits simultaneously.

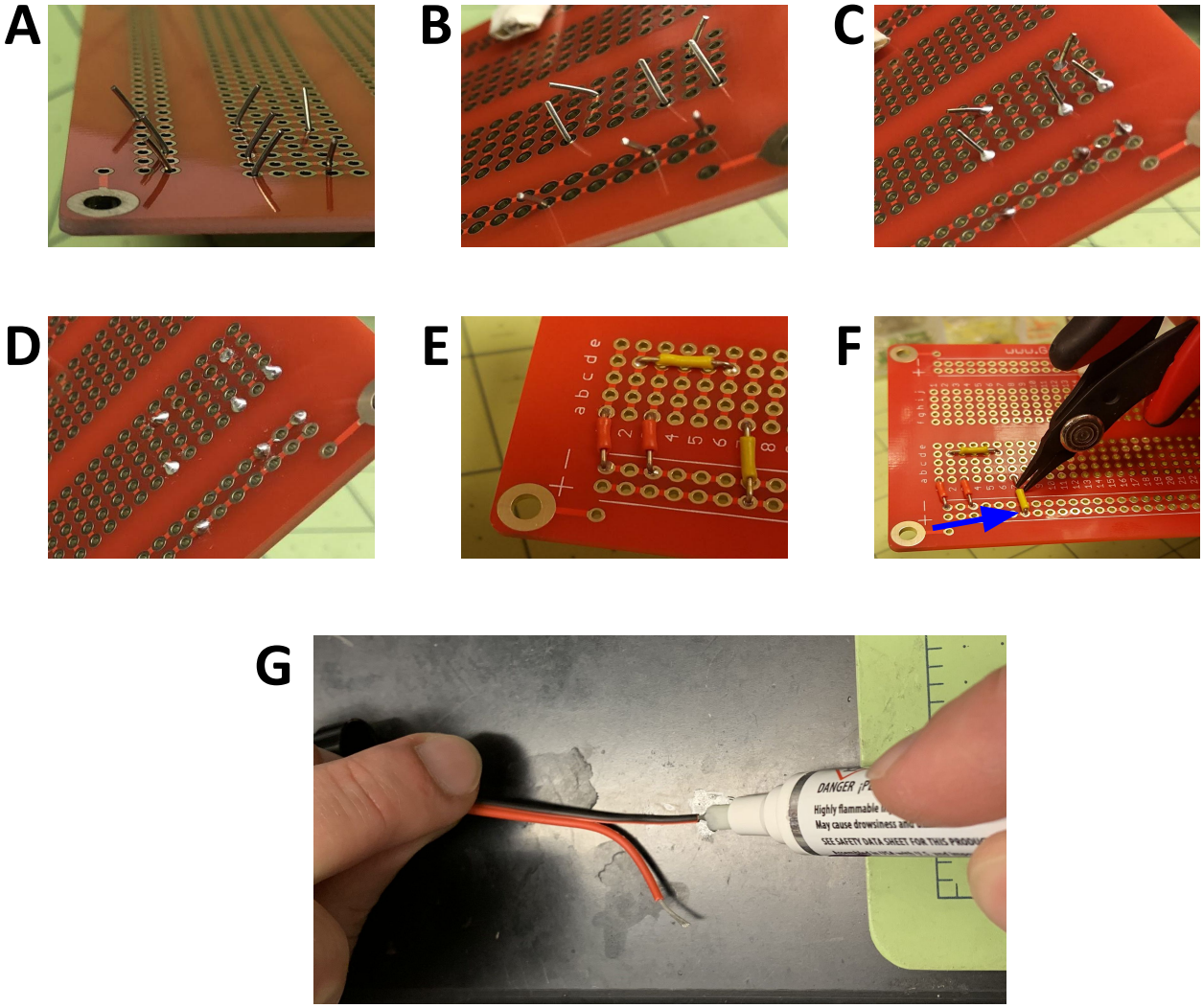


Figure S3: Soldering the wires onto the PCB. (A) Bend jumpers so that they make direct contact with the PCB and stay in place while soldering. (B) Another view of the bent wires. (C) Wires after soldering. (D) Trimmed wires on the PCB. (E) Shrunken insulation after heating with solder. (F) Moving the insulation into position to cover the ground through hole (blue arrow) (G) Adding flux to a wire end or terminal.

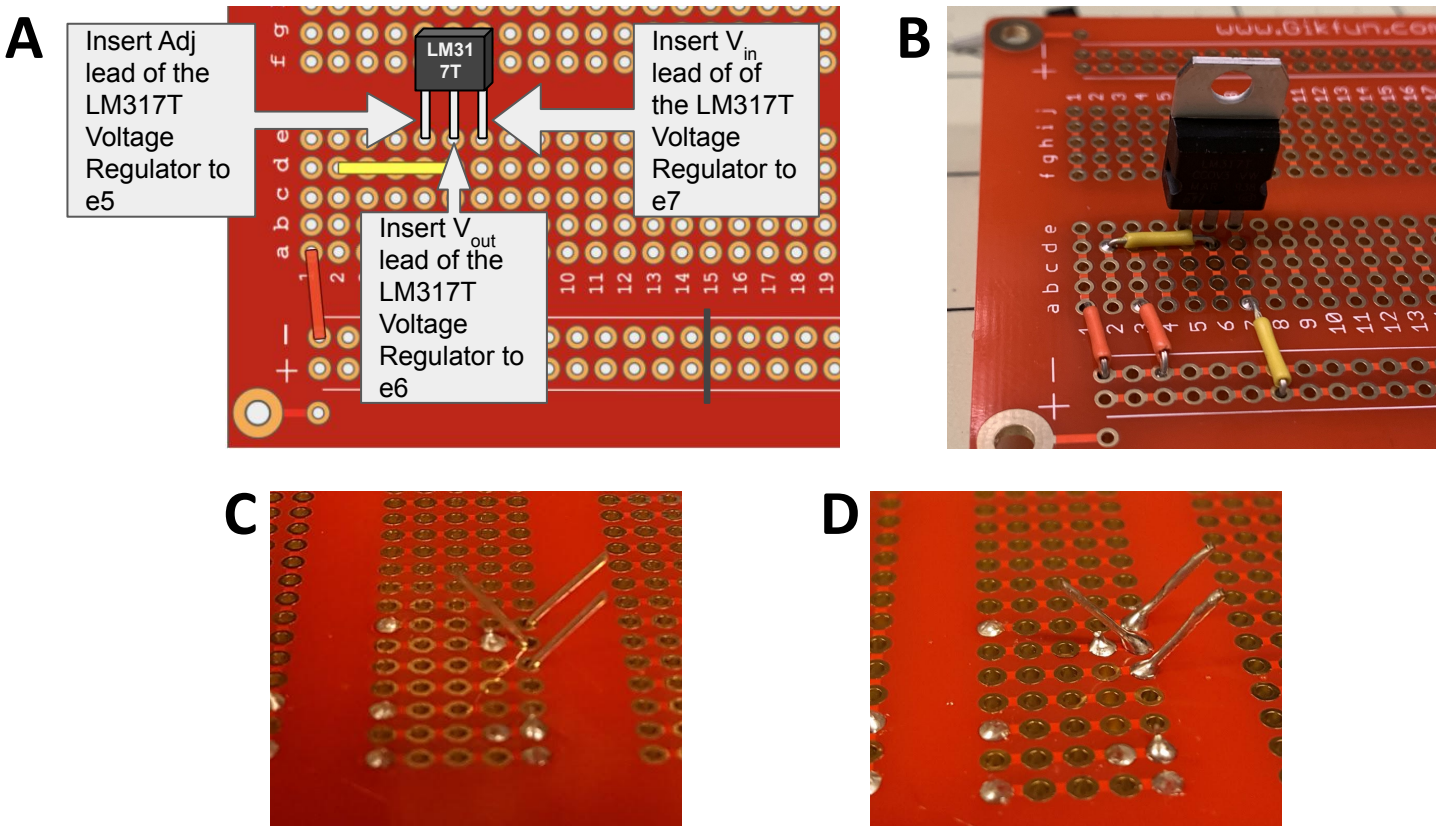


Figure S4: Soldering the voltage regulator into place.(A) Map of the voltage regulator coordinates. (B) Placement of the voltage regulator. (C) Bent voltage regulator leads. (D) Voltage regulator terminals after soldering.

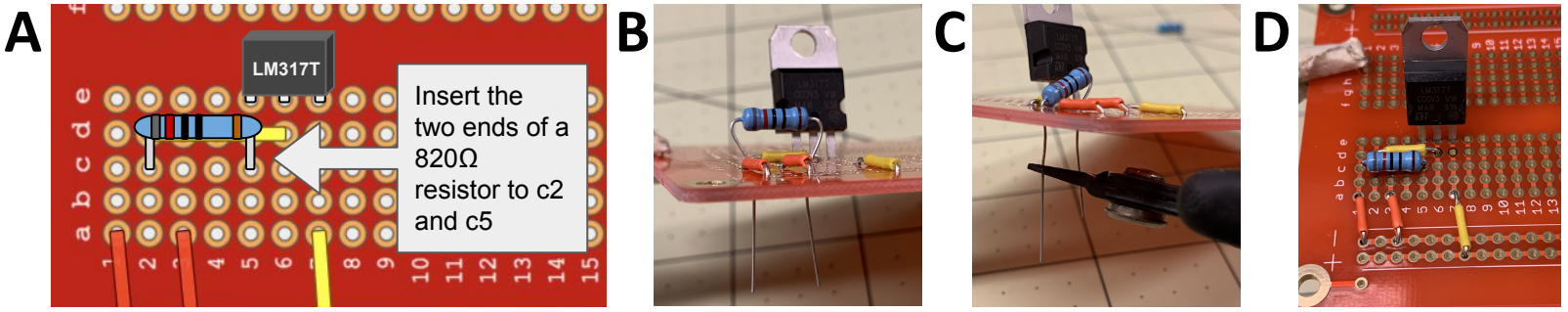


Figure S5: Soldering the R1 resistor into place. (A) Map of the R1 resistor (820Ω) coordinates. (B) Pulling the resistor through by the lead using pliers (C) The pulled resistor close to PCB. (D) The soldered resistor close to PCB.

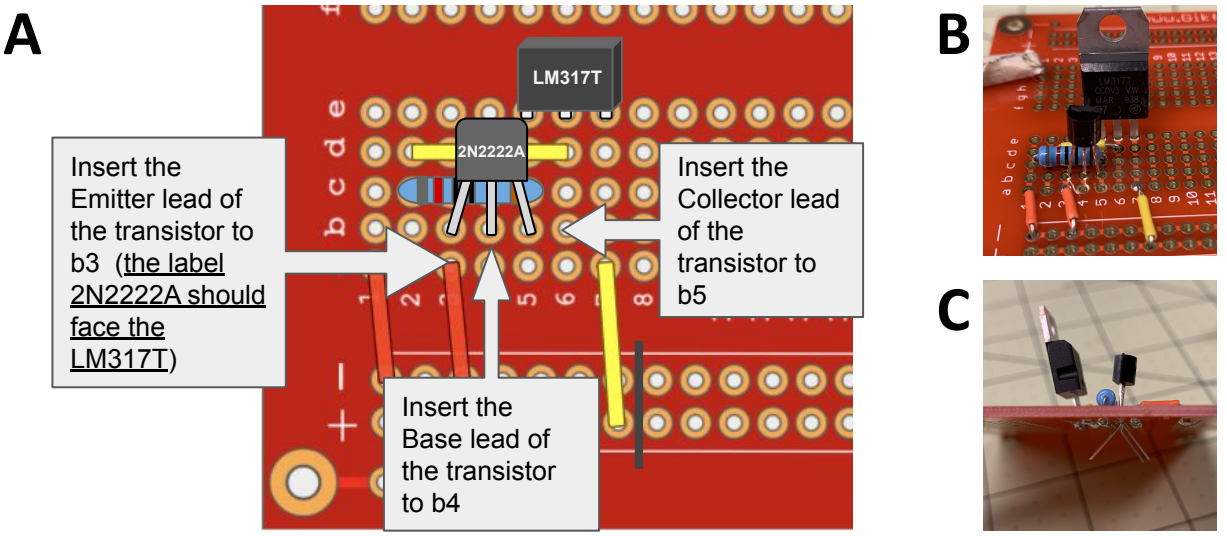


Figure S6: Soldering the transistor into place. (A) Map of the transistor coordinates and orientation. (B) Note the orientation of the transistor, the label in this model is facing the voltage regulator (LM317T). Double check the specification of the transistor to make sure the “Emitter”, “Base”, and “Collector” are in the correct holes. (C) The transistor with the terminals bent prior to soldering.

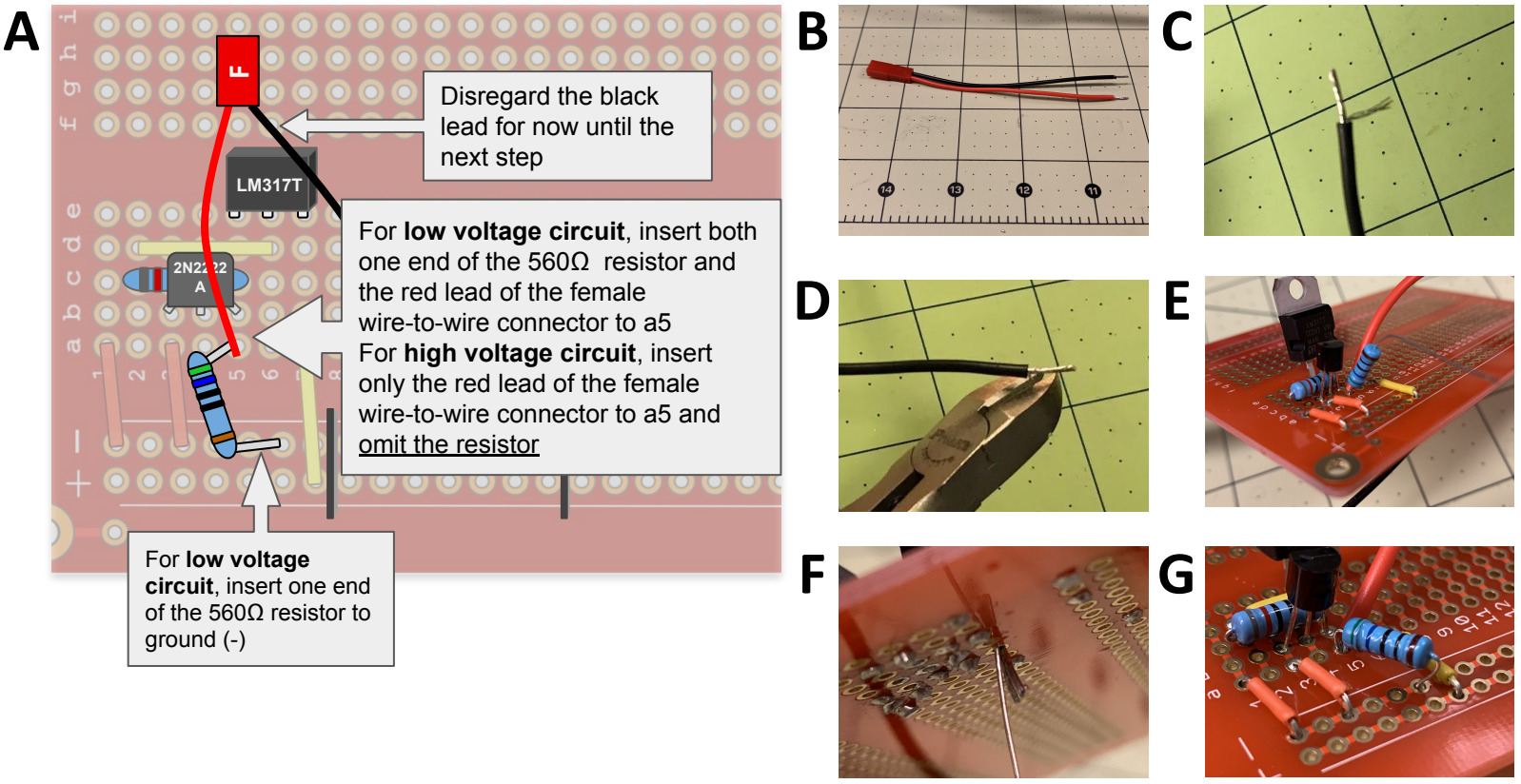


Figure S7: Soldering the wire-to-wire connector for the potentiometer into place (plus a 560Ω resistor for the low voltage circuit). (A) Map of the coordinates of the wire-to-wire connector (plus the R3-560Ω if building the low voltage circuit, the wire-to-wire connector is placed in the hole before the resistor.) (B) A female wire-to-wire connector. (C) To facilitate fitting the resistor along with the wire-to-wire connector, 3-5 strands of the braided wire are bent. (D) The strands are cut off with wire cutters as close to the insulation as possible. (E) Inserted red wire of a female wire-to-wire connector through the a5 through hole (For the low voltage circuit insert R3 through the same through hole.) (F) Underside view of the resistor and wire-to-wire connector before soldering. (G) Image of the soldered R3 resistor connected to ground. (F = Female)

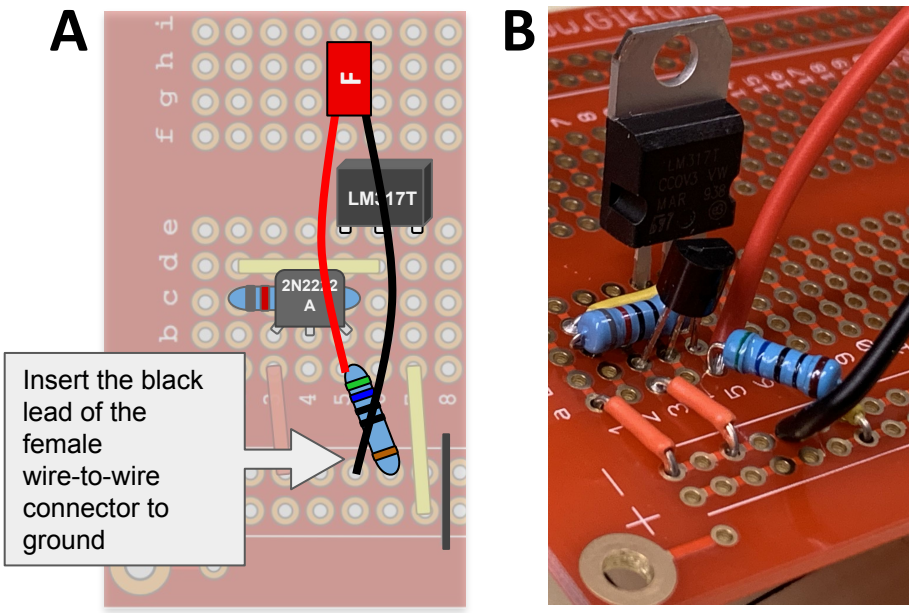


Figure S8: Soldering the wire-to-wire connector for the potentiometer to the ground. (A) Map of the coordinates of the ground connection for the potentiometer wire-to-wire connector. (B) Top view of the potentiometer wire-to-wire connector in parallel with R3. (F = Female)

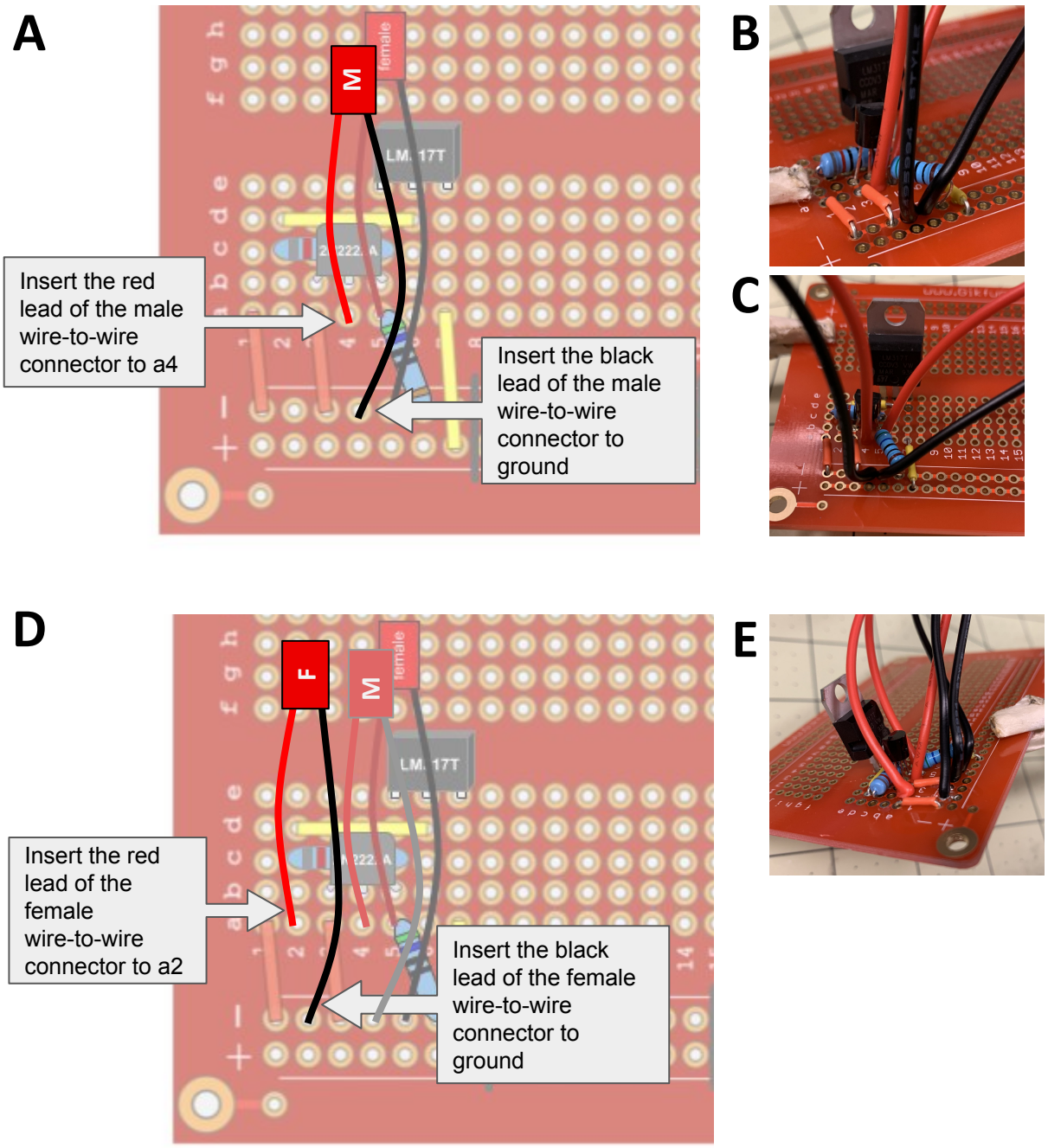


Figure S9: Soldering the microcontroller and LED wire-to-wire connectors. (A) Map of the coordinates of the wire-to-wire connector for connecting the 2N222A and the ground to the microcontroller. (B) Soldered male wire-to-wire connector. (C) Top view of (B). (D) Map of the coordinates of the female wire-to-wire connector for connecting the input of the circuit and ground to the LED. (E) Soldered female wire-to-wire connector. (F = Female, M = Male)

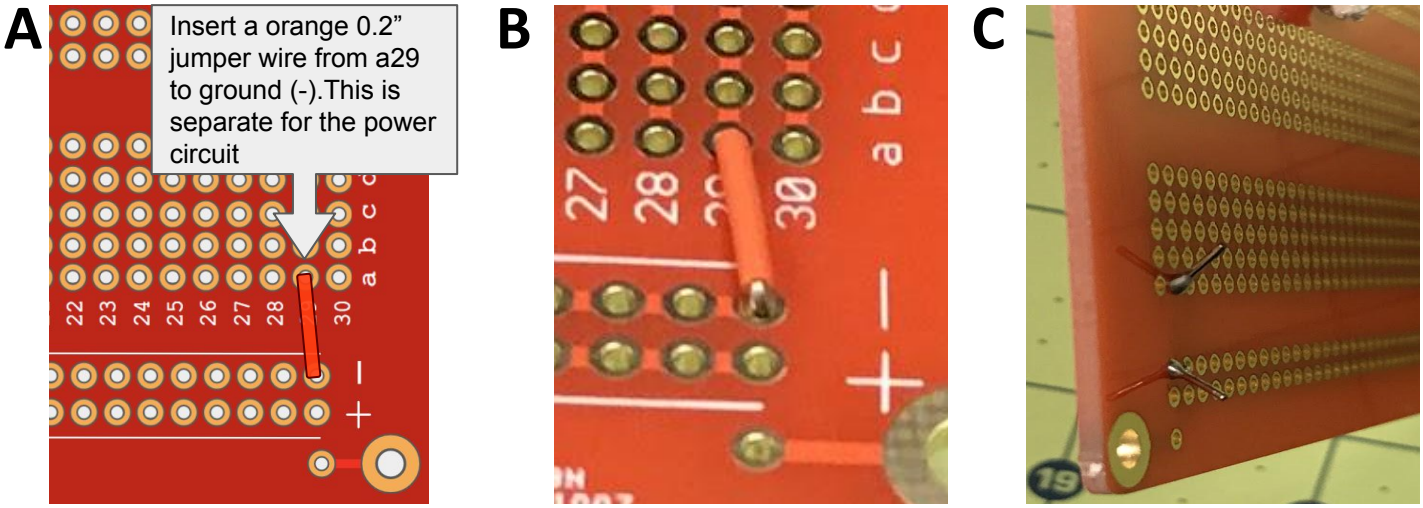


Figure S10: Soldering the jumper for the power supply circuit. (A) Map of the coordinates of the orange jumper for connecting the power supply to ground. (B) The orange jumper soldered in place. (C) The underside view of the jumper soldered in place.

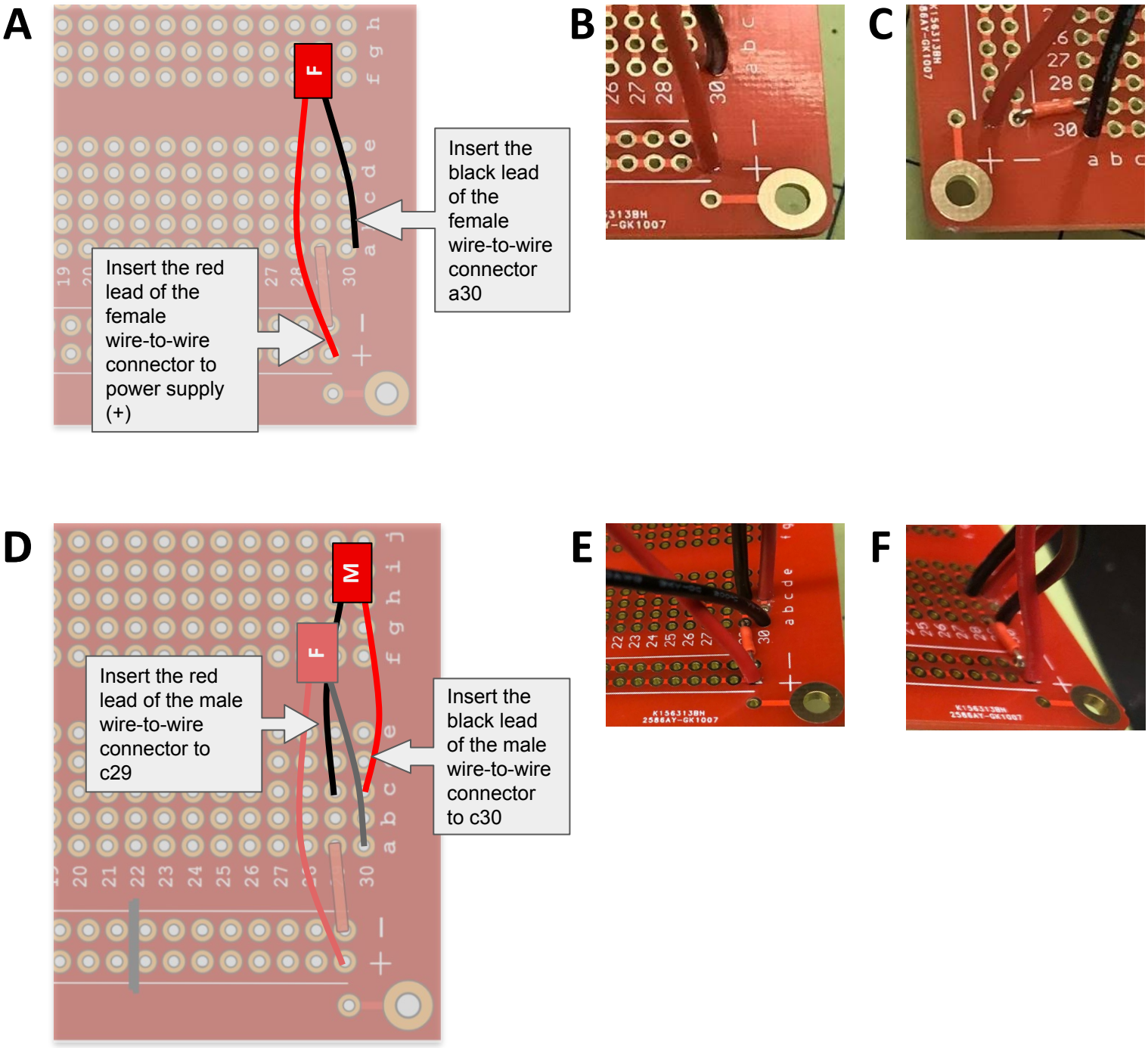


Figure S11: Soldering the power switch and power source wire-to-wire connectors. (A) Map of the coordinates of the female wire-to-wire connector for connecting the power switch. (B) The female wire-to-wire connector soldered in place. (C) Another view of (B). (D) Map of the coordinates of the male wire-to-wire connector for connecting the power source. (E) Soldered male wire-to-wire connector. (F) Another view of (E). (F = Female, M = Male)

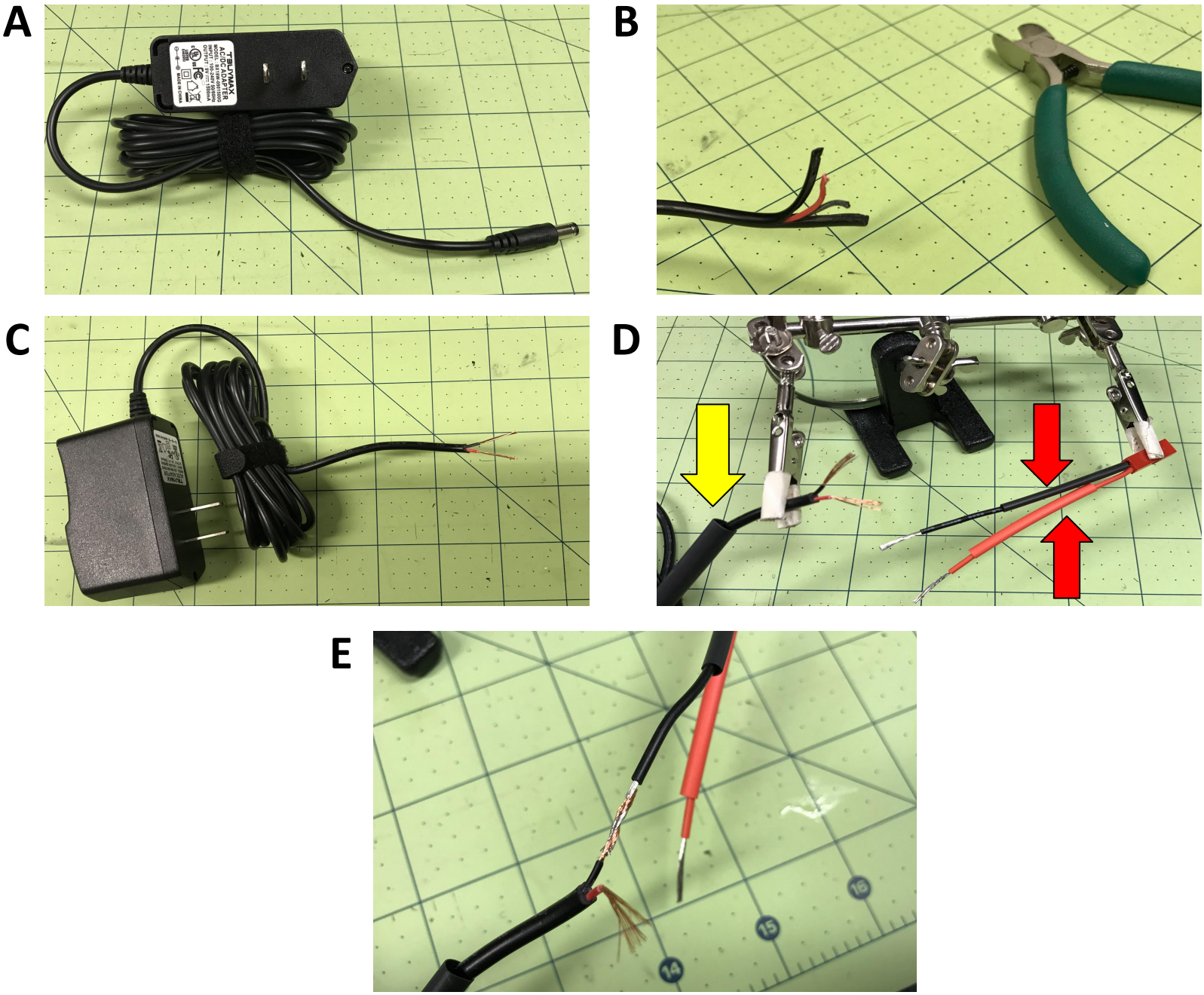


Figure S12: Connecting the power supply to a male wire-to-wire connector. (A) The unmodified power supply. (B) Cutting of the power supply wires. (C) The power supply wires stripped and with excess insulation cut away. (D) Placement of shrink tube around power supply wires. Tubing separating the two connections (red arrows) and tubing to hold the separated wires (yellow arrow). (E) Twisted wires connecting the power supply to the female wire-to-wire connector.

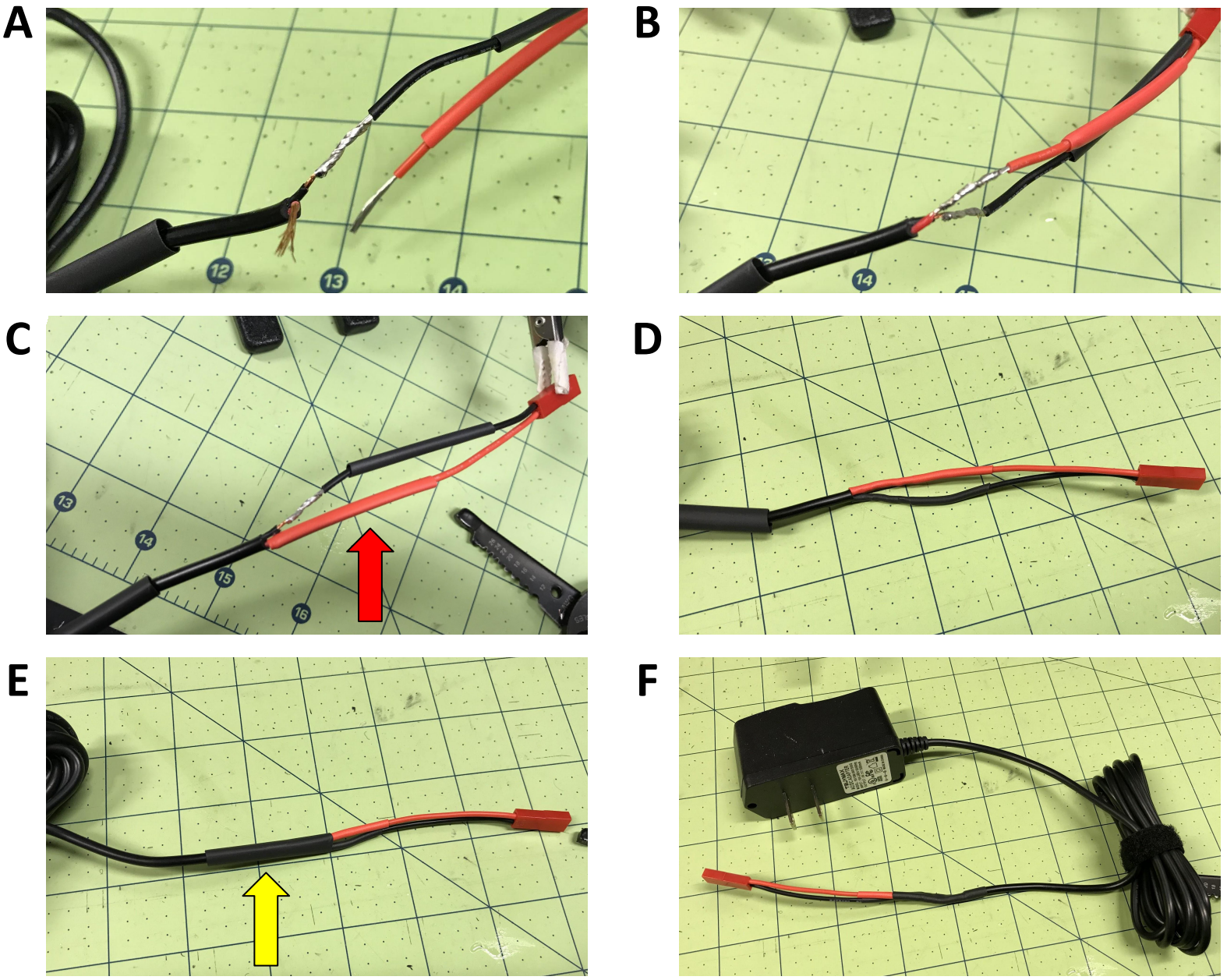


Figure S13: Soldering and insulating the power supply connection to a male wire-to-wire connector. (A) Soldered connection between the power supply ground and a female wire-to-wire connector. (B) Soldered connection between the positive terminal of the power supply and a female wire-to-wire connector. (C) Shrink tube pulled over the soldered individual connections (red arrow). (D) Both power supply connections soldered and with heat treated shrink tube. (E) Placement of shrink tube over individual connections (yellow arrow). (F) Completed power supply.

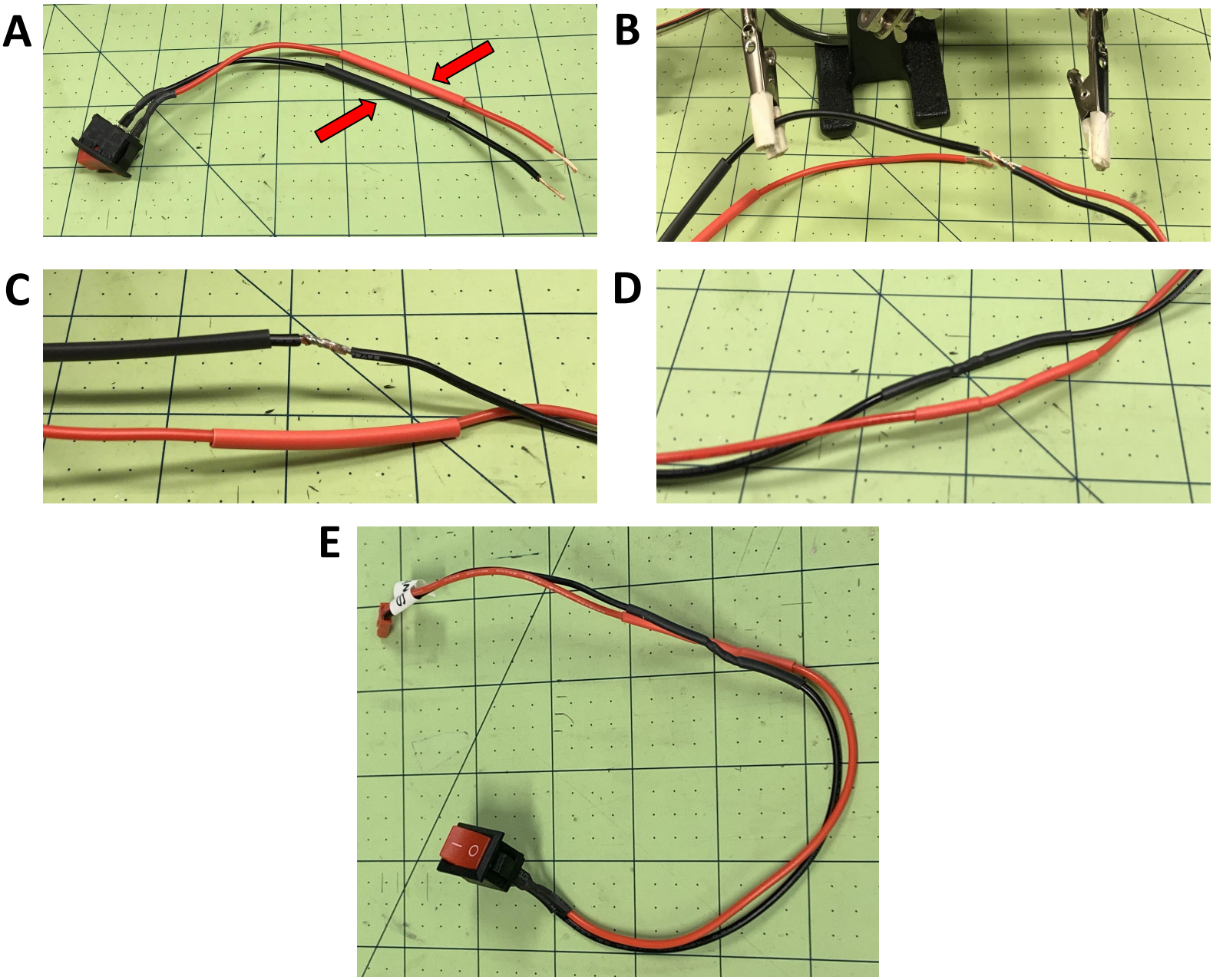


Figure S14: Soldering the power switch to a male wire-to-wire connector. (A) Power switch with stripped wires and shrink tube placed over the wires (red arrows). (B) Wires connecting the switch and male wire-to-wire connector twisted together before soldering. (C) Placing the shrink tube over the soldered connections. (D) Connections covered with the heat treated shrink tube. (E) A powerswitch assembled with a male wire-to-wire connector.

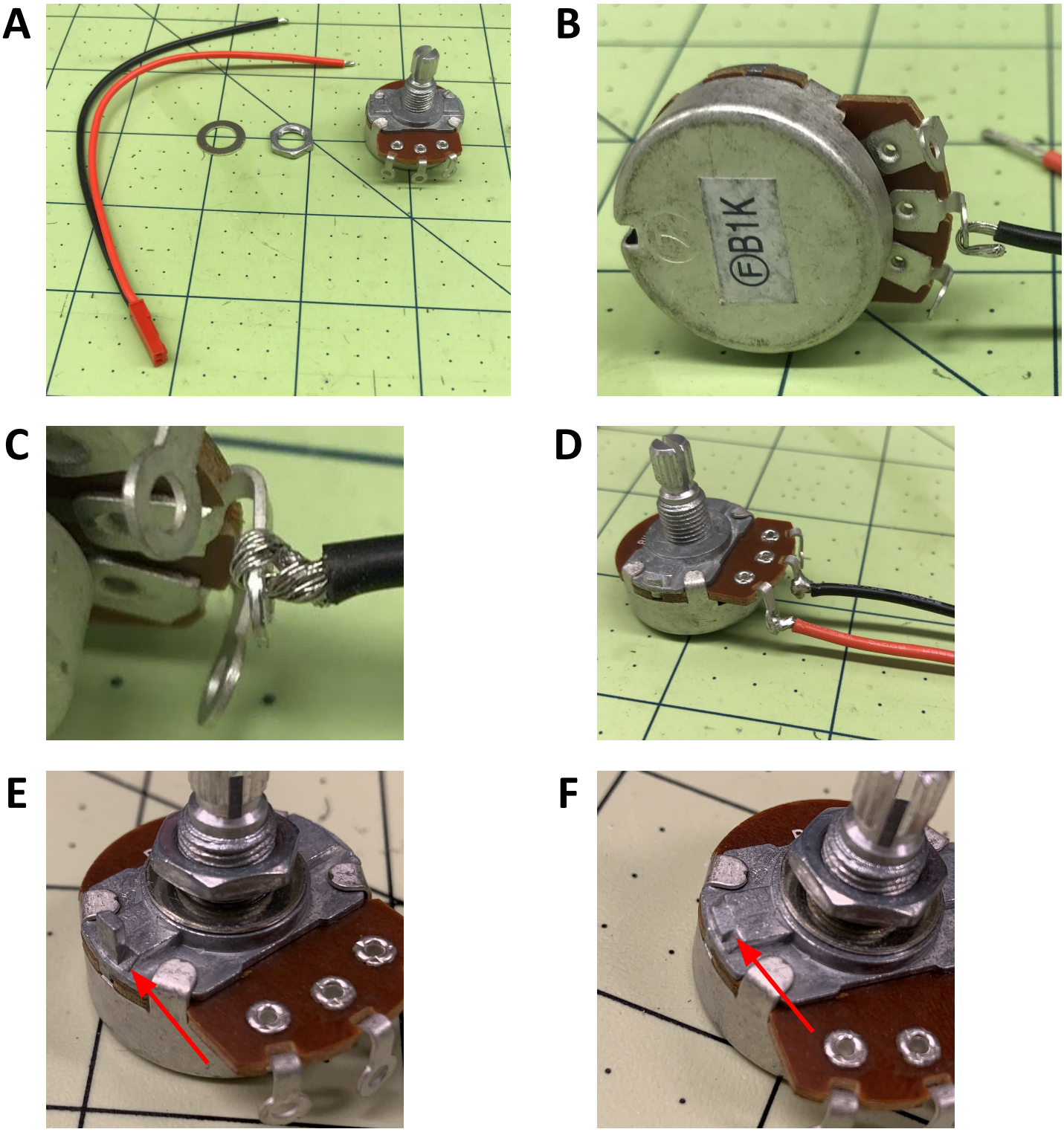


Figure S15: Wiring a potentiometer to a male wire-to-wire connector. (A) The potentiometer parts. (B) A male wire-to-wire connector twisted and bent to hook around the middle terminal of the potentiometer. (C) Male wire-to-wire connector twisted around the middle terminal of the potentiometer. (D) Soldered wire-to-wire connections. (E) Red arrow pointing to the metal tab prior to removal. (F) The potentiometer after metal tab removal.

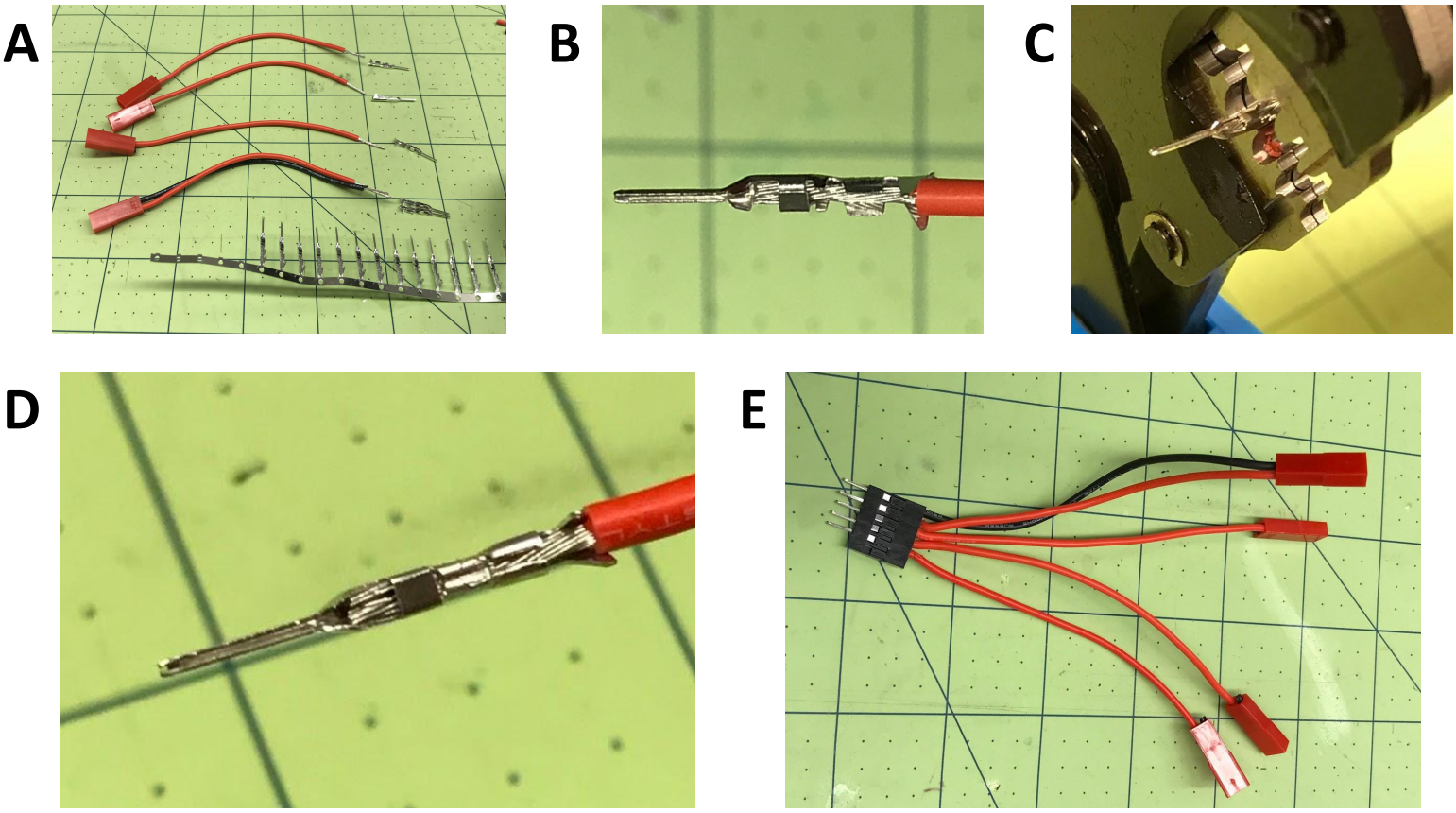


Figure S16: Wiring the microcontroller connection. (A) Wires for female wire-to-wire connectors stripped and cut in preparation for crimpring. (B) Placement of the crimp on the wire-to-wire connector. (C) Crimping of the wire-to-wire connector. (D) Crimped wire-to-wire connector. (E) Fully assembled microcontroller connection.

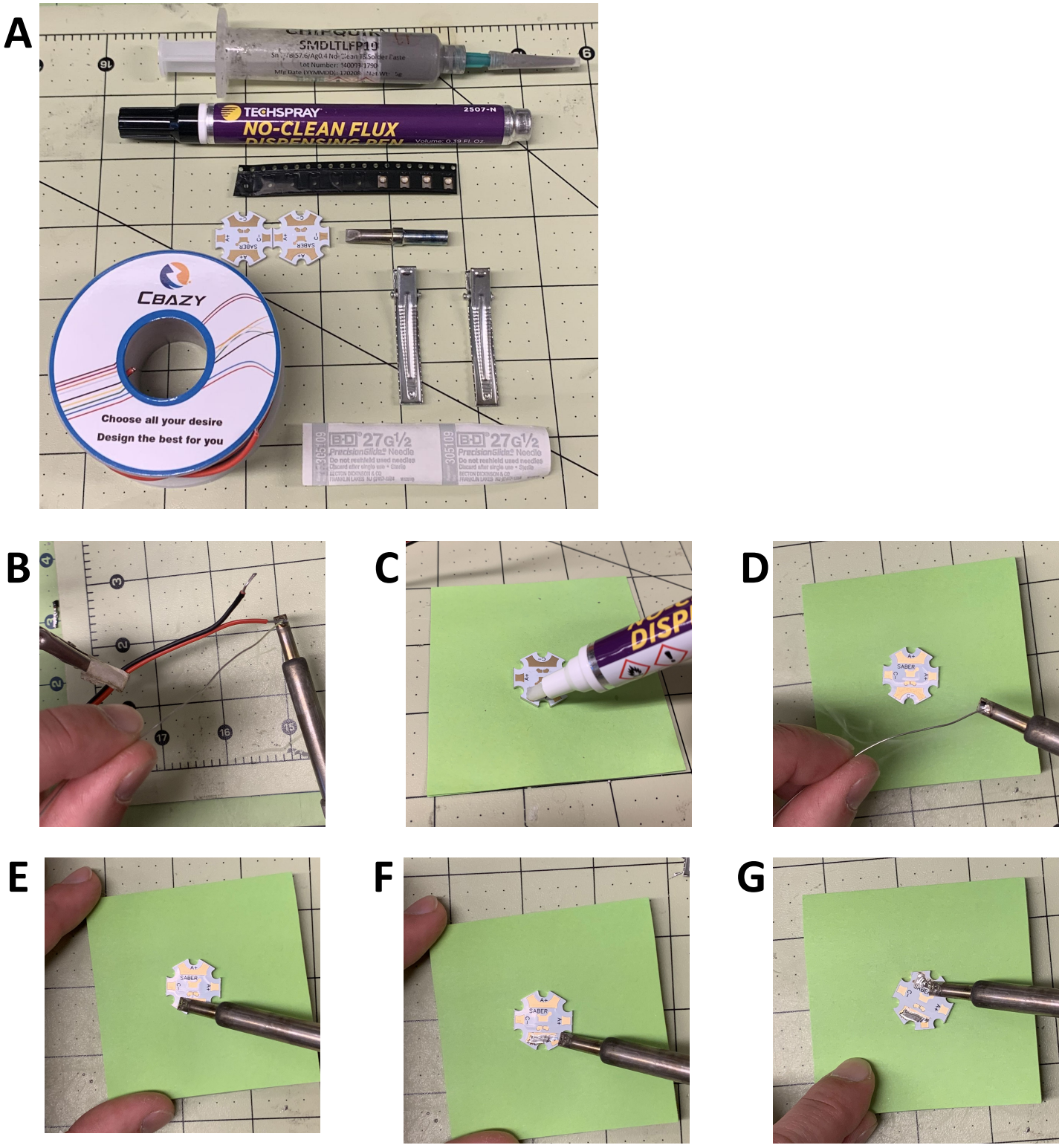


Figure S17: Soldering wires and LED onto the LED base Part 1. (A) Materials needed to solder the LED to the LED base. (B) Tinning the tip of the stripped wire. (C) Applying flux onto the contact of the LED base. (D) Adding solder to the large soldering tip to tinning the LED base. (E) Placement of solder onto the contact to heat the LED base. (F) The LED base after dragging the soldering tip across the contact. (G) The same procedure on the other contact.

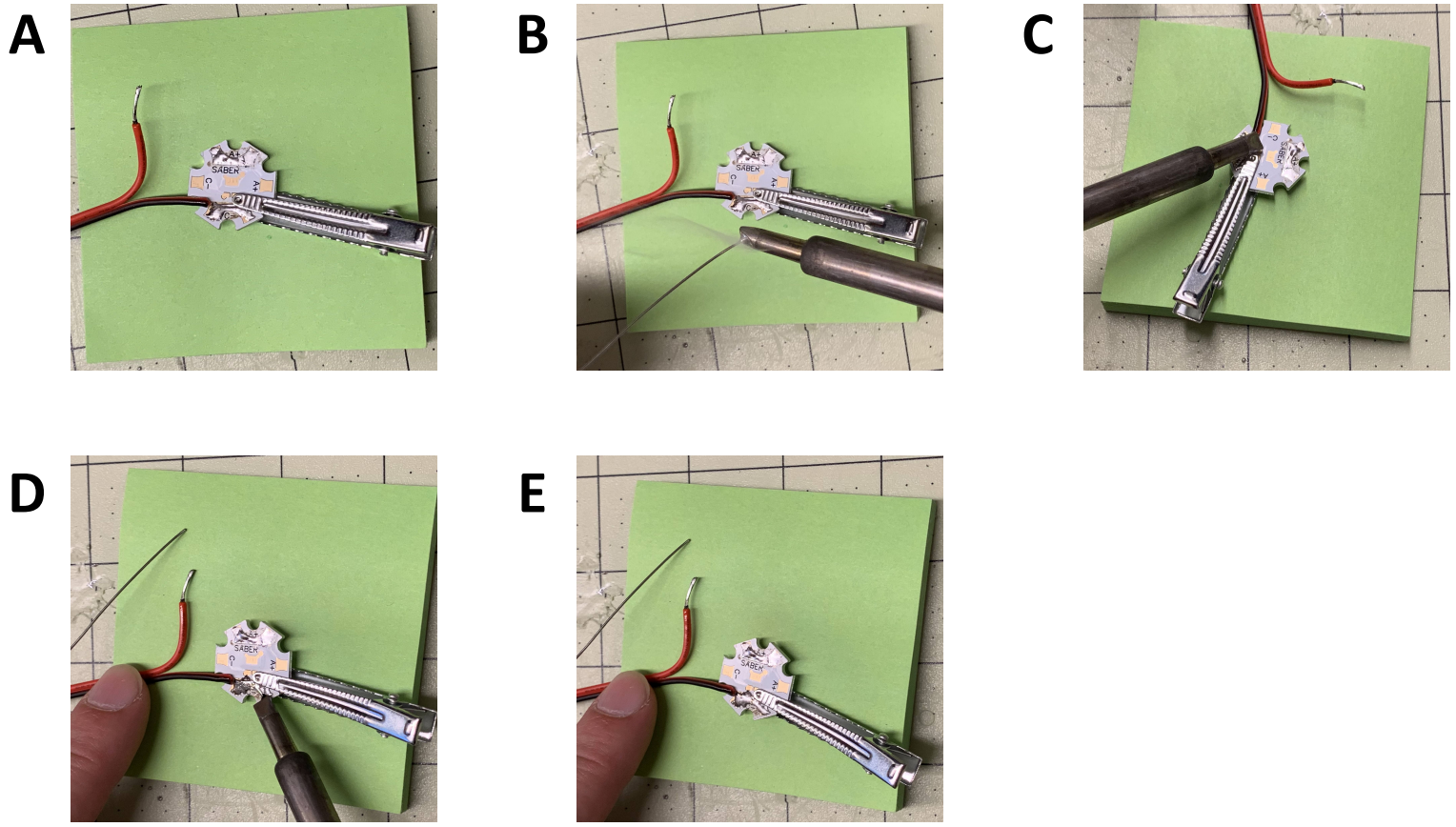


Figure S18: Soldering wires and LED onto the LED base Part 2. (A) A tinned wire clipped to the contact using a hair clip. Note that the black wire is soldered to the cathode "C-" (B) Addition of a generous amount of solder to the the soldering tip. (C) The soldering tip pressing down on the wire, melting the solder on the LED base and the wire. (D) Holding down the wire so that it stays put when the soldering iron is removed. (E) Holding the wire in pace until the solder hardens.

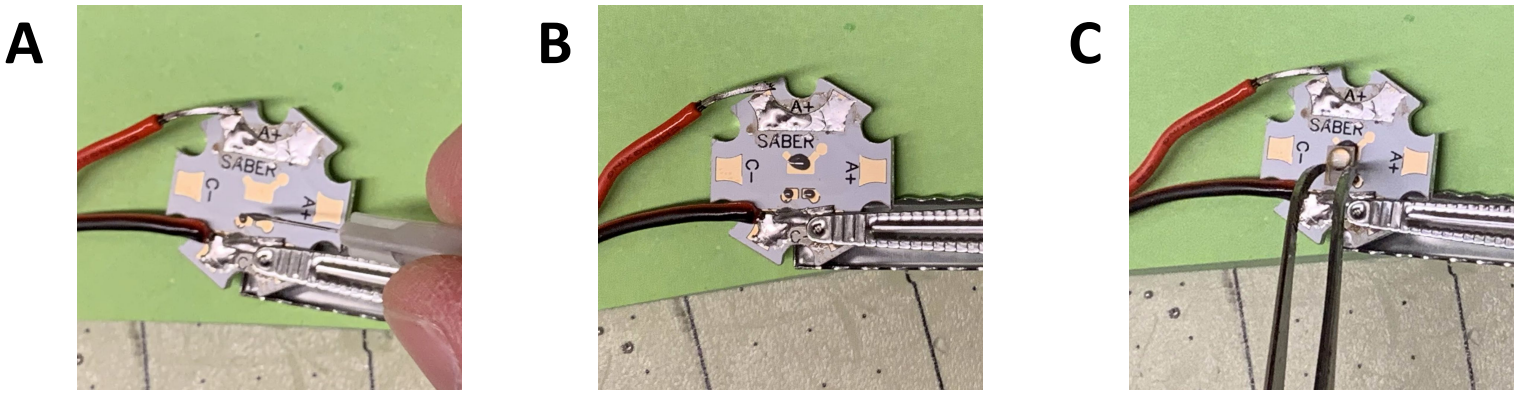


Figure S19: Soldering wires and LED onto the LED base Part 3. (A) Using a sharp tip to place solder paste onto the LED base for mounting the LED. (B) The LED base with the soldering paste in place. (C) Placement of the LED onto the LED base such that the contacts of the LED and LED base match.

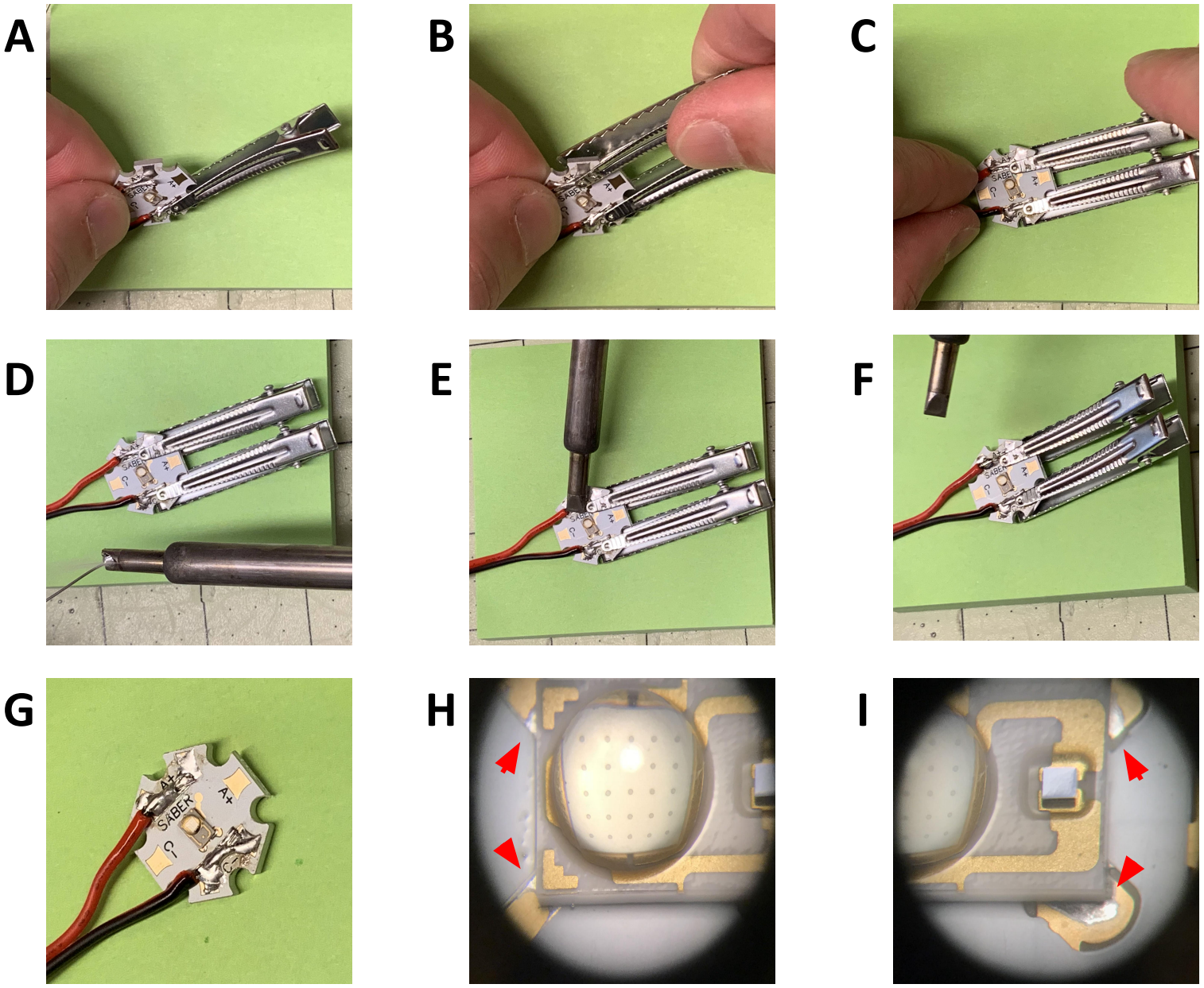


Figure S20: Soldering wires and LED onto the LED base Part 4. (A) The black wire still clipped to the contact by the hair clip. (B,C) Using a second hair clip, the red wire is held into place. Note that the red wire is soldered to the anode "A+". (D) Addition of a generous amount of solder to the the soldering tip. (E) The soldering tip pressing down on the wire, melting the solder on the LED base and the wire as well as the solder paste under the LED. (F) The hot LED base cooling after soldering. (G) The LED base with the wires and LED soldered on. (H,I) Red arrows point to soldering pads. After soldering, the solder appears metallic/shiny (Compared to grey before soldering (Figure S16D)).

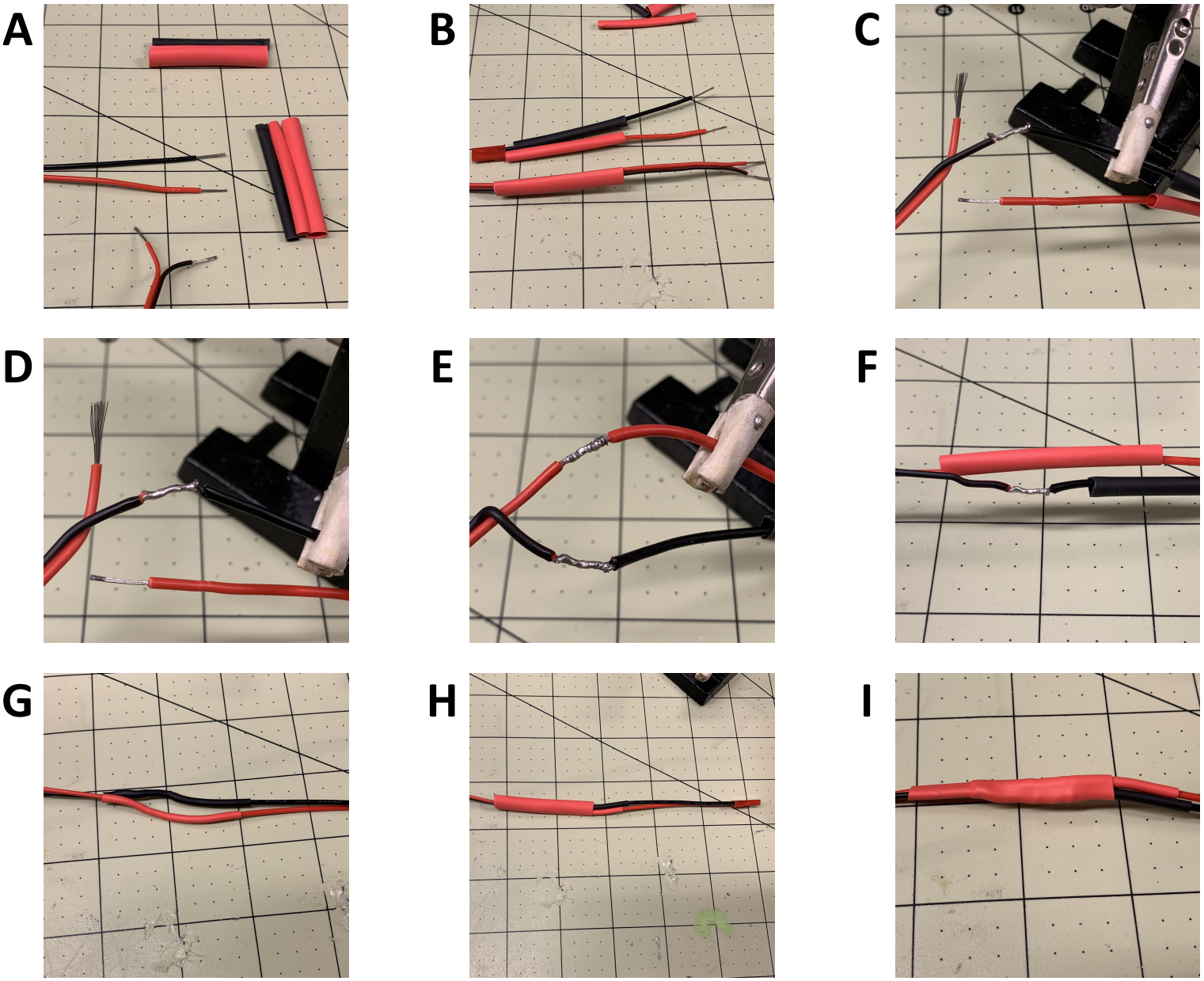


Figure S21: Connecting the LED wire to a male wire-to-wire connector. (A) Stripped wires and male wire-to-wire connector next to the shrink tube cut in half (1/8'' and 3/16''). (B) Shrink tube placement over the wires prior to soldering. (C) Wires twisted together before soldering. (D) The soldered connection from the wire to wire-to-wire connector. (E) Both the red and black wires soldered together. (F) Placement of the 1/8 ''shrink tube over the soldered connection. (G) The shrink tube after shrinking with the heat gun. (H) Placement of the 3/16'' shrink tube over the smaller shrink tube. (I) The connection soldered and sealed with shrink tube.

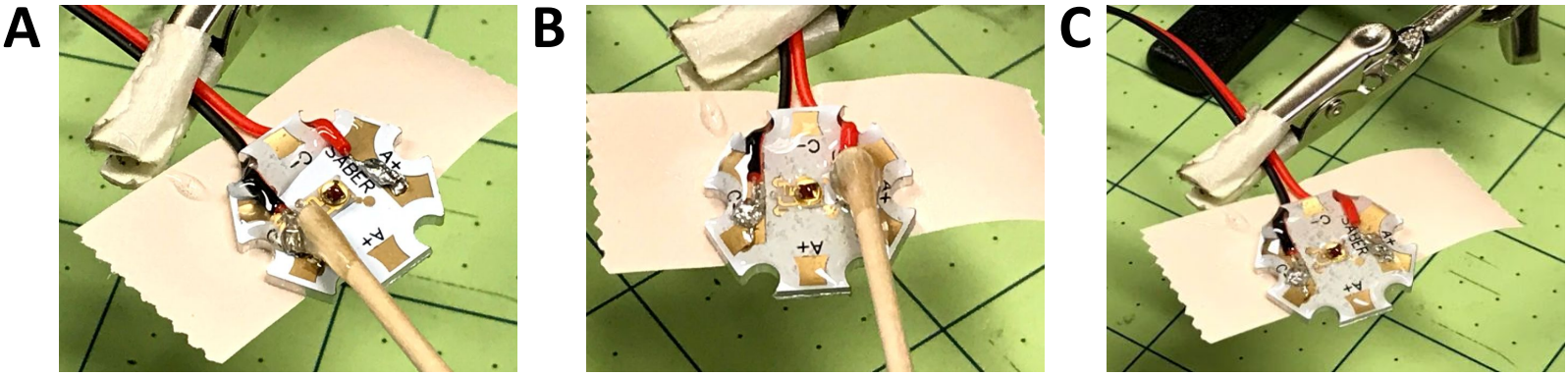


Figure S22: Securing the wires and LEDs to the LED base using epoxy. (A) Using wooden applicator to place epoxy into the LED base. Tape is placed below to catch any dripping epoxy. (B) Epoxy is spread evenly over the entire surface. (C) The LED is left overnight to cure.

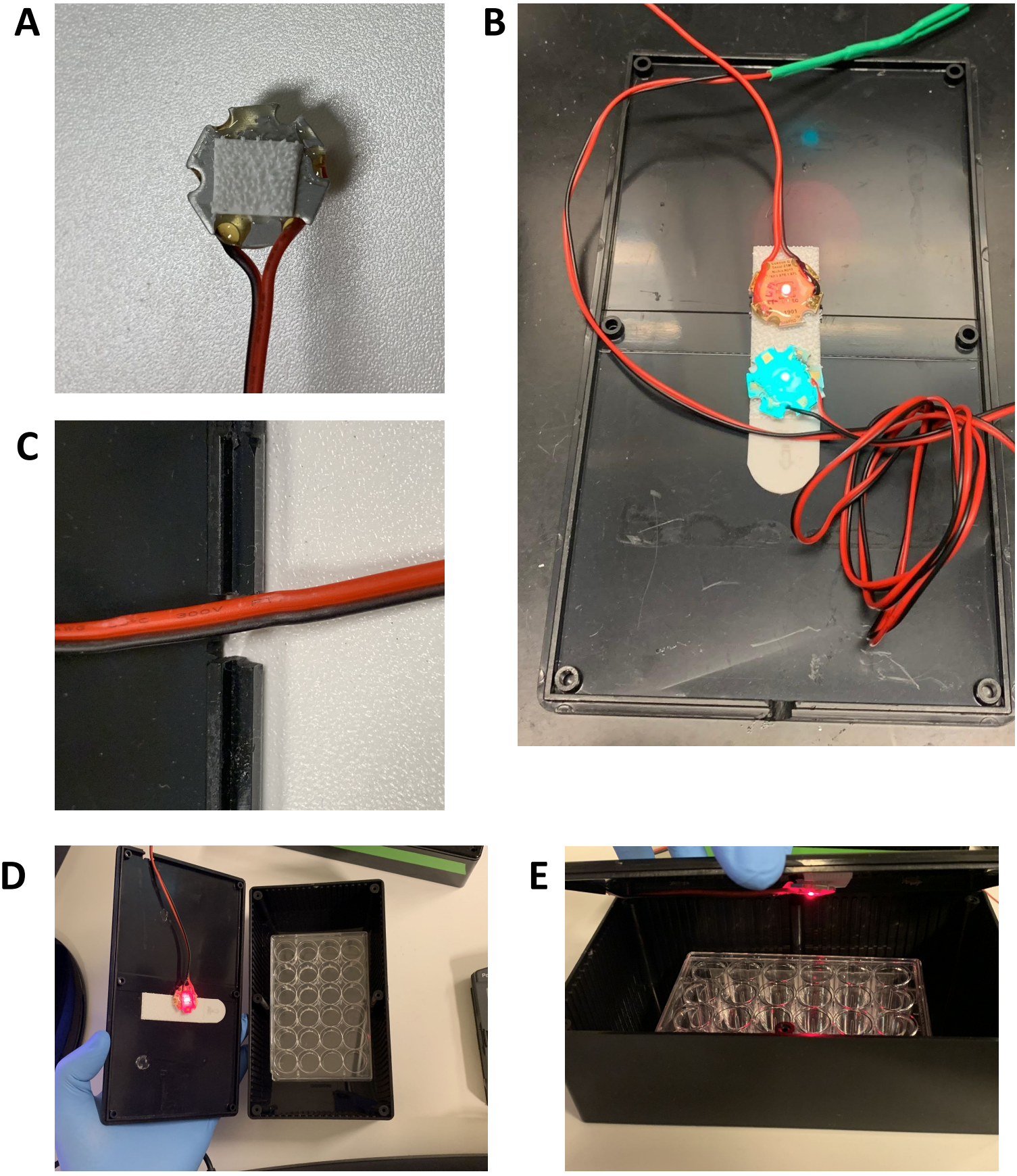


Figure S23: Mounting LEDs inside a box lid. (A) An LED with a command strip piece attached for easy mounting. (B) Different color LEDs mounted on the inside of a black box using a command strip. (C) A notch on the lid of the black box made by a Dremel to make room for the LED wire. (D) A black box for stimulating the cells with touch fasteners for mounting the LED. (E) Placement of a multiwell dish inside of the touch fastener version of the LED box.

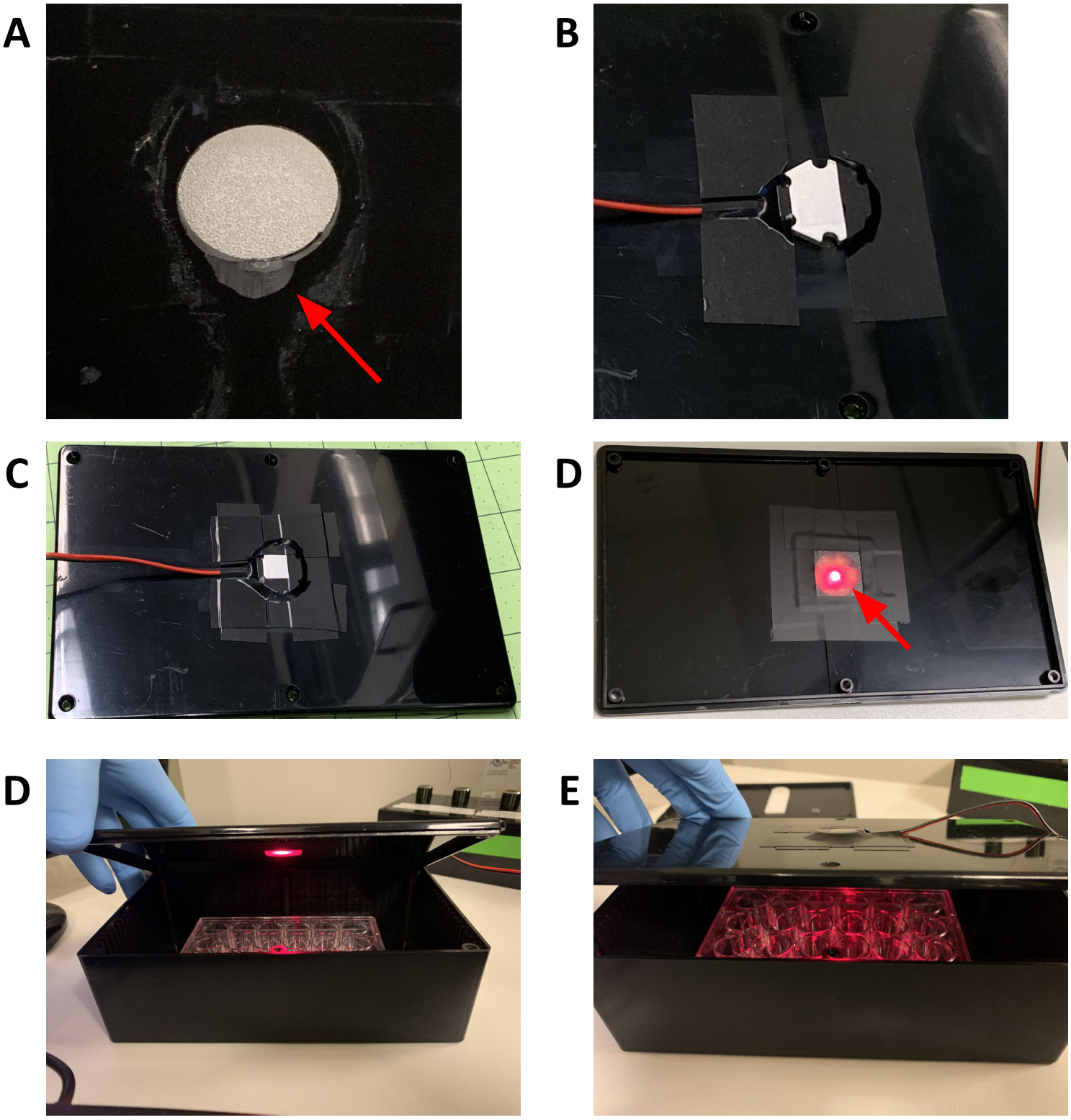


Figure S24: Mounting LEDs outside a box lid. (A) Hole drilled into the lid of the black box with a notch from the Dremel to make room for the wire (red arrow). (B) LED placed into the hole with the wire in the notch, held in place with electrical tape. (C) Two more pieces of tape are used to secure the LED. The backside of the heat sink is exposed to maximize heat exchange. (D) Privacy film taped over the hole where the LED will be placed. The red arrow points to the privacy film. (E) A black box for stimulating the cells with an LED mounted outside the box and with privacy film for diffusing the illumination. (F) Placement of a multiwell dish inside of the external LED+privacy film version of the LED box.

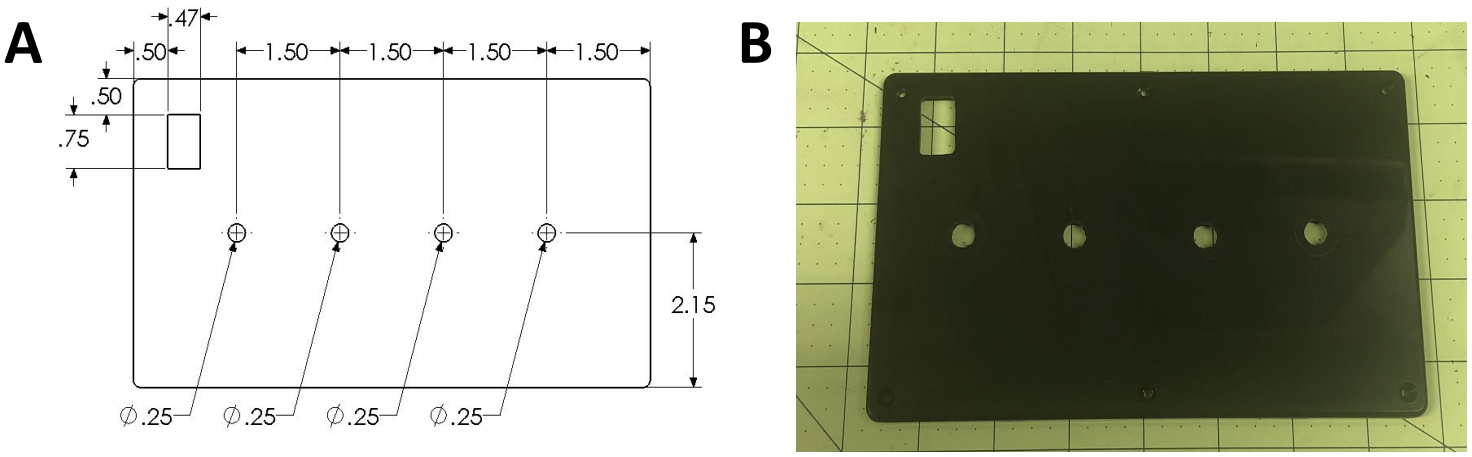


Figure S25: Drilling holes on the box lid for the power switch and potentiometers. (A) A CAD drawing with annotated dimensions of the box lid. (B) The box lid with the potentiometer and power switch holes.

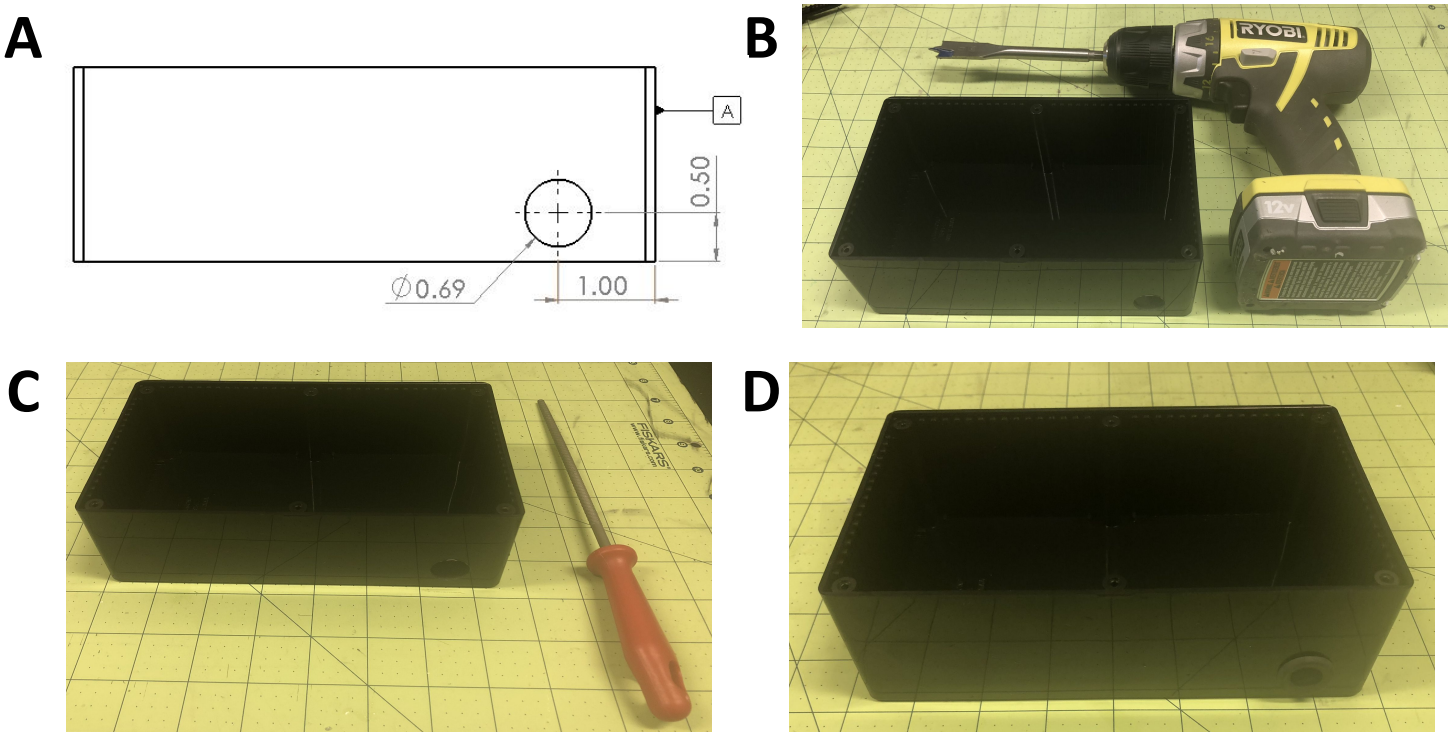
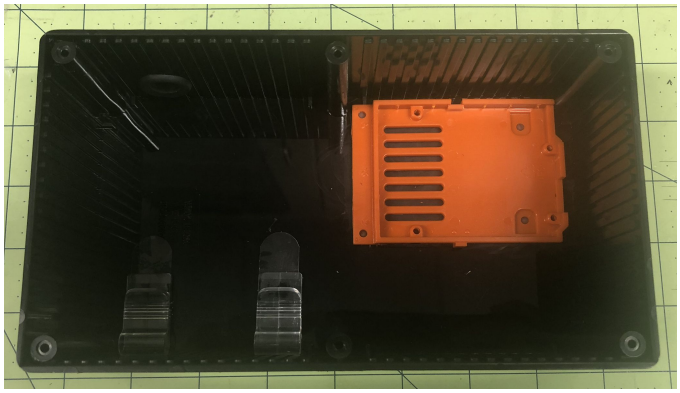


Figure S26: Preparing the wire outlet hole. (A) A CAD drawing with annotated dimensions. (B) Image of the drilled hole with the drill bit. (C) Smoothing of outlet hole with Dremel or filing tool. (D) Placing grommet in outlet hole.

A



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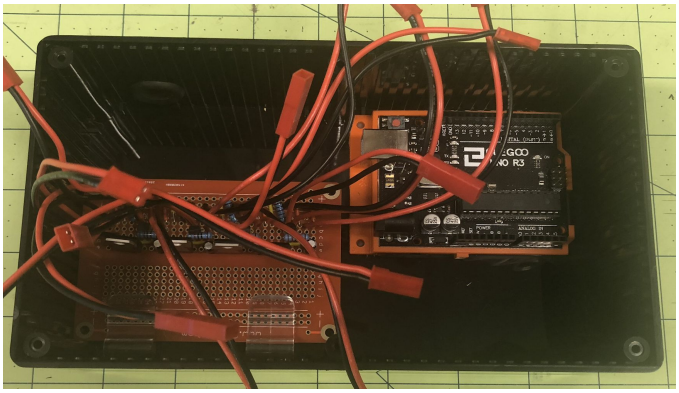


Figure S27: Placement of the microcontroller and the PCB in the box. (A) The microcontroller holder (orange) and PCB holders inside the box. (B) The microcontroller and PCB secured into the box.

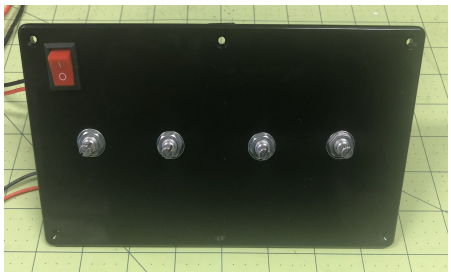
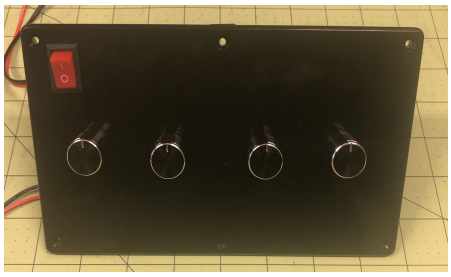
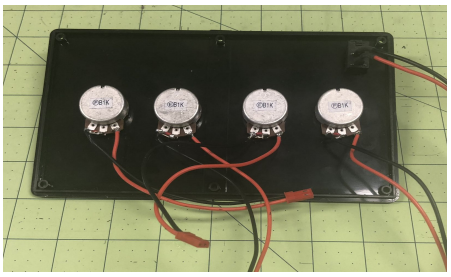
A  B  C 

Figure S28: Placement of the potentiometers and the power switch. (A) A front view of a box lid with a power switch and four POTs. (B) A front view of box lid with potentiometer knobs added. (C) A rear view of box lid with attached components.

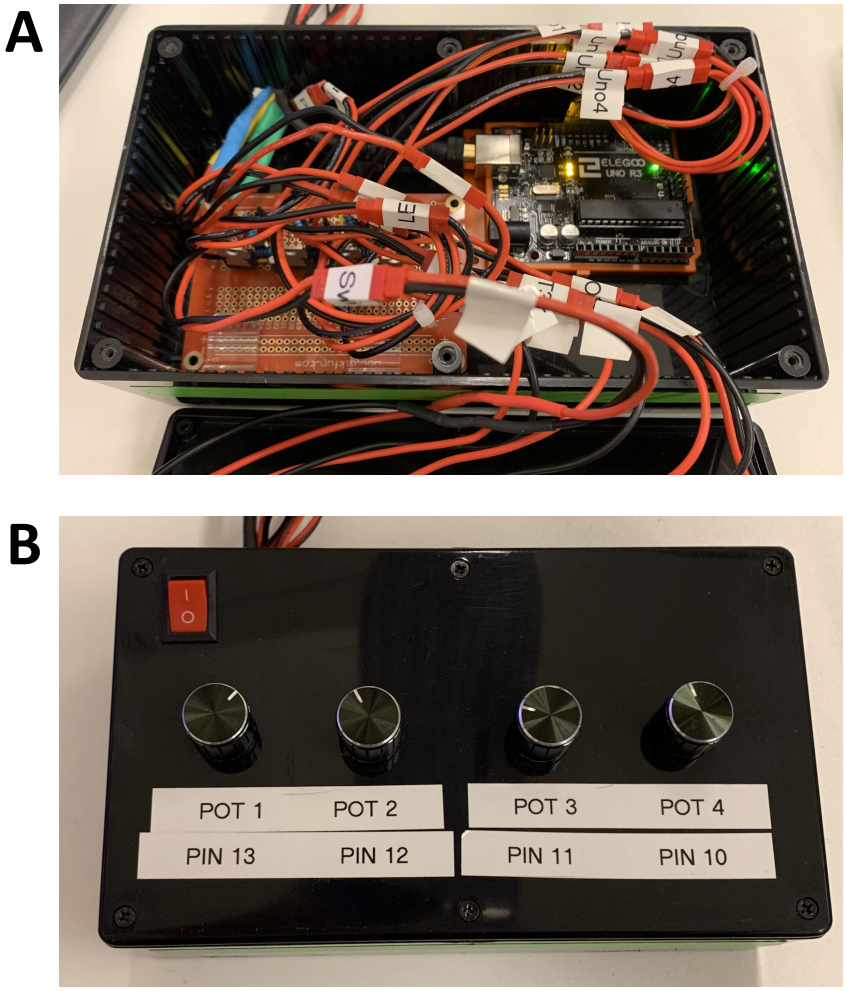
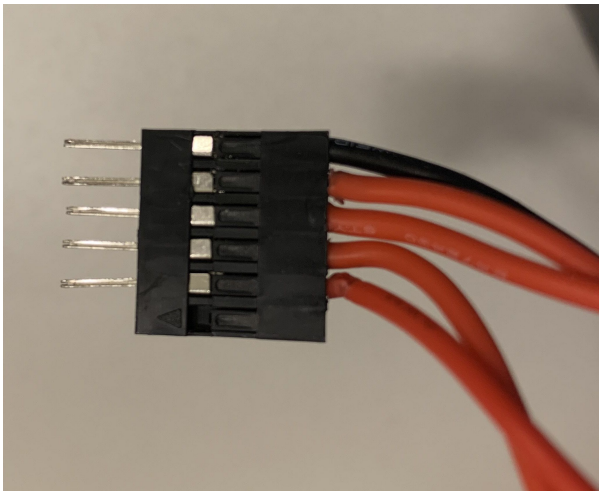


Figure S29: The assembled LED control system. (A) An open control box with the wires labeled with a label printer and zip tied for organization. (B) The box once fully assembled with each POT labeled along with the PIN it is connected to.

A



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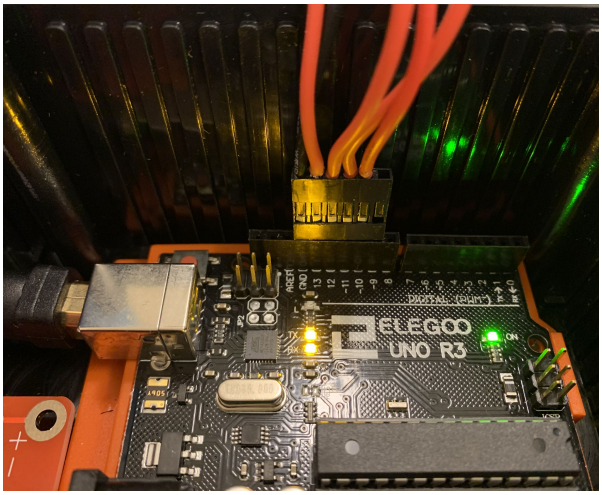

Figure S30: Placement of the crimped wire-to-wire connector. (A) Picture of the crimped wire-to-wire connectors for a four LED-microcontroller system. (B) Placement of the crimped connector into the microcontroller ports.

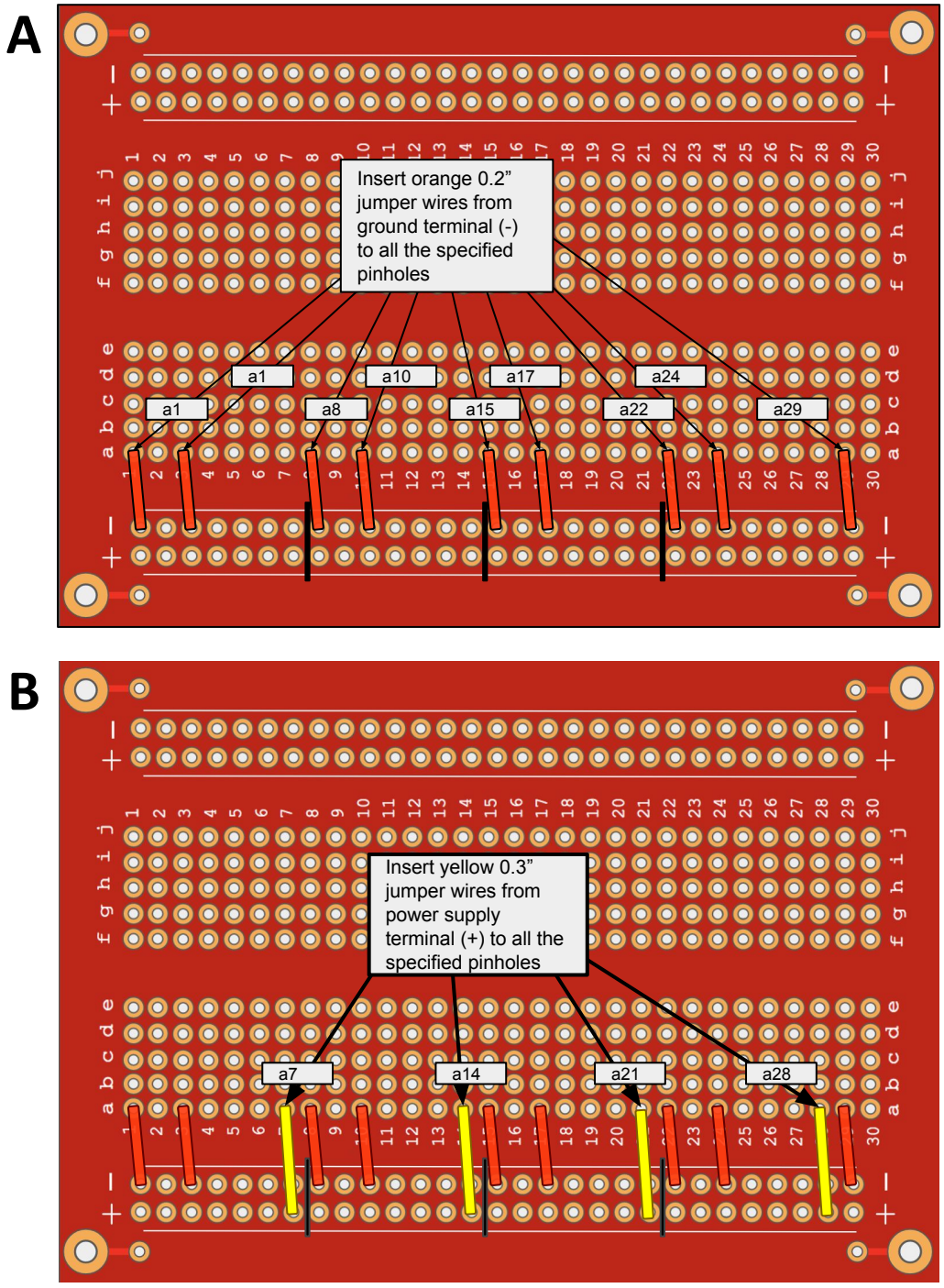

Figure S31: Placing the jumper wires. (A) A circuit board with the coordinates of the red jumper wires labeled. (B) A circuit board with the coordinates of the yellow jumper wires labeled.

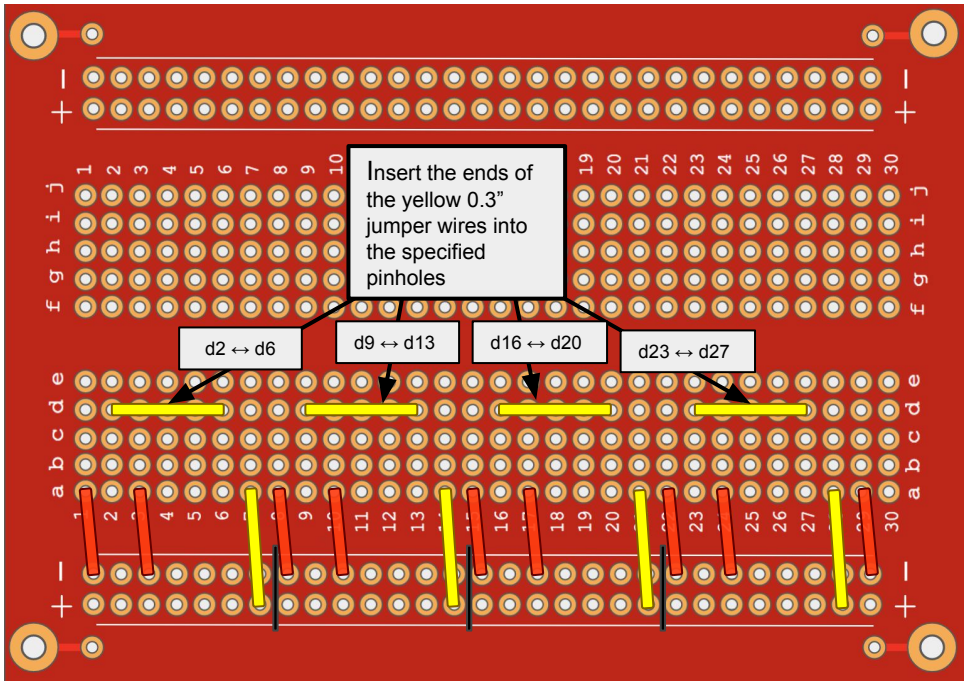

Figure S32: Placing the jumper wires. A circuit board displaying coordinates of the yellow jumper wires.

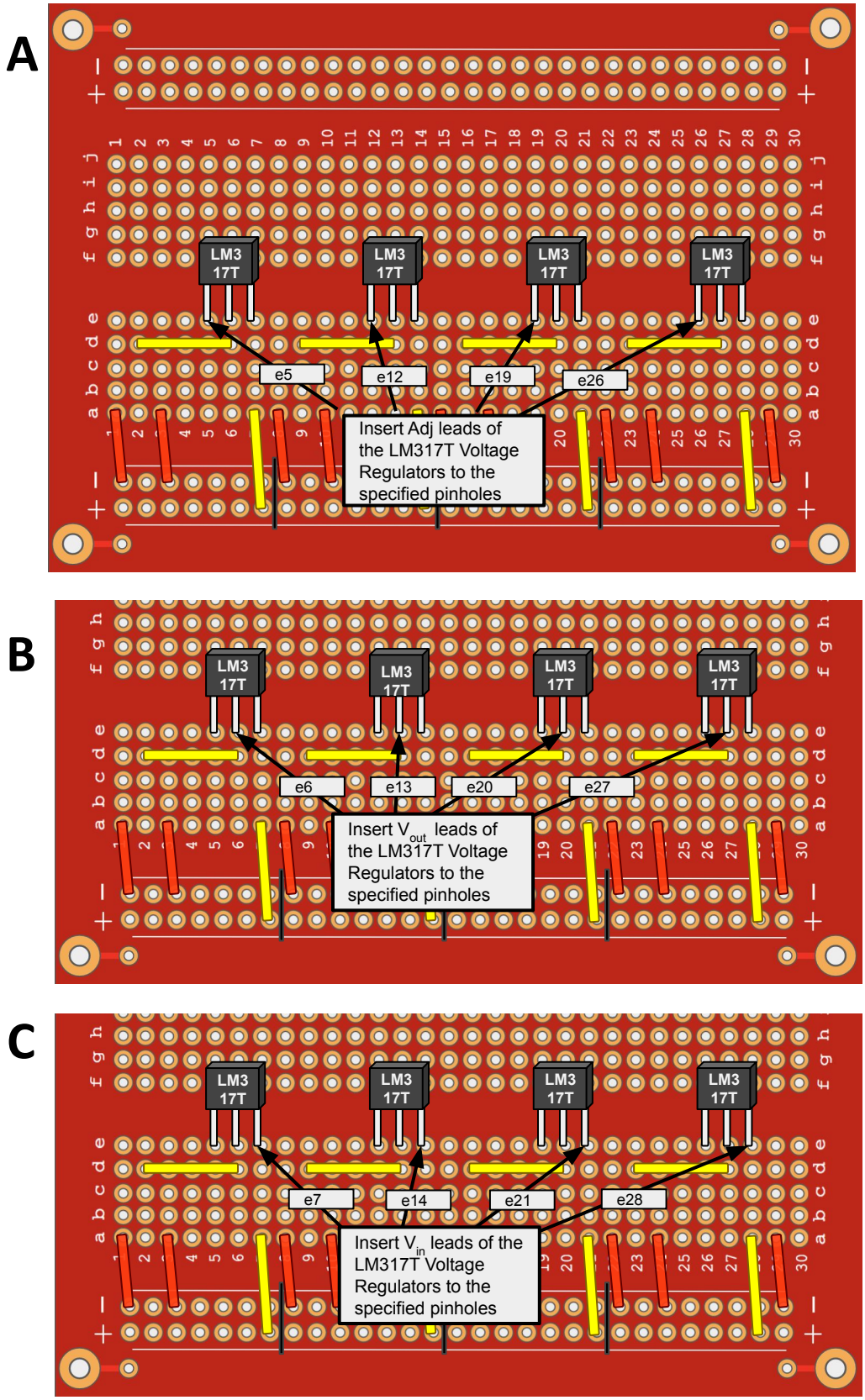

Figure S33: Adding the voltage regulators. The LM317T voltage regulators are added to the circuit with their coordinates labeled in the diagrams.

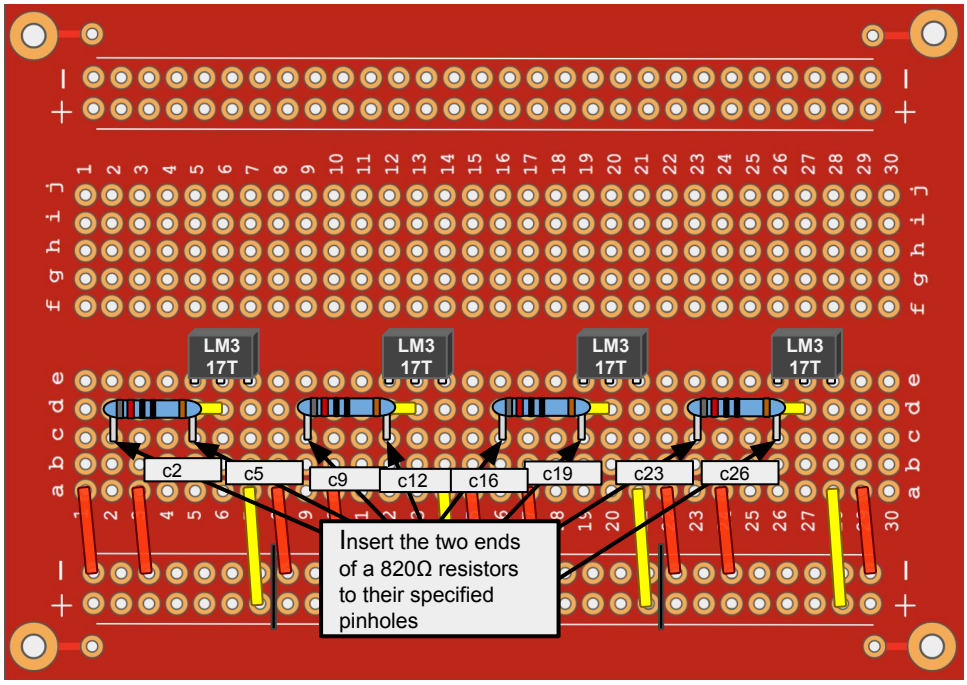

Figure S34: Inserting the 820Ω resistors. The R1 resistors are added to the circuit with their coordinates labeled in the diagrams

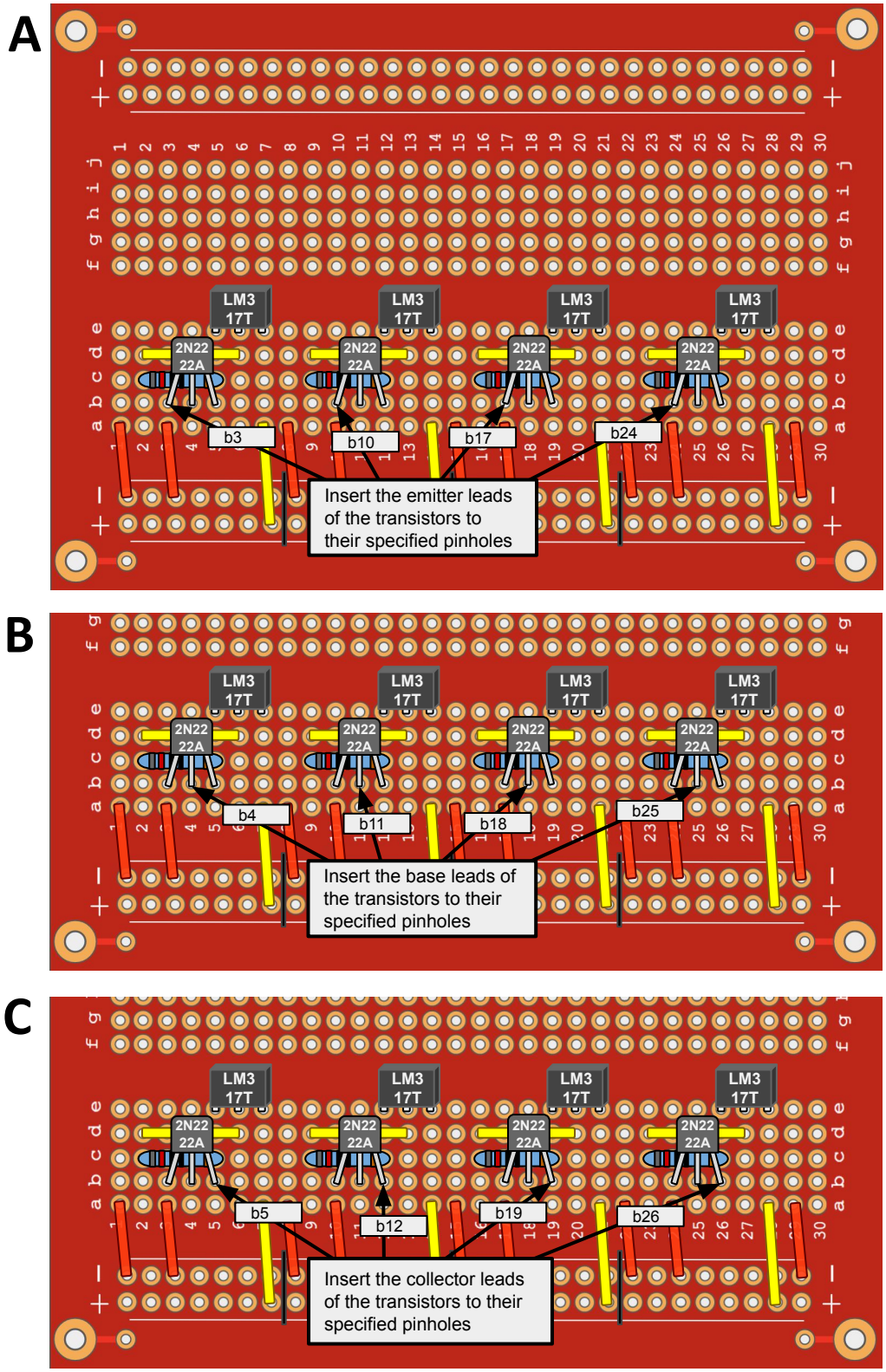

Figure S35: Inserting the transistors. The 2N2222A transistors are added to the circuit with their coordinates labeled in the diagrams

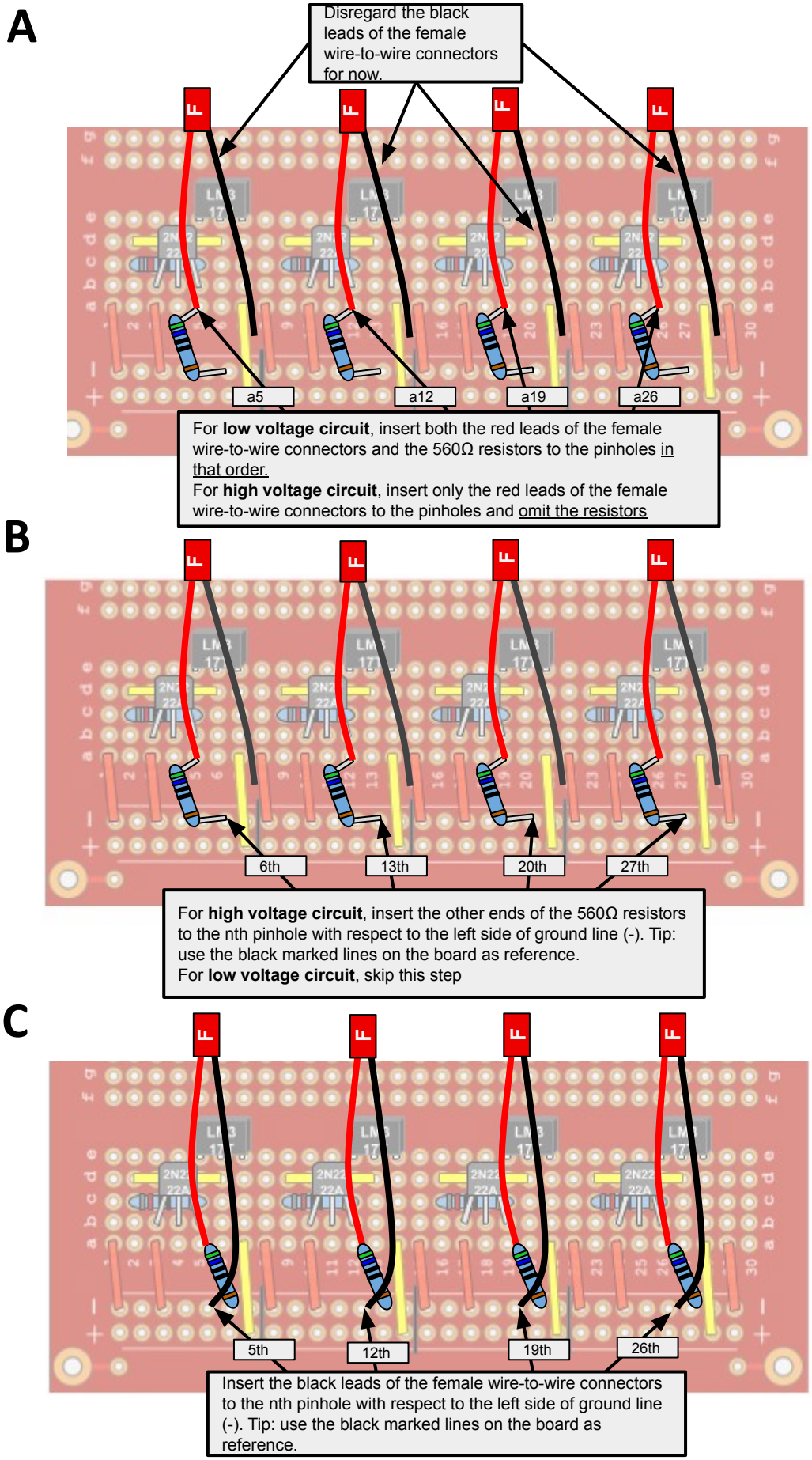

Figure S36: Inserting the Female wire-to-wire connectors and Resistors (optional) for the POT connection. The wires and resistor are added to the circuit with their coordinates labeled in the diagrams. (A) Insert the red wire, followed by the R2 resistor (560Ω) (for the low voltage circuit only). (B) Insert the other end of the resistor into the indicated ground hole. (C) Insert the black wires into the indicated holes to connect to ground. Note: R2 (560Ω) is in parallel with the potentiometer.

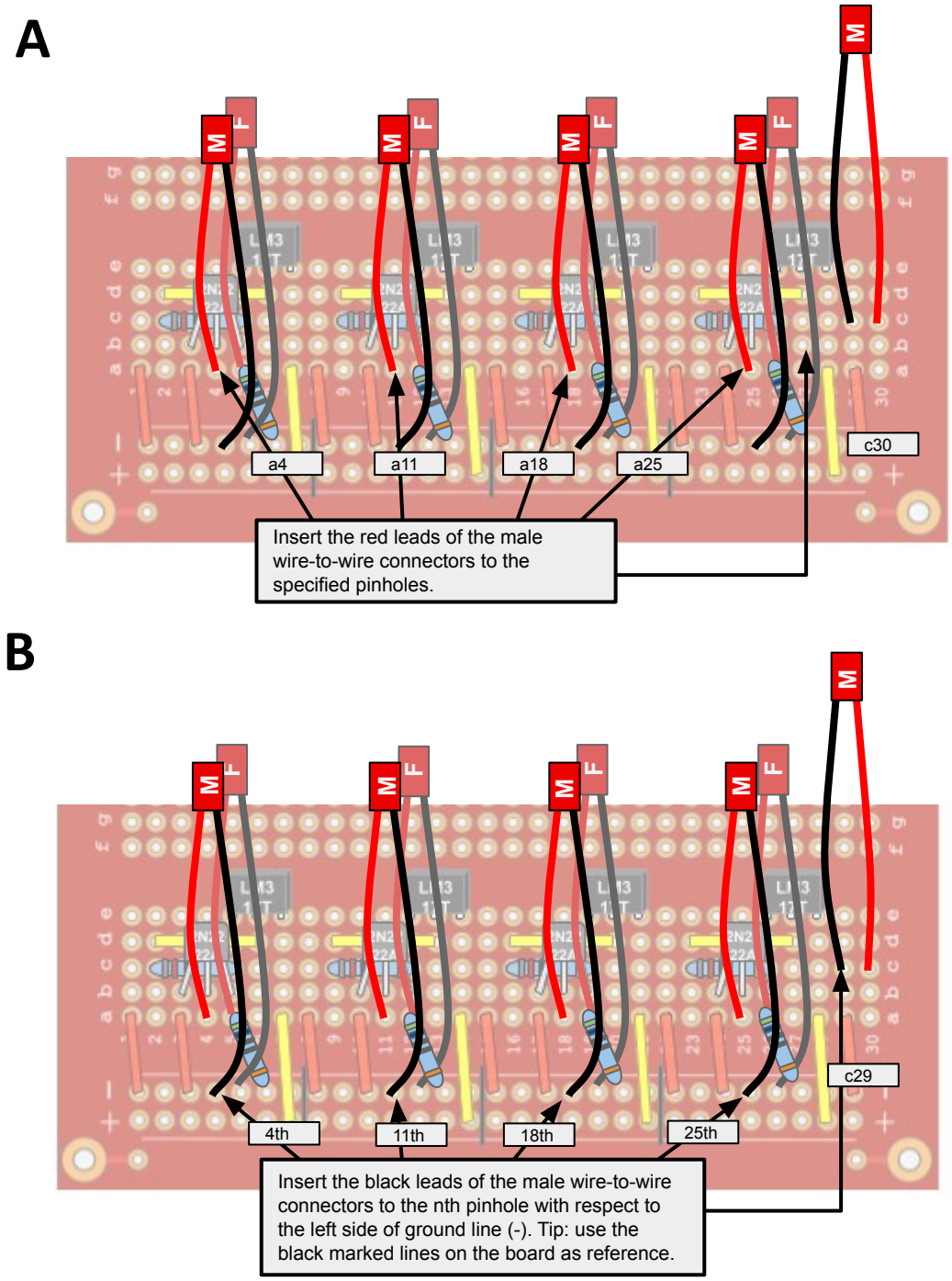

Figure S37: Inserting male wire-to-wire connectors for the microcontroller connection and power supply. The wires are added to the circuit with their coordinates labeled in the diagrams. (A) Insert the red wires into the indicated holes. (B) Insert the black wires into the indicated holes.

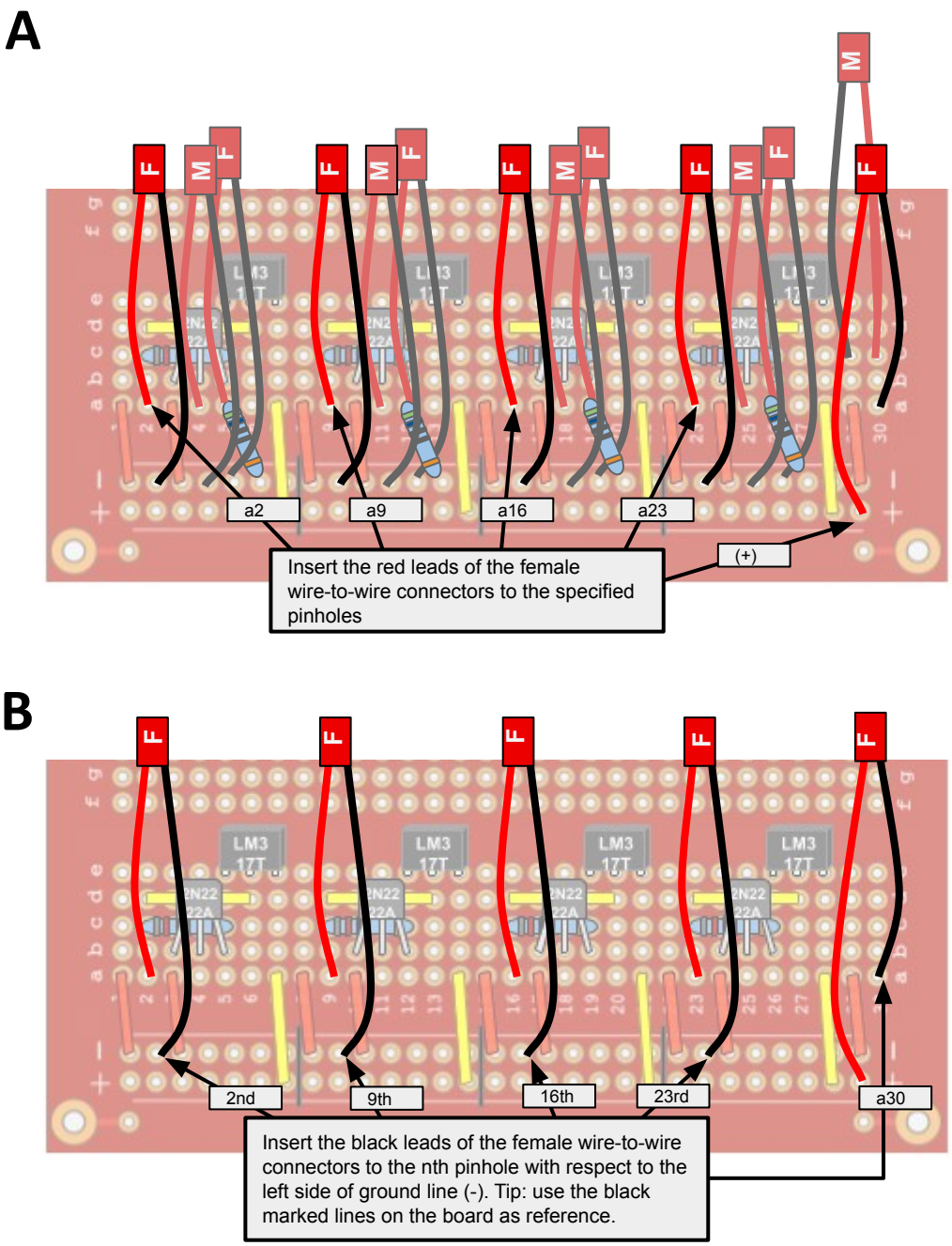

Figure S38: Adding LED wire-to-wire connectors. (A) Female wire-to-wire connectors with the red lead coordinates highlighted. (B) Female wire-to-wire connector with the black lead coordinates highlighted.

A

Do this first:						
Wells using Master mix (MM)		20				
Master Mix DNA	Conc.	% of MM	DNA Mass	DNA Vol/well	DNA Vol all wells	10% extra
pPKm-121	723	4.0000	20	0.028	0.553	0.609
	Total			0.028	0.553	0.609
			MM Optimem	Optimem/well	Optimem all wells	
			MM Optimem	19.972	199.723	219.696
				Vol/ condition		20
Aliquot the mastermix:		Volume per condition (ul)	20			

B

							# of wells	2.2
A	Condition	Plasmid	Construct	DNA ratio	DNA mass	Conc. ng/ul	Vol/well (ul)	FV
	3:1 Phy:PCB	Master Mix DNA		4	20.0			20.00
		pPK-351	A-CMV-PIF3-MTAD-IRES-PhyB-G4	66	330	422.4	0.78	1.72
		pPK-352	DNA-CMV-PCYA-IRES-HO1-FD-FN	22	110.0	234.4	0.47	1.03
		pPKm-202	UAS-Luciferase	8	40.0	92.69	0.43	0.95
			Total	100			Optimem	3.80
							# of wells	2.2
	Condition	Plasmid	Construct	DNA ratio	DNA mass	Conc. ng/ul	Vol/well (ul)	FV
B	Leaky	Master Mix DNA		4	20.0			20.00
		pPK-351	A-CMV-PIF3-MTAD-IRES-PhyB-G4	66	330.0	422.4	0.78	1.72
		pPK-178	ABE-EGFP-P2A-mito-his-sfGFP-Pu	22	110.0	106.1	1.04	2.28
		pPKm-202	UAS-Luciferase	8	40.0	92.69	0.43	0.95
			Total	100			Optimem	2.55
							# of wells	2.2
	Condition	Plasmid	Construct	DNA ratio	DNA mass	Conc. ng/ul	Vol/well (ul)	FV
C	3:1 Phy:PCB	Master Mix DNA		4	20.0			20.00
		pPK-351	A-CMV-PIF3-MTAD-IRES-PhyB-G4	66	330.0	422.4	0.78	1.72
		pPK-352	DNA-CMV-PCYA-IRES-HO1-FD-FN	22	110.0	234.4	0.47	1.03
		pPK-178	ABE-EGFP-P2A-mito-his-sfGFP-Pu	4	20.0	106.1	0.19	0.41
		pPKm-202	UAS-Luciferase	4	20.0	92.69	0.22	0.47
			Total	100			Optimem	3.86
							# of wells	2.2
	Condition	Plasmid	Construct	DNA ratio	DNA mass	Conc. ng/ul	Vol/well (ul)	FV
D	Leaky	Master Mix DNA		4	20.0			20.00
		pPK-351	A-CMV-PIF3-MTAD-IRES-PhyB-G4	66	330.0	422.4	0.78	1.72
		pPK-178	ABE-EGFP-P2A-mito-his-sfGFP-Pu	26	130.0	106.1	1.23	2.70
		pPKm-202	UAS-Luciferase	4	20.0	92.69	0.22	0.47
			Total	100			Optimem	2.61

C

Calculations	Volume *per* well	Volume for *all* wells
PEI	1.5	30
Optimem	11	220
Number of wells	20	
Make this	20% extra	Volume to add to each master mix
PEI	36	27.5
Optimem	264	
Volume (ul) of master mix per w	25	<---- Add this to each cell culture well

D

Figure S39: Setting up a PhyB-PIF3 gene switch experiment. (A) An example table of a mastermix containing Renilla for the internal control. (B) An example table for setting up the DNA mixture for a Dual Luciferase Reporter Assay of a PhyB-PIF3 optogenetic experiment. (C) An example table for setting up PEI transfection reagent and aliquoting the mixture onto cells (dropwise). (D) Placement of the light meter for setting the LED brightness.