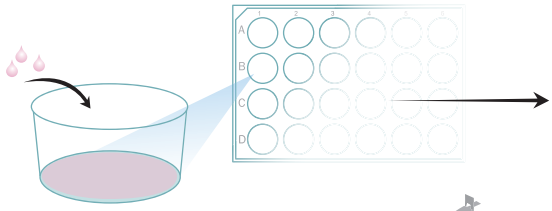
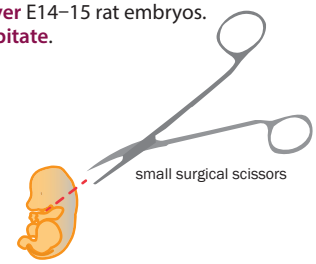
Day 1

Prepare cell culture plates by coating with Poly-D-Lysine.



Day 2

Recover E14–15 rat embryos. **Decapitate**.



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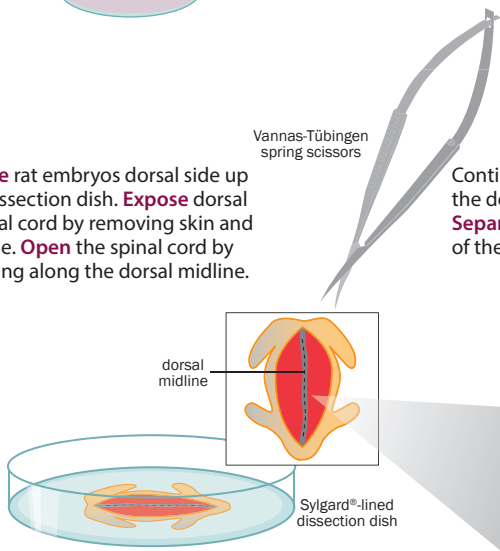
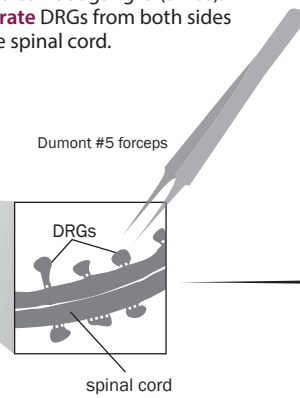
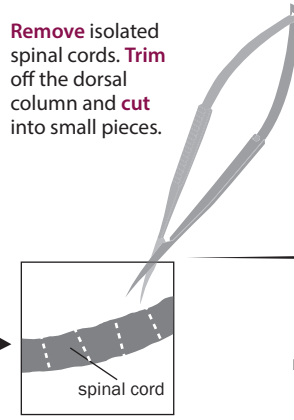
Place rat embryos dorsal side up in dissection dish. **Expose** dorsal spinal cord by removing skin and tissue. **Open** the spinal cord by cutting along the dorsal midline.



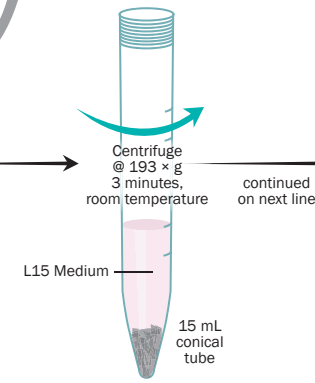
Continue to **remove** tissue to expose the dorsal root ganglia (DRGs). **Separate** DRGs from both sides of the spinal cord.



Remove isolated spinal cords. **Trim** off the dorsal column and **cut** into small pieces.



Transfer dissected spinal cord tissue. **Centrifuge**. **Decant** supernatant.



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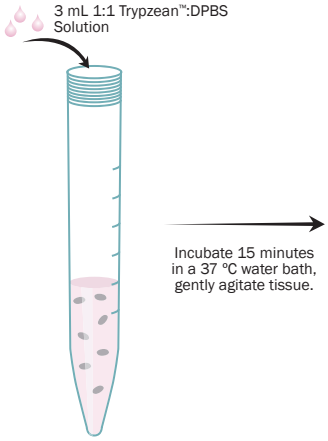
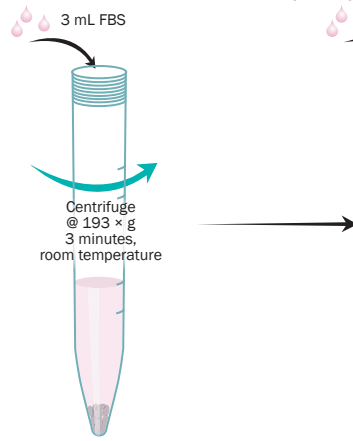
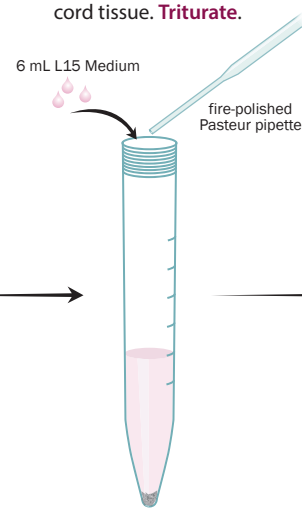
Digest the spinal cord tissue.



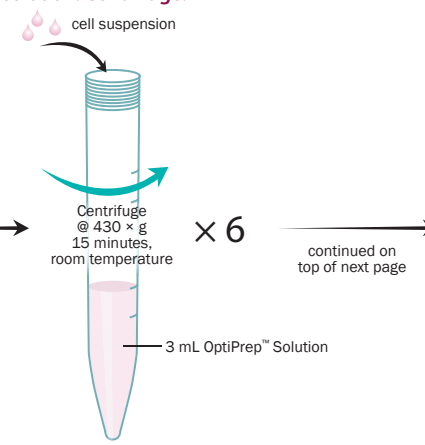
Stop the tissue digestion. **Centrifuge**. **Decant** supernatant.



Resuspend the spinal cord tissue. **Triturate**.



Divide the homogenized solution among 6 tubes containing the 9% OptiPrep™ solution. **Centrifuge**.



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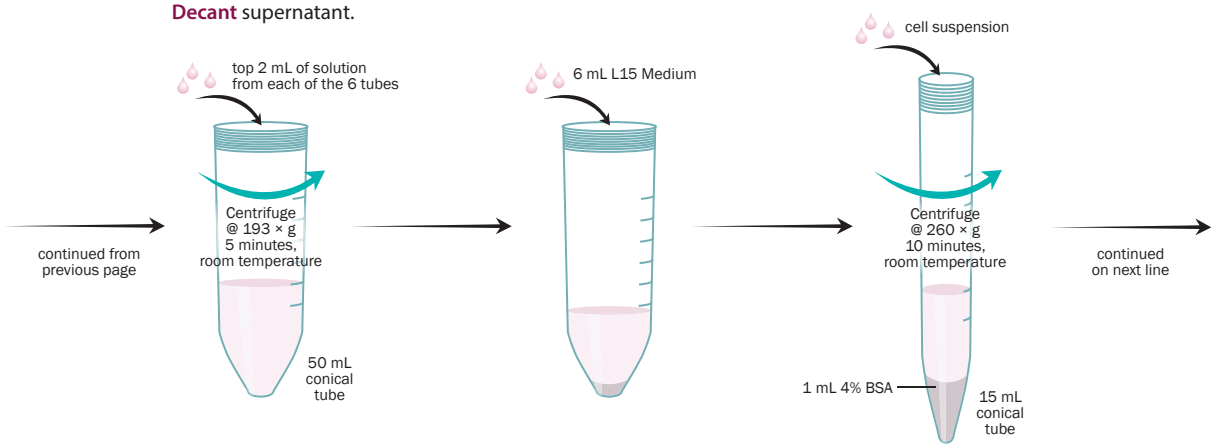
Transfer the top 2 mL of solution from each tube to one 50 mL conical tube. **Centrifuge**. **Decant** supernatant.

Resuspend the spinal motor neurons.

Transfer the cell suspension to a new 15 mL conical tube, layering it on top of the 4% BSA solution. **Centrifuge**. **Decant** supernatant.



Resuspend the spinal motor neurons in 250–500 µL of culture medium. **Count** cells.

Seed neurons onto Poly-D-Lysine-coated cell culture plates.

Add culture media to each well of the plate. **Culture** spinal motor neurons for desired amount of time. **Exchange** media every 3–4 days.