**Protocol: OPC isolation**

**In advance (or during tissue dissociation) coat flasks with Poly-D-Lysine (PDL) or Poly-L-Lysine (PLL)**

* Coat flat bottomed 75cm flasks, with filter caps
* Stock concentration of PDL/PLL = 1 mg/ml 🡪 aliquot and store in freezer
	+ Use 50 µl from stock and dilute in 30ml sterile MQ
* Add enough to cover bottom of flask (6-10 ml) and place for at least 1 hour in incubator
* Wash once with water before use (it is not necessary to wait for the flasks to dry)

**Dissection (P0 pups preferentially)**

* Work in dissection room, wipe area with alcohol and sterilize instruments
* Required material
	+ Dissection material (scissors, pincers,..)
	+ Petridish for decapitation
	+ Petridish for brain isolation
	+ Petridish filled with DMEM placed under the microscope
	+ 15ml tube with 1 ml DMEM/2 mice brains 🡪 place on ice
* Decapitate animals and spray head with ethanol
* Remove the ears and pull skin over skull (until level of the eyes)
* Place one tip of scissors just under the skull, cut up from the back of the neck to the eyes (either side), avoid cutting into the brain
* Peel back skull and scoop out brain using small curved pincers and transfer to dish with cold DMEM
* With fine forceps, diagonally pinch the cerebral hemisphere away from the hindbrain/midbrain to collect the cortices (if necessary, transfer to new dish)
* Under dissection microscope, remove meninges with fine forceps (tip: start at hippocampus)
* Transfer cortices to 15ml tube on ice





**Papain solution for dissociating tissue**

* Storage papain (20 Units/ml) in -80°C 🡪 when dissecting last brain, place papain in warm water bath (stored in aliquots of 1ml, 1 ml is enough for up to 8 brains)
* Add DNase to papain solution (final concentration of DNase = 40 µg/ml)
* Place papain – DNase solution in incubator until needed
* Remove medium from cortices up to +/- 1ml on top of cortices
* Homogenise cortices by resuspending with P1000
* Centrifuge homogenised cortices for 5 min @ 300g
* Remove supernatant from pellet
* Add papain – DNase solution and place in warm water bath for 30 minutes (shake every 10 min)
* Inactivate digestion by adding 9 ml cold DMEM
* Centrifuge 5 min @ 300g
* Carefully remove most of supernatant, and resuspend in 2-5ml culture media (depending on number of brains)
* Triturate (gently!) a few times with a 21g needle
* Add 10 ml media
* Dilute cells as necessary to divide evenly between PDL-coated flasks (need to be 2 brains/flask)
* Replace medium at day 4, day 7 and day 11
	+ Starting from day 7, add 5µg/ml insulin

**Shake-off for OPC enrichment**

* At day 14, the separation can be done of OPCs from monolayer of astrocytes
* Tighten cap of flask and tape shut with parafilm
* Agitate on 37°C heated orbital shaker for 45 min @ 75 rpm
* Aspirate and discard medium and add 10 ml fresh culture medium. This step removes the lossely adherent microglia
* Return flasks to shaker and continue shaking overnight (aim for 16-18h) @ 280 rpm
* Coat plates/glasses/wells with PDL/PLL where your experiments will be conducted in
* Remove medium next morning and place them on plastic petridish
* Incubate for 25-30 minutes and shake after 10-15 min. This step removes remaining microglia- they attach to plastic quickly, OPCs do not. Do not leave longer as OPCs will stick to plate and reduce yield in solution
* Collect media from dishes and pellet cells by centrifuging 5 min @ 300g
* Resuspend in appropriate culture media (proliferation or differentiation media)
* Triturate (gently, avoid bubbles) cells to break apart clumps with a 21g needle, followed by a 23g needle if necessary – it is important to have single cells for plating!
* Count cells
* Cells prefer high density for survival but too high and they differentiate prematurely and will not make myelin processes/membrane sheets
	+ ICC: 150.000 cells/well – 24 well plate (coverslips)
	+ qPCR: 250.000 cells/well – 24 well plate
	+ WB: 500.000 cells/well – 6 well plate

**Media & reagents**

* Culture media
	+ DMEM, high glucose (Life Tech 41966-029)
	+ 1% Pen/strep
	+ 10% FCS
	+ (5µg/ml insulin )
* OPC proliferation medium (prevents differentiation: Sato + growth factors) (also ‘resets’ all cells to same starting phase)
	+ 50 ml SATO **without B27**
	+ 10 ng/ml PDGF (1000x, peprotech 100-13A-100µg)
	+ 10 ng/ml FGF2/bFGF (1000x peprotoch 100-18B-250µg)
* Add growth factors (PDGF & FGF2 fresh, just before use)
* **Need to add fresh growth factors daily (change media). Only keep cells for a few days in this media before using cells or switching to differentiation media (2-3 days)**
* OPC differentiation medium (Sato - growth factors)
	+ 50 ml SATO
* **After 6-8 days, cells are differentiated in this media, don’t keep cells longer in here**
* SATO
	+ DMEM, high glucose 48 ml
	+ Transferinne 120µl
	+ Putrescine 50µl
	+ Insuline 25µl
	+ Progesterone 50µl
	+ Sodium Selenite 37µl
	+ TIT 50µl
	+ L-Thyroxine 52µl
	+ Pen/strep 250µl
	+ Heat inactivated horse serum 1ml
	+ B27 (growth factor) 1ml
* **Media needs to be filtered afterwards (0.22µm)**