Cultivating Cerebral Organoids

This is intended as a guide only; for full experimental details please read the reference provided.

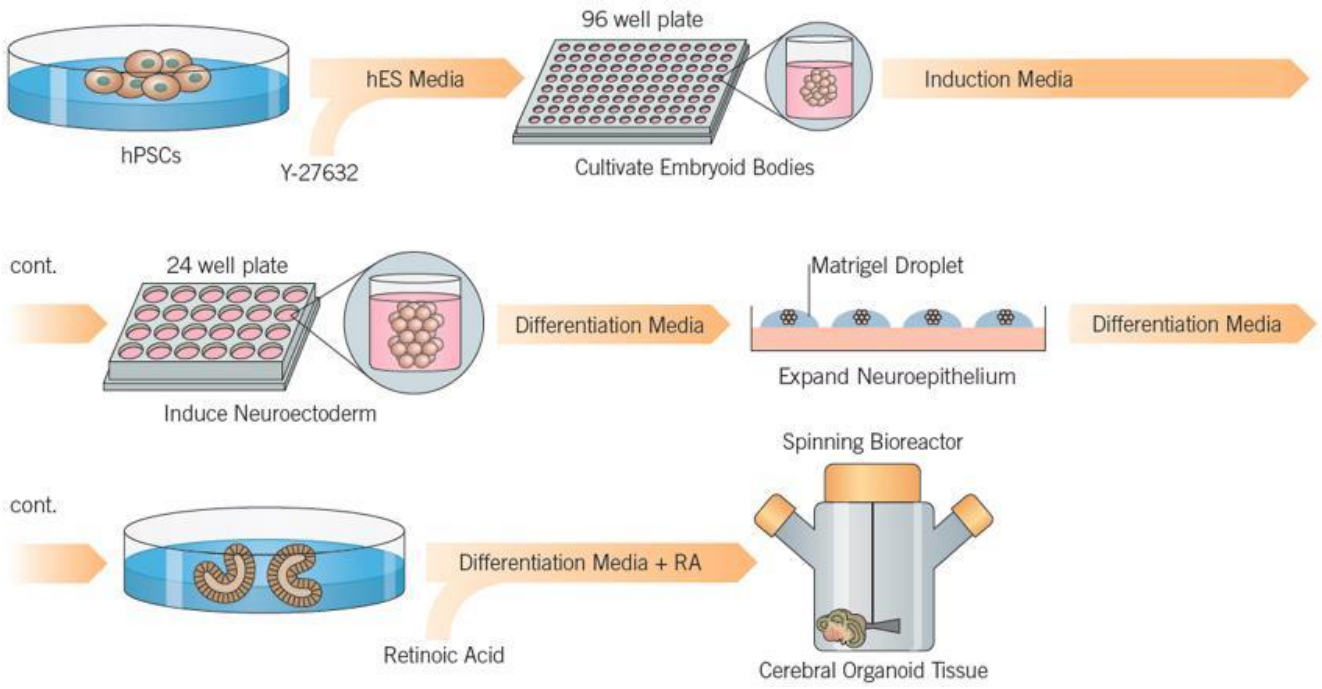
In Brief

Lancaster *et al.* describe a protocol to generate cerebral organoids from H9 ES cells.

On Day 0 ES cells or iPSCs were dissociated from mouse embryonic stem cells (MEFs) and separated to make single cells. Cells were then plated in 96-well plates in human ES media containing 50 µM Y-27632. Embryoid bodies were fed every other day for 6 days.

On Day 6 cells are transferred to 24 well plates and grown in induction media. They are fed every other day for 5 further days.

On Day 11 tissue is transferred to droplet Matrigels containing differentiation media. After 4 days of stationary growth the tissue droplets are moved to a spinning bioreactor, and grown in differentiation media containing retinoic acid (RA).



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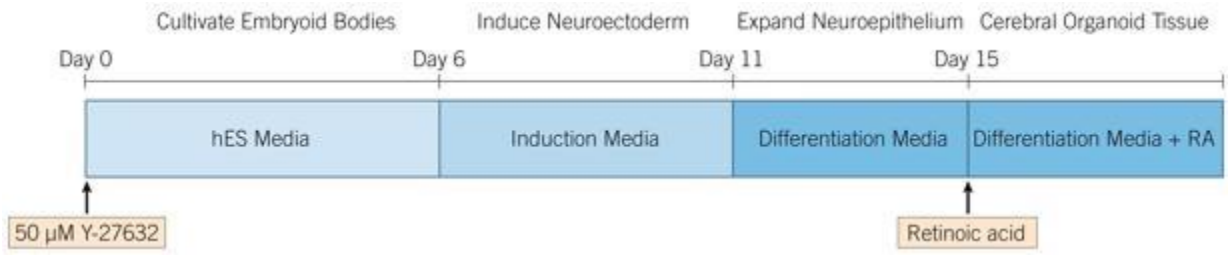
Cocktails

ES Media		Neural Induction Media		Differentiation Media		Differentiation Media + RA	
FGF	4 ng ml^{-1}	DMEM:F12 medium		1:1 DMEM/F12 and neurobasal containing N2 supplement (1:200)		Same as differentiation +	
Y-27632 (Cat.No. 1254)	50 µM	N2 Supplement	1:100	B 27 supplement w/o vitamin A	1:100	B 27 supplement with vitamin A	1:100
		Glutamax		2 Mercapotoethanol	3.5 $µl^{-1}$	Retinoic Acid (Cat.No. 0695)	
		MEM-NEAA		Insulin	1:4000;		
		Heparin	1 mg/ml	Glutamax	1:100		
				MEM-NEAA			



Reference

Lancaster *et al.* (2013) Cerebral organoids model human brain development and microcephaly. Nature **501** 373 PMID: 23995685