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Human Primary Astrocyte (hPA) CRISPRa reprogramming protocol

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol describes methods for CRISPRa reprogramming of human primary astrocytes.

Materials

	A	B	C
	AGM	**unit**	**500 mL**
	AGM (Sciencell 1801)	mL	remove 60ml from bottle
	FBS	mL	50 mL
	AGM supplement (Sciencell 1801 supp)	mL	5 mL
	P/S (Sciencell 1801 supp)	mL	5 mL

	A	B	C	D	E
	NDM (Guo 2014)	**unit**	**100 mL**	**250 mL**	**500 mL**
	DMEM/F12 (GIBCO)	mL	97.5	243.75	487.5
	0.5% FBS (GIBCO)	mL	0.5	1.25	2.5
	3.5 mM glucose (Sigma)*	mg	63.056	157.64	315.28
	penicillin/streptomycin (GIBCO)	mL	1	2.5	5
	N2 supplement (GIBCO)	mL	1	2.5	5
	Filter sterilize after combining				

Supplements

Aliquot prep

BDNF (Peprotech 450-02-50UG)

- Dilute 50ug in 250ul H2O (final conc 200 ug/ml)
- Add 2.25ml 0.1% BSA (final: 2.5ml @ 20 ug/ml).
    - This should be 1000x of what most protocols call for (20 ng/ml)
- Make 50ul aliquots, store at -80C

NT-3 (Peprotech 450-03)

- Dilute 10ug in 100ul H2O (final conc. 100 ug/ml)
- Add 900ul sterile PBS + 0.1% BSA (final: 1ml @ 10ug/ml).
    - This should be 1000x of what most protocols call for (10 ng/ml)
    - Make 50ul aliquots, store at -80C

- To make BSA PBS
    - 0.1g BSA in 10ml PBS, filter sterilize (0.22um filter)

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	A	B	C
	Day	**Action**	**Notes**
	-1	Coat plates with PDL	PDL is stored at -20 at 100x in 400ul aliquots (dilute to 40ml with sterile H2O)
	0	Prepare plates and transduce cells	
	1	Change media (AGM)	
	2	Change media (AGM + 1ug/ml Puro)	
	3	Change media (NDM + 1ug/ml Puro)	
	4	Check cells, refresh media if there is lots of debris	I usually change media here
	5	Check cells, refresh media if there is lots of debris	I usually change media here
	6	Change media to NDM without puro	
	7	Check cells, refresh media if there is lots of debris	I usually do not change media here

	A	B	C
	8	Change media to NDM + BDNF (20ng/ml) + NT-3 (10ng/ml)	BDNF and NT3 stocks are both 1000x and are stored at -80C in 50ul aliquots
	9	Check cells, refresh media if there is lots of debris	I usually do not change media here
	10	Harvest. Proceed to sorting or RNA extraction	
	Be very gentle with all media changes and rinses		