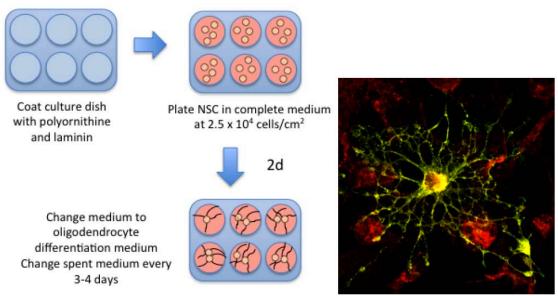
Title	Differentiating Neural Stem Cells into Oligodendrocytes	
Date Submitted	May 5, 2012	
Submitted by -	Efthymiou, Anastasia - anastasia.efthymiou@nih.gov	
Adapted from -	Gibco Protocol	
Contributors -	Efthymiou, Anastasia	
Affiliation(s) -	r) - NIH CRM - NIAMS – Laboratory of Stem Cell Biology	

! Introduction:



Oligodendrocyte stained for PLP (green) and an intracellular marker (red) - Prof. Klaus-Armin Nave, Max-Planck-Institute for Experimental Medicine

❖ Protocol:

Neural stem cells (NSCs) will proliferate as progenitors a few times even after the complete growth medium is replaced with the appropriate differentiation medium. If the cells reach 90% confluency, it might be necessary to split the cells at a 1:2 ratio. However, do not split the cells once they reach day 9-10 of differentiation when they can get damaged during the passaging process.

- 1. Plate the NSCs on a polyornithine and laminin- coated culture dish in complete StemPro NSC SFM at 2.5×10^4 or 5×10^4 cells/cm2.
- 2. After 2 days, change the medium to oligodendrocyte differentiation medium. Change the spent medium every 3 to 4 days.

❖ Materials:

polyornithine and laminin-coated culture dish
StemPro NSC SFM

oligodendrocyte differentiation medium				
StemPro NSC SFM Complete Media				
Component Fina	al concentration	Amount		
KnockOutTM D-MEM/F-12	1X	97 mL		
GlutaMAXTM-I Supplement	2 mM	1 mL		
bFGF (prep as 100 μg/mL stoo	ck) 20 ng/mL	20 μL		
EGF (prep as 100 μg/mL stock)	20 ng/mL	20 μL		
StemPro® Neural Supplement	2%	2 mL		
Oligodendrocyte Differentiation Medium				
Component Fina	l concentration	Amount		
Neurobasal® Medium	1X	97 mL		
B-27 [®] Serum-Free Supplement	2%	2 mL		
GlutaMAXTM-I Supplement	2 mM	1 mL		
T3	30 ng/mL	0.1 mL		

Troubleshooting:

***** References: