Supplemental Table 1. Observations of reagents tested during co-culture optimization

	Variables Tested	Observations	Final Selected
Matrix	Matrigel (1:50)	Cells from embryonically and postnatally sourced tissue adhere well, but thick substrate makes imaging and assessing the development of neurons difficult	Not used
	Poly-d-lysine (100µg/ml)	Cells from both sources adhere well but develop slowly	Not used
	Poly-d-lysine (100µg/ml) and Laminin (4µg/ml)	Cells adhere as well as in higher laminin concentration and develop faster than PDL alone. Develop in near mono-layer, able to image neurons easily	Selected as final matrix due to good adherence, survival, development, and imaging
Cell Source	P1-3 striatum dissected from coronal slice	Initial cell numbers are high, but cultures fail to survive to three weeks *in vitro*	Not used
	E16 "striatum" dissected as whole ganglionic eminence	This technique provides a large number of cells that survive well in culture, but also contributes more non-MSNs than the postnatal source	Despite the increase in non-MSN cells from this source, it consistently produces MSNs that can survive past three weeks in culture
Cell Density	$1*10^6$ cells/35mm dish	This density was used in attempt to overcome the failure of postnatal tissue to survive *in vitro*. In embryonically sourced tissue, this density was far too high for imaging.	Not used
	$2*10^5$ cells/35mm dish	In cultures from postnatal tissue this density was initially promising, but cultures failed to survive to three weeks in culture. In embryonically sourced tissue this density gave sufficient for good MSN survival and ease of imaging.	This density, in a 2:3 striatum to cortex ratio, produces embryonic cultures that survive past three weeks in culture with high numbers of mature MSNs
Media	Glial Conditioned Neurobasal Growth Media	Despite the presence of glia in the cultures, the conditioning of growth media on confluent glial plates improved the survival and development of cultures above unconditioned media.	All cultures are maintained in glial conditioned growth media that is supplemented weekly
Supplements	BDNF	The addition of BDNF to glial conditioned growth media did not improve survival or development of MSNs	Not used
	Anti-mitotic agents (67.5µm 5-Fluoro-2'-deoxyuridine and 137µm uridine at 3, 7, and 14div)	Although anti-mitiotic agents reduced the number of proliferating glia in the cultures they produced a pronounced loss of neurons that were associated with the glial cells. The addition of glial conditioned media did not augment this effect, indicating that the presence of glia may be helping MSNs to survive in culture	Not used
	Antibiotics	Antibiotics are not needed in cultures sourced from embryonic tissue but required to reduce contamination seen in postnatally sourced tissue.	Not used
Digestion Solution	Papain Digestion	Digested cells well, produced cells that were easily dissociated manually	Not used
	Trypsin Digestion	Digested cells well, produced cells that were easily dissociated manually	Although both digestion techniques worked well, trypsin digestion was already in routine use and was selected for future experiments
Transduction	Calcium Phosphate	Effective at transducing the cortical pyramidal neurons but failed to transduce MSNs	Not used
	Adeno-virus	Infected the high levels of glia in the culture with greater efficiency than neurons and non-MSNs with greater effciency than neurons	Not used
	Electroporation (AMAXA)	Effective in transducing ~10-20% of MSNs in mature culture when introduced at plating. Electroporation of striatal cells in culture precludes transduction of pyramidal neurons.	Only effective method at consistently transducing an appreciable number of MSNs in culture.
Coverslip Preparation	German Glass (ex. Bellco)	Improves adherence and survival after 5div	Used
	Acid and Ethanol washing	Reduces fasiculation of neurites during development. Nitric acid can also be used, but is more difficult to dispose of	Used
	Maintained in 200˚C oven	Eliminates seasonal air quality differences that impact cell adherence	Used