Determination of population doubling (PD) time, cell death and differentiation in O-2A cells by clonal analysis

- Plate purified O-2A cells at clonal density: 500-1000 cells/slide flask or 1000-2000 cells/T25 flask.
- Cells are cultured in PDGF or PDGF plus bFGF for 7 d. Duplicate or triplicate flasks are set up for each time point. Precursors and oligodendrocytes are identified by their characteristic morphologies.
- On day 7, the numbers of live, dead, and differentiated cells are counted for each clone; a total of 200 – 400 clones are randomly counted for each time point. The proportions of cell death and spontaneous differentiation are determined by dividing the number of dead or differentiated cells by the total number of cells (including live, dead, and differentiated) in each clone.
- The approximate population doubling (PD) time is determined by using the formula \( d = t/\log_2 N \), where \( d \) is the population doubling time, \( t \) is the time of cells in culture, and \( N \) is the total number of cells in the clone.

Alternatively, PD time can be determined as follows, \( 2x = \text{cell number at harvest/cell number initially plated} \). \( x \) refers to how many doublings have undergone during a total of \( y \) days (i.e., 7 days); so PD time \( d = x/7 \) (x 24 hr)