D721 Medulloblastoma Cell Line Parameters

1. Source of cells: Duke University Medical Center, surgical resection from a patient with medulloblastoma as described by S Bigner (1997). Requests for D721 cells should be directed to Darrell Bigner (bigne001@mc.duke.edu).
2. Lineage of cells: tumor-derived cancer cells. Cell origin of medulloblastoma is the neuron or a neural precursor cell
3. Donor information: 2 year old male
4. Karyotype: abnormal
5. Media for cell lines: 1X Zinc Option Media (prepared from Gibco Improved MEM Zinc Option Media 5X Concentrate, formula# 86-0194DJ). Each liter of medium is supplemented with 10 mL Hepes and 40 mL 5.5% $NaHCO_3$. Cells are grown in media supplemented with 10% FBS (Gibco Certified FBS, Cat#16000-044).
6. Culture conditions: Cells grow suspended in a flask and should be incubated at 37º in the presence of 5% CO_2. When disaggregated (i.e. during passage) and grown in ZO media D721 cells will eventually form clumps visible to the naked eye (particularly once cells reach a density of ~200K cells/mL). Cells are grown in T-75 or T-225 filter cap flasks set at an angle (i.e., resting on a sheathed 25 mL pipette, in order to increase area of gas exchange) with up to 150 or 400 mL of media, respectively.
7. Cell Line Maintenance: Cells for passage are transferred to 50 mL conical tubes and centrifuged at 1260 rpm (319 rcf) for 3 min in a standard benchtop centrifuge with swing-out rotor (both from Eppendorf). Media is aspirated and the cells are resuspended at a density of ~200K cells/mL. After 4-5 days or when the media begins to turn orange (whichever occurs first) add 1 volume of media to the cells. After another 3-4 days the cells will have formed large (4-5mm) clumps and at this point they are ready for passage (expect about 500K cells/mL).

   Cells may be frozen at a density of 10^6 cells/mL in ZO medium supplemented with 50% FBS and 20.5% DMSO. To freeze cells, pellet cells as if passaging, then resuspend in freezing medium and freeze at a rate of 1 ºC/min, then store in the vapor phase of liquid nitrogen. Frozen cells are recovered by thawing in a 37 ºC water bath, washing once with PBS or ZO medium, and resuspending in 8 mL of 20% ZO medium in a T-25 flask. Once the medium has begun to turn orange, replace with 2 volumes of 10% ZO medium and maintain as above.

8. Cell passage#: unknown

   Notes: D721 cells were selected for study due to 1) neural cell origin, 2) generally diploid karyotype as revealed by digital karyotyping, and 3) activation of oncogenic transcriptional networks (Siu et al., 2003; Di et al., 2005).

   References:

- Bigner SH, McLendon RE, Fuchs H, McKeever PE, Friedman HS. Chromosomal characteristics of childhood brain tumors. Cancer Genet Cytogenet. 1997 Sep;97(2):125-34.
- Di C, Liao S, Adamson DC, Parrett TJ, Broderick DK, Shi Q, Lengauer C, Cummins JM, Velculescu VE, Fults DW, McLendon RE, Bigner DD, Yan H. Identification of OTX2 as a medulloblastoma oncogene whose product can be targeted by all-trans retinoic acid. Cancer Res. 2005 Feb 1;65(3):919-24.
- Siu IM, Lal A, Blankenship JR, Aldosari N, Riggins GJ. c-Myc promoter activation in medulloblastoma. Cancer Res. 2003 Aug 15;63(16):4773-6.