Cell line: GCGR-NS16CT_A

Description: Human primary cell line derived from regionally dissected developing human brain tissue and expanded as an adherant monolayer

- **Specimen age (week+day of gestation)**: 16+3
- **Region**: Cortex
- **Molecular data available:** RNA-seq, EPIC methylation arrays

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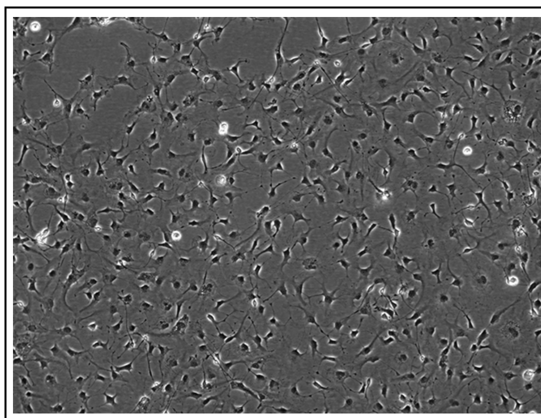
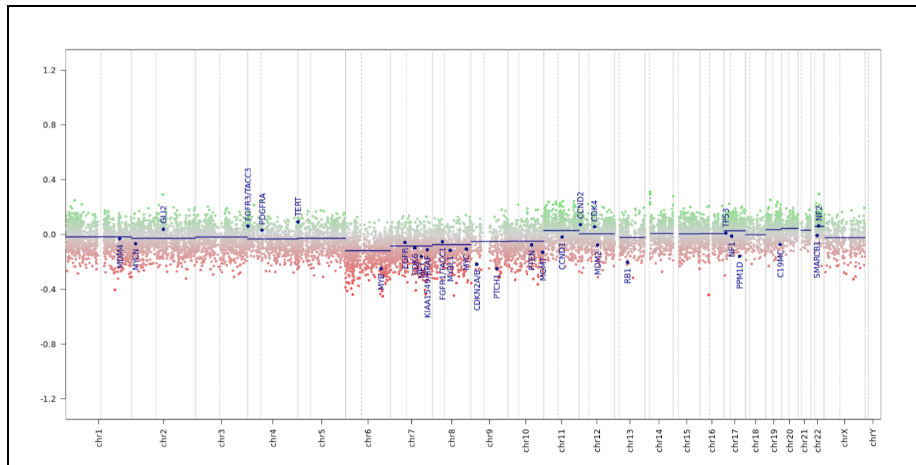
Live image, passage 1



STR profiling

STR locus	genotype
AMEL	X
CSF1PO	12
D13S317	12
D16S539	10
D18S51	13, 16
D21S11	28
D3S1358	15, 16
D5S818	12
D7S820	9, 11
D8S1179	13, 16
FGA	22
PENTA D	2.2, 12
PENTA E	12, 15
THO1	6, 9.3
TPOX	8, 11
vWA	16

Copy number variation profile [(1)]



Acknowledgement Please use the following in any publication using GCGR materials:

The GCGR-xx cell lines were made available through the Glioma Cellular Genetics Resource funded by Cancer Research UK Accelerator Award (A21992) and also reference Pollard *et. al.* [(2)]

Contact information: gcgr@ed.ac.uk

(1) https://www.molecularneuropathology.org
(2) Pollard *et al* Cell Stem Cell. 2009 Jun 5;4(6):568-80

Cell culture protocol for GCGR human Neural Stem Cell lines

General

Cell lines are shipped on dry ice and if not cultured immediately should be transferred to -80ºC (short-term storage, for up to 1 week) or vapor phase of LN_2 for long-term cryostorage.
All work should be carried out in Class II containment hoods.
Incubators: 37°C, 5% CO_2.

Materials:

hNSC culture plastics, media and substrates

We recommend Corning cell culture wells, flasks and dishes (T25: 430639, T75: 430641U)
Cryovials (Nunc 377224).

Important note regarding Laminin use:

All GCGR hNSC lines require laminin as a substrate for optimal growth as adherent monolayer.
All lines grow well on laminin pre-coated plastic; however, we have found that some hNSC lines grow as an adherent monolayer without pre-coating by supplementing the media directly with laminin.
The dependency of the cell lines on laminin pre-coating for optimal growth should be tested in-house following receipt of the cells.

Laminin preparation:

5 ml Cultrex® 3-D Culture Matrix Laminin I (6 mg/ml) (R&D Systems 3446-005-01) is thawed overnight at 4ºC, then carefully added to a 50 ml Falcon tube containing 25 ml ice cold PBS, mix gently by inverting then aliquot 30 x 1 ml at 1 mg/ml. Work quickly and do not allow laminin to rise in temperature.
Store aliquots at -20°C.
Thaw aliquots at 4°C, store at 4°C and use within 1 month.
We have also successful used Laminin supplied by Sigma (L2020) at the same concentrations.

Growth Factor preparation:

rhFGFbasic 500 ug (Peprotech: 100-18b) reconstituted in 5 ml PBS/0.1% BSA = 100 ug/ml.
Aliquot 80 x 60ul store at -20°C short term or -80°C for longer-term storage.
Thawed aliquots are stable at 4°C for up to 4 weeks.

rmEGF 500 ug (Peprotech: 315-09) reconstituted in 5 ml PBS/0.1% BSA = 100 ug/ml.
Aliquot 80 x 60ml store at -20°C short term or -80°C for longer term storage.
Thawed aliquots are stable at 4°C for up to 4 weeks.

Media Preparation

Complete Media (with recommended reagents):
Notes: Bring all reagents to room temp.
Add the N2 supplement last, slowly swirling bottle to avoid precipitation.

Add to a 500 ml bottle of basal DMEM/HAMS-F12 (Sigma D8437) media:

- 7.25 ml Glucose (Sigma G8644)
- 5 ml MEM NEAA 100x (Gibco 11140-035)
- 5 ml Pen-Strep (Gibco 15140-122)
- 800 ul BSA Soln 7.5% (Gibco 15260-037)
- 1 ml bMercETOH 50mM (Gibco 31350-010)
- 5 ml B27 Supplement 50x (LifeTech/Gibco 17504-044)
- 2.5 ml N2 Supplement 100x (LifeTech/Gibco 17502-048)

Complete media can be stored at 4°C up to a month or -20°C for longer.

Before use, complete media is supplemented with:

- Mouse EGF to final concentration 10 ng/ml
- Human FGF-2 to final concentration 10 ng/ml
- Laminin-1 to a final concentration 2 ug/ml

This media (termed, '**Complete+EFL'**) should be stored at 4°C and used within 4 weeks.

Wash Media:

To a 500ml bottle DMEM/HAMS-F12 (Sigma D8437) media, add:

- 5 ml Pen-Strep (Gibco 15140-122)
- 1 ml BSA 7.5% (Gibco 15260-037)

Freeze Mix:

Wash Media + 10% final conc. DMSO.

Cell recovery, Expansion & Cryopreservation

Recovering Cells:

Important note: the number of cells frozen should be optimal to seed a T25 flask. However, we recommend thawing hNSC cells into two wells of a 6 well plate (¼ vial into one well and 3/4 vial into the other) to avoid any seeding density issues.

Laminin coating of cell culture plastic:

Add ice cold laminin stock to ice cold PBS to a final concentration of 10 ug/ml (1:100 dilution of 1 mg/ml stock). Immediately coat the surface of plastic with the Laminin solution and allow to gel for minimum of 2 hr at 37°C. Remove laminin immediately before plating cells, avoiding washing.

Thawing cells:
Once removed from the freezer or liquid nitrogen storage, keep the vial on dry ice until ready to thaw.
Add 10 ml of prewarmed wash media to a 15 ml conical tube.
When ready the vial should be thawed quickly either in a 37°C water bath (hold cap above water line) or in a gloved hand.
Gently transfer thawed cells to pre-warmed wash media.
Centrifuge 3 min @1200 rpm to pellet cells.
Aspirate supernatant and resuspend cells in 8 ml of Complete media + EFL and transfer to a T25 flask (or wells, see note above).
Replace the media the next day to ensure that all residual DMSO and dead cells are removed.

Passaging Cells:
Ideally passage cells at 80-90% confluency and split 1 in 4 to 1 in 6.
Aspirate media and add sufficient Accutase (Sigma A6964 or BioLegend 424201) to cover surface (approx.1 ml for T25). Return to incubator.
Cells should detach within a few minutes.
Flasks may be tapped to encourage detachment.
Add wash media and pipette cell suspension.
Transfer appropriate volume (depending on splitting ratio) to a 15 ml conical tube, centrifuge 3 min @1200 rpm to pellet cells.
Aspirate supernatant then resuspend pellet in complete media + EFL and transfer to new flask.

Freezing Cells:
Cells are detached and collected as above.
Different cell lines vary in numbers of cells per flask and we freeze based on confluency, 2 vials from 80-90% confluent T25 and 6 vials from a T75. If you wish to count cells before freezing, 0.5 - 1 million cells will be sufficient to seed a T25 flask.

Resuspend cells gently in Freeze Mix (1 ml/vial) and transfer to cryovials. Transfer tubes to a specialised freezing chamber (Biocision CoolCell or similar) and place in -80°C freezer immediately. hNSC lines can be maintained at -80°C short-term only (1-2 weeks max). For longer-term storage we recommend storing cryopreserved cells in the vapor phase of LN_2.

Important additional notes:

- Accutase must be removed by aspiration, dilution is not enough.
- Media should be replaced every 7 days.
- We recommend passaging hNSC lines when 80 – 90% confluent.
- Do not split cells at too high a ratio. Cells plated at low density often decrease/stop proliferating.
- We have occasionally observed cells around the edges of the flask forming spheres. These can detach.
- We find GCGR hNSC lines do not proliferate optimally in flasks larger than T75.
- The growth rate of different hNSC lines varies. As a rough guide, an average hNSC line requires a 1:6 split every 7-14 days.

MATERIAL SAFETY DATA SHEET FOR GCGR FROZEN CELL CULTURES

Product name:
Human Neural Stem Cell (hNSC) lines supplied by the Glioma Cellular Genetics Resource.

Established by:

Glioma Cellular Genetics Resource
Centre for Regenerative Medicine
IRR North
The University of Edinburgh
5 Little France Drive
Edinburgh EH16 4UU
UK
Tel: +44 (0) 131 6519575
Email: gcgr@ed.ac.uk

Appearance: Frozen fluid in small plastic containers (vials).
Solid/liquid/gas: Solid (frozen state).

The product is provided as a frozen culture of human cells. Yellow or pink solid for frozen cultures. Aqueous pH 6-8. The frozen components may include but are not limited to: water, inorganic salts, vitamins, amino acids, carbohydrates, lipids, proteins (animal-derived) and cryoprotectant (dimethyl sulphoxide 10% v/v), phenol red.

Chemical Hazards: Frozen cultures contain 10% (v/v) dimethyl sulphoxide (DMSO). DMSO may be harmful and toxic if in contact with skin or ingested and may also irritate eyes and respiratory system. Thawed contents of vials should not come into contact with skin, eyes or digestive and respiratory epithelium and should be diluted upon use with culture media. Persons handling vials of frozen cells containing DMSO should wear a laboratory overall, protective glasses (if not using a safety hood) and gloves.

Biological hazards: Patients donating tissue to derive the human foetal NS cell lines have been screened for HIV; Hep B; Chlamydia and gonorrhoea. Although a human cell line may not be known to contain any agents capable of harm to healthy adult humans the possibility of a contaminant, adventitious virus can rarely be excluded. Therefore, it is recommended that all human cell lines are handled as Containment level 2 (hse.gov.uk).

Health Effects: Eyes: Not known; Skin: Not known; Ingestion: Not known; Inhalation: Not known

Physical Hazards: Where cell lines are shipped as frozen vials there is a small risk that the vial may be pressurised, due to the expansion of trapped liquid nitrogen and could explode on warming. It is recommended that persons handling vials of frozen cells should wear a laboratory overall, protective glasses (if not using a safety hood) and protective laboratory gloves. This sheet does not constitute an assessment as required by the Control of Substances Hazardous to Health Regulations 1994. The information contained in this publication is given in good faith and is accurate to the best of our knowledge.

First aid measures: If accidental contact with material occurs laboratory staff must follow the local first aid procedures that are normally applied following exposure to organisms of containment level 2 (hse.gov.uk). Eyes: Irrigate with physiological saline or water. Seek medical advice immediately. Skin: Wash thoroughly with soap and water. Seek medical advice immediately. Ingestion: Seek medical advice immediately. Inhalation: Seek medical advice immediately.

Accidental release measures: avoid direct contact with the thawed material. Do not open the primary containers unless authorised to do so. Wear a laboratory overall, protective laboratory gloves and safety glasses (if not using a safety hood). If spillage occurs wear a laboratory coat, protective laboratory gloves and protective glasses (if not in hood), place absorbent material over the spillage. Pour disinfectant over spillage to saturate and leave for 30 minutes prior to cleaning and disposal. The preferred disinfectant is 10% v/v sodium hypochlorite (10,000 parts per million available chlorine). This should not be used in combination with other disinfectants, see your local risk assessment or contact the manufacturer of the disinfectant for additional information.

Handling and storage: Personal protective equipment comprised of laboratory coat, protective laboratory gloves and safety glasses should be worn when handling (unpacking) human and animal cell lines. The dry ice (solid carbon dioxide) used to ship frozen vials should be allowed to evaporate in a well-ventilated area. Do not dispose of dry ice in a sealed container. Vials or flasks containing human and animal cells should be opened in a Class II microbiological safety cabinet under conditions of containment level 2.

Exposure controls/Personal protection: Vials containing human and animal cells should be opened in a Class II microbiological safety cabinet under conditions of containment level 2. Personal protective equipment comprised of laboratory coat and protective laboratory gloves should be worn. Avoid aerosol production and inhalation. Handle as for containment level 2.

Stability and reactivity: Stable. Hazardous polymerization will not occur.

Toxicological information: In its thawed liquid state this material is not normally toxic but avoid aerosol formation and inhalation. Vials contain dimethyl sulphoxide 10% v/v which is an irritant that readily penetrates the skin.

Disposal considerations: Follow established procedures for Containment Level 2. Follow all national, regional and local regulations.

Methods for disposal for thawed contents:

Spillage: wear a laboratory coat, safety glasses and protective laboratory gloves. Place paper towels or other absorbent material over the spillage. Pour disinfectant over spillage to saturate and leave for 30 minutes prior to cleaning and disposal. The most appropriate disinfectant is 10% v/v Sodium hypochlorite (10,000 parts per million available chlorine). This should not be used in combination with other disinfectants. See your local risk assessment or contact the manufacturer of the disinfectant for additional information.

Waste disposal: Decontaminate prior to disposal with a 10% sodium hypochlorite solution and dispose of decontaminated liquid waste down a designated sink with running water. Solid waste should be placed in a sealed bag and labelled and destroyed by incineration.

Transport information: GCGR cell lines are not classified as dangerous goods as they are considered non-infectious and non-hazardous to humans or animals.

The following categories apply and GCGR will ensure the outer packaging indicates the appropriate packaging requirements:

UN no: 1845- Dry Ice. Dry ice not deemed dangerous by road transport only air.

Biological Substance Category B UN3373 – packed in compliance with IATA packing instruction 650.

Other information: The above information is correct to the best of our knowledge. All materials and mixtures may present unknown hazards and should be used with caution.

The user should make independent assessments and decisions regarding the completeness of the information based on all sources available.

As per MTA, the GCGR (University of Edinburgh, UCL and Cancer Research UK) shall not be held liable for any damage resulting from handling or contact with the above product.