Q: How do I obtain cell lines from the GCGR?
Navigate to **Obtain cell lines** using the link on the Home page.

Q: How do I grow the GSC and hNSC cell lines?
We supply detailed cell culture protocols. Navigate to **Additional Info** using the link on the Home page and select **Protocols.**

Q: What level of biological containment is required for culturing GSC and hNSC cell lines?
Biosafety Level/Containment level 2 laboratory and Class II biological safety cabinet.

Q: Have the cell lines been screened?
All GCGR cell lines have tested negative for Mycoplasma on the first sub-culture. We recommend that all lines coming into your laboratory are quarantined until they have been re-tested for mycoplasma in-house.
Patients donating tissue to derive the human foetal NS cell lines have been screened for HIV; Hep B; chlamydia and gonorrhoea. All were negative. Glioma patients were not screened prior to surgery; however, the samples are from low-risk individuals.
Most adventitious agents only present a risk in the human cell cultures prior to their first sub-culture. All GCGR cell lines have been sub-cultured multiple times. Selected GSC lines have been screened for HIV, Hep B and Hep C. All were negative. 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 (https://www.hse.gov.uk).

Q: How do I access the molecular data associated with the cell lines?
RNA-seq, WGS and Infinium EPIC methylation array data may be accessed via the links on the cell line inventory tables. Navigate to **Resources** using the link on the Home page and select cell line inventory of interest.

Q: Are GSC and hNSC lines classified as human tissue and subject to HTA regulations?
No. Our GSCs and hNSCs have divided and cell lines have been sub-cultured multiple times.
Cells that have divided in culture are not considered to be 'relevant material' by the Human Tissue Authority. Only human cell lines intended for human application may require a HTA license.
Please note: Our lines may be referred to as primary cell lines. The term 'primary' is used to indicate that they have not been genetically modified for continuous passage in vitro. Cell lines, including primary cell lines, that have been sub-cultured, are not considered relevant material by the HTA. The term primary cell lines should not be confused with the term primary cell culture.
https://www.hts.gov.uk/guidance-professionals/hts-legislation/relevant-material-under-human-tissue-act-2004.

Q: How do I select the best cell lines for my research?
Our cell lines have all been subject to extensive characterisation. Details of individual lines may be found in the **Resources** section, navigate to Resources using the link on the Home page.
The data files (RNA-seq, Infinium EPICmethylation arrays and WGS) can be accessed using the links in the cell line inventory tables or browse our cell line data on cBioportal, also accessed via the Resources section.
You may select your cell lines based on genetics (i.e. specific somatic mutations, chromosomal amps/dels), epigenetics, transcriptional subclass, expression levels of specific genes of interest, tumour initiation capacity, cell growth rates, etc.
If you are interested in obtaining cell lines that cover the broad spectrum of GBM then we suggest obtaining a selection of lines from different transcriptional subclasses.

Q: How have the GSC lines been verified as tumour cells?
We have performed deep molecular profiling on all our cell GSC lines and confirmed they have GBM associated chromosomal aberrations and driver mutations.

Q: Do the cell lines have the same mutations as the primary tumour tissue?
The same chromosomal aberrations are found in the primary tissue and derived-cell line. Sub-clonal changes may occur in the cell lines, as occurs in the disease. Please see our resource publication for more details on primary tissue and cell line comparisons.

Q: Do the cell lines stop proliferating? / What cell line passage number should I use for my research?
All GSC lines were expanded beyond 10 passages (~25 doublings) and all lines tested could be sustained beyond passage 30.
We have found the GSC lines to be stable, with similar chromosomal copy number and molecular profiles at high passage and following xenotransplantation. As mentioned above, genetic and transcriptional changes may occur in the cell lines, as occurs in the disease. As with all primary cell lines it is good practice to make low passage number stocks of the cell lines and use the lowest passage number possible for your experiments.
hNSC lines proliferate well over many passages (lines tested grow for over 30 passages) however, they are primary cell lines with normal karyotype, they have not been immortalised and do not have the GBM associated driver mutations, therefore it is unlikely they will have the same proliferative capacity as GSCs. We advise using the lowest passage number possible for your experiments.

Q: What is the doubling rate of the cell lines?
This is variable between lines. Average doubling time data is detailed in the cell line inventory tables found in the **Resources** section, navigate to Resources using the link on the Home page

Q: Do you have any IDH mutant glioma cell lines?
Yes. We have four Astrocytoma_High grade_IDHmutant cell lines in our collection. These are detailed in the **Glioma Stem Cell lines** inventory in the **Resources** section, navigate to Resources using the link on the Home page.

Q: Do you have any low-grade Glioma cell lines?
All our GSC lines are classified as Glioblastoma_IDH wildtype or Astrocytoma_IDH mutant_high grade. It was not possible to establish tumour cell lines from low grade gliomas using our derivation protocol. Please see our resource publication for more details.

Q: How can I check cell line authenticity?
We have performed STR analysis on all our cell lines. This data is available on the **cell line datasheets** accessed from the **cell line inventories** in the Resources section, navigate to Resources using the link on the Home page.

Q: Do you have any patient/clinical data?
No. The GCGR samples are anonymised, and we do not hold any patient data.

Q: Do you have any GSC lines from recurrent surgery?
Yes, 4 lines are from recurrent surgery. We do not have matched lines from first and recurrent surgery due to the limited timeframe of this project.

Q: Can cell lines be engineered?
Yes. Validated engineered GSC and hNSC lines are available from our resource. We also supply a detailed transfection protocol in the Additional Info section. Navigate to **Additional Info** using the link on the Home page and select **Protocols.**

Q: Do you have any isogenic normal adult NSC cells or any normal adult NS/neural cell line?
No. See next question below.

Q: What do you recommend as a normal reference control to GSC lines?

We recommend our GCGR hNSC lines derived from human foetal brain tissue. Our hNSC lines are derived and expanded in the same conditions as GSC lines with a molecular profile very similar to GSCs but they are not tumorigenic on transplantation. We recommend cortex or striatum-derived hNSCs for controls for GBM models, but you can also choose to select your own hNSC lines from our extensive range of temporal and regionally distinct lines. These are detailed in the **Human Neural Stem Cell lines** inventory in the **Resources** section, navigate to Resources using the link on the Home page.

Q: Are these patient-derived xenograft (PDX) lines?

No – the GCGR GSC lines were derived from direct in vitro culture of dissociated primary tumour tissue.

Q: Are the GSC lines clonally derived?

No. We aim to maintain the GSC lines as heterogenous populations to reflect the heterogenous nature of the primary tumour tissue. We do not plate at low density, sort or preselect any cells from the primary tumour or apply any deliberate selective pressure on the cultures.

Q: Can the GSC lines make tumours?

Data on tumour generation and lethality of the transplanted GSC-GFP-Luc reporter lines is detailed in the Glioma Stem Cell lines inventory and individual cell line datasheets in the **Resources** section. Navigate to Resources using the link on the Home page.