Thawing and amplifying the GWDI-pool mutant library

The key to handling the library effectively is not to bottleneck the cells. To retain library complexity, never transfer fewer than 10^6 cells. Avoid unnecessary growth of the library to mitigate enrichment or depletion of mutants with growth phenotypes.

A. Materials
- *Frozen GWDI-bank stock, stored at -80°C*
- *Media: Filter-sterilised HL-5 media including glucose (Formedium, Cat# HLG0102) supplemented with PVS (100,000 units of penicillin, 100 mg streptomycin sulphate, 200 µg folate, 600 µg vitamin B12 per litre of media*)*

B. Equipment
- *Flow cabinet*

C. Consumables
- *Sterile 10cm tissue culture plates.*
- *Cryotubes*
- *Sterile pipettes*

1) Thaw the frozen aliquot of cells at room temperature
2) Pour the entire contents of a single aliquot directly into a sterile 10cm dish containing 10mL sterile HL5 media with PVS (see 'Materials' above). It is recommended that the HL5 media is filter sterilised as opposed to autoclaved (autoclaving caramelises the media somewhat).
3) Leave the cells to settle and recover overnight. The cells should be confluent on the dish between 24-36 hours after plating. If the plate is not confluent after 48hours the library mutant cell viability following thaw-out is too low, the library has been bottlenecked and should be discarded.
4) Once the plate is confluent (1-2 x 10^7 cells in total), collect the cells and split them into 5 plates (~2 x 10^6 cells/plate). The following day you should have 5 plates of confluent cells.
5) Freeze down cells from 4 of these plates each in 1mL freezing media (50% FBS, 42.5% filter-sterile HL5, 7.5% DMSO) to generate a total of 4 stocks as back-ups of the starting library. Alternatively, the cells can be split again and grown up to generate more stocks, just beware that the more the library is grown the greater the reduction in library complexity due to growth-mutant enrichment
6) Use cells from the final plate to start the screen

NB. The library can also be amplified by growing the cells on bacteria (e.g. *Klebsiella aerogenes*) on SM agar plates (Formedium SMA0102).

*We usually make up 500X PVS stock that we keep at 4deg and dilute into autoclaved or filter sterilised media as needed