Nanobody Library Verification, Representative Results

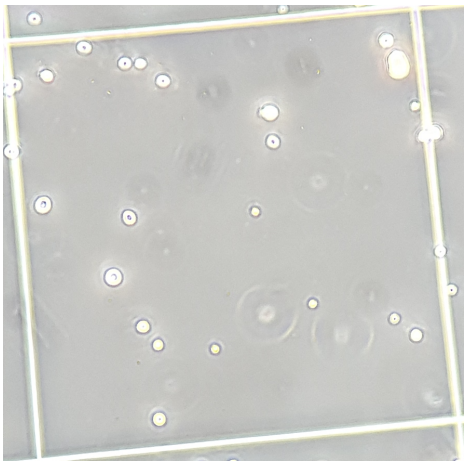
Following initial expansion of the yeast nanobody library and freezing down of aliquots, the library was verified by testing for cell viability and contamination.

Cell Viability

- Following resuspension of a thawed library aliquot ($2x10^{10}$ cells) in 1L –Trp + glucose, serial dilutions from this culture were used to plate 1000, 100, and 10 cells (each in 100ul –Trp + glucose)
- After 2 days at 30°C, cell colonies were counted: 184 out of 1000, 22 out of 100, and 3 out of 10 cells survived.
- After a third day at 30°C, the final colony count was 186 out of 1000, 22 out of 100, and 3 out of 10.
- This corresponds, on average, to a cell viability of ~24%, implying a total of ~$4.8x10^9$ viable cells per library vial.
- This is sufficiently close to the optimal viability of $5x10^9$, i.e. 10-fold over the library diversity

Contamination

- After taking cells from the 1L culture to estimate viability, the remaining culture was shaken at 30°C, 230 rpm, for 48 hours.
- **Contamination check I**: A small sample was taken from the culture, with 10^6 cells placed on a hemocytometer for observation under the microscope. No contamination present (see right).
- From the 1L culture, 5 ml were passaged to 50 ml –Trp + glucose and let grow at 30°C for 24 hours.
- **Contamination check II**: 10^6 cells were again observed on the hemocytometer to check for contamination. No contamination present.
- From this 50 ml culture, 5 ml were passaged to another 50 ml –Trp + glucose and let grow at 30°C.
- **Contamination check III**: 10^6 cells were again observed on the hemocytometer to check for contamination. No contamination present.



A sample of uncontaminated yeast cells

Checklist:

- No contamination visible under microscope **PASS**
- No contamination following each passage of culture (3 passages total) **PASS**
- Cell viability is ~10-fold library diversity **PASS**

This batch of the library may now be put to use.