Nanobody Library FAQ

Thawing the library

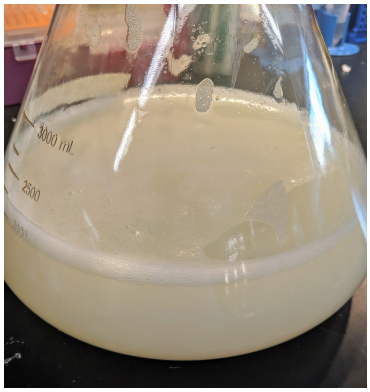
Q: Can I use only half of a library aliquot and refreeze the rest for later?

No. You should thaw and use an entire aliquot to ensure no loss of library diversity. Refreezing will reduce viability. We recommend following the protocol for expansion as closely as possible.

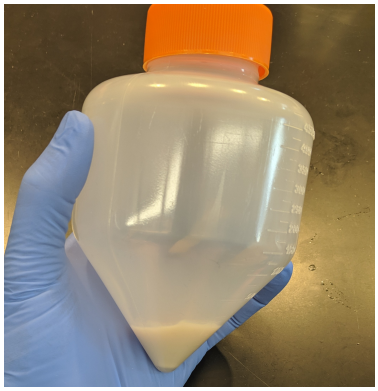
Optical density

Q: My OD_{600} measurement is not as high as the expected 10-20 for a saturated yeast culture; is there something wrong?

Possibly, but there may not be. After 48-72 hours of growth the cell culture should require dilution by a factor of at least 50 prior to OD measurement. Your measured OD value after dilution should be less than 1 for an accurate measurement with most spectrophotometers. The culture should appear opaque with an off-white/slightly yellow colour, and should yield a ~15 ml cell pellet per liter of culture when centrifuged (see pictures below). If an OD value seems low but the culture appears saturated, then plating cells to test for viability will inform more accurately as to the library quality. Counting cells under a microscope with a hemocytometer can also be done to verify cell density.



2 litre saturated yeast culture


Cell pellet from 500 ml saturated culture

Media components

Q: What are the specific products used to make the various media?

Trp Dropout: US Biological D9531
Yeast Nitrogen Base: Himedia M878
Glucose: Sigma G8270
Galactose: Difco 216310
Pen/Strep: Gibco 15140-122
Sodium Citrate: Ward's Science 470302-530
Citric Acid: MP Biomedicals 150699

Q: Can I use SDCAA or another medium instead of the recipe in the protocol?

No. The media described in the protocol are designed to supplement for all auxotrophies in the BJ5465 cell line. If you choose to use a different medium composition you should carefully check to ensure necessary nutrients are included.

Passage volume

Q: What volume of -Trp + galactose should be used for inducing expression in the naïve library?

1 litre is suitable for induction of the naïve library. A saturated culture will have a cell density of ~4x10^8 cells/ml. You should passage this into induction media such that the starting cell count for this expression culture is between 1x10^7 and 2x10^7 cells/ml. So to 1 litre of induction media you should add 25 ml of saturated culture, for instance. The two main things to consider are the starting cell density as mentioned, but also to make sure that at least 10-fold the library diversity is passaged for expression. For instance in the above example, 25 ml of culture contains ~1x10^{10} cells, which is roughly 20-fold the library diversity.

Plating cells

Q: Should I plate cells to test for viability immediately after thawing the vial?

Yes. This should be done immediately after resuspending the thawed vial in –Trp + glucose. At this moment, the cell density is known (2x10^7 cells/ml in 1 L). One may take 1 ml of this, and perform serial dilutions to get three tubes of 20000, 2000, and 200 cells. From each tube, 100 µl can be plated (giving plates of 2000, 200, and 20 cells). The number of colonies that grow (after 48 hours) can be used to determine an average percent viability for the cell culture.

My cells give large colonies in just 24 hours, why?

If colonies are appearing substantially faster they are likely bacteria or wild yeast contamination. If your liquid cultures grow very quickly and show no nanobody expression this may also indicate contamination. Use aseptic technique and include antibiotic to minimize risk of contamination. Wild yeast contamination can be cleared by the addition of 5’ FOA since BJ5464 cells lack the URA3 gene.