

Please find below a selection of testimonials from some of the first researchers who used our Hybribody services.

## Cell biology

### Improved nanobody using a yeast display system

One of the aims of the project of our team is to develop low-cost protein therapy for rare bleeding disorders using single domain antibody fragments (nanobodies). Nanobodies have several advantages, including a low (if any) immunogenicity when applied to humans. Further, their small size ( $\pm 17$  kDa) facilitates their molecular engineering.

We obtained by immunizing a llama with our target of interest, a nanobody that has demonstrated in *in vitro* and *in vivo* experiments its ability to improve a rare haemorrhagic phenotype.

Fusions to the C-terminal end of the Aga2p mating adhesion of *Saccharomyces cerevisiae* has been used in many studies for the selection of affinity reagents by yeast display followed by flow cytometric analysis. Here, thanks to the expertise of Hybrigenics and using an improved yeast display system for the screening of nanobody immune libraries, we have been able to obtain a variant of our nanobody of interest with an affinity improved 50 fold compared to the molecule of origin. This variant has kept all its therapeutic potential and is now one of our lead product in our pipeline of products in pre-clinical stage.

— Dr Olivier Christophe (INSERM U1176, Le Kremlin Bicêtre, France) - Europe

My lab has previously used Hybrigenics services to perform Yeast two-hybrid screens. The data that they provided initiated a new direction in our research. They also used Y2H screening to select nanobodies working as intrabodies against our protein of interest. Hybrigenics team have put a large effort to provide us with several candidate clones. All the clones were provided as plasmids for mammalian expression and went through the initial validation. After a more in-depth validation, we now have reagents that work in several different assays. I am very impressed with the customer service and dedication of the scientific team at Hybrigenics.

— Dr. Emilia Galperin (University of Kentucky, Lexington, KY, USA) - North America

We asked Hybrigenics to select intrabodies against four different proteins involved in vesicular trafficking. They quickly provided us with several candidates per protein, in the form of ready-to-use plasmids for mammalian expression. We already validated some of these intrabodies. Fused to a red fluorescent protein, they will be extremely valuable tools to block our target proteins in live cells

— Dr. Thierry Galli (Jacques Monod Institute, Paris, France) - Europe

## Neuroscience

As part of a collaboration under FP7-HEALTH AgedBrainSYSBio program, Hybrigenics selected and validated anti-Fyn VHH nanobodies working inside cells - intrabodies. Our lab currently explores these very exciting novel tools in primary neurons.

— Prof. Michel Simmoneau (Centre for Psychiatry and Neuroscience, Paris, France) - Europe

We received many VHH nanobodies from Hybrigenics, selected and validated against Tau and phospho-Tau. The biochemical and functional characterization of those binders is ongoing but we can already say that this service opens new avenues for our research.

— Dr. Luc Buée (Jean-Pierre Aubert Research Centre, Lille, France) - Europe

## Plant Biology

My group has been using the services provided by Hybrigenics to perform Y2H screens from

*Arabidopsis thaliana* libraries using viral proteins as baits at several occasions, and has been very satisfied.

Not only Hybrigenics teams have a great expertise in bait design, in performing high-coverage screens, and most importantly in scoring the candidates obtained and pinpointing the technical false-positives - which are invaluable assets, but I also found very enjoyable to work with them. They are friendly, supportive, and highly competent, and I always appreciated the discussions we had together.

I will for sure never ever perform a yeast two-hybrid screen myself again and I strongly recommend their high-quality services, which are really worth the price.

Moreover, following a very successful yeast two-hybrid screen using one of our viral protein as a bait, our group also got the chance to make use of their newly developed Hybribody service, i.e. synthetic single chains nanobodies. Hybrigenics selected several VHH intrabodies, which they further validated in mammalian cells using fluorescence microscopy. Our lab is now currently validating them in plant cells and the preliminary data we obtained appear very promising. In addition to their use as basic research tools to detect our proteins in living cells, the intrabodies obtained may as well turn useful to inhibit key steps in the viral multiplication process, thus opening new possibilities for designing antiviral strategies.

— Dr. Isabelle Jupin (Jacques Monod Institute, Paris, France) - Europe