Antibodies are of utmost importance for research and therapy but their generation is laborious and time consuming. We established a novel streamlined workflow for obtaining antibodies by incorporating all natural steps such as antibody maturation, selection and production in one genetic system implemented into a eukaryotic cell line. We stably transfect an antibody construct into CHO cells and mimic maturation by using the enzyme AID (activation-induced deaminase), which is known to induce somatic hypermutation.

For selection, we are testing and deploying a versatile and continuous viral system as well as magnetic beads and cell sorting. Finally, a genetic switch enables the transition from surface expression to production of soluble antibodies. In addition, we pursue phage display with an antibody fragment to study mutation rate and evolution by AID in prokaryotes. Our system supercedes animal immunization and the smooth process will increase the ready availability of antibodies in various formats.

**Antibody Module**

The aim of the antibody module was to design and assemble antibody constructs that would demonstrate the principle of our generation system. The antibody constructs need to enable the directed maturation by the enzyme AID, the demethylated and specific selection by the selection module, especially by virus molecules, and need to permit a switch from cell surface to soluble expression. All steps require constant expression over longer periods maintaining stable transfection (i.e., chromosomal integration) of the antibody constructs. We worked with a Flp-FRT CHO cell line to achieve stable integration in the CHO cell genome by using the Flp recombinase-mediated recombination.

**Advantages**

- Animal friendly
- Speed
- Lab to Industry Scale
- Mono-/Polyclonal Diverse Formats
- Human Antibodies

**Potsdam Standard – RFC 91**

For the Potsdam Standard, we designed a new cloning antibody library system, which can be directed by an anti-AID control and two new restriction sites, in the protease A site and in the engulf sphi I in psiRBCS. For the cloning process, we cut the vector with Apa I and Sph I.

**SocialBricks**

The concept of SocialBricks is to modularize human practice routines in order to provide adapted versions of activities to various target audiences.

**Modulation Module**

Activation induced Cytidine Deaminase (AID) is a 24 kDa small enzyme and plays an important role in mammalian immune system. The AID is a central controlling point of antibody maturation in B lymphocytes. We used the AID to mutate the antibody sequences in CHO cells. It was shown, that the AID mutates actively transcribed, single stranded DNA. Therefore, the AID has to be localized to the nucleus to mutate single stranded DNA.

**Mutation Reaction**

Activation induced Cytidine Deaminase (AID) is a 24 kDa small enzyme and plays an important role in mammalian immune system. The AID is a central controlling point of antibody maturation in B lymphocytes. We used the AID to mutate the antibody sequences in CHO cells. It was shown, that the AID mutates actively transcribed, single stranded DNA. Therefore, the AID has to be localized to the nucleus to mutate single stranded DNA.

**Testing**

Selection system of higher affinity antibodies by recombinant virus labelled with antigen

**Software**

With our app you can:
1. Browse/follow many protocols at the same time
2. Make sure that all the devices, solutions and materials are ready – the checkbox option is there for you
3. Scan the checksum that you used via Barcode Scanner
4. Comment on every step
5. Thanks to the programmed timer, you don’t need to search for the timer in the lab space - you just use it on your mobile phone

**Achievements**

- 17 new and improved submitted Biobricks
- New Biobricks work like expected
- New Biobricks distributed
- Predictive model designed
- Help another team by characterizing a part (Freiburg)
- New Standard – RFC 91

**References**

[1] Surprisingly, this system is relatively simple: it only needs the enzyme AID (EC 3.1.11.63) and an antibody sequence with both N- and C-terminal expression sequences. Therefore, the AID has to be localized to the nucleus to mutate single stranded DNA.

**Fig. 1.** In the presence of the AID, a transmembrane, scFv MCS and a C-terminal 5xHis was found enabling the ability to diffuse into the nucleus and, therefore, modulate. We then used, in a modified form of the AID, with NLS and without 5xHis, modulate more efficiently. Therefore, we, constructed a single, the well-known AID (uAID) and the modified AID (mAID) without NLS fused with GFP to investigate the intracellular localization.

**Fig. 2.** Association of modified AID-FRET with Nucleofactor in living CHO cells. The green fluorescence signal (dF/dt) shows that the NLS is functional and that the modified AID is located in the nucleus.

**Fig. 3.** To determine the mutation rate, we co-expressed CHO cells with set gene sequences and DNA pools labeled with different fluorophores. For every part of the sequence the frequency of the fluorophore was determined. The results show that the mutation rate of our AID is three times lower than the ectopic gene expression. Surprisingly, the residual wild-type of modified AID is only twice lower than the control group.

**Fig. 4.** Using the intracellular localization of AID we can control gene expression by using an antibody construct. We show that the antibody can be reprogrammed by using the AID in the nucleus. Intriguingly, the antibody was reprogrammed with an efficiency similar to the AID in the cytoplasm.