Short timeline for LBA set-up with an anti-drug specific antibody: How to quickly generate high affinity monoclonal antibodies tools?

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Introduction

In bioanalysis, Ligand-Binding Assays (LBA) have now become keys methods to detect or quantify biotherapeutic molecules (proteins, antibodies...). They give critical information to determine the safety and efficacy of the biotherapeutics. Typically, they are set-up with the presence of anti-drug antibodies to enable a specific immunocapture.

Generally, LBA methods are widely used, for example, for immunogenicity, pharmacokinetics and receptor assays... which required high sensitivity. Hence, one of the key success variable will be the high affinity and specificity of the developed anti-drug antibodies. These antibodies-tools can be generated by several ways: polyclonal generation using rabbits as the most common host (the classical method in positive controls generation for immunogenicity assays), or by monoclonal generation offering benefits of specificity and sustainable production.

Another essential variable for the project success is the required time for development and validation of the method. The anti-drug antibody generation can sometimes be the most time-consuming and complicated part. For this purpose, the use of a high-throughput single cell cloning method to generate high-affinity antibodies, can be decisive in the challenge of tight deadlines. Here, a case study of mAbs generation for 2 LBA methods is summarized.

Program steps

The aim of this project seeks to develop a panel of specific mAbs against a therapeutic protein (110 kDa), that can be used in several LBA methods, such as PK (sandwich format) and neutralizing assay. Thus, it requires to develop paired-antibodies (Pk assay) and specific antibodies targeting the binding site involved in the interaction with the target (neutralizing assay).

Immunization & fusion

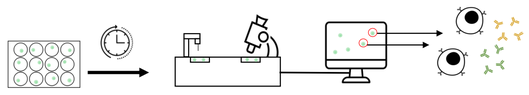
Mice are immunized with the whole protein, ELISA controls of the immune response are performed. Then, lymphoid organs from the best mouse are taken and the fusion of B lymphocytes and myeloma cells is performed in order to obtain several hybridomas

- Cohort of 4 mice
- Control: indirect ELISA with coating of the therapeutic protein

Mouse #1	Mouse #2	Mouse #3	Mouse #4
5 923	4 317	4 005	20 937

Mouse 4 is selected for fusion (highest titer) ☑

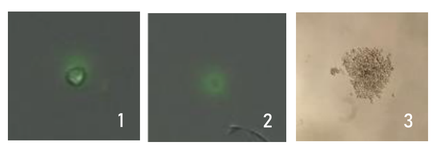
Cell selection on high-throughput platform



Hybridomas are placed on 96 wells plates and tested on a fully automatized platform which allows screening, selection and isolation of single cells. Screening is made thanks to fluorescent halo detection (specific antibodies secretion). Cells of interest are picked by a robotized arm with a needle and cloned.

Secreting cells:

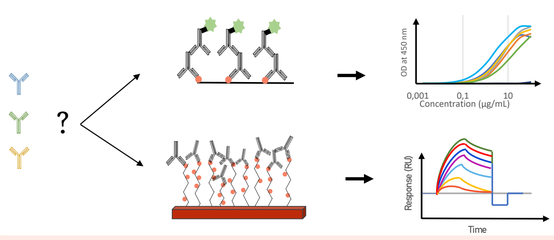
Coating: antigen on plate



1 Before picking
2 After picking
3 After growing

Needle-picked hybridomas, depending on the fluorescence intensity
→ criteria evaluated with an internal scoring

Secondary ELISA screening & affinity ranking by SPR



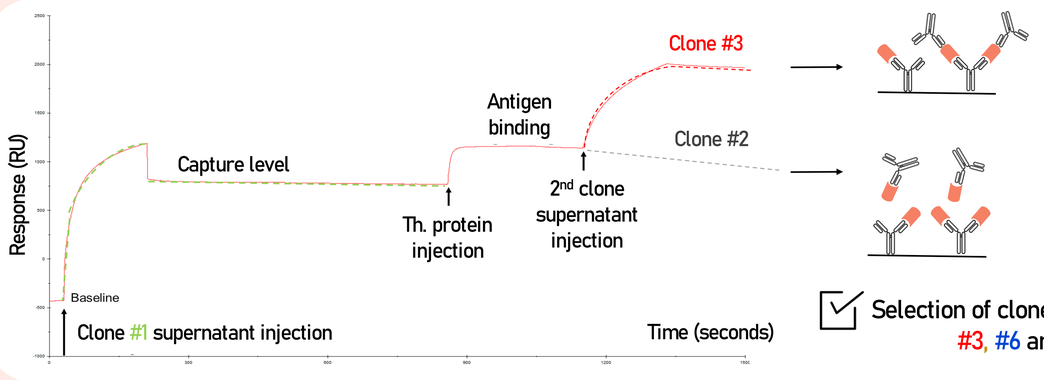
ELISA screenings are performed to control if the clones are still secreting specific antibodies (selection depending on the OD).
Ranking analysis is performed by SPR technology to select the high-affinity clones.

Affinity ranking: ☑ 5 clones are selected

Clones:	#1	#2	#3	#4	#5	#6	#7	#8	#9
K_D (M):	2.39E-10	6.16E-10	5.32E-09	7.40E-09	3.86E-08	8.57E-08	6.45E-08	3.30E-08	1.68E-07

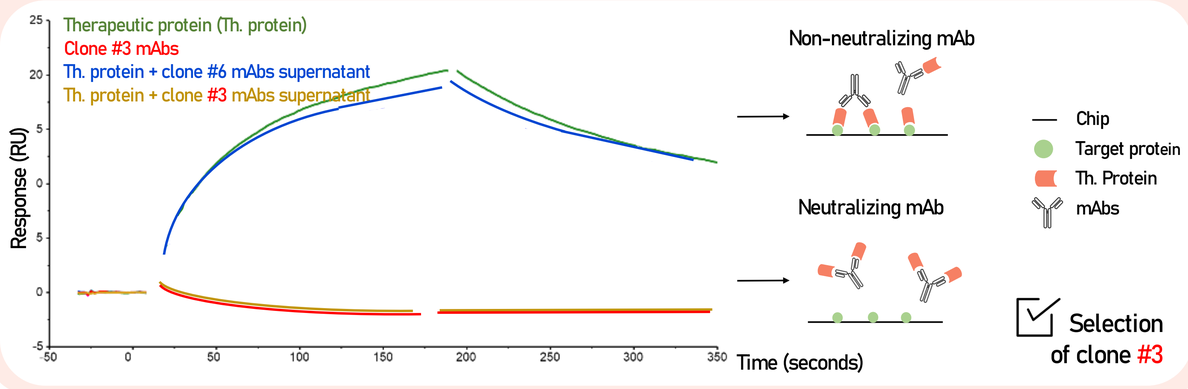
Conformatory testing

Pairing test: performed using SPR technology.



☑ Selection of clones #1, #3, #6 and #8

Neutralizing test: performed using SPR technology with immobilized target protein and injection of mix therapeutic protein and mAbs.

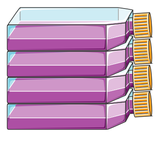


☑ Selection of clone #3

Life cycle management: production & securization

After conformatory testings, 4 clones were selected for pairing with one for the neutralization.

Production



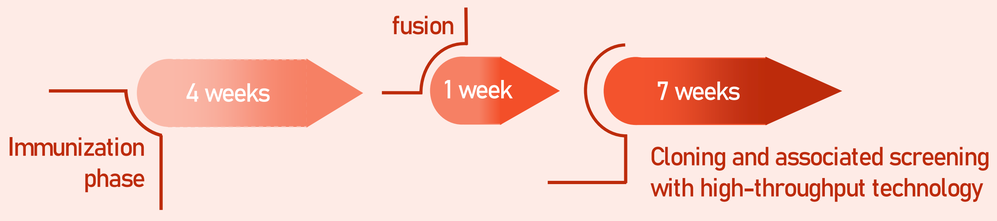
Production in T75 flasks

Production with 500 mL of supernatant:
Clone #1: 7.40 mg
Clone #3: 10.3 mg
Clone #6: 5.70 mg
Clone #8: 8.60 mg

Securization

Securization in recombinant format for animal-component free antibodies variants or fragments production, in accordance with 3Rs principle.

Associated timelines



	Classical methodology	High-throughput single cell cloning methodology
Timelines	~5 Months	~3 Months
Experimental schedule	Immunization > Fusion > Screenings > Cloning	Immunization > Fusion > Screenings > Cloning
Cloning overall procedure	Cells cloning is performed by successive limiting dilutions and screenings	Enables simultaneous screening and cloning of positive cells directly after fusion
☑ Pros	Classical & mastered	Faster, more candidates

Conclusion

The development of neutralizing antibodies is always difficult because it requires to be highly specific (targeting only a few amino acids) while having from the outset many candidates to maximize the success rate. Another challenge in this type of project is the timeframe required for antibodies development. This protocol combined with the high-throughput platform allowed to develop in approximately 3 months 2 pairs of monoclonal antibodies selected for PK method and one monoclonal antibody retained for neutralizing format assay. Following this development, these antibodies will need to be validated directly in the final neutralizing and PK assays.

- ☑ Large number of hybridomas and clones
- ☑ Easier selection with microscopy and robotization
- ☑ Faster cloning, facilated isolated grow

Agro-Bio, good antibodies for good biotherapeutics