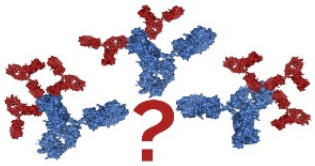
The Antibody Society presents:



Antibody Validation Webinar Series

WEBINAR 9: Getting to Recombinant Antibodies that Guarantee Reproducible Research

Moderator: Dr. Simon Goodman, The Antibody Society

Speaker: Dr. Andrew Bradbury, Specifica Inc.

First Broadcast: February 12, 2020

Now available On Demand

Questions and Answers from the live Webcast on February 12, 2020

Question	Answer
How long would it take to sequence, clone, and test all possible combinations?	It all depends on how many different chains you have in the hybridoma. Starting from cDNA, it takes about a couple of months to get to a functional antibody.
Please define a "bad" antibody.	A bad antibody is one that doesn't bind to the expected target, and/or binds to an unexpected target. A good antibody is the opposite - binds to the expected target and has no off-target binding.
Follow on: what is a "good" antibody?	**SG**: I'd add to that- fit for purpose in your experimental context, and with a public domain sequence, and defined epitope. That would be very good.
Are you recommending transient or stable recombinant expression?	I think transient is faster for testing and you can get a lot of antibody from a transient. However, if it's an antibody that you plan to use a lot, it's worth taking the trouble to make a stable cell line.
Any recommendations for cell lines, e.g., HEK or CHO?	HEK is better for transient, CHO for stable expression, although you can buy CHO systems suitable for transient as well.
Are recombinant antibodies from vendors worse than sequence verified, in your opinion?	Recombinant antibodies from vendors are good as far as functionality is concerned (providing they are appropriately validated). Vendors selling recombinant antibodies have the sequences - i.e., they are sequence verified, but don't want to release them. If sequences were publicly available it would allow everyone to be on the same page.
Similarity can be approximated by mass measurement and/or sequencing.	Accurate DNA sequencing is vital in establishing (and comparing) the identity of antibodies, bearing in mind that even a single amino acid change in a CDR can significantly change antibody specificity.
How many monoclonals should one sequence for a particular target? And if variations are seen in sequencing? Which sequence to trust? Make all and test the function?	Yes, you need to test all VH VL combinations, unfortunately.

When would you use DNA sequences vs. protein mass spec sequencing?	DNA sequencing (after mRNA isolation and cDNA synthesis) is available to all scientists. MS is less widely available, and MS sequencing of CDRs is challenging. Furthermore, it doesn't overcome the problem of multiple chains.
What are your views on clinical-grade mAb reproducibility? Thanks for the nice seminar.	Many clinical-grade antibodies are recombinant, and sequences are often available. The validation process for clinical antibodies is significantly more stringent than research antibodies
A downstream issue is production of recombinant Abs and establishing their biophysical properties. It is not trivial. So, not only transparency in sequence is important, but how the Ab is produced and the lot-specific characterization. It all does speak to the need for a centralized source or sources for as many Abs as feasible.	I agree. That said, I would argue that the differences between different non-recombinant antibodies is greater than different lots of the same recombinant.
Is there was a way to capture multiple chains and accurately sequence the CDRs? Would that be a way of validating commercial antibodies?	Antibody validation is required for both recombinant and normal antibodies. Once validated, obtaining the sequence is the way to ensure reproducibility between researchers. However, it would be difficult to obtain CDR sequences for research antibodies except with MS
Thank you for an interesting and useful webinar.	You are extremely welcome! Thanks for viewing and participating.
When is the next episode?	**SG**: The final webinar in this series, Episode #10, will be broadcast on February 19, 2020, 9am ET / 15:00 CET. In Episode #10, Prof. Andreas Plückthun, University of Zürich, will discuss the use of recombinant binding tools derived from diversified libraries of small and versatile protein scaffolds that are not antibody-based, which opens a wide range of previously-inaccessible approaches in cell biology and biochemistry.

All episodes in this webinar series will be made On Demand after the initial broadcast.

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