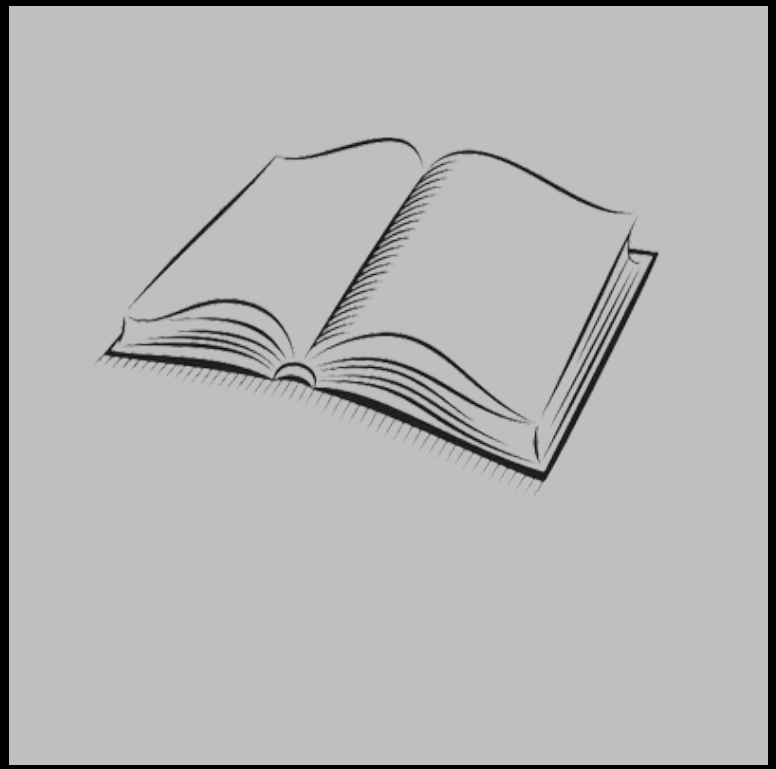
RECOMBINANT ANTIBODIES
A USER GUIDE FOR ACADEMIA



Pierre Cosson
University of Geneva
Pierre.Cosson@unige.ch

The life science crisis (1)

Select 53 high-impact original preclinical research studies

Try to reproduce the results at Amgen

Begley 2012 Nature 483:531

The life science crisis (1)

Select 53 high-impact original preclinical research studies

Try to reproduce the results at Amgen

-6 confirmed (11%)

Begley 2012 Nature 483:531

The life science crisis (2)

BLAME IT ON THE ANTIBODIES

Antibodies are the workhorses of biological experiments, but they are littering the field with false findings. A few evangelists are pushing for change.

BY MONYA BAKER

Baker Nature (2015), 521:274

The life science crisis (2)

50 % of commercial antibodies do not recognize their intended target and/or lack specificity

Baker Nature (2015), 521:274

The life science crisis (2)

50 % of commercial antibodies do not recognize their intended target and/or lack specificity

Baker Nature (2015), 521:274

Standardize research antibodies !
Switch to recombinant antibodies !

Bradbury Nature (2015), 518:27

There are many reasons to use recombinant antibodies

-Defined reagents
-Availability (unlimited amounts, no possible loss)
-Selection in controlled conditions

-Reduce the use of animals in biomedical research

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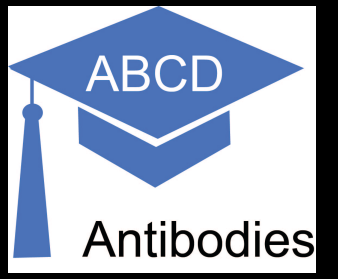
How do you get access ?

The ABCD project
Antibodies for academia

Academia to Academia
Not for profit
Open access

Animal-free

THE ABCD DATABASE



ABCD: AntiBodies Chemically Defined
21'000 entries, 3'000 antigens

Unique identifier
Linked to Uniprot or ChEBI
Detailed information
Direct order

Are there antibodies against your favorite protein ?

ABCD database:
https://web.expasy.org/abcd/

ABCD Antibodies

Expasy ABCD Home

The ABCD (AntiBodies Chemically Defined) Database

The ABCD (AntiBodies Chemically Defined) database is a manually curated depository of **sequenced antibodies**, developed by the Geneva Antibody Facility at the University of Geneva, in collaboration with the CALIPHO and Swiss-Prot groups at SIB Swiss Institute of Bioinformatics.

Search by antibody name, species or target (UniProt or ChEBI ID)

Search Clear

Example searches: 9E10, P07766, 37926, Escherichia coli, Protein tag, Nanobody

The ABCD database is part of a broader project, with the mission of promoting the widespread use of **recombinant antibodies** by academic researchers and, ultimately, the replacement of animal-produced antibodies. This concerted effort also includes the Geneva Antibody Facility (for discovery and production of antibodies) and the scientific journal Antibody Reports (publishing technical articles on antibody characterization).

Release information: Version 9.0 (August 2020)
21'543 sequenced antibodies, against 3'671 different targets

If you'd like to cite the ABCD database: Lima WC, Gasteiger E, Marcatili P, Duek P, Bairoch A, Cosson P. The ABCD database: a repository for chemically defined antibodies. Nucleic Acids Res. 2019, pii: gkz714

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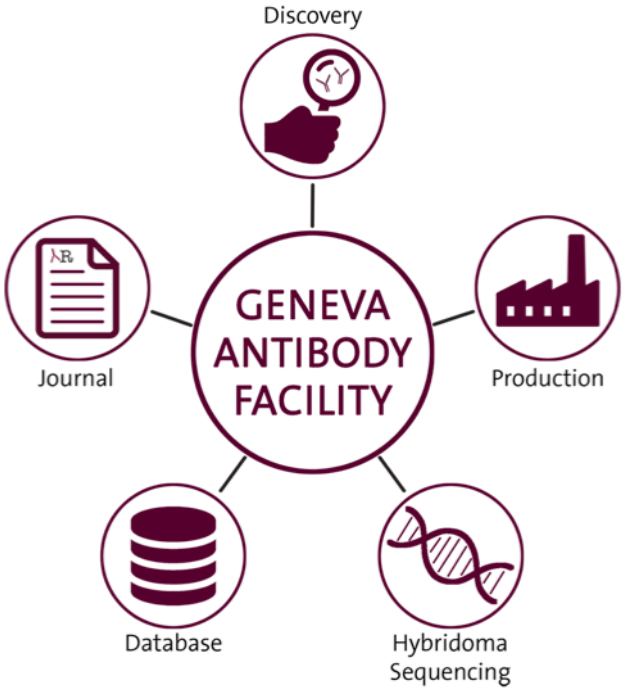

Expasy is operated by the SIB Swiss Institute of Bioinformatics | Terms of Use

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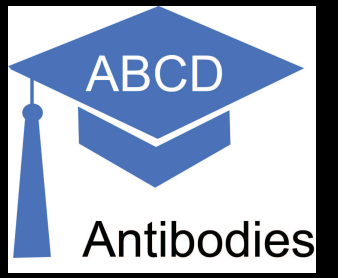

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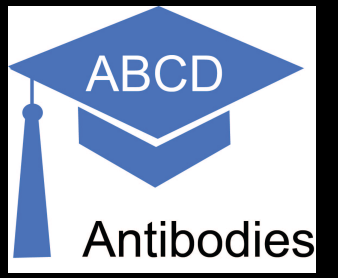
SPIKE COV-2 Search Clear

Example searches: 9E10, P07766, 37926, Escherichia coli, Protein tag, Nanobody



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P0DTC2 Search Clear

Example searches: 9E10, P07766, 37926, Escherichia coli, Protein tag, Nanobody

Expasy

P0DTC2 Search Clear

ABCD (AntiBodies Chemically Defined) Database result: 662 hits for P0DTC2

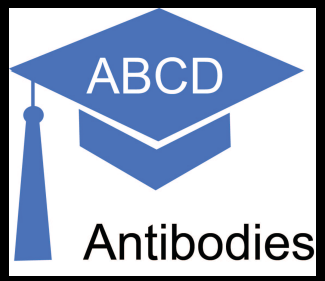
Identifier	Antibody name	Target
ABCD_AI334	anti-SARSCoV-CR3022	S, Spike protein, Spike glycoprotein
ABCD_AQ806	SARS VHH-72	S, Spike protein, Spike glycoprotein
ABCD_AR209	anti-SARS-CoV-2-Sb#1	S, Spike protein, Spike glycoprotein
ABCD_AR210	anti-SARS-CoV-2-Sb#2	S, Spike protein, Spike glycoprotein
ABCD_AR211	anti-SARS-CoV-2-Sb#3	S, Spike protein, Spike glycoprotein
ABCD_AR212	anti-SARS-CoV-2-Sb#4	S, Spike protein, Spike glycoprotein
ABCD_AR213	anti-SARS-CoV-2-Sb#5	S, Spike protein, Spike glycoprotein
ABCD_AR214	anti-SARS-CoV-2-Sb#6	S, Spike protein, Spike glycoprotein
ABCD_AR215	anti-SARS-CoV-2-Sb#7	S, Spike protein, Spike glycoprotein
ABCD_AR216	anti-SARS-CoV-2-Sb#8	S, Spike protein, Spike glycoprotein
ABCD_AR217	anti-SARS-CoV-2-Sb#9	S, Spike protein, Spike glycoprotein
ABCD_AR218	anti-SARS-CoV-2-Sb#10	S, Spike protein, Spike glycoprotein
ABCD_AR219	anti-SARS-CoV-2-Sb#11	S, Spike protein, Spike glycoprotein
ABCD_AR220	anti-SARS-CoV-2-Sb#12	S, Spike protein, Spike glycoprotein
ABCD_AR221	anti-SARS-CoV-2-Sb#13	S, Spike protein, Spike glycoprotein
ABCD_AR222	anti-SARS-CoV-2-Sb#14	S, Spike protein, Spike glycoprotein
ABCD_AR223	anti-SARS-CoV-2-Sb#15	S, Spike protein, Spike glycoprotein
ABCD_AR224	anti-SARS-CoV-2-Sb#16	S, Spike protein, Spike glycoprotein
ABCD_AR225	anti-SARS-CoV-2-Sb#17	S, Spike protein, Spike glycoprotein

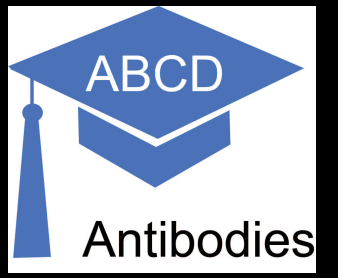
ABCD Antibodies

ABCD database:
https://web.expasy.org/abcd/

↓

Detailed info





Expasy

ABCD

Search Clear

ABCD_AI334 in the ABCD (AntiBodies Chemically Defined) Database

	Antigen information
Target type	Protein
Target link	UniProt: Q1T6X6 SARS coronavirus Frankfurt 1 UniProt: P0DTC2 Severe acute respiratory syndrome coronavirus 2 (2019-nCoV) (SARS-CoV-2)
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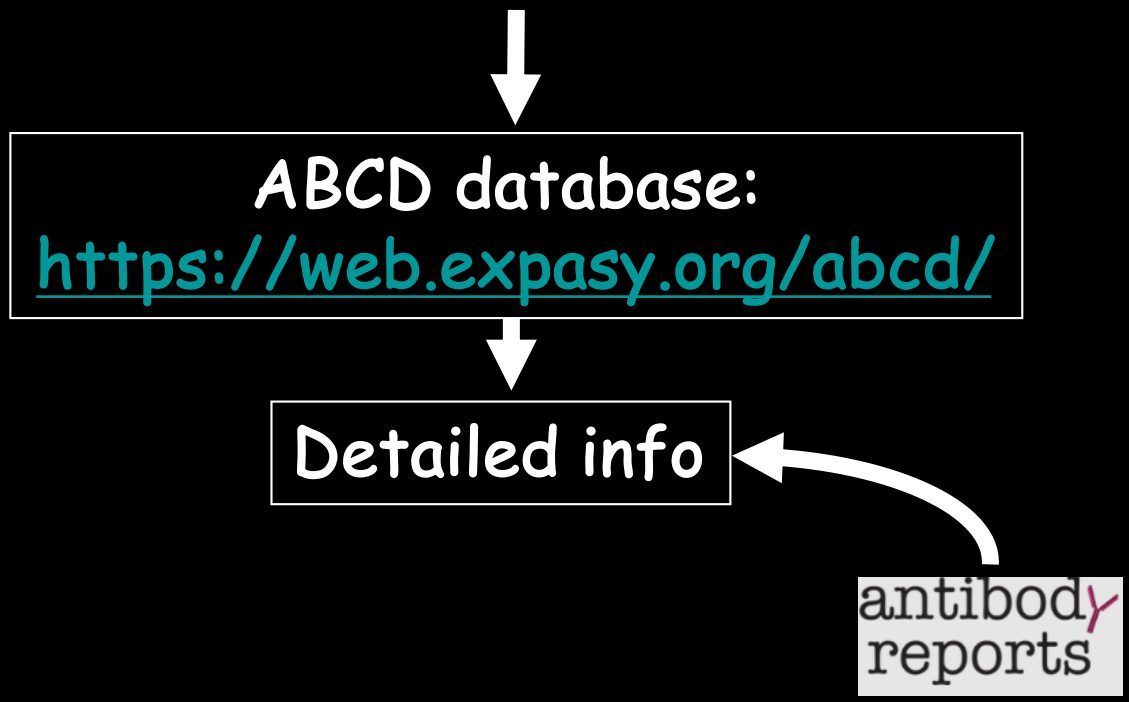
Epitope	Spike protein S1 (YNSTFFSTFKCYGVSATKLNDLCF+DDFM+FEL) This antibody binds to SARS-CoV RBD with a 100x higher affinity than to SARS-CoV-2 RBD.
	Antibody information
Antibody name	anti-SARSCoV-CR3022
Antibody synonyms	anti-SARS-CoV SC03-022
Applications	ELISA, Immunofluorescence, Neutralization, Surface plasmon resonance, X-ray crystallography
Cross-references	PDB: 6W41
Publications	Patent: US43781050 PMID: 32245784 PMID: 16796401 DOI: 10.24450/journals/abrep.2020.e186 DOI: 10.24450/journals/abrep.2020.e219



ABCD
Antibodies

antibody reports

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AI334, AQ806 and RB596 antibodies recognize the spike S protein from SARS-CoV-2 by immunofluorescence

doi:10.24450/journals/abrep.2020.e219 *Antibody Reports*, 2020, vol. 3, e219

AI334, AQ806 and RB596 antibodies recognize the spike S protein from SARS-CoV-2 by immunofluorescence

Anna Marchetti[1], Philippe Hammel[1], Frederic Zenhausern[2,3,4]

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[2] *Center for Applied NanoBioscience and Medicine, The University of Arizona, Phoenix, AZ 85004, USA*
[3] *Whitespace Enterprise Corporation, 1303 Auto Drive, Tempe, AZ 85284, USA*
[4] *School of Pharmaceutical Sciences, University of Geneva, 1 rue Michel Servet, CH-1211, Geneva, Switzerland*

Abstract

The recombinant antibodies AI334, AQ806 and RB596 detect by immunofluorescence the spike S protein from SARS-CoV-2.

Introduction

The spike (S) glycoprotein mediates attachment of coronaviruses to the host ACE2 receptor and fusion with the host cell membrane (Yan *et al.*, 2020). Three recombinant antibodies (AI334, AQ806 and RB596) successfully detect by immunofluorescence the S protein from SARS-CoV-2 (UniProt P0DTC2) expressed in Vero-B4 cells.

Materials & Methods

Antibodies: ABCD_AI334, ABCD_AQ806 and ABCD_RB596 antibodies (ABCD nomenclature, https://web.expasy.org/abcd/) were produced by the Geneva Antibody Facility (http://www.unige.ch/medecine/antibodies/) as mini-antibodies with the antigen-binding scFv portion fused to a mouse IgG2A Fc. The synthesized scFv sequences (GeneArt, Invitrogen) of antibodies AI334 and AQ806 correspond to the sequences of the variable regions of the clones CR3022 (ter Meulen *et al.*, 2006) and VHH-72 (Wrapp *et al.*, 2020), respectively. Antibodies RB596 was raised via phage display against the SARS-CoV-2 S protein (Hammel *et al.*, 2020). HEK 293T suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected with the vector coding for the scFv-Fc of each antibody. Supernatants (30-100 mg/L) were collected after 5 days.

Antigen: Vero-B4 adherent cells (growing in DMEM, Gibco #11960044, supplemented with 10% FBS), were transiently transfected 24 h before the experiment with a vector coding for the full-length SARS-CoV-2 S protein (BEI Resources, NR-52310, pCAGGS vector containing the full-length SARS-CoV-2/Wuhan-Hu-1 S glycoprotein coding sequence). Transfected cells were then seeded on multi-test glass slides (Thermo Fisher #15546375), and used to detect the viral protein. Non-transfected cells were used as a negative control.

Protocol: Transfected Vero-B4 cells were fixed with ice-cold Acetone/Methanol (ratio 1:1) for 10 min, and slides rehydrated for 10 min in PBS + 0.1% Tween20 (w/v) (PBS-T). Cells were then blocked in PBS-T + 0.2% BSA (w/v) for 30 min, and then incubated with the anti-S antibodies (final concentration 5 mg/L in PBS-T + BSA) for 1 h. After 3 washes with PBS-T, cells were incubated for 30 min in PBS-T + BSA with secondary goat anti-mouse IgG conjugated to AlexaFluor-488 (1:400, Molecular Probes, #A11029). After 3 washes with PBS-T, slides were briefly rinsed with dH_2O, and mounted with Mŏwiol (Hoechst) + 2.5% (w/v) DABCO (Fluka #33480). Pictures were taken using a Zeiss LSM700 confocal microscope, with a 63x Neofluar oil immersion objective.

Results

AI334, AQ806 and RB596 antibodies specifically detected a signal in Vero-B4 cells transfected with the SARS-CoV-2 S protein (Fig. 1). The distribution observed is consistent with a presence mostly in the early secretory pathway (endoplasmic reticulum and Golgi apparatus). The specificity of the signal was verified by the absence of staining in non-transfected cells (Fig. 1).

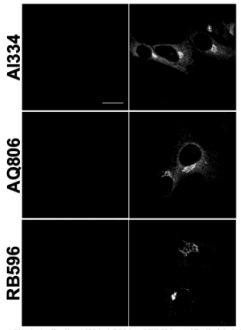

Fig. 1. Antibodies AI334, AQ806 and RB596 specifically labeled Vero-B4 cells expressing the SARS-CoV-2 S protein. No labeling was seen in non-transfected cells. Scale bar: 20 µm.

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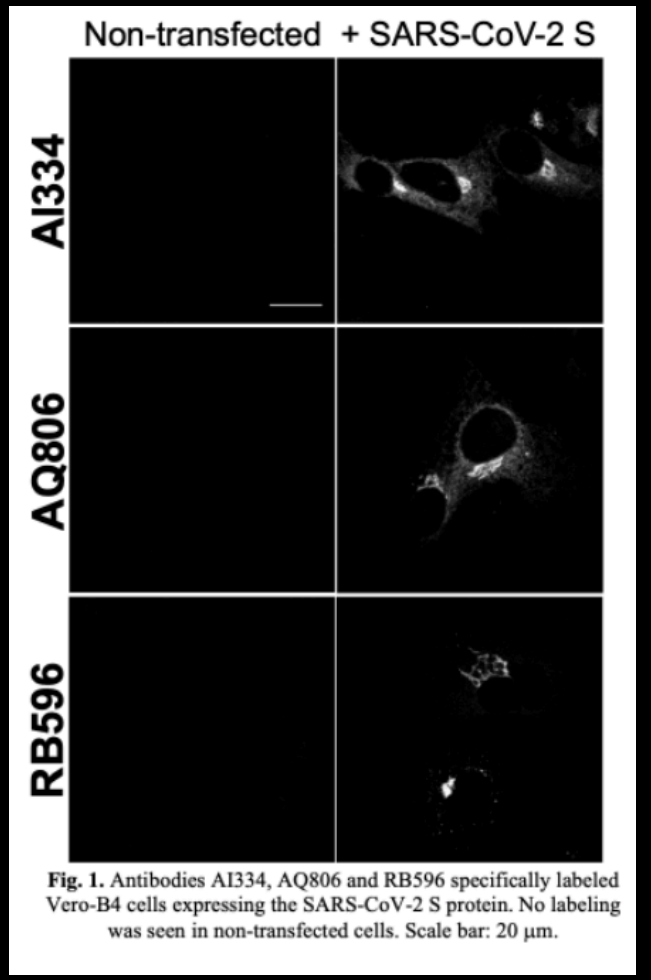

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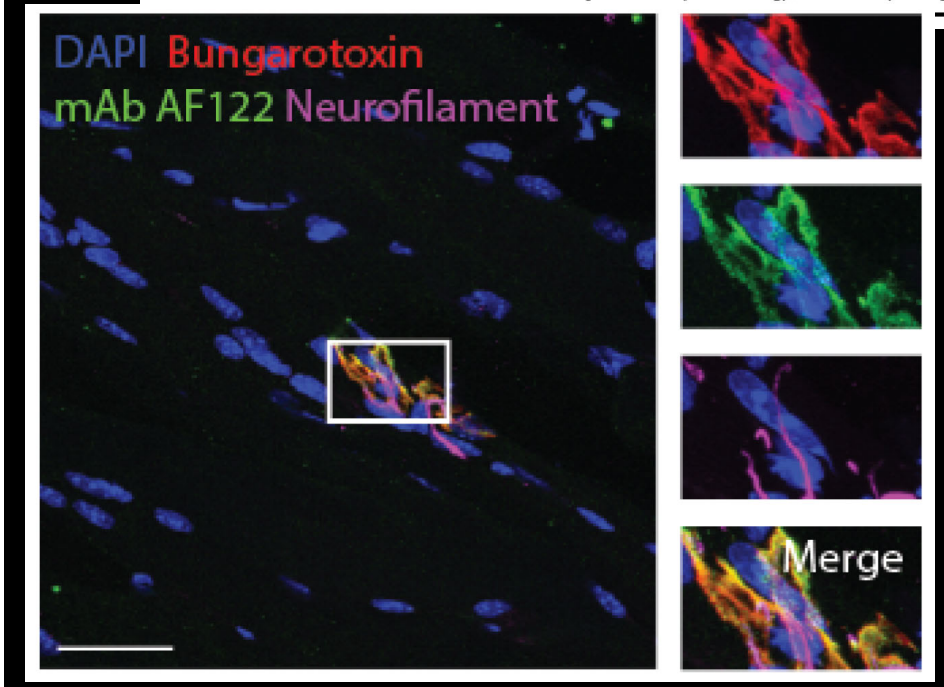
antibody reports

The AF122 antibody recognizes the AChRα subunit in murine muscle endplates by immunofluorescence

Florian Ingelfinger[1,2], Dilay Cansever[1], Bettina Schreiner[1,2]

[1]*Institute of Experimental Immunology, University of Zurich, Zurich, Switzerland*
[2]*Department of Neurology, University Hospital Zurich, Zurich, Switzerland*



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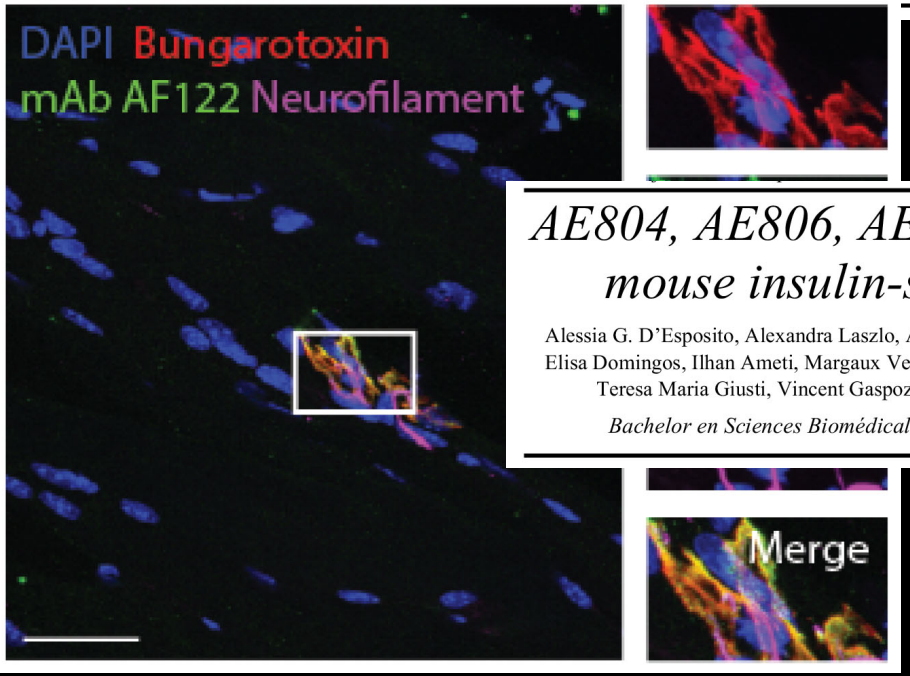
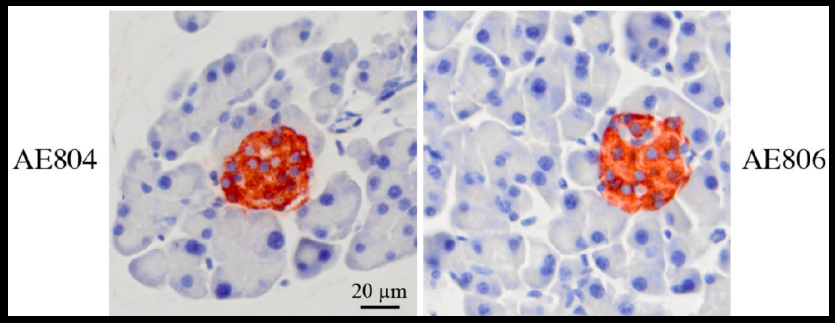


AE804, AE806, AE999, AF017, AF041 and AF131 antibodies label mouse insulin-secreting beta cells by immunohistochemistry

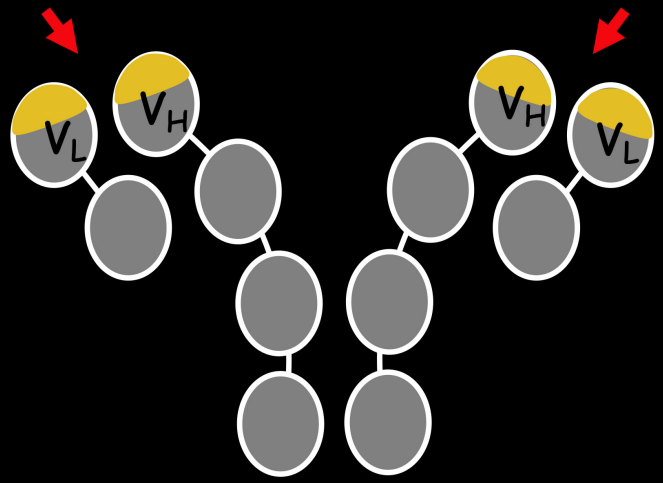
Alessia G. D'Esposito, Alexandra Laszlo, Arec Manoukian, Aurélie Cino, Boris R. Gueorguiev, Cyril Pirek, David Arsimoles, David Celeny, Eliott Bosshard, Elisa Domingos, Ilhan Ameti, Margaux Verdon, Maxime Richard, Mélissa Longepierre, Mustafa Haraj, Orlane L. Maxit, Sara Hussami, Sébastien Spedaliero, Teresa Maria Giusti, Vincent Gaspoz, Marie Ebrahim-Malek, Laura De Luca, Zoé Ropiot, Ali Sassi, Monica Bulla, Cyril Guilhen, Priscilla Soulié

Bachelor en Sciences Biomédicales, Département de Physiologie Cellulaire et Métabolisme, Faculté de Médecine, CH-1211 Genève 4

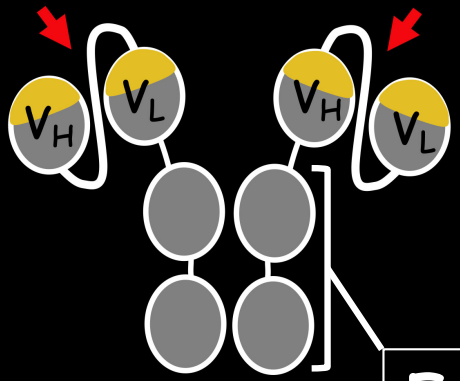


ABCD antibodies
Production format

IgG antibody



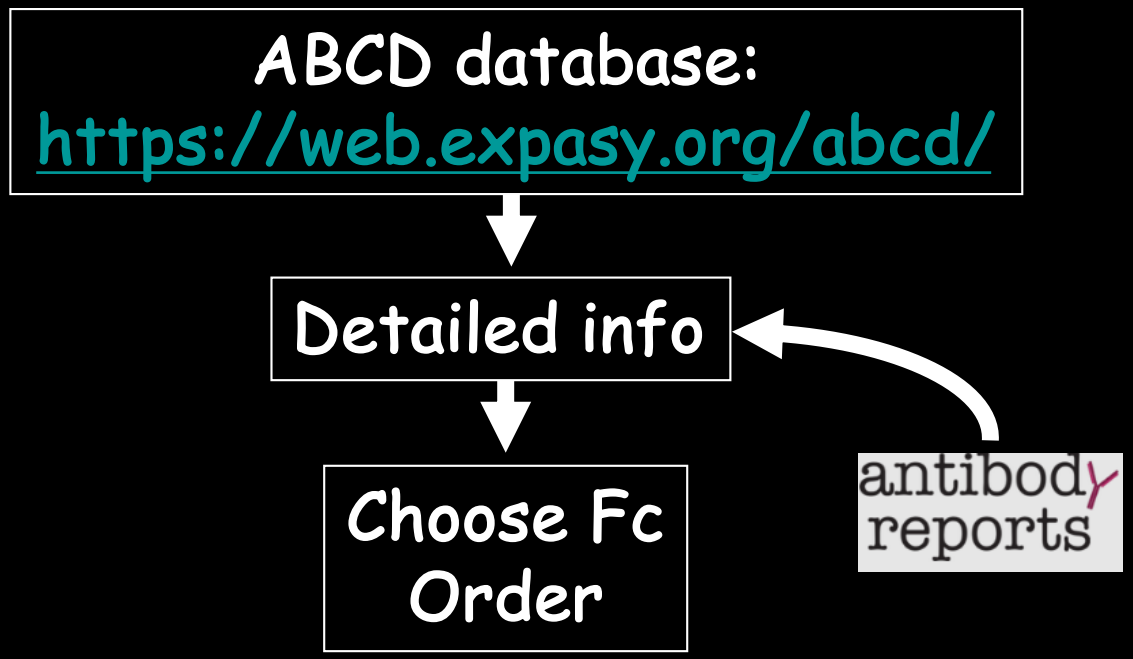
Minibody



5ml antibody
50μg/ml (variable)
2-4 weeks

Fc portion

Rabbit
Mouse
Human
Guinea pig
Others…



ABCD
Antibodies

ABCD_AI334 in the ABCD (AntiBodies Chemically Defined) Database

Antigen information	
Target type	Protein
Target link	UniProt: Q1T6X6 SARS coronavirus Frankfurt 1 UniProt: P0DTC2 Severe acute respiratory syndrome coronavirus 2 (2019-nCoV) (SARS-CoV-2)
Target name	S, Spike protein, Spike glycoprotein
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Antibody synonyms	anti-SARS-CoV SC03-022
Applications	ELISA, Immunofluorescence, Neutralization, Surface plasmon resonance, X-ray crystallography
Cross-references	PDB: 6W41
Publications	Patent: US43781050 PMID: 32245784 PMID: 16796401 DOI: 10.24450/journals/abrep.2020.e186 DOI: 10.24450/journals/abrep.2020.e219
Antibody sequence	
If you want to have the protein sequence of this antibody, please check the Publications and Cross-references links (a more comprehensive step-by-step can be found here). If you have trouble finding it, just send us an email using the contact form.	
Would you like to obtain this antibody, or the plasmid coding for it?	
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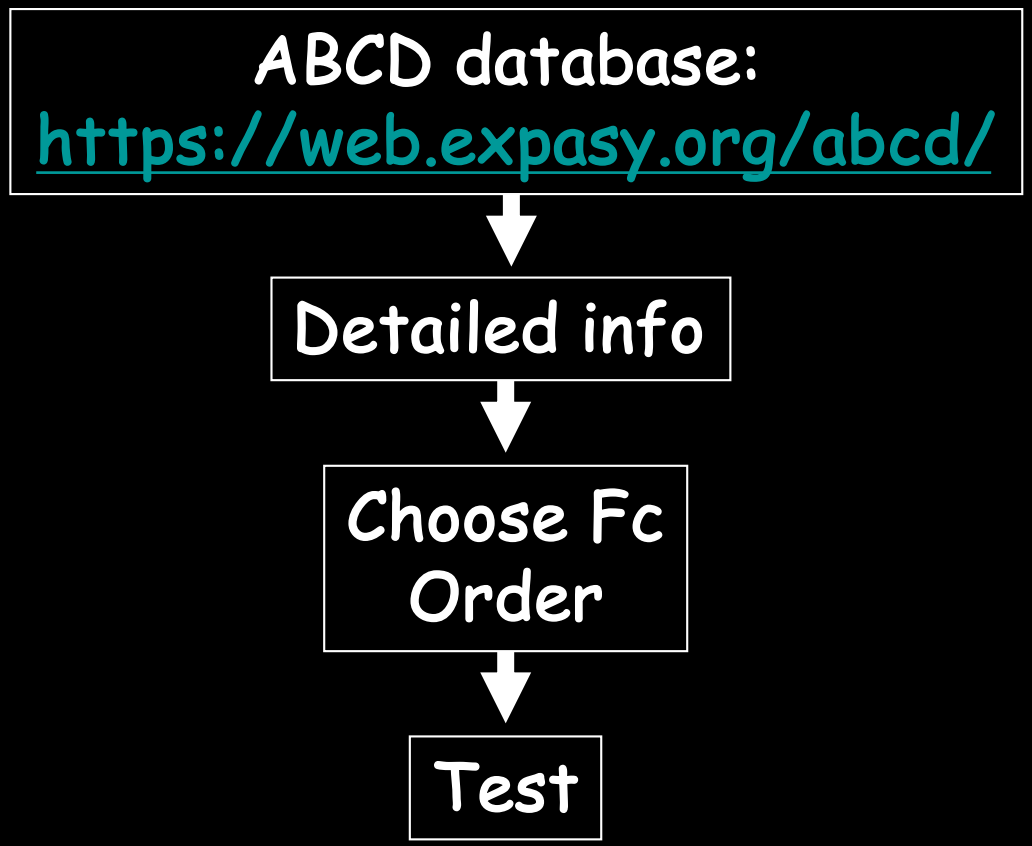
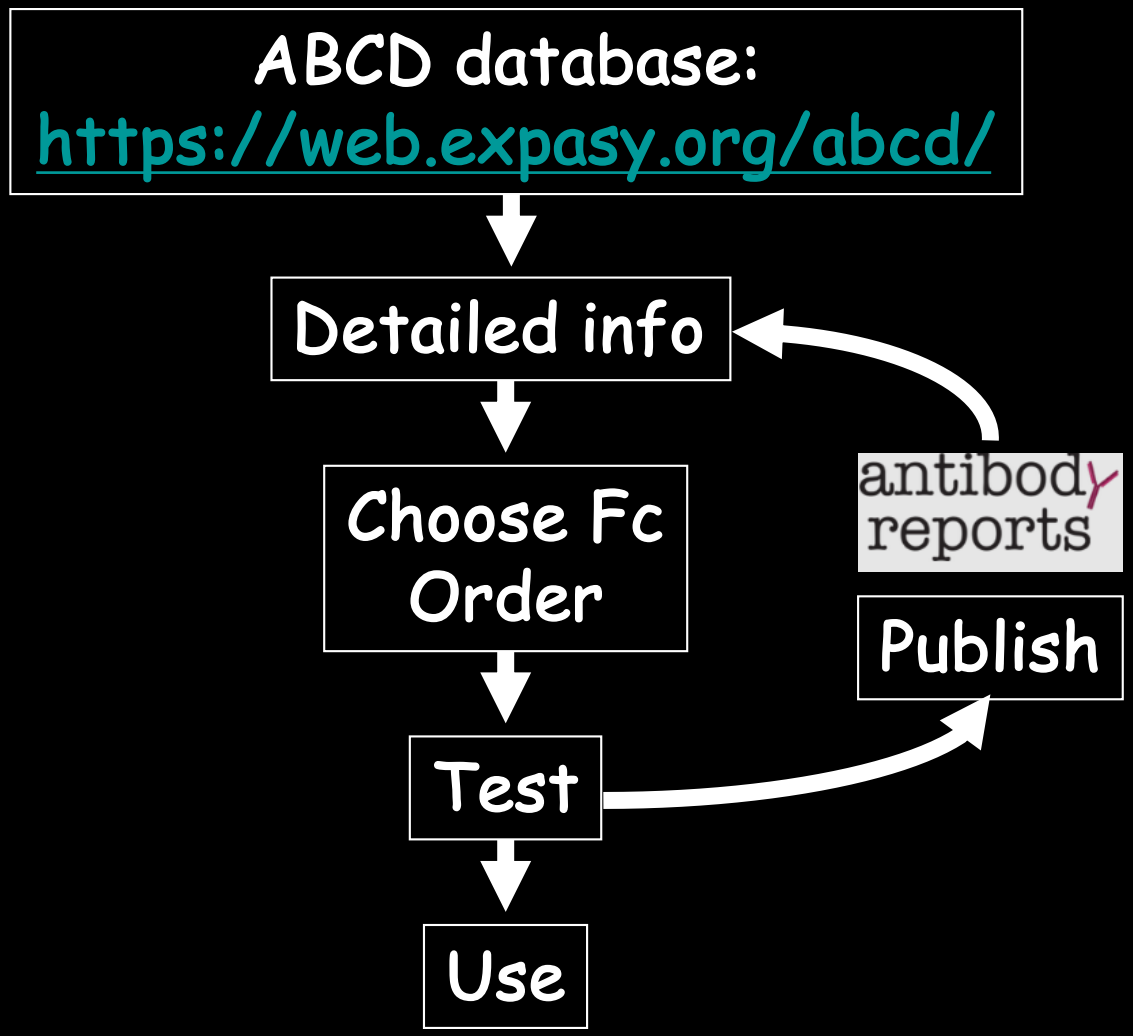
→ Direct order (credit card) 200CHF≈200€≈200$


ABCD database:
https://web.expasy.org/abcd/
Detailed info
Choose Fc
Order
Test



ABCD
Antibodies

What if there are no antibodies against your favorite protein ?

ABCD database:
https://web.expasy.org/abcd/

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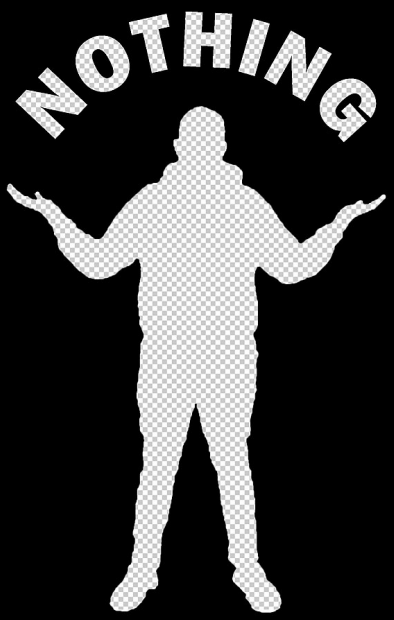

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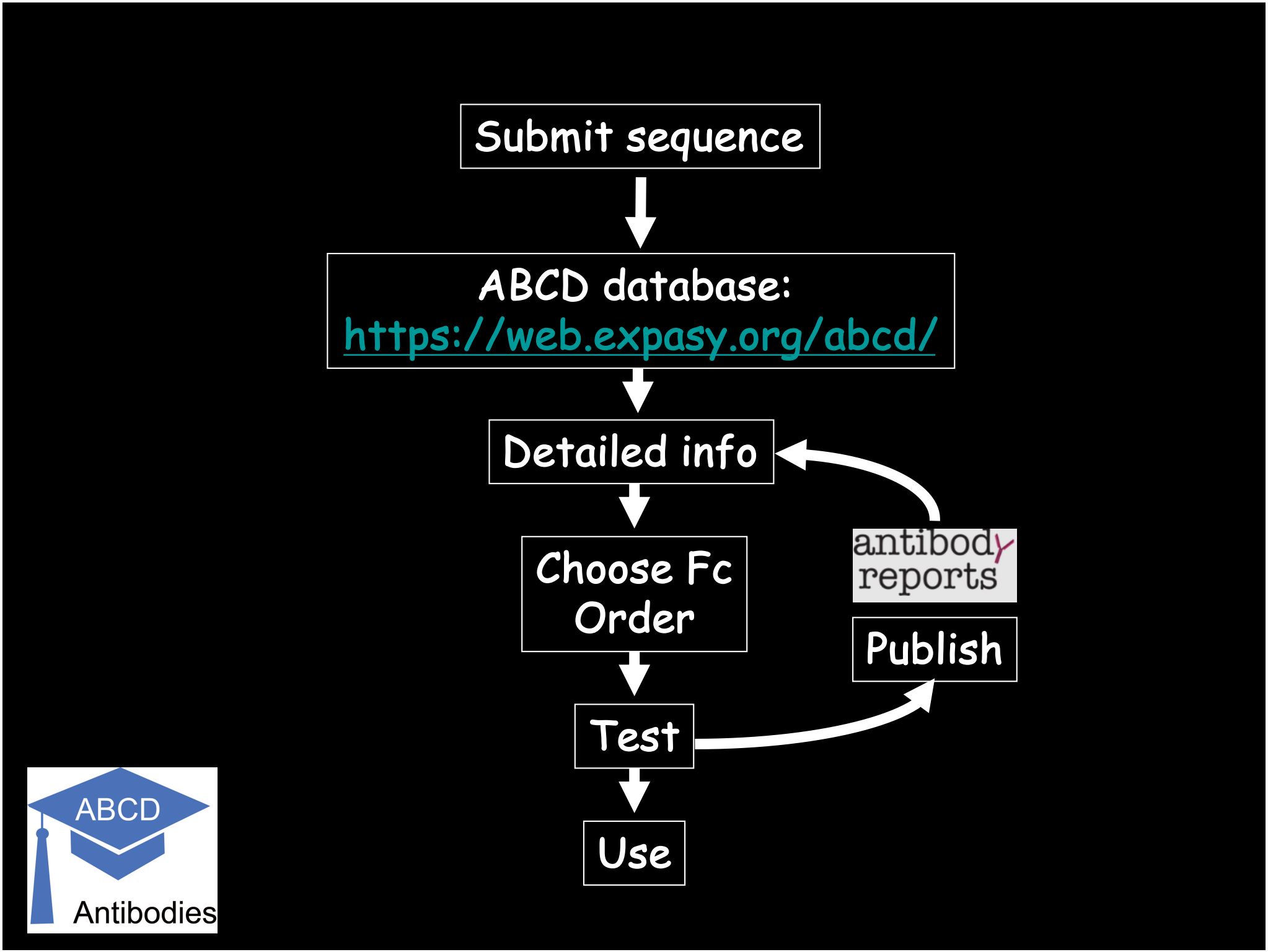
ABCD
Antibodies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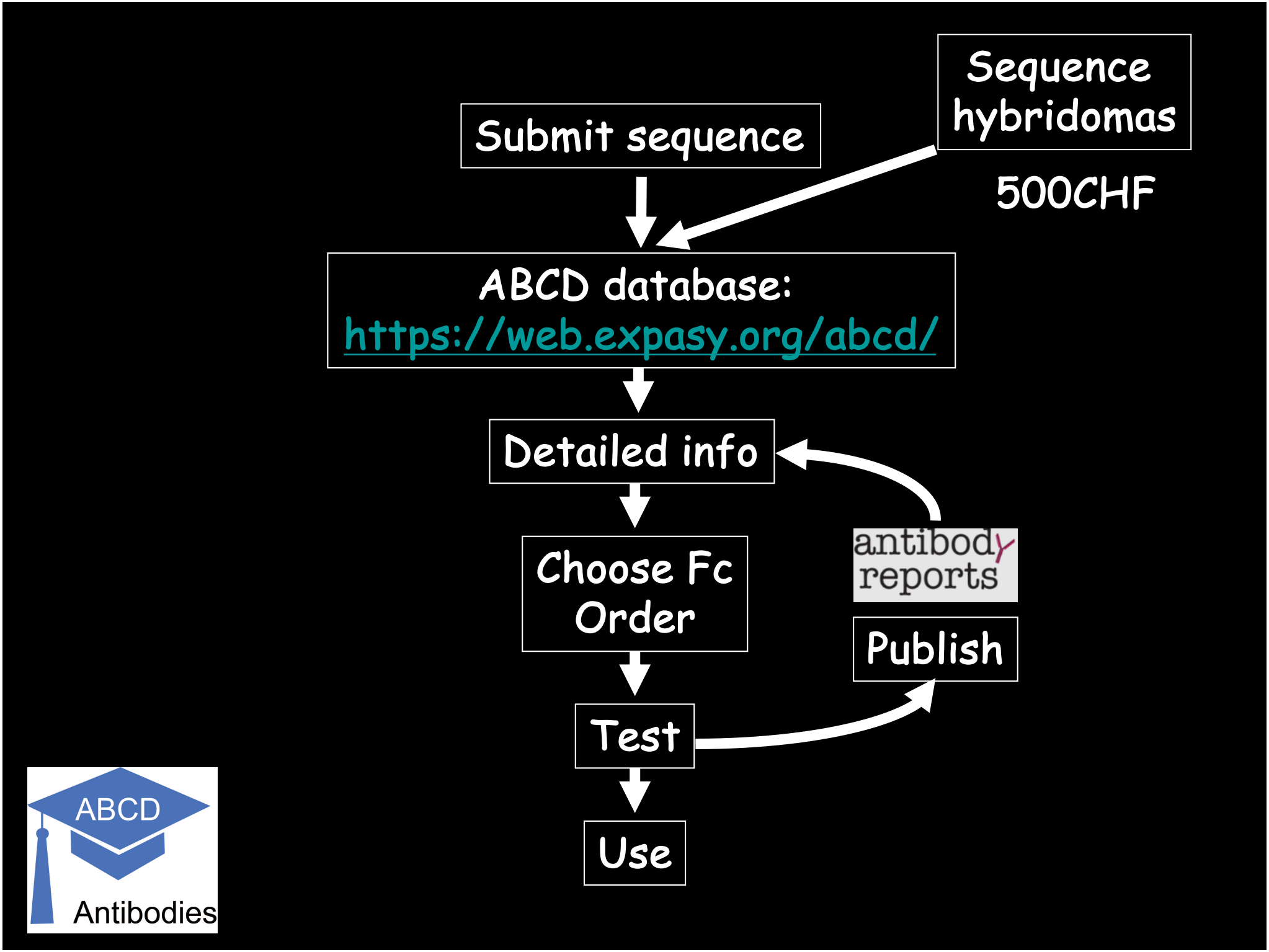
ABCD database:
https://web.expasy.org/abcd/
Detailed info
Choose Fc
Order
Test
Use
antibody
reports
Publish
ABCD
Antibodies


Sequence hybridomas
500CHF
Submit sequence
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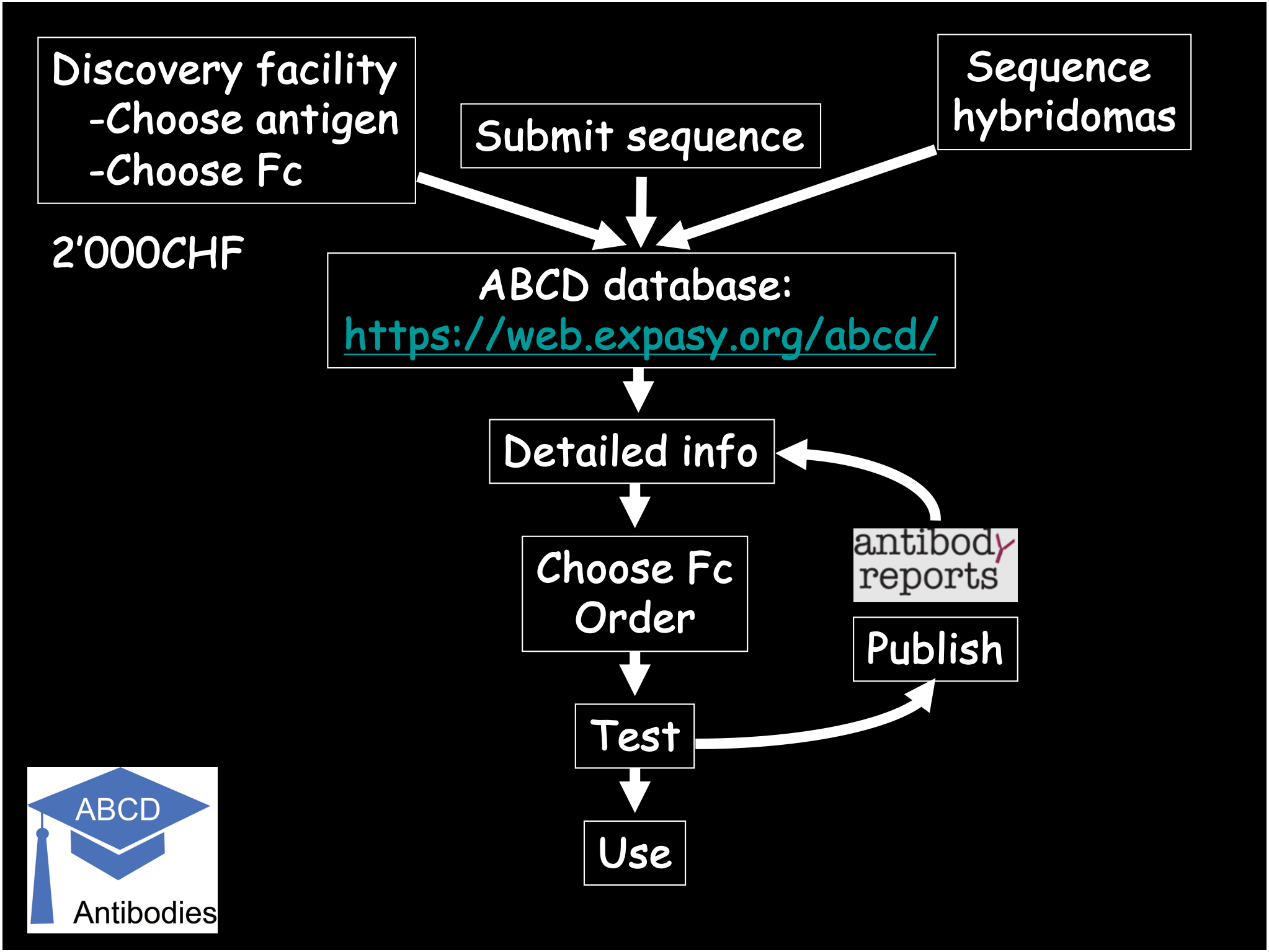
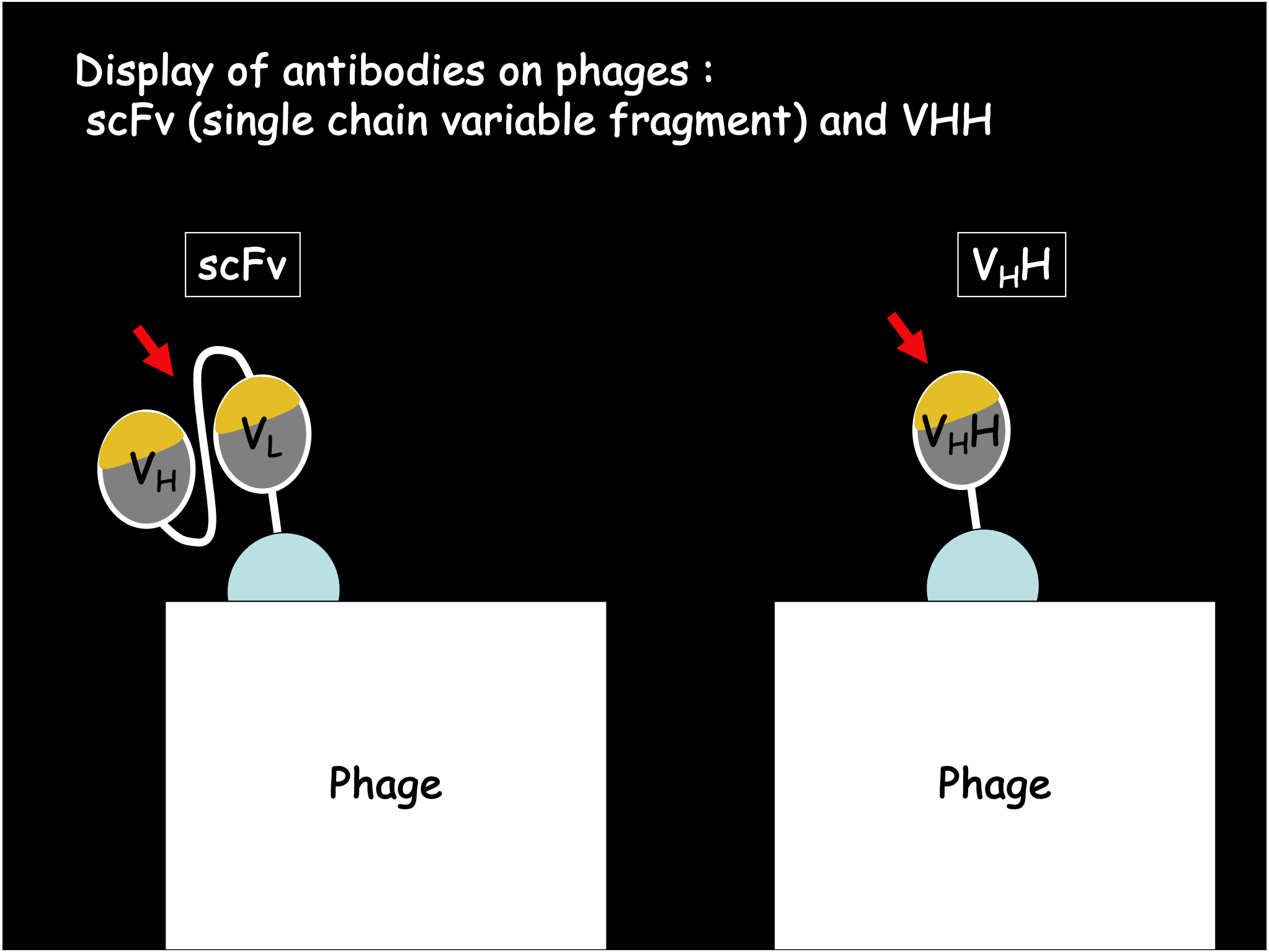
Detailed info
Choose Fc Order
Test
Use
antibody reports
Publish
ABCD
Antibodies


Discovery facility
-Choose antigen
-Choose Fc
2'000CHF
Submit sequence
Sequence hybridomas
ABCD database:
https://web.expasy.org/abcd/
Detailed info
Choose Fc
Order
Test
Use
antibody reports
Publish
ABCD
Antibodies

ABCD discovery service

Library
-Human Abs
-Lama nanobodies

Display of antibodies on phages :
scFv (single chain variable fragment) and VHH



ABCD discovery service

Library
-Human Abs
-Lama nanobodies

→

Select
Antigen-
binding
phages

Selection is done in vitro:
-You control selection conditions
-You can deplete some antibodies before selection

ABCD discovery service

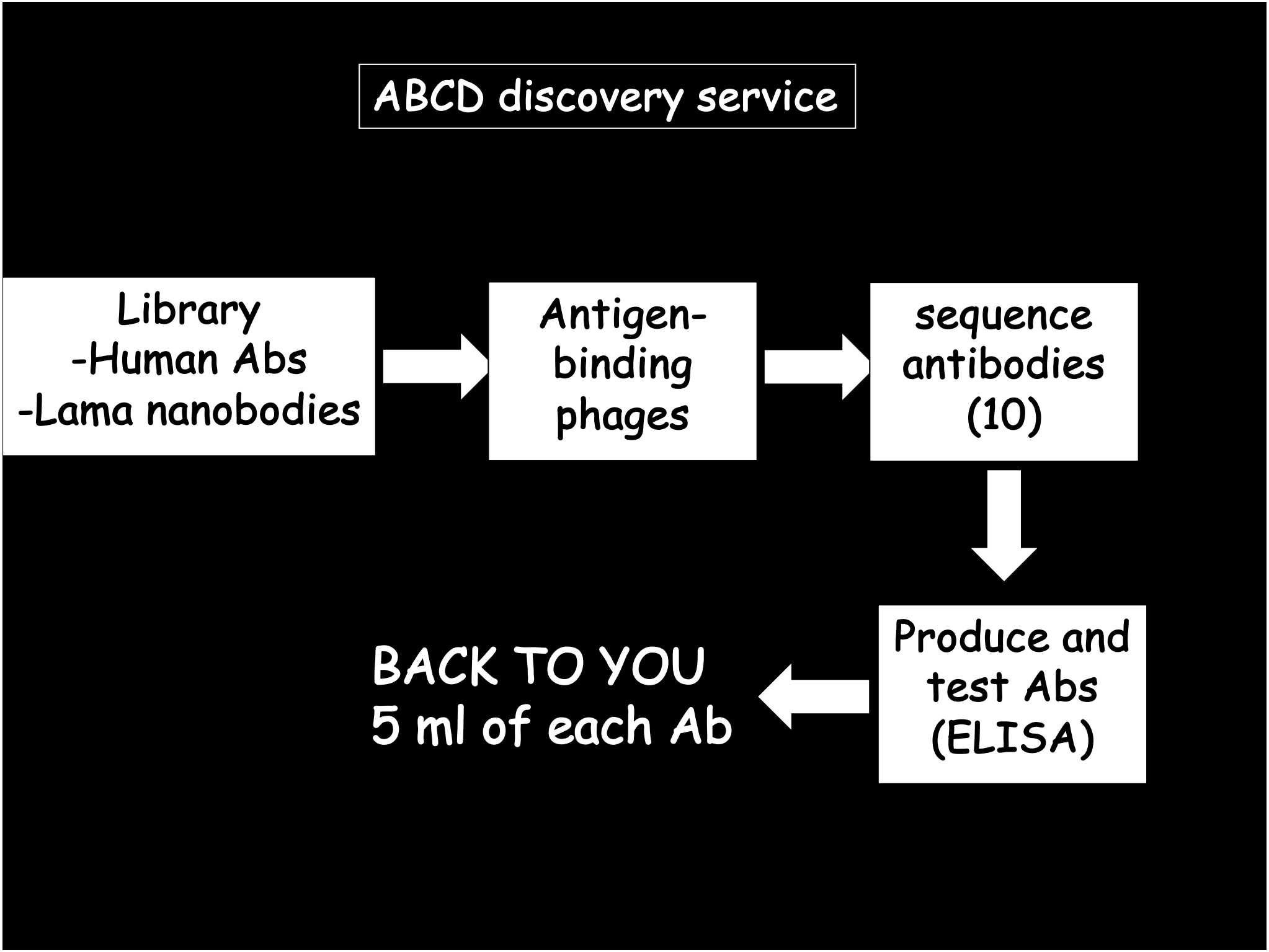
Library
-Human Abs
-Lama nanobodies

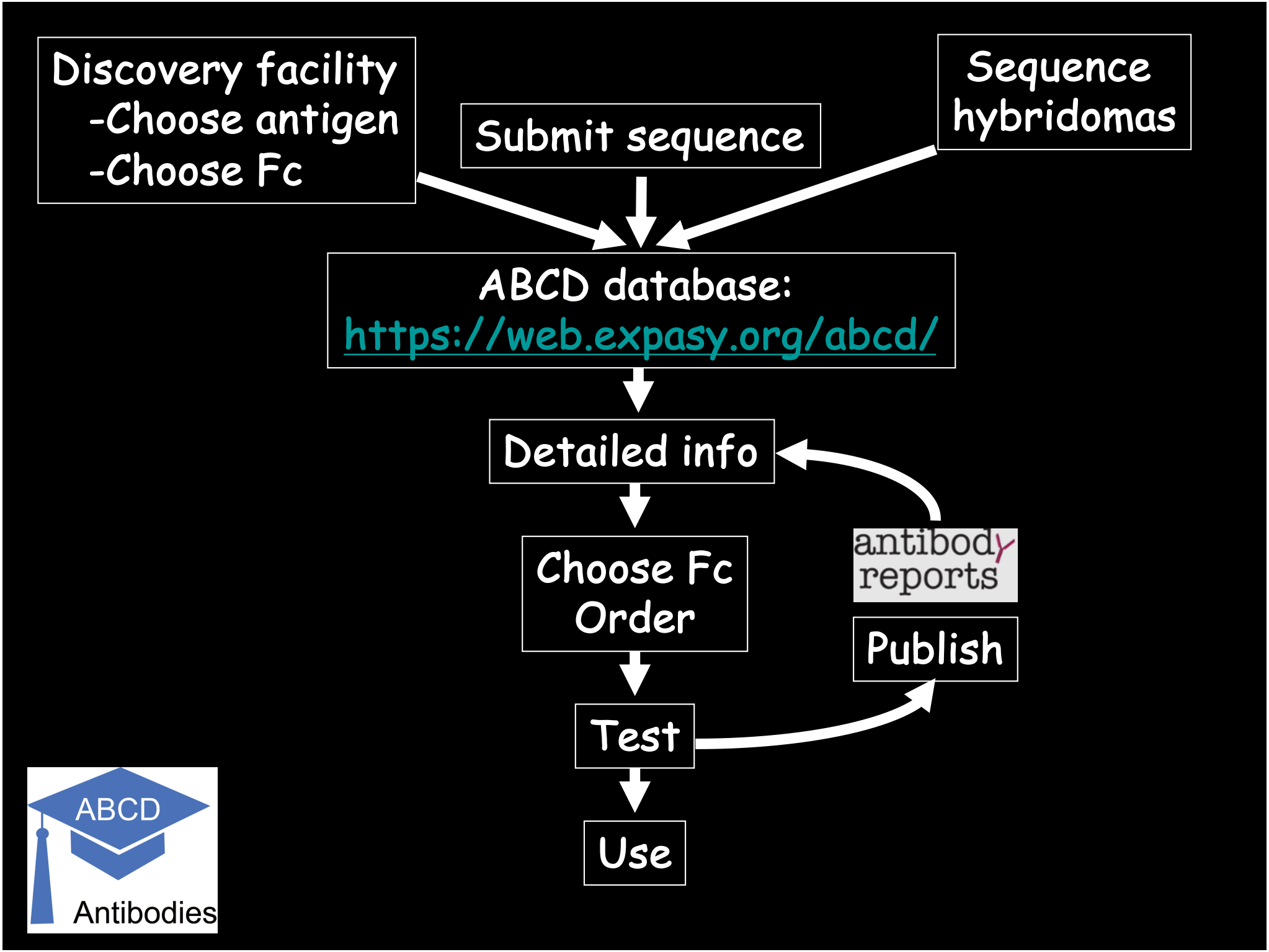
→

Select
Antigen-
binding
phages

Selection is done in vitro:
-You control selection conditions
-You can deplete some antibodies
-You must choose the right antigen: peptide, modified peptide, full protein…

ABCD discovery service


ABCD discovery service
Library
-Human Abs
-Lama nanobodies
Antigen-
binding
phages
sequence
antibodies
(10)
Produce and
test Abs
(ELISA)
BACK TO YOU
5 ml of each Ab


Discovery facility
-Choose antigen
-Choose Fc
Submit sequence
Sequence hybridomas
ABCD database:
https://web.expasy.org/abcd/
Detailed info
Choose Fc
Order
Test
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antibody reports
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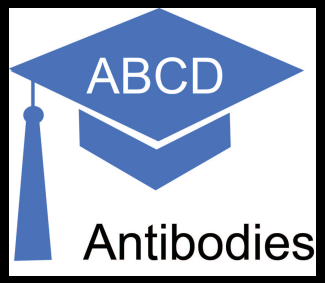
ABCD
Antibodies

1-Recombinant antibodies will replace classical antibodies. The sooner the better.



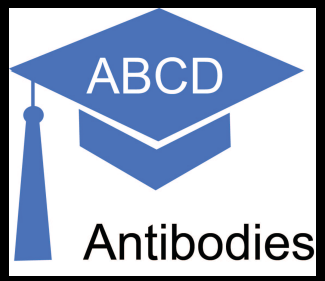
https://web.expasy.org/abcd/

antibodies@unige.ch

1-Recombinant antibodies will replace classical antibodies. The sooner the better.

2-The ABCD project is a community project

- Academia to academia
- Not for profit
- Open access



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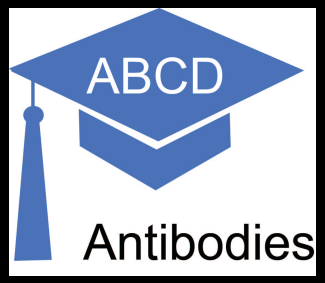
antibodies@unige.ch

1-Recombinant antibodies will replace classical antibodies. The sooner the better.

2-The ABCD project is a community project

- Academia to academia
- Not for profit
- Open access

3-We need you, you need us. Contact us



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