Development of VHH Anti-murine IFNg: First Lama project success at Diaclone

Best practices meeting, 20th of December, 2022

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Contents

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Context of study

Context

- Diaclone's murine IFNg Elispot Kit

Murine IFN-γ ELISpot Kit

PRODUCT SPECIFICATIONS

Target species	Murine
Specificity	Recognizes natural murine IFN-g
Incubation	3h after cell stimulation
Cross Reaction	No cross reactivity with other murine cytokines
Kit Content	Diaclone Pre-coated ELISpot kits include precoated PVDF plates, Detection antibody, Alkaline phosphatase conjugate, BSA, BCIP/NBT ready-to-use substrate buffer.
Synonym	IFN-g IFN-gamma

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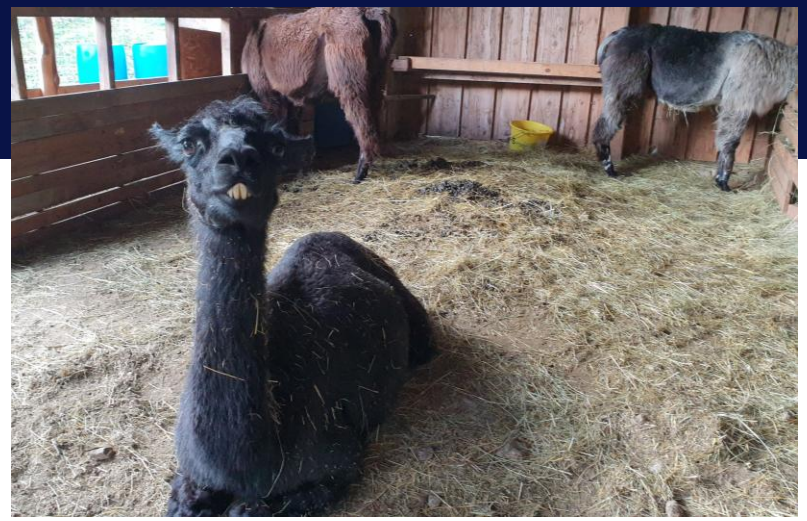
Context - Diaclone’s murine IFNg Elispot Kit

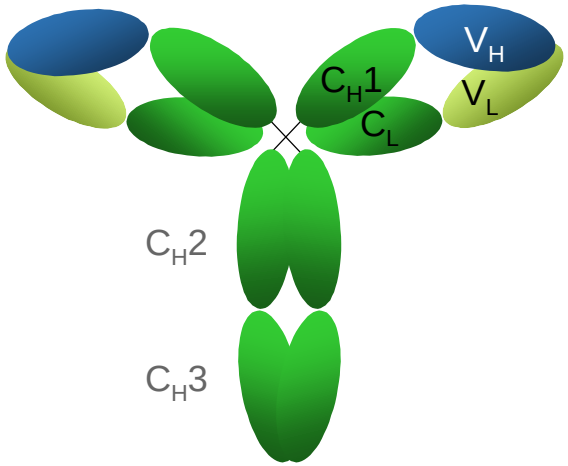
- Work with commercial Mab DB-1 (coating) and polyclonal serum from Rabbit (detection)
- Stock of polyclonal decrease (< 2 years)
  - new batches of polyclonal haven't same quality than older
- Emergency to find a substitute for Elispot kit

Generalities

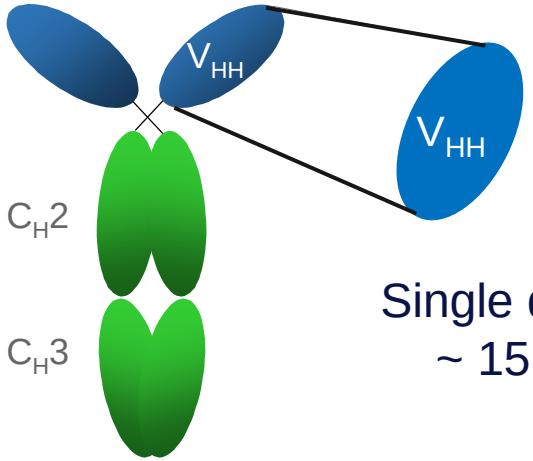
Lamas (Camelidae)



- Convential and HC IgG



Conventional~150 kDa



HC ~ 80 kDa

Single domain
~ 15 kDa

- Phage Display
- Focusing on V_{HH}
- Called ”Nanobodies”

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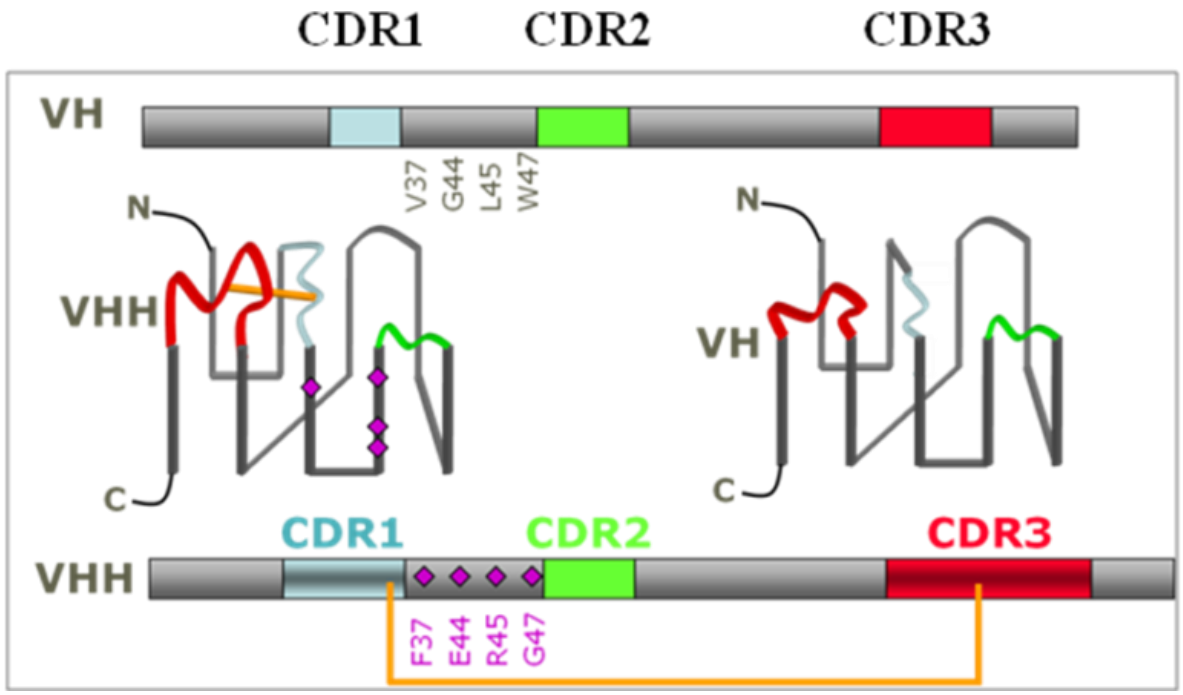
V_{HH} nanobodies

- Used in medical fields: oncology, infection, immunity, Imagery

- Structure



Single domain
~ 15 kDa

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Goal

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- Develop Mabs against mIFNg to replace the Rabbit polyclonal in the Diaclone's Elispot Kit

- Use the Lama model to find V_{HH} (First project at Diaclone)

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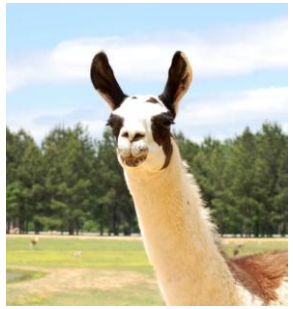
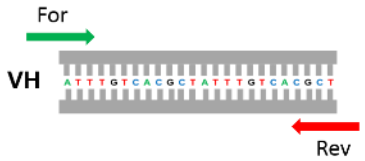
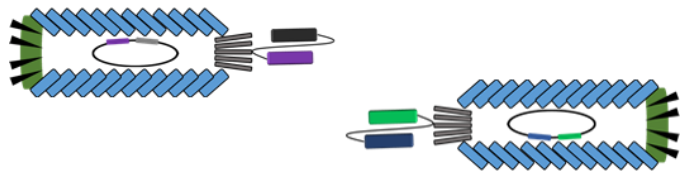
Work flow

Phage Display

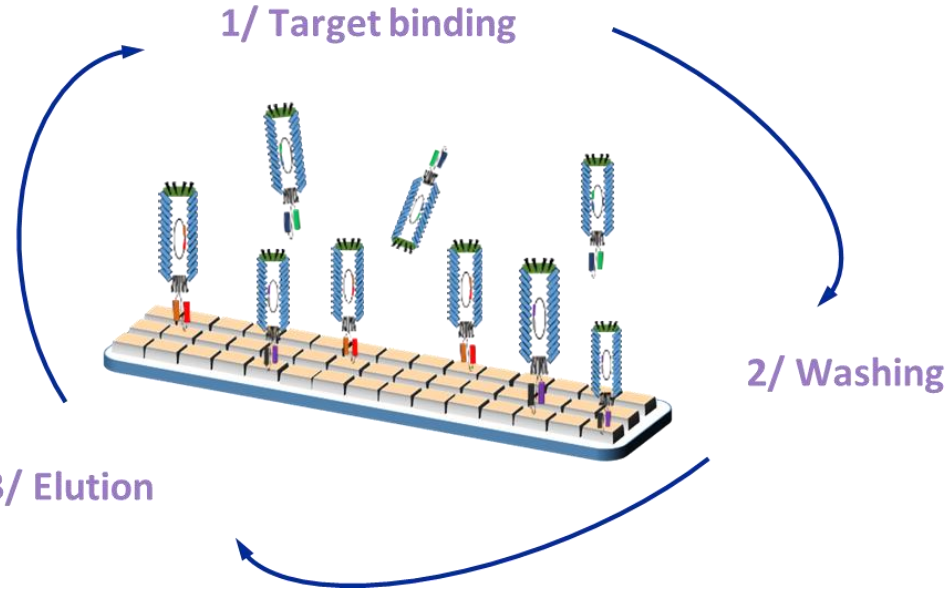


(6 weeks)





Recombinant Phages



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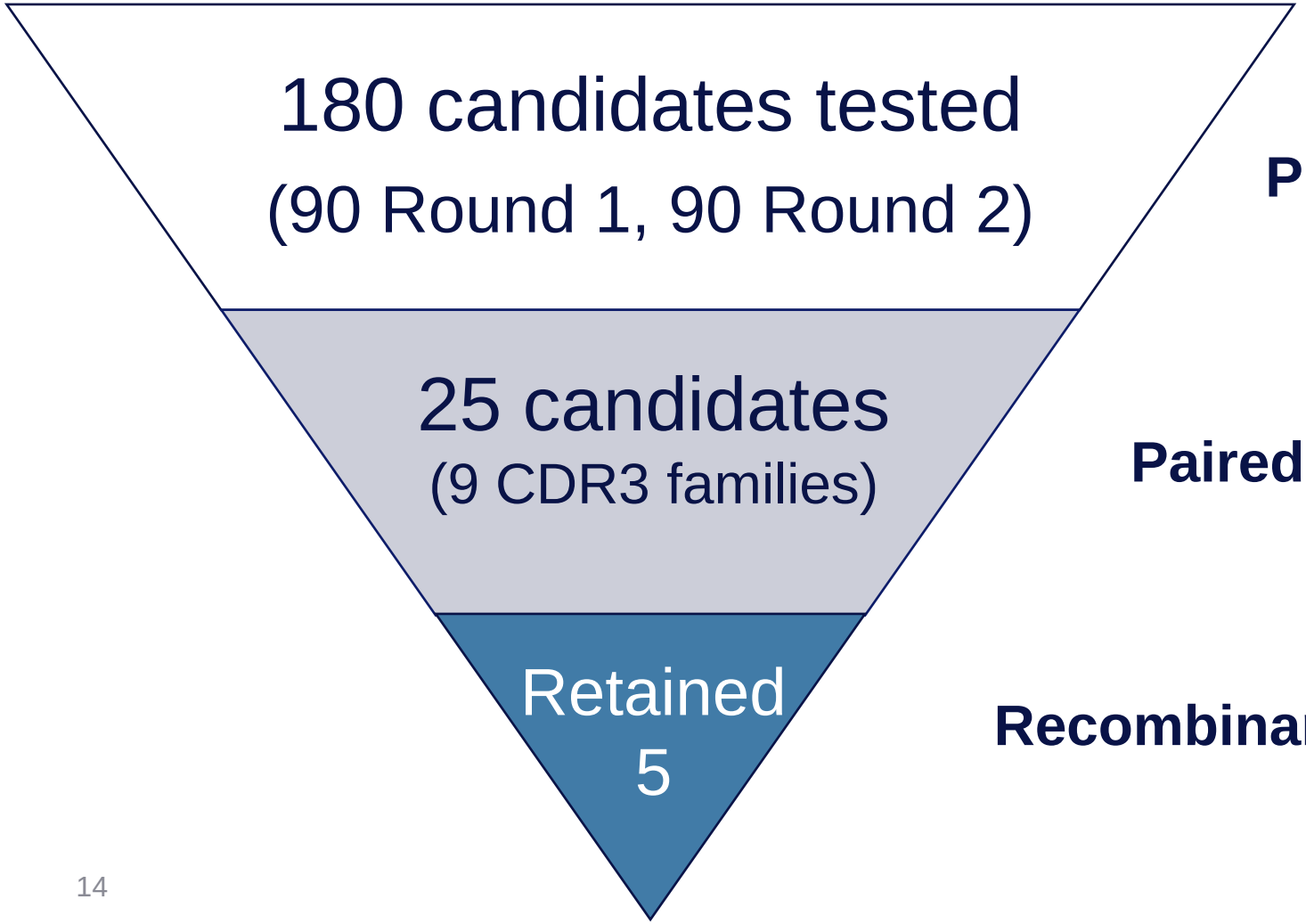
Screening



Production Periplasmic extracts

Paired with the DB-1 in ELISA

Recombinant step (Engineering)

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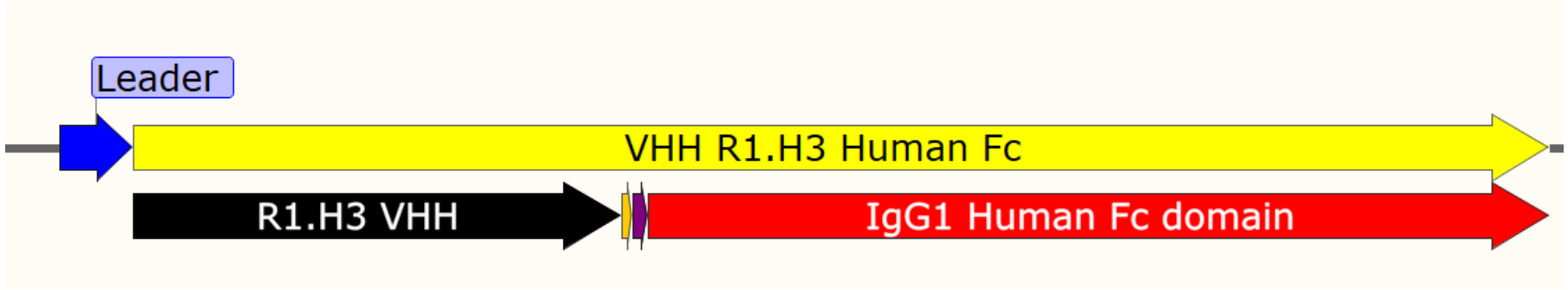
Engineering

- Optimized vector for mammalian expression (CHO)

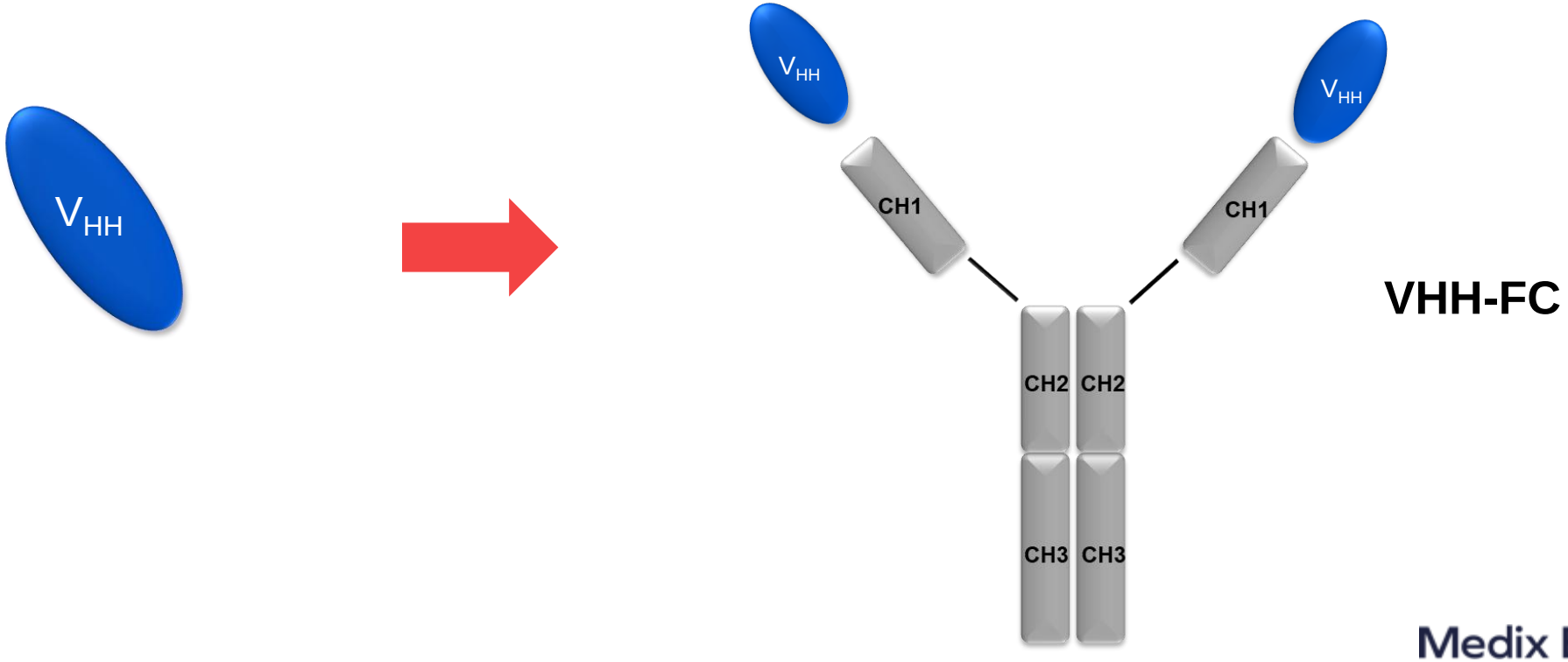


- Construction in fusion with IgG1 Human Fc

- 4 of 5 candidates were good produced

Engineering

- Construction in fusion with IgG1 Human Fc



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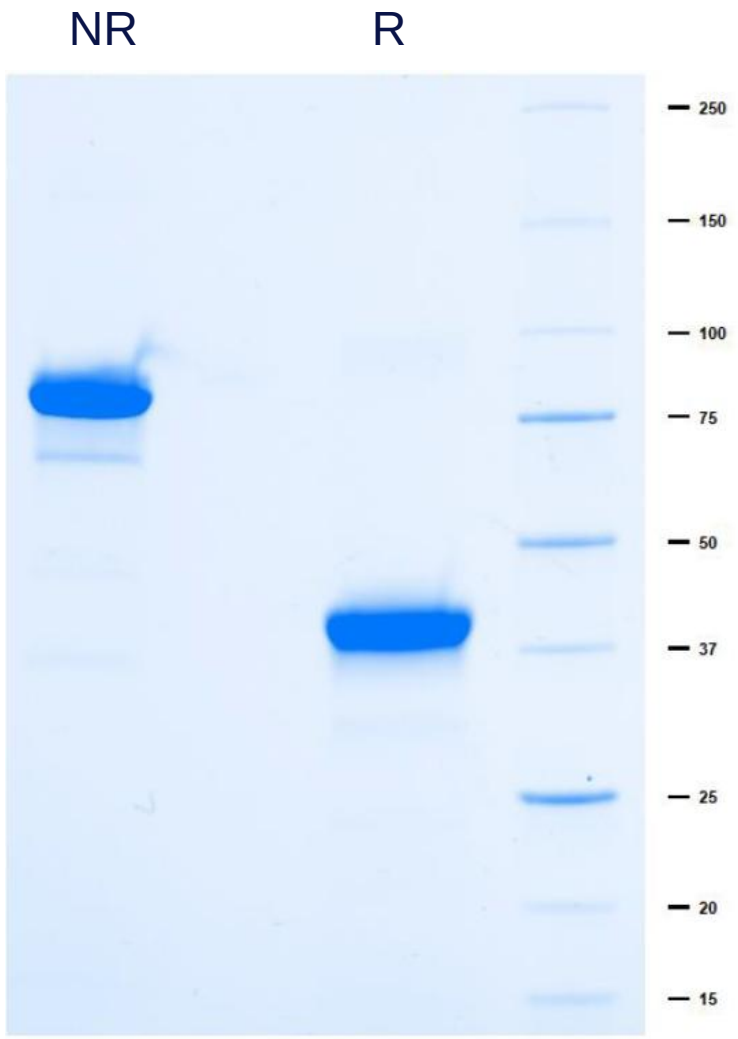
Recombinant production



- Good yield of production (> 100 µg/ml)
- Good purity (> 90 %)
- Transfer to IC lab (Harmonie) for test in Elispot kit

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ELISpot assays

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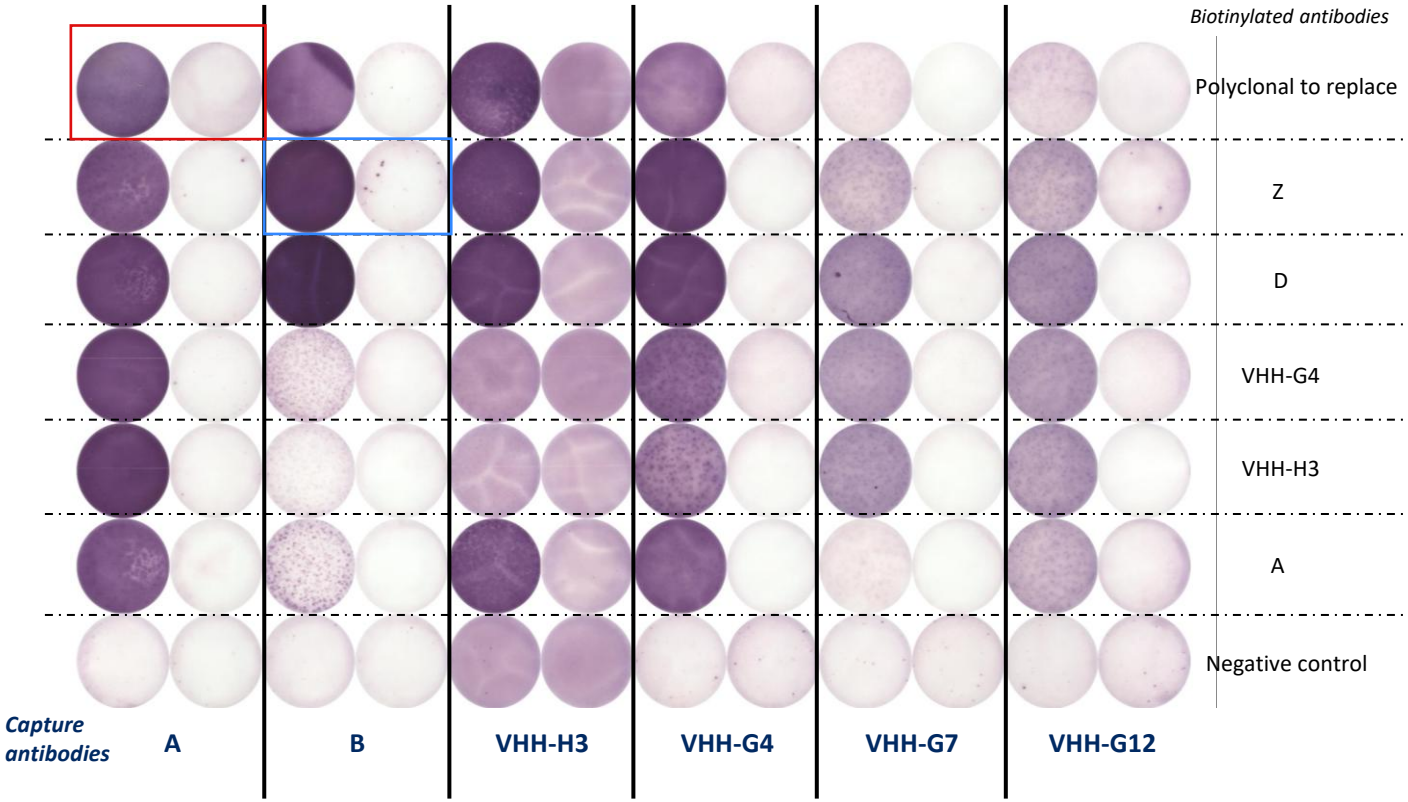
Pairs Screening

Reference (Antibody pairs used in Diaclone kits)
Competitor



Capture Antibody	A		B		C		VHH H3		VHH G4		D	
murine IFNg	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
Detection Antibody												
A	1,5	0,1	0,4	0,0	1,2	0,2	5,0	0,1	5,0	0,2	0,7	0,1
Polyclonal to replace	5,0	0,1	5,0	0,1	5,0	0,1	5,0	0,2	5,0	0,2	5,0	0,0
Z	1,5	0,0	5,0	0,1	1,0	0,1	5,0	0,1	5,0	0,2	0,8	0,1
VHH H3	5,0	0,1	0,1	0,1	5,0	0,1	0,2	0,2	0,3	0,3	5,0	0,1
VHH G4	5,0	0,3	0,2	0,2	5,0	0,1	2,9	2,8	0,4	0,4	5,0	0,2
D	2,3	0,1	5,0	0,1	1,9	0,1	5,0	0,4	5,0	0,3	1,6	0,1
Negative control	0,1	0,0	0,1	0,0	0,0	0,0	0,1	0,1	0,1	0,1	0,0	0,0

A / B / C / Z / D are commercial antibodies

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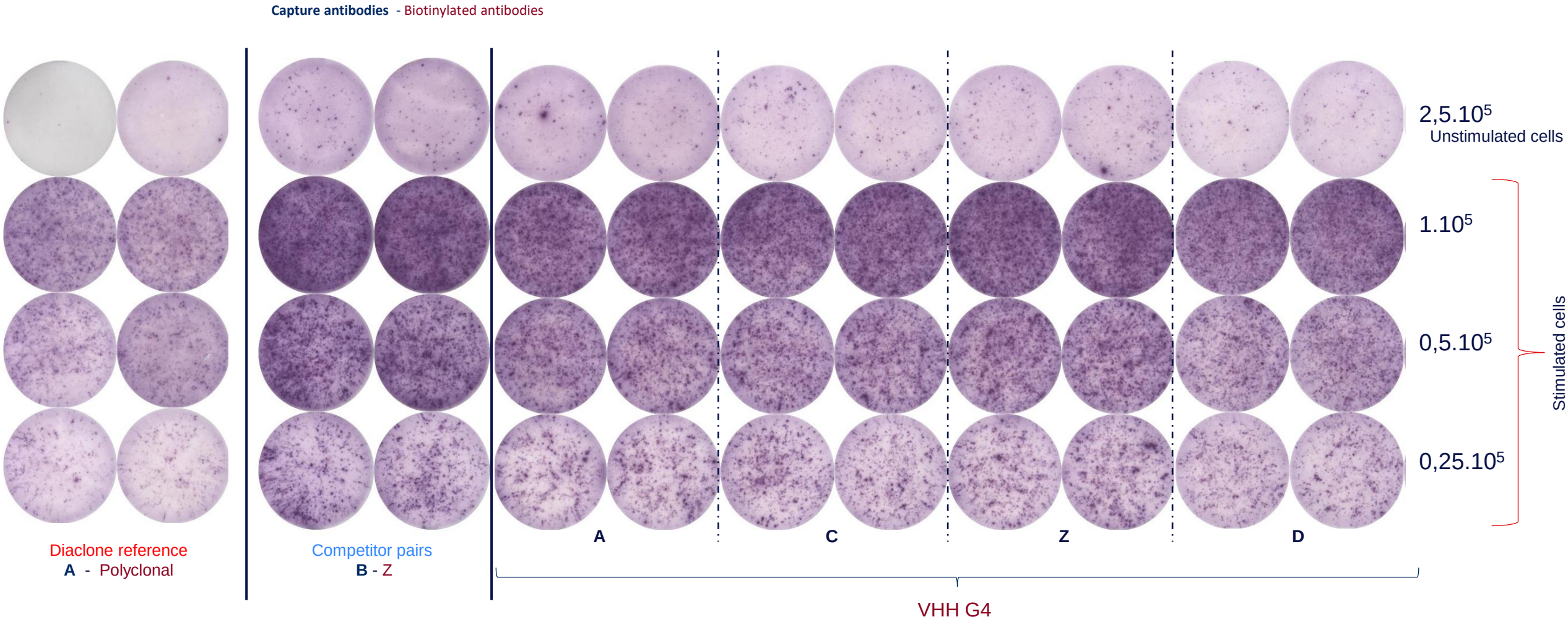
Pairs Comparison



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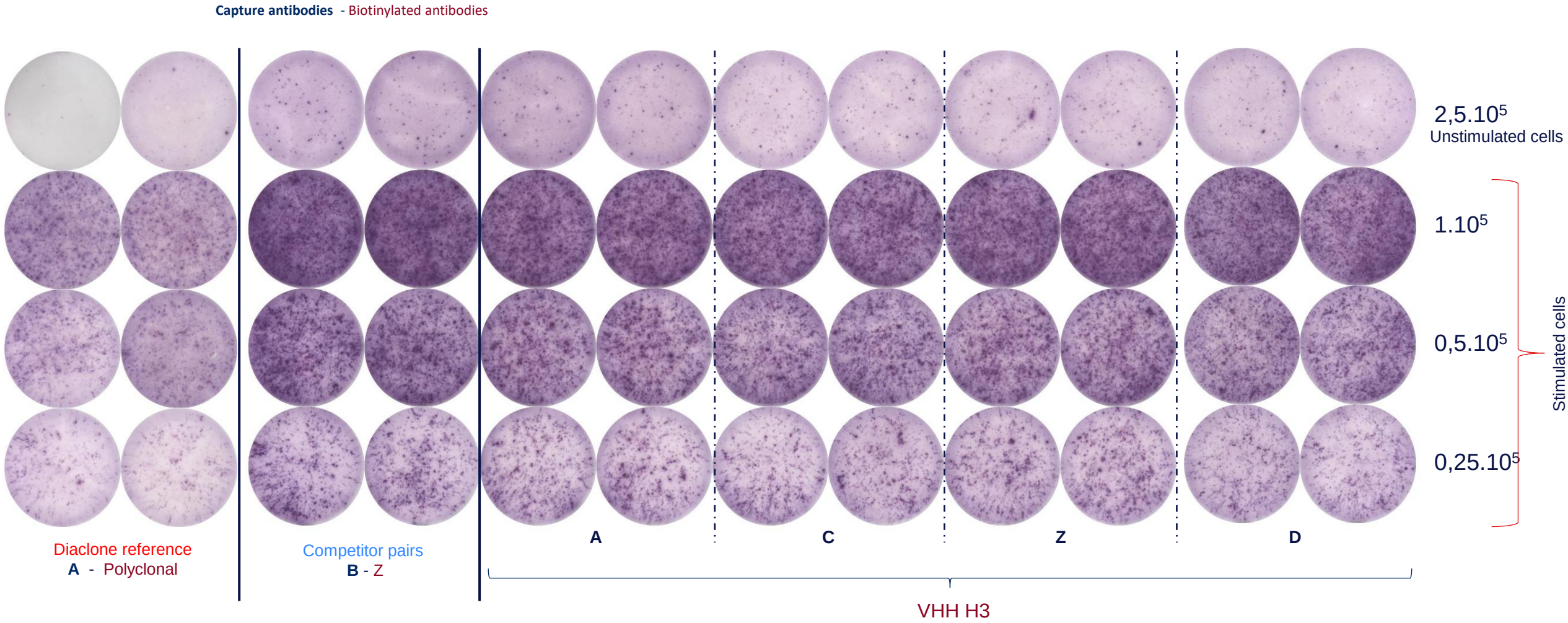
Pairs Comparison



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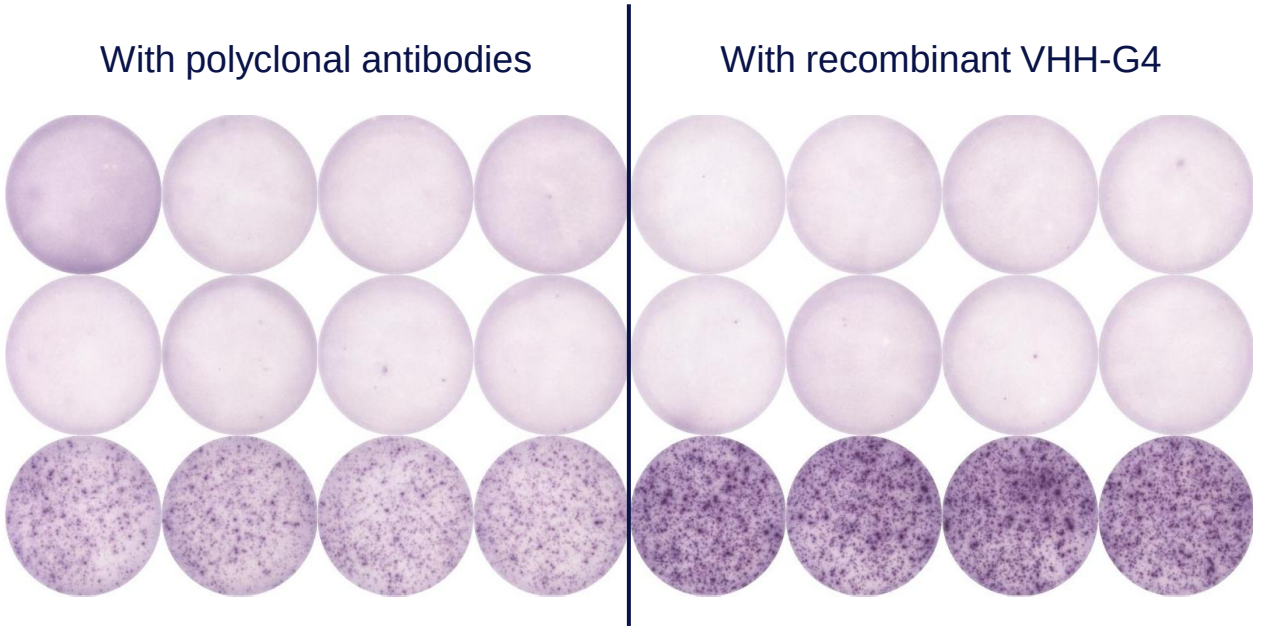
Recombinant VHH choice

Evaluation of different criterion :

- Stability
- Sensitivity
- Spot size and intensity
- Noise background
- Reproducibility

→ VHH-G4



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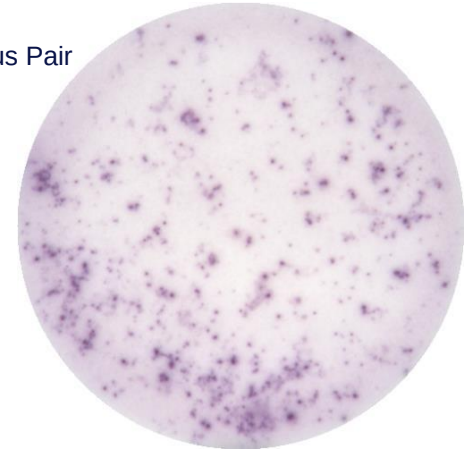
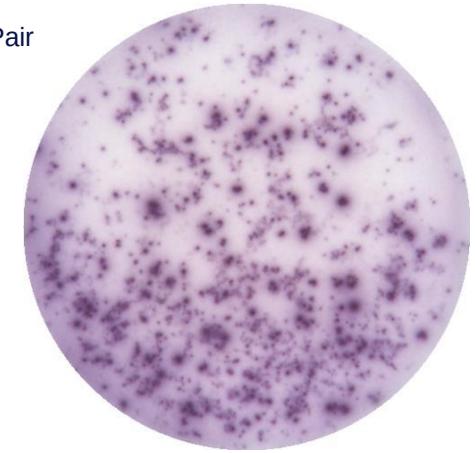
ELISpot mIFNg improvement

Previous Pair



New Pair



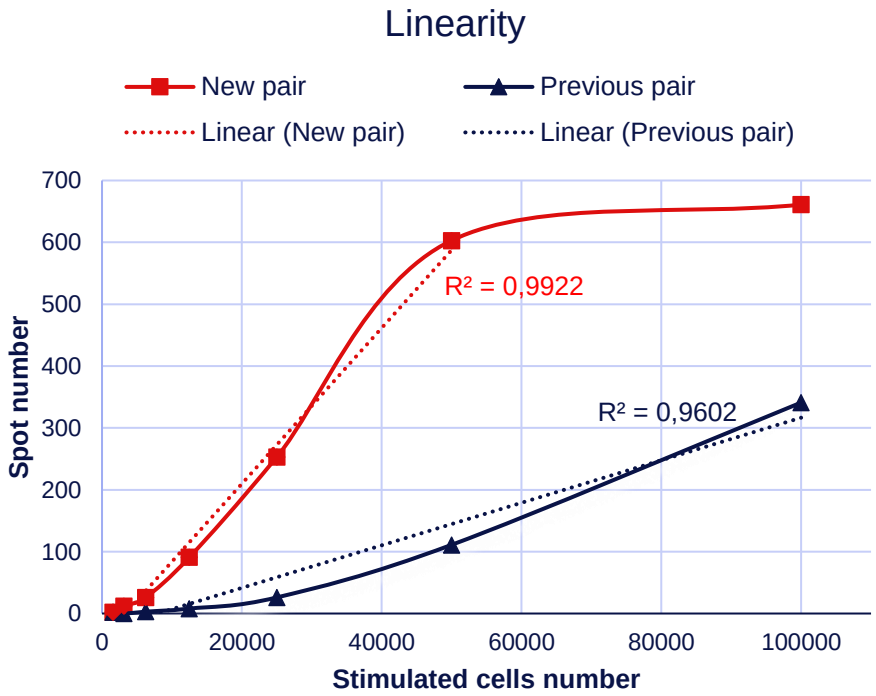
6 250 stimulated cells

Rabbit polyclonal

Cells / well	n	Mean number of spots per well	Min	Max	CV%
100000	12	TNTC	NA	NA	NA
50000	12	828	748	889	4,2
25000	12	746	654	817	7,4
12500	12	344	235	521	19,1
6250	12	89	41	113	26,4

VHH recombinant

Cells / well	n	Mean number of spots per well	Min	Max	CV%
100000	12	TNTC	NA	NA	NA
50000	12	TNTC	NA	NA	NA
25000	12	887	841	920	2,8
12500	12	669	568	725	7,5
6250	12	215	178	278	13,8



→ Better linearity, sensitivity and reproducibility

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Conclusions

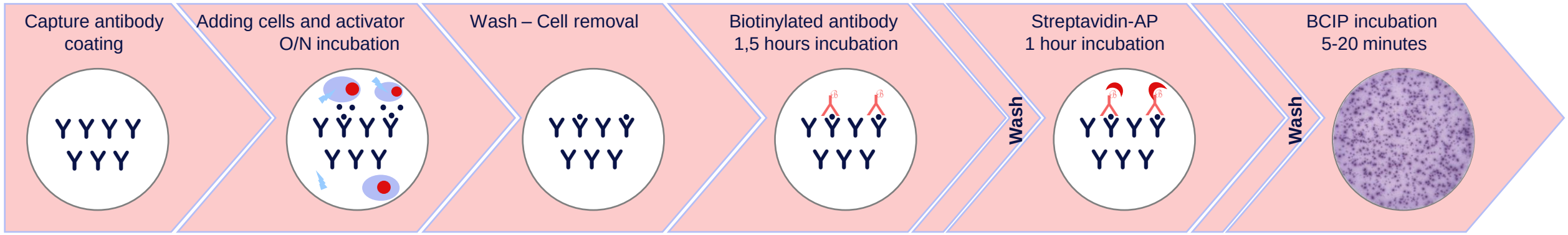
Summary

- Lama phage display approach is another alternative to develop mab against difficult targets (with low immunogenicity)
- Fast track development (no more than 2 months after immunization end to have recombinant candidates to test)
- V_{HH} can be good substitutes to polyclonal rabbit serum in ELISpot applications

Workflow – Screening

- Production of 95 candidates from panning Round 1 & 2 (190 in total)
- Periplasmic extracts tested in ELISA to select best candidates paired with the DB-1.
- 25 positives candidates (from 9 different CDR3 families)
- 5 candidates retained for recombinant step

ELISpot Assay



Well:

Capture antibody:

Cell:

Activator:

Analytes:

Biotinylated antibody:

Streptavidin-AP:

BCIP substrate:

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