Generation of a Diverse VHH Phage Display Library from Naive Llamas

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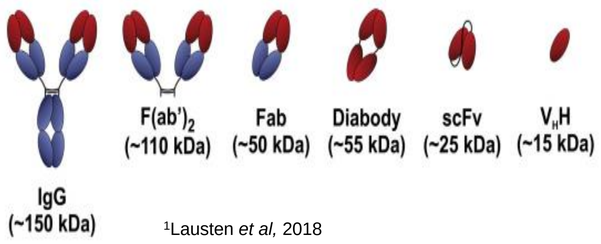
ABSTRACT

VHH antibodies (or nanobodies) have been very useful for numerous antibody discovery applications. Due to their small size (12-15 kDa), oftentimes longer H3-CDRs, ease of expression in E. coli, and increased thermostability, VHH fragments are ideal for antibody discovery against challenging targets. VHH fragments have reportedly been able to recognize cryptic epitopes of GPCRs, one of the most medicinally relevant family of antibody targets. A large, high-quality VHH library will prove to be very helpful for the discovery of such potent antibodies. We describe the construction of a VHH library from naive llamas to capture the potential diversity of antibodies for target discovery. NGS sequencing of the constructed library was used to determine repertoire diversity and CDR lengths. Finally, validation of the library is underway using multiple targets.

INTRODUCTION

- Small size (15 kDa) and monomeric
- Easy to clone and express, with generally better yield than scFvs/Fabs
- Very stable and soluble
- High specificity and affinity to targets
- Long CDR3 loops can potentially access different epitopes
- Ready-to-use phage display library
- Diagnostic and therapeutic indications
  - Can recognize a variety of antigens
    - Viral particles (current flu indication)
    - Toxins
    - Multi-span TM proteins



[1]Lausten *et al*, 2018

RESULTS

LakePharma's Naive VHH Phage Display Library Construction

- **Isolation**
  - PBMCs isolated from 13 naive llamas
- **Library generation**
  - Llama VHH phage library constructed using primer sets from RNA samples derived from naive animals
  - 2.05 x 10^9 library size (theoretical diversity of library: 2.5 x 10^7)
  - Rescued titer: 1.16 x 10^{12} cfu/mL
- **Sequence validation underway**
  - NGS analysis of library
- **Panning validation underway**
  - 3-4 rounds of panning selections against various targets
    - Target B: recombinant antigen
    - Target C: viral coat protein
    - Target D: GPCR

Figure 2: Naive llama VHH library generation

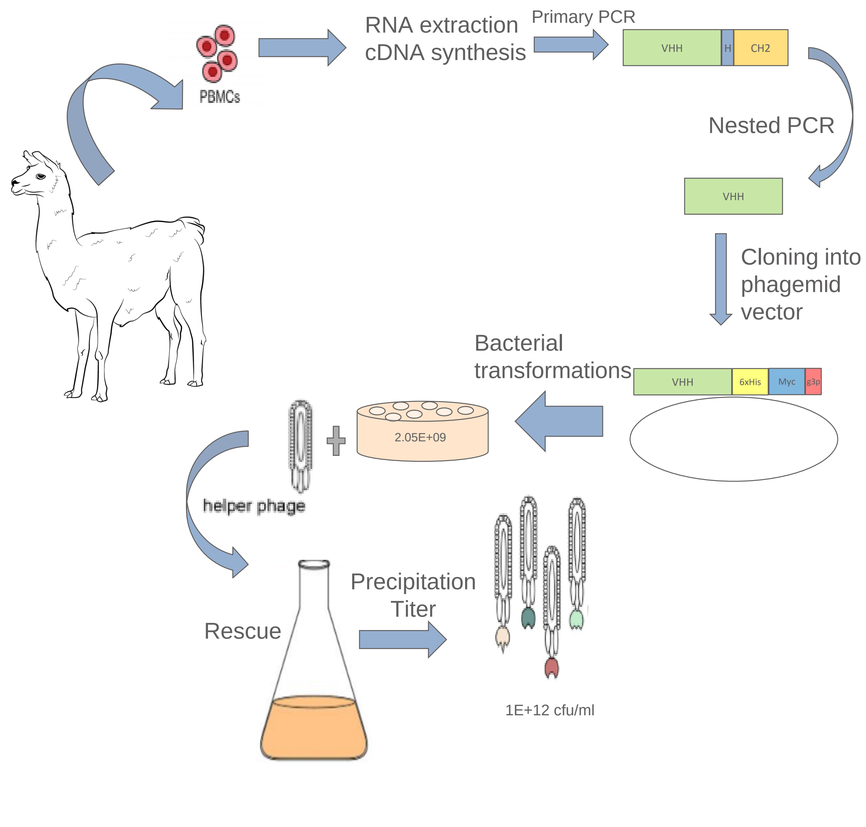
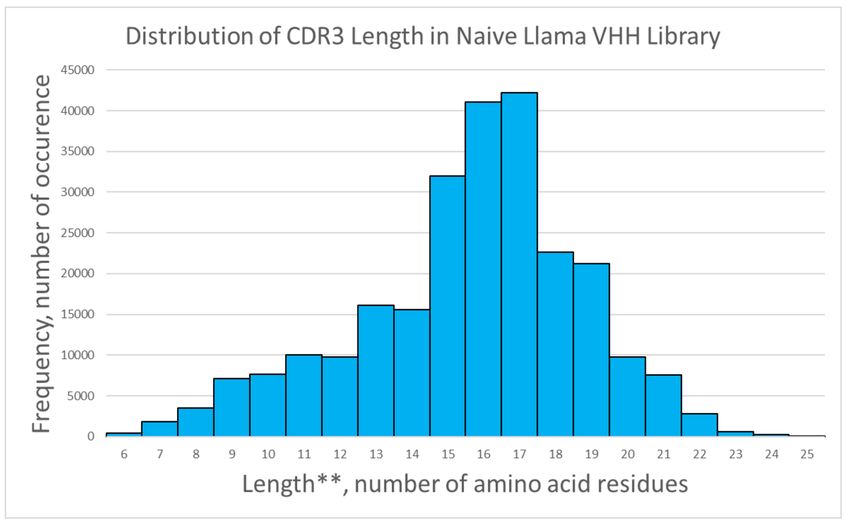


Figure 3. NGS sequencing overview

A. NGS sequencing data overview	
Number of QC-ed reads	359,816
Number of unique clones	227,031
Number of unique CDR sets	167,592
Number of unique CDR1	16,052
Number of unique CDR2	54,595
Number of unique CDR3	40,098

B. CDR3 length distribution



** IMGT annotation

Figure 4. Phage display panning strategy

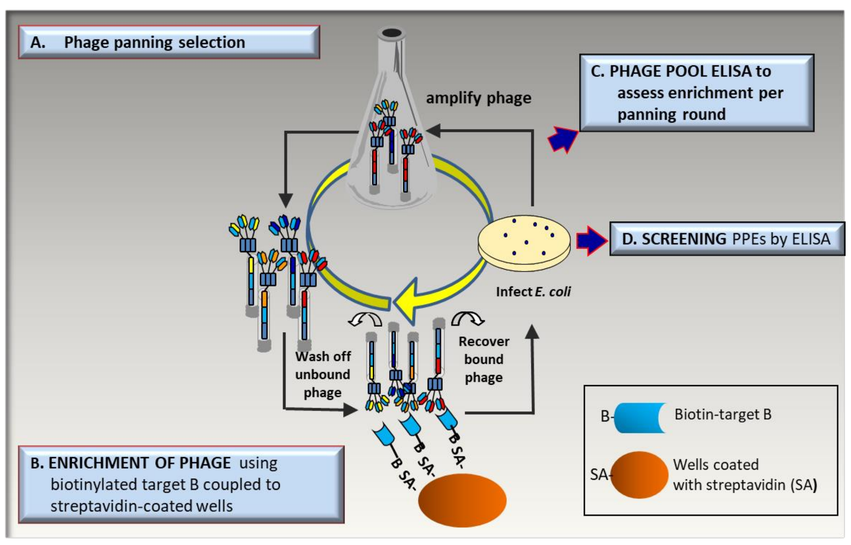
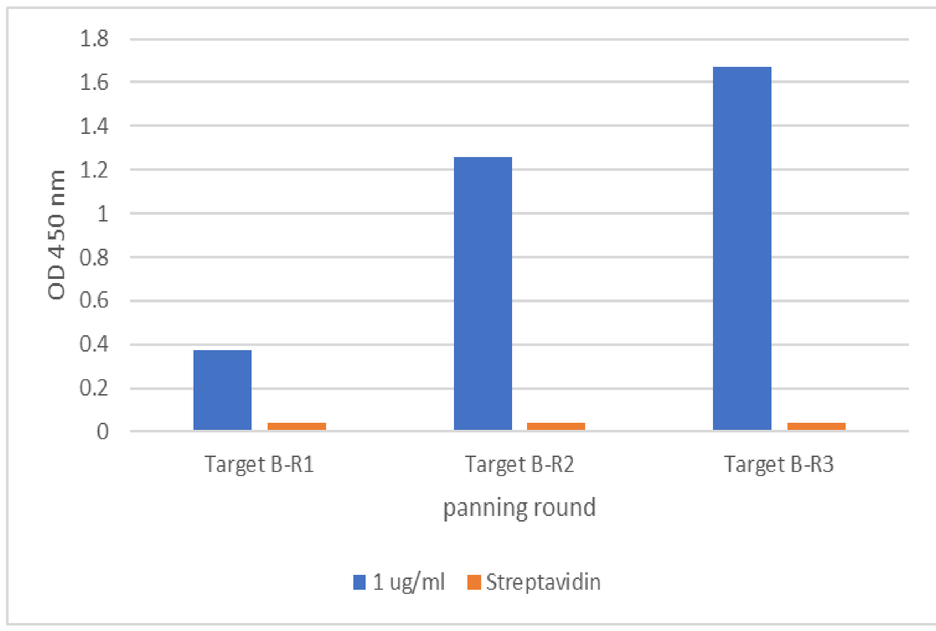


Table 1. Panning output titers against Target B

Panning Rd	Target B (ug/ml)	Washes	Input titer	Output Titer	Enrichment
R1	10	3X PBST + 3X PBS	5.80E+11	1.85E+05	3.19E-07
R2	5	5X PBST + 5X PBS	1.10E+10	5.83E+07	5.3E-03
R3	2	10X PBST +10X PBS	1.20E+10	8.8E+08	7.33E-02

Figure 5. Screening validation of Target B panning (Phage Pool ELISA)



Antigen: 1 ug/ml Target B coated overnight at 4°C
Phage titer: ~1 x 10^{10} pfu
Detection with 1:5000 HRP anti-M13 mAb and detection with TMB

Figure 6. Screening and sequence validation of Target B panning

A. Screening summary

Ag	Panning Strategy	PPEs screened	Positive Binders (3x background)	# Sequenced	# Uniques	# CDR uniques	# Overall Uniques	# Overall CDR Uniques
Target B	Immobilized	186 (R3)	180	24	8	3	11	3
		93 (R2)	38	38	8	3		
		93 (R1)	6	6	5	2		

B. Sequence variability of 3 clones identified against Target B

	FR1			FR2								FR3					J-Region	
IMGT positions	1	14	15	39	40	44	45	46	48	49	51	55	66	78	82	83	88		
Unique clone 1	E	Q	P	M	N	Q	A	P	G	Q	E	D	K	M	N	V	S	Q	L
Unique clone 2	Q	Q	A	M	G	R	P	P	A	Q	D	T	E	I	I	A	Y	K	L
Unique clone 3	Q	K	A	M	G	Q	P	P	A	Q	Y	T	E	I	I	A	H	K	Q

CONCLUSIONS

- Naive VHH phage display library has been generated from 13 llama donors
- Library diversity was assessed by NGS
- Unique binders against target B were identified after initial validation
- Validation against other antigen formats such as viral proteins and GPCRs are being initiated

REFERENCES

[1] A.H. Lausten *et al*. Toxicon 146 (2018) 151-175