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.

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 10^12 library size (theoretical diversity of library: 2.5 x 10^12)
  - 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

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