(12) UK Patent Application (19) GB (11) 2616707 (13) A

(43) Date of A Publication 20.09.2023

(21) Application No: 2300352.8

(22) Date of Filing: 10.01.2023

(71) Applicant(s):
RxBiologics Limited
(Incorporated in the United Kingdom)
The Dorothy Hodgkin Building,
Babraham Research Campus, Babraham CB22 3FH,
Cambridgeshire, United Kingdom

(72) Inventor(s):
Jonathan James William Finlay
Hannah Louise Wall
Christine Joy Rossant
Yun Yun Sanders

(74) Agent and/or Address for Service:
Reddie & Grose LLP
The White Chapel Building,
10 Whitechapel High Street, London, E1 8QS,
United Kingdom

(51) INT CL:
C07K 16/00 (2006.01) ***C12N 15/10*** (2006.01)
C40B 40/10 (2006.01)

(56) Documents Cited:
WO 2014/128628 A1
mAbs, Vol.1, Gupta, A. et al., "Directed evolution of an...", pp.268-280
PLoS One, Vol.7, 2012, Venet, S. et al., "Transferring the characteristics...", Article No.: e43471
mAbs, Vol.11, 2019, Valadon, P. et al., "ALTHEA gold libraries...", pp.516-531
mAbs, Vol.13, 2021, Teixeira, A.A.R., "Drug-like antibodies with high affinity...", Article No.: 1980942

(58) Field of Search:
Other: **WPI, EPODOC, Patent Fulltext, BIOSIS, MEDLINE, XPESP**

(54) Title of the Invention: **Methods**
Abstract Title: **Antibody Library**

(57) A method of identifying and/or selecting an antibody molecule which binds specifically to a target antigen. The method comprises the steps of: (1) providing a single-chain variant fragment (scFv) phage display library; wherein each scFv nucleic acid construct comprises: (a) a first nucleic acid sequence encoding either a human germline VH scaffold amino acid sequence including an HCDR1 and HCDR2, or a modified version thereof having at least 80% sequence identity to the human germline VH scaffold nucleic amino acid 10 sequence; (b) a second nucleic acid sequence encoding either an scFv human germline VL scaffold amino acid sequence including an LCDR1, LCDR2 and LCDR3, or a modified version thereof having at least 80% sequence identity to the human germline VL scaffold nucleic amino acid sequence; and (c) a third nucleic acid sequence encoding either a human B-cell donor-derived amino acid sequence including an HCDR3, or modified version thereof having at least 15 80% sequence identity to the human B-cell donor-derived amino acid sequence; (2) screening the scFv phage display library against the target antigen and selecting one or more binding scFv molecules; (3) identifying an enriched nucleic acid sequence encoding an HCDR3 from the one or more binding scFv molecules; (4) generating a fragment antigen-binding (Fab) display library of a population of engineered Fab nucleic acid constructs encoding Fab molecules including the enriched nucleic acid sequence or a modified version thereof; and (5) screening the Fab display library against the target antigen and selecting one or more binding Fab molecules.

GB 2616707 A

Figure 1.

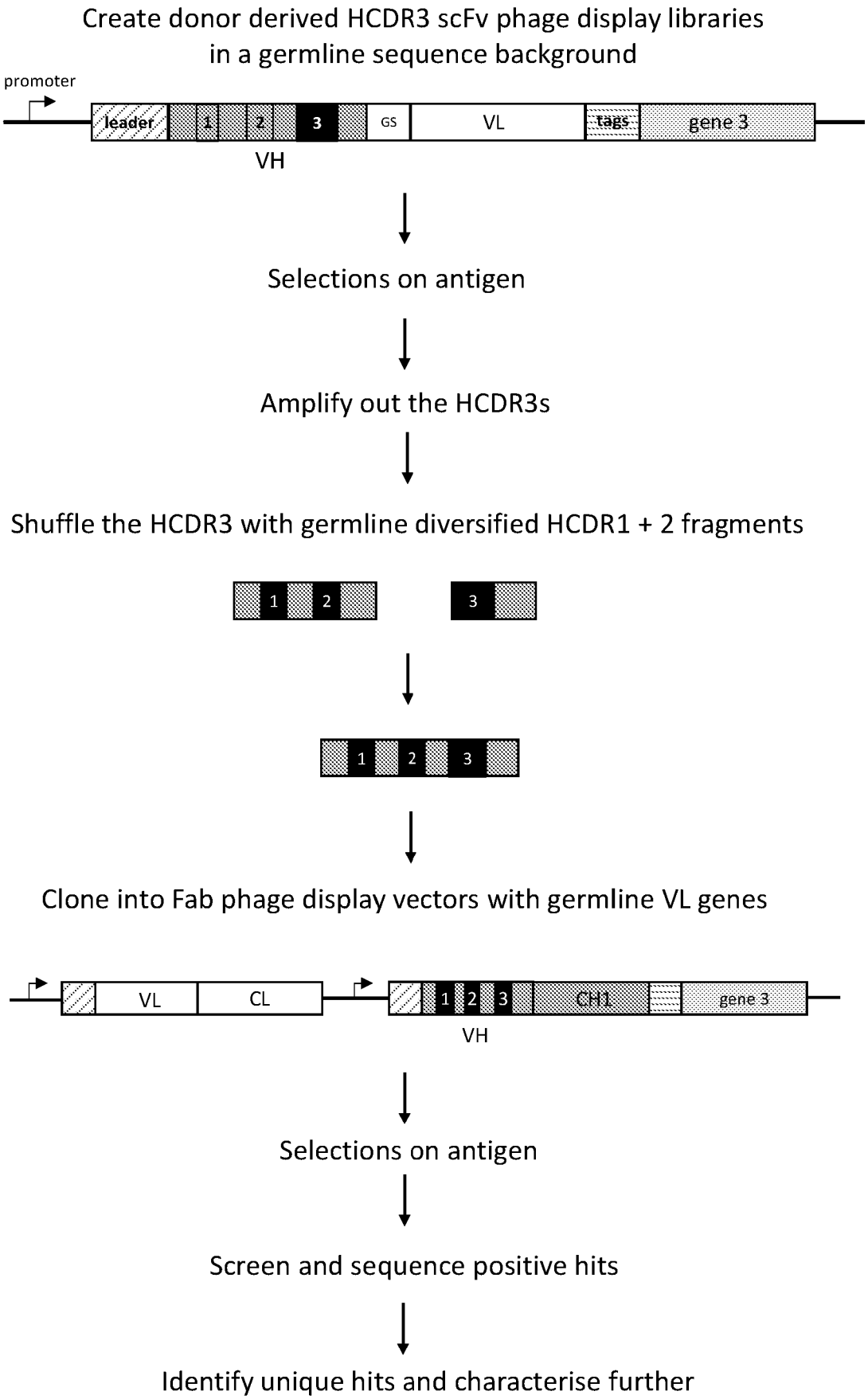

Create donor derived HCDR3 scFv phage display libraries in a germline sequence background
promoter
leader
1
2
3
GS
VL
tags
gene 3
VH
Selections on antigen
Amplify out the HCDR3s
Shuffle the HCDR3 with germline diversified HCDR1 + 2 fragments
1
2
3
1
2
3
Clone into Fab phage display vectors with germline VL genes
VL
CL
1
2
3
CH1
gene 3
VH
Selections on antigen
Screen and sequence positive hits
Identify unique hits and characterise further

Figure 2.

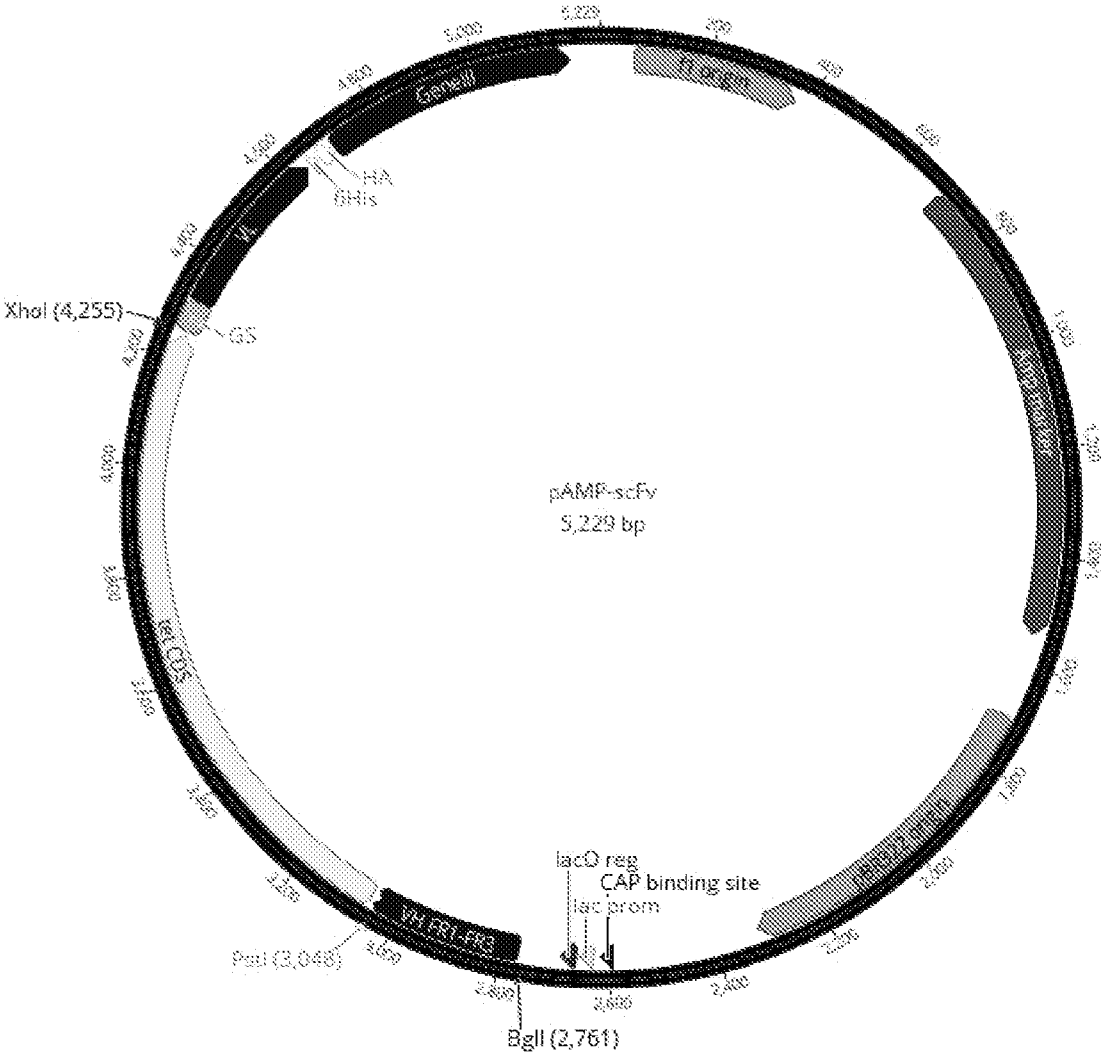

pAMP-scFv
5,229 bp
GeneIII
HA
6His
L
XhoI (4,255)
GS
ori COS
lacO reg
CAP binding site
lac prom
VH FR1-FR3
PstI (3,048)
BglII (2,761)

Figure 3.

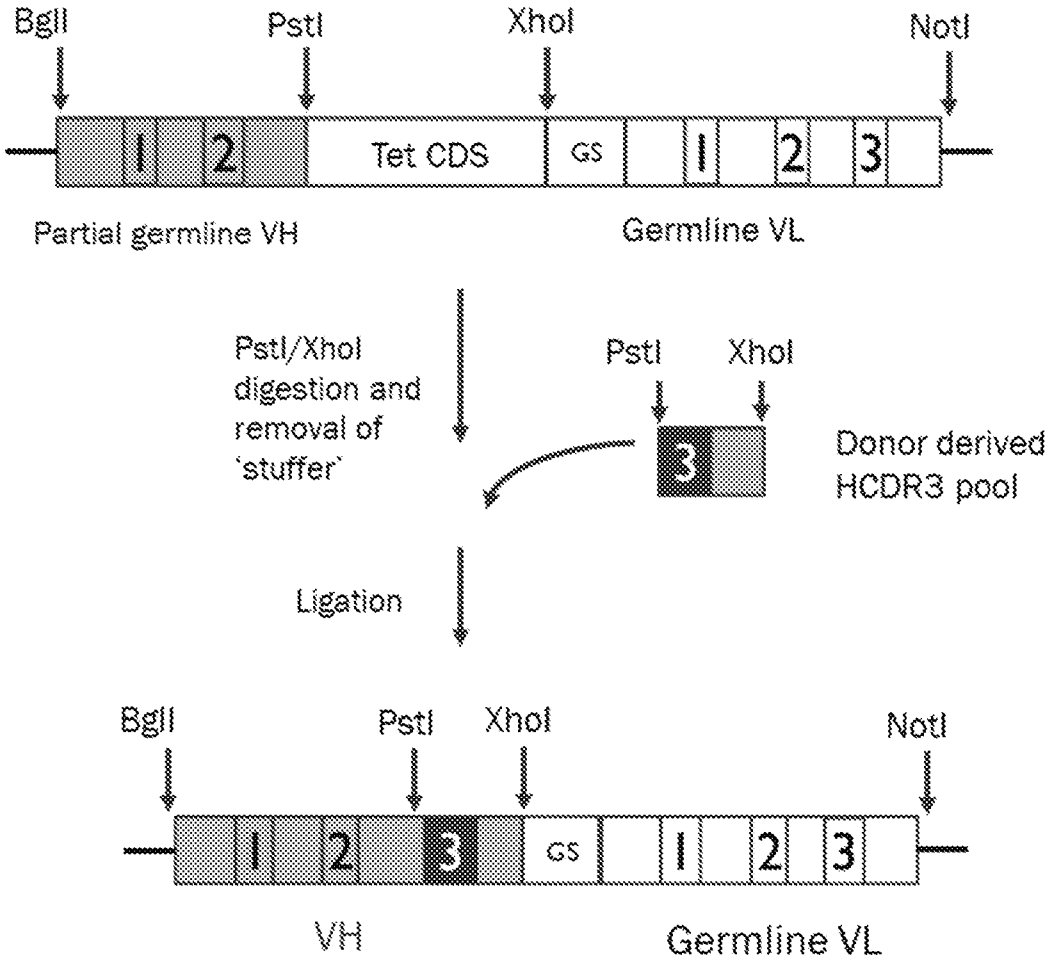
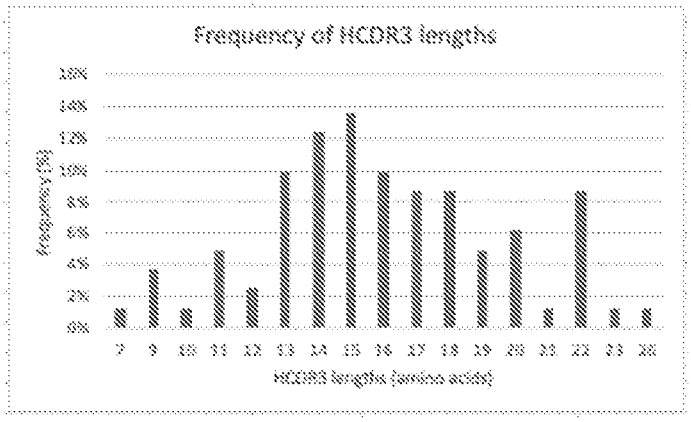
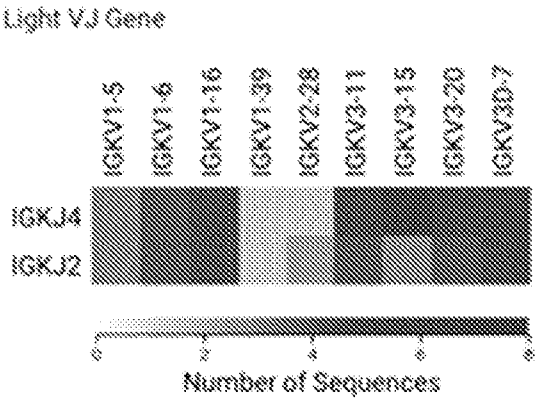

BglI
PstI
XhoI
NotI
1
2
Tet CDS
GS
1
2
3
Partial germline VH
Germline VL
PstI/XhoI
digestion and
removal of
'stuffer'
PstI
XhoI
3
Donor derived
HCDR3 pool
Ligation
BglI
PstI
XhoI
NotI
1
2
3
GS
1
2
3
VH
Germline VL

Figure 4.

A.


Frequency of HCDR3 lengths
HCDR3 lengths (amino acids)

B.


Light VJ Gene
IGKV1-5
IGKV1-6
IGKV1-16
IGKV1-39
IGKV2-28
IGKV3-11
IGKV3-15
IGKV3-20
IGKV3D-7
IGKJ4
IGKJ2
Number of Sequences

C.

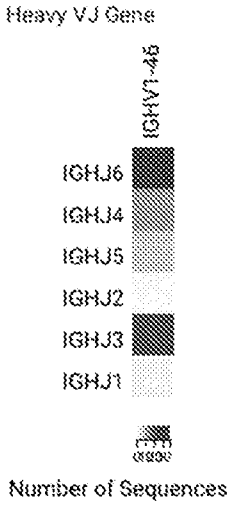

Heavy VJ Gene
IGHV1-46
IGHJ6
IGHJ4
IGHJ5
IGHJ2
IGHJ3
IGHJ1
Number of Sequences

Figure 5.

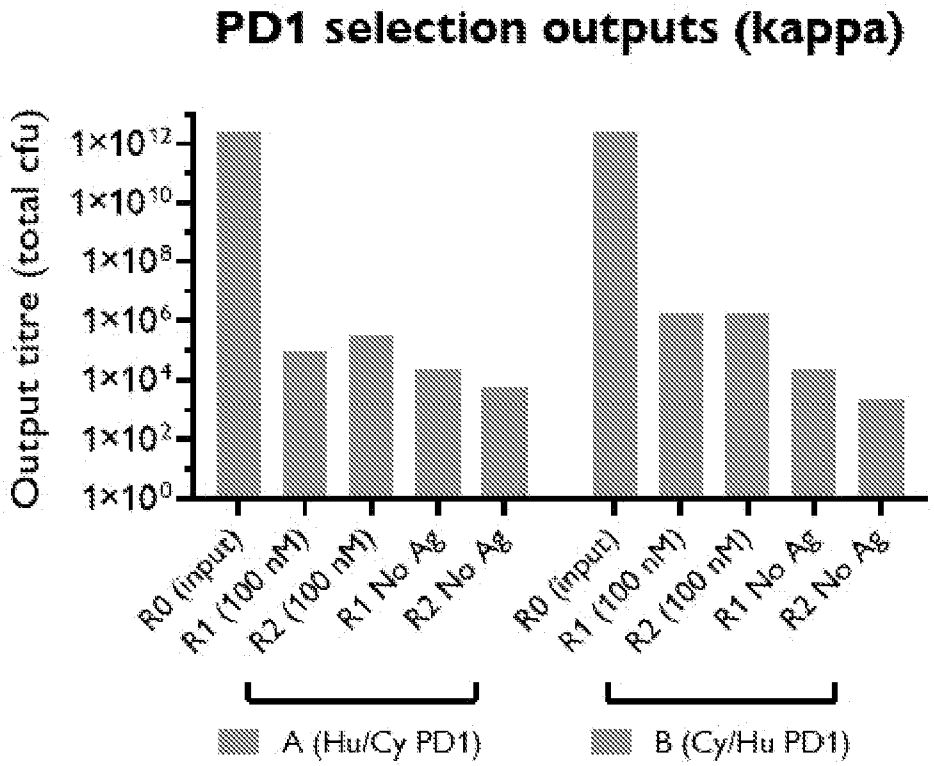

PD1 selection outputs (kappa)
Output titre (total cfu)
1×10^12
1×10^10
1×10^8
1×10^6
1×10^4
1×10^2
1×10^0
R0 (input)
R1 (100 nM)
R2 (100 nM)
R1 No Ag
R2 No Ag
R0 (input)
R1 (100 nM)
R2 (100 nM)
R1 No Ag
R2 No Ag
A (Hu/Cy PD1)
B (Cy/Hu PD1)

Figure 6.

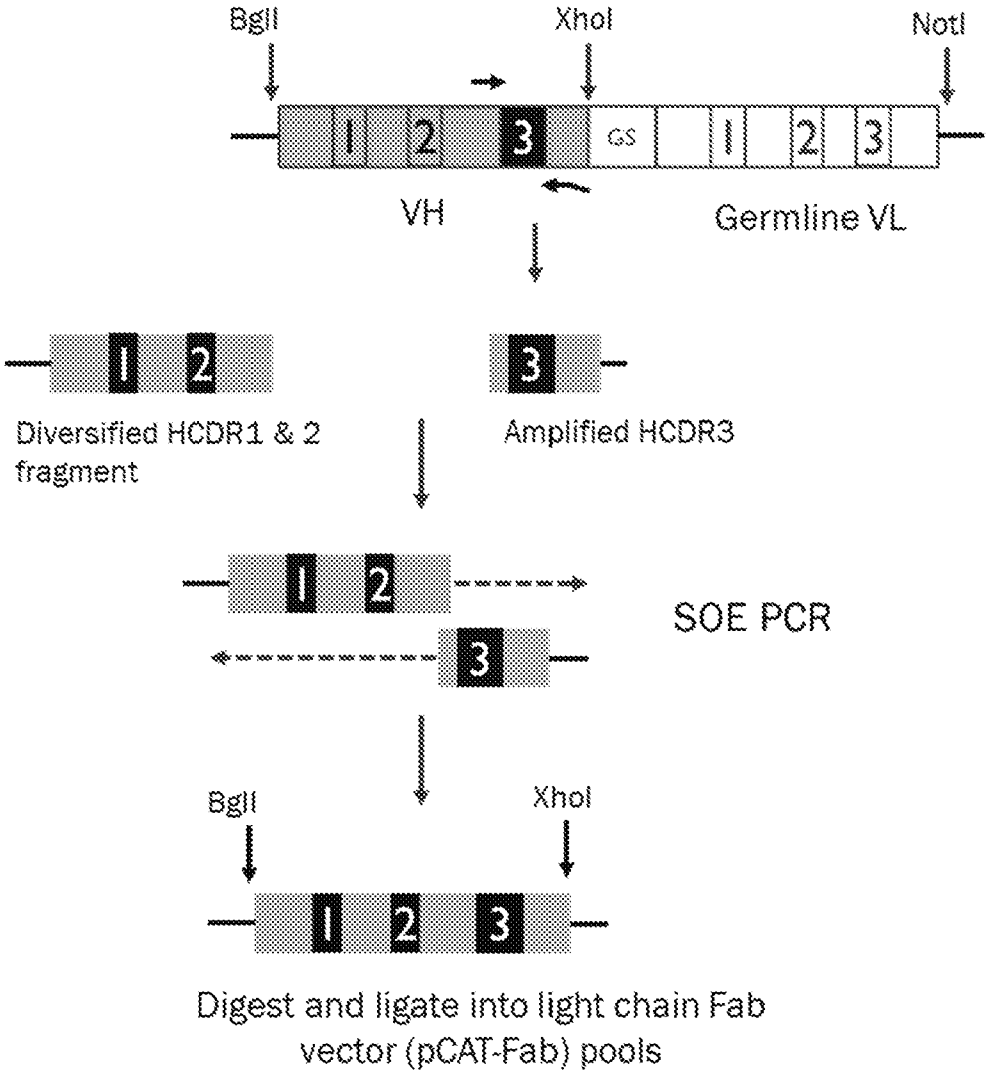
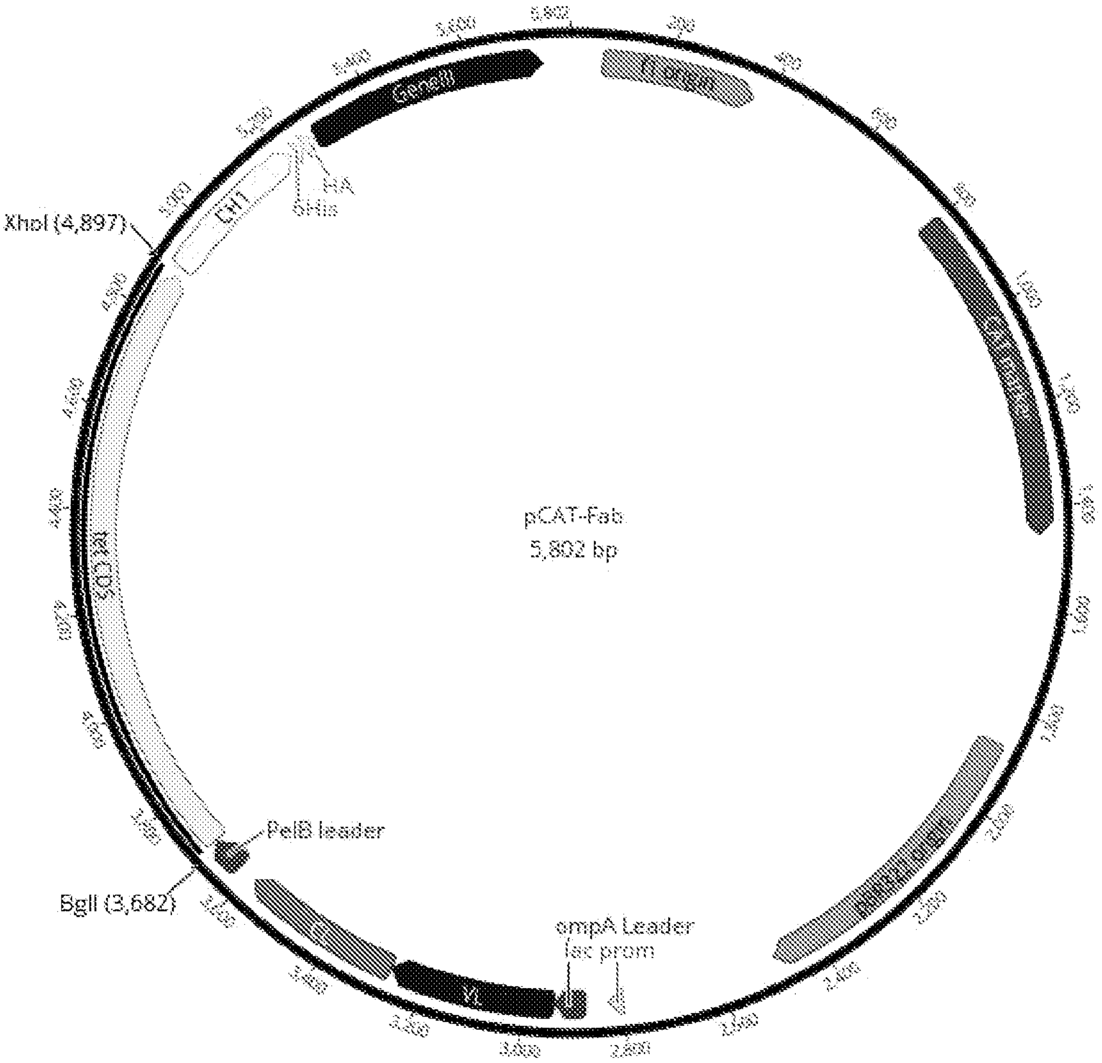

BglI
XhoI
NotI
1
2
3
GS
1
2
3
VH
Germline VL
1
2
3
Diversified HCDR1 & 2 fragment
Amplified HCDR3
1
2
3
SOE PCR
BglI
XhoI
1
2
3
Digest and ligate into light chain Fab vector (pCAT-Fab) pools

Figure 7.



pCAT-Fab
5,802 bp
GeneIII
HA
6His
CH1
XhoI (4,897)
PelB leader
BglI (3,682)
ompA Leader
lac prom

Figure 8.

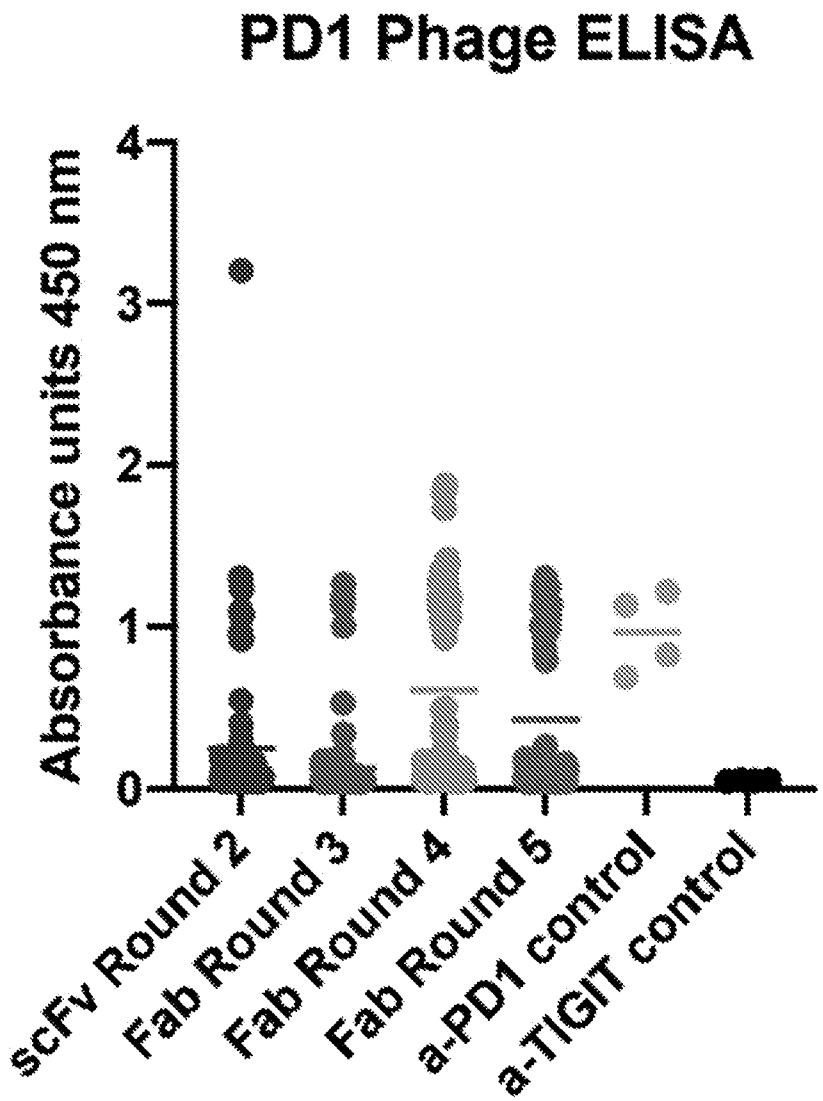
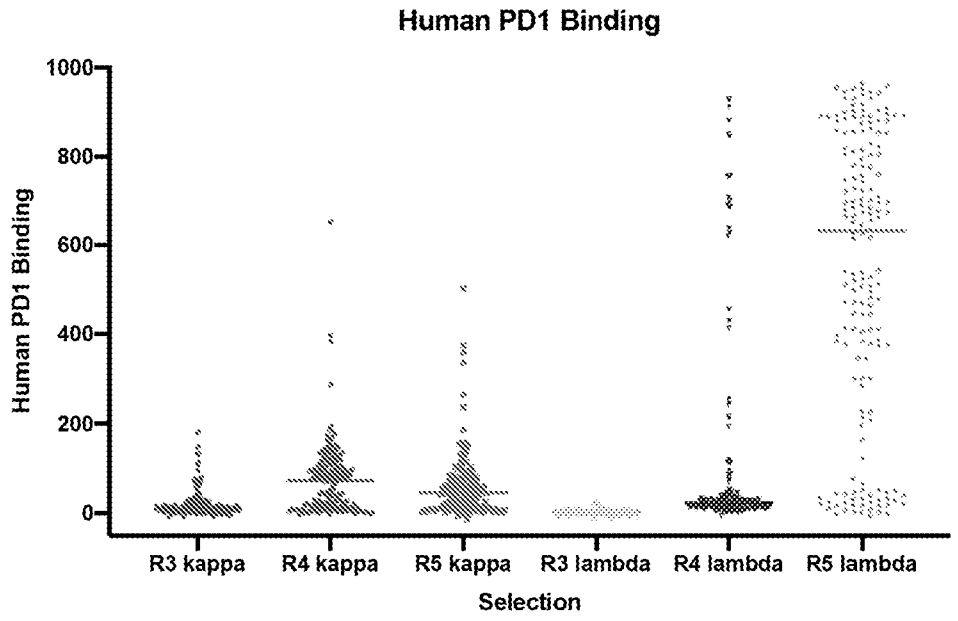

PD1 Phage ELISA
Absorbance units 450 nm
0
1
2
3
4
scFv Round 2
Fab Round 3
Fab Round 4
Fab Round 5
a-PD1 control
a-TIGIT control

Figure 9

A.


Human PD1 Binding
Human PD1 Binding
1000
800
600
400
200
0
R3 kappa
R4 kappa
R5 kappa
R3 lambda
R4 lambda
R5 lambda
Selection

B.

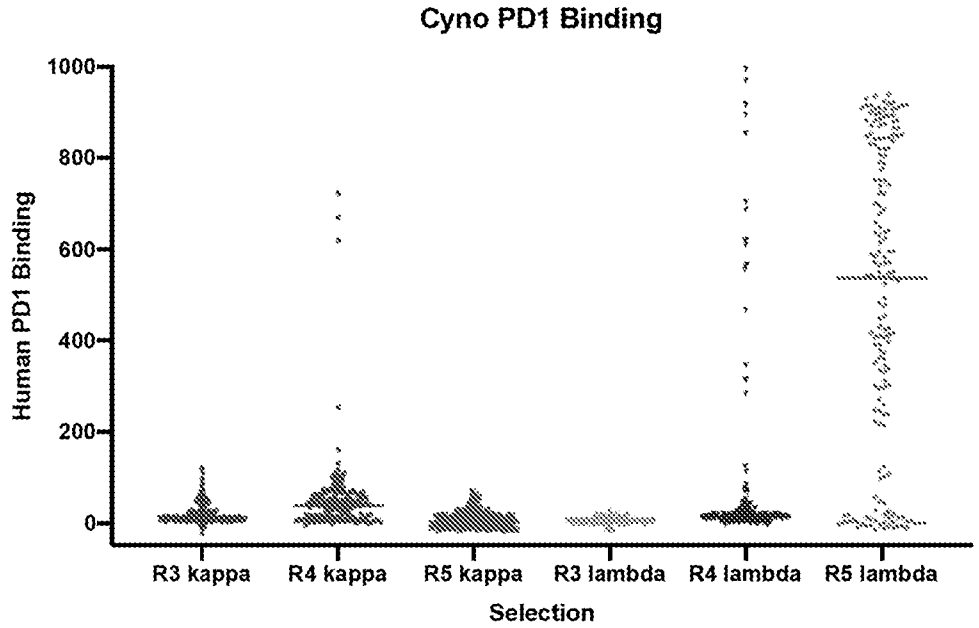
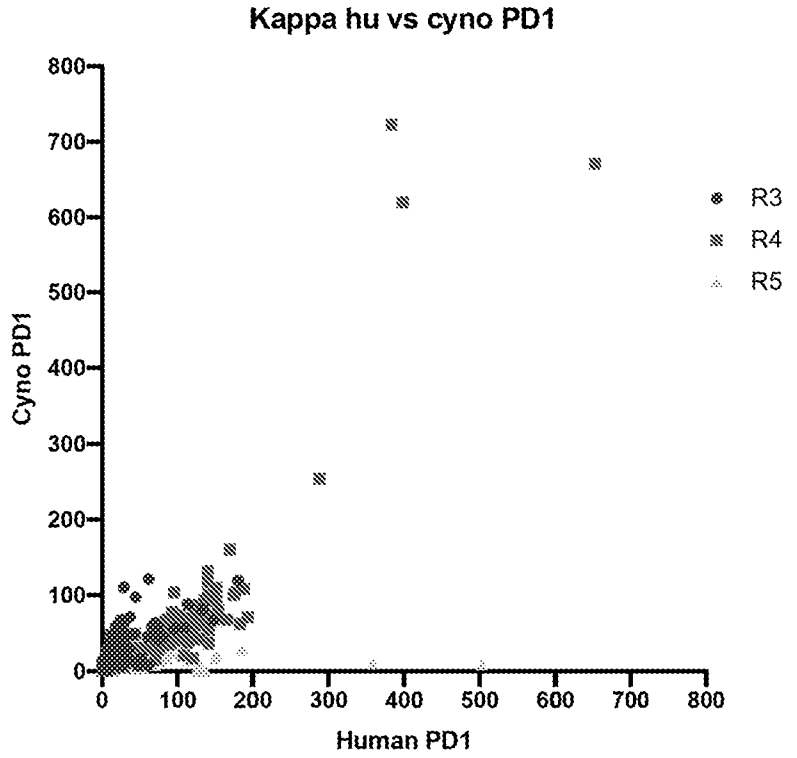

Cyno PD1 Binding
Human PD1 Binding
1000
800
600
400
200
0
R3 kappa
R4 kappa
R5 kappa
R3 lambda
R4 lambda
R5 lambda
Selection

Figure 10

A.


Kappa hu vs cyno PD1
Cyno PD1
Human PD1
R3
R4
R5
0
100
200
300
400
500
600
700
800

B.

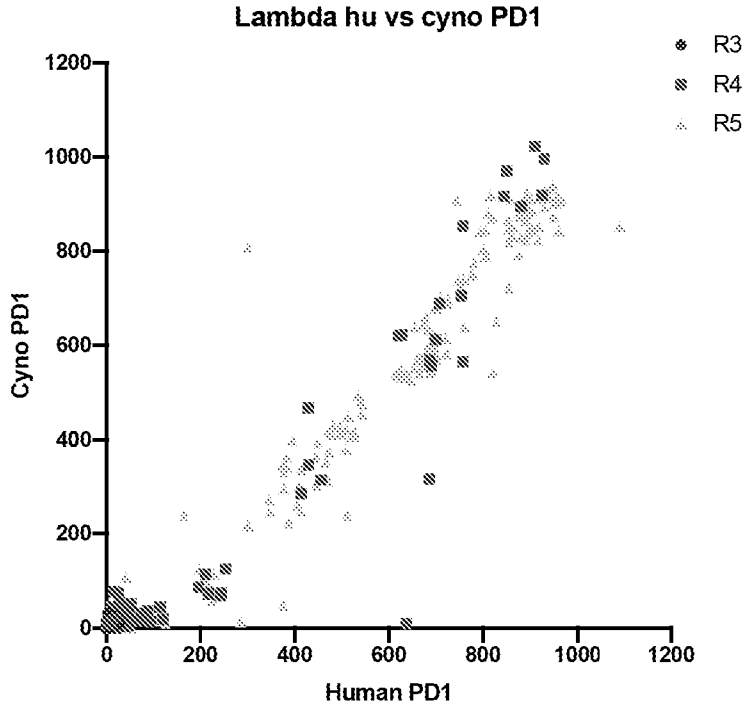

Lambda hu vs cyno PD1
Cyno PD1
Human PD1
R3
R4
R5
0
200
400
600
800
1000
1200

Figure 11

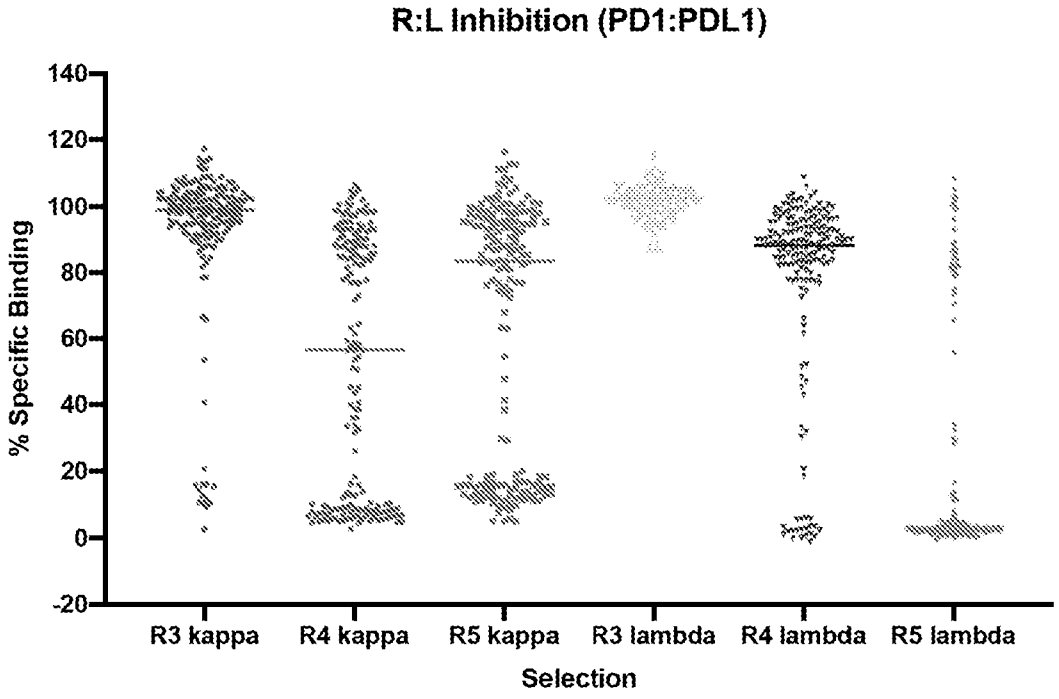

R:L Inhibition (PD1:PDL1)
% Specific Binding
140
120
100
80
60
40
20
0
-20
R3 kappa
R4 kappa
R5 kappa
R3 lambda
R4 lambda
R5 lambda
Selection

Figure 12

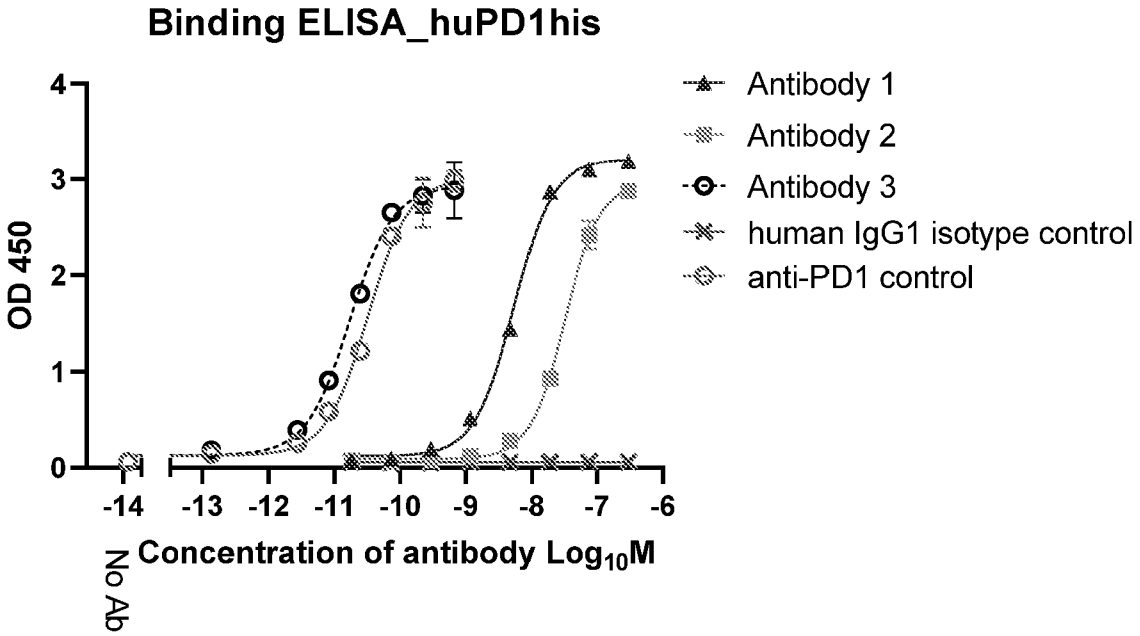
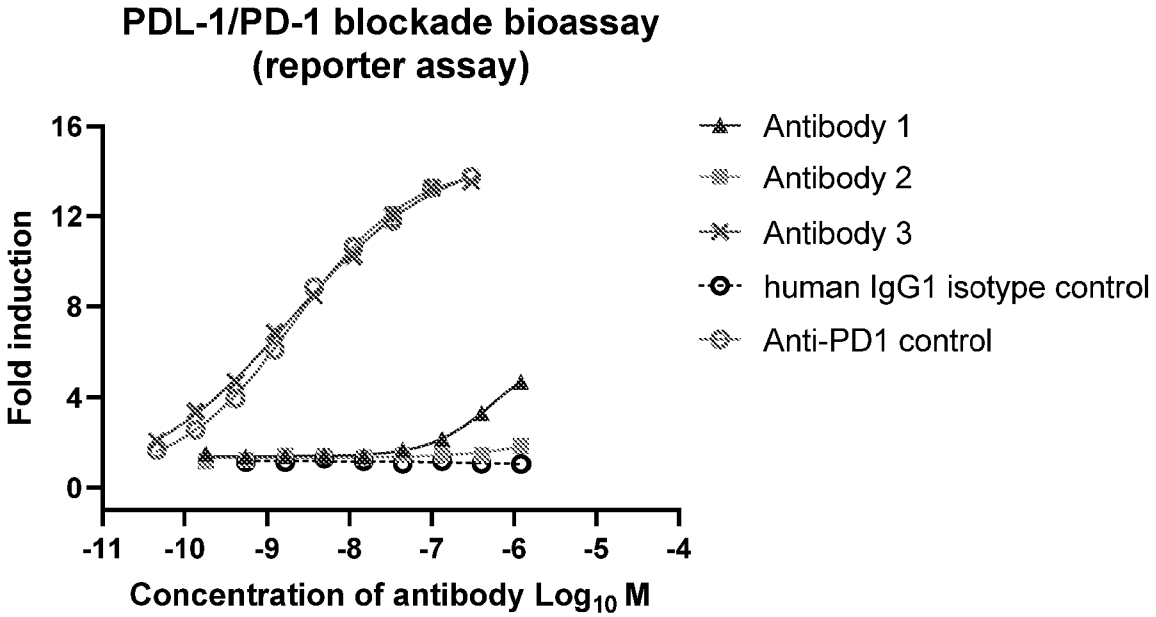

Binding ELISA_huPD1his
OD 450
4
3
2
1
0
-14
-13
-12
-11
-10
-9
-8
-7
-6
No Ab
Concentration of antibody Log10M
Antibody 1
Antibody 2
Antibody 3
human IgG1 isotype control
anti-PD1 control

Figure 13


PDL-1/PD-1 blockade bioassay
(reporter assay)
Fold induction
16
12
8
4
0
-11
-10
-9
-8
-7
-6
-5
-4
Concentration of antibody Log10 M
Antibody 1
Antibody 2
Antibody 3
human IgG1 isotype control
Anti-PD1 control

Methods

Field of the invention

The invention relates to methods of identifying and/or selecting antibody molecules which binds specifically to a target antigen, as well as libraries obtained or obtained according to the methods, and related products.

Background of the invention

Antibody discovery campaigns have employed antibody phage display (APD) technologies or immunisation approaches, including the use of transgenic animals and B cell cloning. APD technologies in particular have become powerful and effective in producing antibody molecules for diagnostic and therapeutic purposes. These technologies include methods for generating and screening single-chain variant fragment (scFv) phage display libraries and fragment antigen-binding (Fab) phage display libraries (see for example: US10,829,541; US8,921,281; US10,954,598; "Antibody Phage Display, Methods and Protocols", 2009, Aitken [Ed], Springer Protocols; "Phage Display, Methods and Protocols", 2018, Hust & Lim [Eds], Humana Press).

Key to the quality of APD libraries are their diversity (also called complexity). Various approaches have been adopted to introduce diversity into such libraries, falling into the following three categories:

(1) natural, using full length variable heavy (VH) / variable light (VL) chain repertoires amplified from donors (either naïve or immune libraries);

(2) synthetic, by generating diversified complementary determining regions (CDRs) according to design; and

(3) semi-synthetic, which relies on a combination of natural and designed CDRs.

The variable regions used in synthetic and semi-synthetic APD libraries have generally been chosen based on H and L chain germline gene frequency in the natural human repertoire and/or have been shown to have favourable properties. For example, Van Blarcom et al. (2018; mAbs 10: 256-268) took a total of four H chain and three L chain human germline frameworks as the backbone for the synthetic library, chosen based on their frequency in the memory compartment of the immune repertoire obtained from 10 donors. Similarly, Venet et al. (2012, PLoS ONE 7(8): e43471) used seven human H chain variable germline genes, five human kappa L chain variable germline genes and two human lambda L chain variable germline genes on the basis of their stability and frequency in the human antibody repertoire, to construct their libraries.

However, the importance of HCDR3 in driving antigen contact has become apparent. As early as 1992, Barbas et al. (1992, Proc. Natl Acad. Sci. USA 89: 4457-4461) generated a semi-synthetic

Fab phage display library with synthetic diversity introduced only in the HCDR3. Subsequently, APD libraries have incorporated the natural diversity of the HCDR3 regions derived from the immunoglobulin genes from B cells (see for example Hoet et al. [2005, Nat. Biotechnol. 23, 344-348]; Venet et al. [2012, supra]; Valadon et al. [2019, mAbs 11: 516-531]; Teixeira et al. [2021, mAbs 13: e1980942-1 to -16]; US8,921,281; US10,829,541; US10,954,508). In those libraries, HCDR3 regions are usually flanked by conserved framework (FW) sequences that allow the isolation of naïve or immune repertoire HCDR3 sequences.

Despite the advances in APD libraries, there remains a need for improvements for example to reduce processing time to generate lead antibodies that have ideal affinity, specificity, developability and/or lack of immunogenicity.

Summary of the invention

The present invention provides *inter alia* a method of identifying and/or selecting an antibody molecule which binds specifically to a target antigen, the method comprising the steps of:

(1) providing a single-chain variant fragment (scFv) phage display library of a population of engineered scFv nucleic acid constructs encoding scFv molecules, wherein each scFv nucleic acid construct comprises: (a) a first nucleic acid sequence encoding either a human germline VH scaffold amino acid sequence including an HCDR1 and HCDR2, or a modified version thereof having at least 80% sequence identity, for example at least 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity, to the human germline VH scaffold nucleic amino acid sequence; (b) a second nucleic acid sequence encoding either an scFv human germline VL scaffold amino acid sequence including an LCDR1, LCDR2 and LCDR3, or a modified version thereof having at least 80% sequence identity, for example at least 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity, to the human germline VL scaffold nucleic amino acid sequence; and (c) a third nucleic acid sequence encoding either a human B-cell donor-derived amino acid sequence including an HCDR3, or modified version thereof having at least 80% sequence identity, for example at least 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity, to the human B-cell donor-derived amino acid sequence;

(2) screening the scFv phage display library against the target antigen and selecting one or more binding scFv molecules;

(3) identifying an enriched nucleic acid sequence encoding an HCDR3 from the one or more binding scFv molecules;

(4) generating a fragment antigen-binding (Fab) display library of a population of engineered Fab nucleic acid constructs encoding Fab molecules, wherein each Fab nucleic acid construct comprises: (a) a fourth nucleic acid sequence encoding either a VH scaffold amino acid sequence including an HCDR1 and HCDR2, each or both of which are human germline and/or diversified, or a modified version having at least 80% sequence identity, for example at least

85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity, thereto; (b) a fifth nucleic acid sequence encoding either a Fab human germline VL scaffold amino acid sequence including an LCDR1, LCDR2 and LCDR3, or a modified version thereof version thereof having at least 80% sequence identity, for example at least 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity, to the Fab human germline VL scaffold nucleic amino acid sequence; and (c) an enriched nucleic acid sequence encoding either an HCDR3 identified in step (3), or a modified version thereof having at least 80% sequence identity, for example at least 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity, to the HCDR3 identified in step (3);

(5) screening the Fab display library against the target antigen and selecting one or more binding Fab molecules; and

(6) optionally, reformatting the one or more binding Fab molecules into an alternative antibody molecule format,

thereby identifying and/or selecting an antibody molecule which binds specifically to the target antigen.

Also provided according to the invention is an scFv phage display library and/or a Fab phage display library obtained or obtainable according to the method of the invention.

Further provided according to the invention is a library of vectors containing the nucleic acid constructs as defined here.

Additionally provided a host cell or library of host cells containing the library of the invention.

A kit containing a library of the invention is also encompassed.

Further provided according to the invention is an antibody molecule obtained or obtainable according to the method of the present invention.

Brief description of the drawings

Figure 1 is a schematic representation of an embodiment of the invention. An illustration of the region encompassing an scFv and gene 3 is shown with donor derived HCDR3 highlighted in black. The LacZ promoter is labelled and identified by a right angled arrow. The position of the VH gene is labelled and shaded in the grey and the VL gene in white. The GS represents the glycine/serine linker region that joins the VH to the VL. After an initial two or three rounds of selections the HCDR3s are amplified out and shuffled with diversified HCDR1 and HCDR2 fragments. This VH fragment is then cloned into the Fab phagemid display vector. A second wave of selections are carried out to identify Fab binders.

Figure 2 is a schematic illustration of the pAMP-scFv phagemid vector used in an embodiment of the invention (see Example 1). The donor derived HCDR3s/FW4 pools are cloned into the PstI/XhoI sites, replacing the tetracycline resistance gene, to generate a scFv which is fused to a 6xHis tag for purification and a HA tag for detection. There is an amber stop codon (not shown) between the tags and a gene III. In an *E. coli* amber suppressor strain the scFv is fused to the gene III protein and displayed on the phage. Expression is under control of a LacZ promoter and a pelB leader directs expression to the periplasm. An ampicillin marker and origins of replication are also shown.

Figure 3 is a schematic illustration of the cloning of the donor derived HCDR3 into the scFv region of the pAMP-scFv vector.

Figures 4A-C depicts HCDR3 length, IGKV-KJ and IGHJ chain usage in a VH1-46 kappa library embodiment of the invention.

Figures 5A&B show phage output titres after each round of selection (**A** depicts selection strategy A; **B** depicts selection strategy B; see Example 2 below) against PD-1.

Figure 6 is a schematic illustration of the isolation of HCDR3s from outputs of scFv phage display libraries and subsequent combination with diversified HCDR1 and HCDR2 by SOE PCR. The internal arrows represent positions of primers used for amplifying the HCDR3 region.

Figure 7 is a schematic illustration of a pCAT-Fab phagemid vector used in an embodiment of the invention. VH gene inserts generated by HCDR3 shuffling are cloned into the BglI/XhoI sites, replacing the tetracycline resistance gene, to enable Fab display.

Figure 8 is a graph showing a monoclonal phage ELISA against PD-1 for clones from the scFv library round 2 selection output and all subsequent rounds from the Fab libraries. An anti-PD-1 positive control and anti-TIGIT negative control are included.

Figure 9A&B are graphs showing the binding of Fabs derived from periplasmic extract samples for the Kappa library and Lambda library R3 to R5 selection outputs to human (**A**) and cyno (**B**) PD-1 in a TR-FRET format.

Figures 10A&B are graphs showing correlations between human and cyno PD1 binding levels by Fabs derived from periplasmic extract samples for the Kappa library (**A**) and Lambda library (**B**) R3 to R5 selection outputs in a TR-FRET format.

Figure 11 is a graph showing the extent of human PD-1 receptor-ligand inhibition by Fabs derived from periplasmic extract samples for the Kappa library and Lambda library R3 to R5 selection outputs in a TR-FRET format.

Figure 12 is a graph showing a binding ELISA for clones in IgG1 format to human PD-1.

Figure 13 is a graph showing the effect of the anti-PD-1 binders in a PDL-1/ PD-1 receptor blocking receptor cell assay.

Detailed description of the invention

According to one aspect of the invention, there is provided a method of identifying and/or selecting an antibody molecule which binds specifically to a target antigen, the method comprising the steps of:

(1) providing a single-chain variant fragment (scFv) phage display library of a population of engineered scFv nucleic acid constructs encoding scFv molecules, wherein each scFv nucleic acid construct comprises: (a) a first nucleic acid sequence encoding either a human germline VH scaffold amino acid sequence including an HCDR1 and HCDR2, or a modified version thereof having at least 80% sequence identity, for example at least 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity, to the human germline VH scaffold nucleic amino acid sequence; (b) a second nucleic acid sequence encoding either an scFv human germline VL scaffold amino acid sequence including an LCDR1, LCDR2 and LCDR3, or a modified version thereof having at least 80% sequence identity, for example at least 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity, to the human germline VL scaffold nucleic amino acid sequence; and (c) a third nucleic acid sequence encoding either a human B-cell donor-derived amino acid sequence including an HCDR3, or modified version thereof having at least 80% sequence identity, for example at least 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity, to the human B-cell donor-derived amino acid sequence;

(2) screening the scFv phage display library against the target antigen and selecting one or more binding scFv molecules;

(3) identifying an enriched nucleic acid sequence encoding an HCDR3 from the one or more binding scFv molecules;

(4) generating a fragment antigen-binding (Fab) display library of a population of engineered Fab nucleic acid constructs encoding Fab molecules, wherein each Fab nucleic acid construct comprises: (a) a fourth nucleic acid sequence encoding either a VH scaffold amino acid sequence including an HCDR1 and HCDR2, each or both of which are human germline and/or diversified, or a modified version having at least 80% sequence identity, for example at least 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity, thereto; (b) a fifth nucleic acid sequence encoding either a Fab human germline VL scaffold amino acid sequence including an

LCDR1, LCDR2 and LCDR3, or a modified version thereof version thereof having at least 80% sequence identity, for example at least 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity, to the Fab human germline VL scaffold nucleic amino acid sequence; and (c) an enriched nucleic acid sequence encoding either an HCDR3 identified in step (3), or a modified version thereof having at least 80% sequence identity, for example at least 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity, to the HCDR3 identified in step (3);

(5) screening the Fab display library against the target antigen and selecting one or more binding Fab molecules; and

(6) optionally, reformatting the one or more binding Fab molecules into an alternative antibody molecule format,

thereby identifying and/or selecting an antibody molecule which binds specifically to the target antigen.

In the method, the scFv phage display library comprises a distinct combination of germline or close to germline sequence H and L chains apart from the HCDR3 which is highly diverse and derived from human B-cell repertoires. The method uses enriched HCDR3 from the binding scFv molecules ("outputs") in step (3) and combines them with germline or diversified VH HCDR1 and/or HCDR2 via "HCDR3 shuffling" in step (4). These VH fragments are then paired with a restricted repertoire of germline sequence L chains as a Fab display library as part of an in-line maturation process. This increases the probability of identifying high affinity antibody molecules during the screening in step (5). Further rounds of selection and screening of the Fab library may be carried out to isolate antibody molecules ("clones") with improved affinities over the original library.

Thus the method of the invention in part mimics natural B cell antibody evolution whereby initial binding is driven primarily through the diversification present in HCDR3 (the scFv library), H and L chain pairing is established, which is then followed by antigen-driven affinity maturation through somatic hypermutation in the HCDR loops (the binding Fab molecules).

The method allows for the identification and/or selecting of suitable antibody molecules as close to the human germline sequence as possible with minimal passenger mutations that may confer sequence liabilities and increase developability risks. This streamlined process reduces the need for multiple iterative cycles of optimisation and liability correction that is required in many existing antibody discovery platforms.

A schematic showing features of a method of the invention is depicted in Figure 1.

In the method of the invention, the scFv molecules encoded by the population of engineered scFv nucleic acid constructs may (collectively) include at least two, for example at least three, four, five, six, seven, eight, nine or ten, human germline VH scaffold amino acid sequences including HCDR1s and HCDR2s, or modified versions thereof as defined above. Thus the scFv molecules may include at least four human germline VH scaffold amino acid sequences including HCDR1s and HCDR2s, or modified versions thereof as defined above. The scFv molecules may include at least five human germline VH scaffold amino acid sequences including HCDR1s and HCDR2s, or modified versions thereof as defined above. The scFv molecules may include at least six human germline VH scaffold amino acid sequences including HCDR1s and HCDR2s, or modified versions thereof as defined above.

In the method of the invention, the Fab molecules encoded by the population of engineered Fab nucleic acid constructs may (collectively) include at least two, for example at least three, four, five, six, seven, eight, nine or ten, human germline VH scaffold amino acid sequences including diversified or modified versions thereof as defined above. Thus the Fab molecules may include at least four human germline VH scaffold amino acid sequences including diversified or modified versions thereof as defined above. The scFv molecules and/or Fab molecules may include at least five human germline VH scaffold amino acid sequences including diversified or modified versions thereof as defined above. The scFv molecules and/or Fab molecules may include at least six human germline VH scaffold amino acid sequences including diversified or modified versions thereof as defined above.

In the method of the invention, the scFv molecules encoded by the population of engineered scFv nucleic acid constructs may (collectively) include at least two, for example at least three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen or fifteen, human germline VL scaffold amino acid sequences including LCDR1s, LCDR2s and LCDR3s, or modified versions thereof as defined above. Thus the scFv molecules may include at least three human germline VL scaffold amino acid sequences including LCDR1s, LCDR2s and LCDR3s, or modified versions thereof as defined above. The scFv molecules may include at least four human germline VL scaffold amino acid sequences including LCDR1s, LCDR2s and LCDR3s, or modified versions thereof as defined above. The scFv molecules may include at least five human germline VL scaffold amino acid sequences including LCDR1s, LCDR2s and LCDR3s, or modified versions thereof as defined above.

In the method of the invention, the Fab molecules encoded by the population of engineered Fab nucleic acid constructs may (collectively) include at least two, for example at least three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen or fifteen, human germline VL scaffold amino acid sequences including LCDR1s, LCDR2s and LCDR3s, or modified versions

thereof as defined above. Thus the Fab molecules may include at least three human germline VL scaffold amino acid sequences including LCDR1s, LCDR2s and LCDR3s, or modified versions thereof as defined above. The Fab molecules may include at least four human germline VL scaffold amino acid sequences including LCDR1s, LCDR2s and LCDR3s, or modified versions thereof as defined above. The Fab molecules may include at least five human germline VL scaffold amino acid sequences including LCDR1s, LCDR2s and LCDR3s, or modified versions thereof as defined above.

According to the method, the scFv phage display library may comprise:
(i) a kappa scFv phage display library (also referred to herein as the "VHVK" phage display library) in which each second nucleic acid sequence is a kappa ("K") light chain sequence or a modified version thereof; and
(ii) a lambda scFv phage display library (also referred to herein as the "VHVλ" phage display library) in which each second nucleic acid sequence is a lambda ("λ") light chain sequence or a modified version thereof.

The kappa scFv phage display library may comprise a first set of vectors having first nucleic acid sequences combined with second nucleic acid sequences containing kappa light chain sequences or derivatives thereof. The lambda scFv phage display library may comprise a second set of vectors having first nucleic acid sequences combined with second nucleic acid sequences containing lambda light chain sequences or derivatives thereof.

In the method of the invention, the Fab phage display library may comprise:
(i) a kappa Fab phage display library in which each fifth nucleic acid sequence is a kappa light chain sequence or a derivative thereof; and
(ii) a lambda Fab phage display library in which each fifth nucleic acid sequence is a lambda light chain sequence of a derivative thereof.

The kappa Fab phage display library may comprise a third set of vectors having fourth nucleic acid sequences combined with fifth nucleic acid sequences containing kappa light chain sequences or derivatives thereof. The lambda Fab phage display library may comprise a fourth set of vectors having fourth nucleic acid sequences combined with fifth nucleic acid sequences containing lambda light chain sequences or derivatives thereof.

The second nucleic acid sequences may be the same or substantially the same as the fifth nucleic acid sequences.

The enriched nucleic acid sequences encoding the HCDR3s may be combined for the Fab display library with either:

the third set of vectors if the enriched nucleic acid sequences are derived from binding scFv molecules obtained using the first set of vectors; or

the fourth set of vectors if the enriched nucleic acid sequences are derived from binding scFv molecules obtained using the second set of vectors.

Depending on which scFv library the enriched HCDR3 were originally extracted from, the VH fragments may be cloned into a pool of Fab phagemid display vectors encoding either kappa or lambda germline L chain genes as used in the scFv library (referred to as a "light chain shuffle"). In other words, if one or more enriched HCDR3s are identified from a kappa scFv phage display library, then a kappa Fab phage display library may be generated for further screening against the target antigen. Additionally or alternatively, if one or more enriched HCDR3s are identified from a lambda scFv phage display library, then a lambda Fab phage display library may be generated for further screening against the target antigen.

In this way, the VH fragment can find an optimal pairing as a Fab format. Accordingly, the natural HCDR3 sequences are combined with sequences as close to the human germline sequence as possible with minimal sequence liabilities.

Switching from scFv to Fab format offers additional advantages. A Fab format is generally considered to be more stable and less prone to form dimers or multimers than scFv.

Furthermore, the monomeric nature of Fab molecules, combined with the typical lower display levels on phage and lower soluble expression levels, acts in favour of selecting and identifying high affinity binders.

Also, because a restricted or defined pool of common light chains may be used, the method allows for the straightforward and rapid generation of bispecific antibodies.

The method of the invention has a particular advantage of being able to isolate and identify by sequence analysis distinct families of antibody molecules derived from a common or related HCDR3. This opens up the possibility to interrogate the sequence space and properties of clones with positive attributes.

In the method of the invention, the population of scFv and/or Fab nucleic acid constructs may include a plurality of first nucleic acid sequences and/or fourth nucleic acid sequences which are one or more (for example, at least two, three, four, five, six, seven, eight, nine or ten) of the

human germline VH gene segment-derived nucleic acid sequences selected from the group consisting of: VH1-2, VH1-46, VH1-58, VH1-69, VH3-7, VH3-11, VH3-13, VH3-23, VH3-30, VH5-51, a modified version of any of these segments encoding HCDR1 and/or HCDR2 and having at least 80% sequence identity, for example at least 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity, to the corresponding unmodified segment, and a combination thereof.

The human germline VH gene segments used for the scFv and/or Fab nucleic acid constructs may exclude the following specific segments or combination of segments:
(1) VH3-23 (as used in Hoet et al. [2015, supra], and Valadon et al. [2019, supra]); and/or
(2) VH1-46, VH1-69 and VH3-23; and/or
(3) IGHV1-24, IGHV3-7, IGHV4-30-4 and IGHV1-18 (as used in Teixeira et al. [2021, supra] and US11,254,931); and/or
(4) VH1-69, VH3-15, VH3-23 and VH5-51 (as used in Van Blarcom et al. [2018, supra]); and/or
(5) VH1-2, VH1-69, VH1-18, VH3-23, VH3-30, VH3-48 and VH5-51 (as used in Venet et al. [2012, supra]).

In the method of the invention, the population of scFv and/or Fab nucleic acid constructs may include a plurality of second and/or fifth nucleic acid sequences which are: (i) one or more (for example, at least two, three, four, five, six, seven, eight or nine) human kappa L chain gene segment-derived nucleic acid sequences selected from the group consisting of: K1-5, K1-6, K1-16, K1-39, K2-28, K3-15, K3-11, K3-20, K3-D7, a modified version of any of these segments encoding LCDR1, LCDR2 and/or LCDR3 and having at least 80% sequence identity, for example at least 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity, to the corresponding unmodified segment, and a combination thereof, combined with a kappa J gene segment-derived nucleic acid sequence selected from the group consisting of: J2, J4, a modified version of J2 or J4 having at least 80% sequence identity, for example at least 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity, to the corresponding unmodified segment, and a combination thereof; or (ii) one or more (for example, at least two, three, four, five or six) human lambda L chain gene segment-derived nucleic acid sequences selected from the group consisting of: L2-8, L2-14, L2-18, L3-27, L7-43, L4-46 a modified version of any of these segments encoding LCDR1, LCDR2 and/or LCDR3 and having at least 80% sequence identity, for example at least 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity, to the corresponding unmodified segment, and a combination thereof, combined with a lambda J gene segment-derived nucleic acid sequence selected from the group consisting of: J1, J2, a modified version of J1 or J2 having at least 80% sequence identity, for example at least 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity, to the corresponding unmodified segment and a combination thereof; or a combination of (i) and (ii).

The human germline VL gene segments used for the scFv and/or Fab nucleic acid constructs may exclude the following specific segments or combination of segments:
(1) VK3-15; and/or
(2) VK3-20 and VK4-1 (as used in Valadon et al. [2019, supra]); and/or
(3) VK1-39, VK3-20 and VL1-47 (as used in Van Blarcom et al. [2018, supra]); and/or
(4) IGKV1-12, IGKV2D-29, IGKV3-11 and IGLV2-14 (as used in Teixeira et al. [2021, supra] and US11,254,931).

In the method of the invention, the fourth nucleic acid sequence may encode either a diversified HCDR1 and/or diversified HCDR2 modified to reflect natural amino acid abundance and variation in VH1 and VH3 human germline families, optionally excluding HCDR1 and/or HCDR2 sequences with sequence liabilities, or a modified version having at least 80% sequence identity, for example at least 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity, thereto.

Sequence liabilities to be excluded may include: deamidation motif NA, NG and/or NS; and/or isomerisation motif DG and/or DS; and/or hydrolysis motif DP and/or DQ; and/or unpaired cysteine C; and/or N-glycosylation motif NXT and/or NXS, where X is any amino acid except P. Additional sequence liabilities which may be excluded are found in Table 1 of Teixeira et al. (2021, supra).

In another aspect of the invention there is provided an scFv phage display library, such as a lambda scFv phage display library or kappa scFv phage display library, obtained or obtainable according to the method of the invention.

Additionally according to the invention there is provided a Fab library, such as a lambda Fab phage display library or kappa Fab phage display library, obtained or obtainable according to the method of the invention.

The present invention also provides a library of vectors containing a nucleic acid construct of the invention as defined herein.

Further provided in an aspect of the invention is a host cell or library of host cells containing a library of the invention.

The invention also encompasses a kit containing the library of the invention.

An antibody molecule obtained or obtainable according to the method of the invention is further encompassed.

An “antibody molecule” is an immunoglobulin molecule capable of specific binding to a target, such as a carbohydrate, polynucleotide, lipid, polypeptide, etc., through at least one antigen recognition site, located in the variable region of the immunoglobulin molecule.

As used herein, the term “antibody molecule” encompasses not only whole or intact polyclonal or monoclonal antibodies, but also any antigen binding fragment (for example, an “antigen-binding portion”) or single chain thereof, fusion proteins comprising an antibody, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site including, for example without limitation, scFv, single domain antibodies (for example, shark and camelid antibodies), maxibodies, minibodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR and bis-scFv. The antibody molecule may be monovalent, bivalent or multivalent.

An “antibody molecule” encompasses an antibody of any class, such as IgG, IgA, or IgM (or sub-class thereof), and the antibody need not be of any particular class. Depending on the antibody amino acid sequence of the constant region of its heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), for example IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. The heavy-chain constant regions that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

The term “antigen binding portion” of an antibody molecule, as used herein, refers to one or more fragments of an intact antibody that retain the ability to specifically bind to a target antigen. Antigen binding functions of an antibody molecule can be performed by fragments of an intact antibody. Examples of binding fragments encompassed within the term “antigen binding portion” of an antibody molecule include Fab; Fab'; F(ab')2; an Fd fragment consisting of the VH and CH1 domains; an Fv fragment consisting of the VL and VH domains of a single arm of an antibody; a single domain antibody (dAb) fragment, and an isolated complementarity determining region (CDR).

The term “Fc” or “Fc region” is used to define a C-terminal region of an immunoglobulin heavy chain. The Fc region may be a native sequence Fc region or a variant Fc region. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The numbering of the residues in the Fc region is that of the EU index as in Kabat. The Fc region of an immunoglobulin generally comprises two constant

domains, CH2 and CH3. As is known in the art, an Fc region can be present in dimer or monomeric form.

A "variable region" of an antibody refers to the variable region of the antibody light chain or the variable region of the antibody heavy chain, either alone or in combination. As known in the art, the variable regions of the heavy and light chain each consist of four framework regions (FRs) connected by three complementarity determining regions (CDRs) also known as hypervariable regions, contribute to the formation of the antigen binding site of antibodies. When choosing FR to flank CDRs, for example when humanizing or optimizing an antibody, FRs from antibodies which contain CDR sequences in the same canonical class are preferred. Such FRs may nevertheless be modified for example by mutation or deletion, including by increasing or shortening their length if required.

Sequence identity between nucleotide or amino acid sequences can be determined by comparing an alignment of the sequences. When an equivalent position in the compared sequences is occupied by the same base or amino acid, then the molecules are identical at that position. Scoring an alignment as a percentage of identity is a function of the number of identical amino acids or bases at positions shared by the compared sequences. When comparing sequences, optimal alignments may require gaps to be introduced into one or more of the sequences to take into consideration possible insertions and deletions in the sequences. Sequence comparison methods may employ gap penalties so that, for the same number of identical molecules in sequences being compared, a sequence alignment with as few gaps as possible, reflecting higher relatedness between the two compared sequences, will achieve a higher score than one with many gaps. Calculation of maximum percent identity involves the production of an optimal alignment, taking into consideration gap penalties.

Suitable computer programs for carrying out sequence comparisons are widely available in the commercial and public sector. Examples include MatGat (Campanella et al., 2003, BMC Bioinformatics 4: 29), Gap (Needleman & Wunsch, 1970, J. Mol. Biol. 48: 443-453), FASTA (Altschul et al., 1990, J. Mol. Biol. 215: 403-410), Clustal W 2.0 and X 2.0 (Larkin et al., 2007, Bioinformatics 23: 2947-2948) and EMBOSS Pairwise Alignment Algorithms (Needleman & Wunsch, 1970, supra; Kruskal, 1983, In: Time warps, string edits and macromolecules: the theory and practice of sequence comparison, Sankoff & Kruskal (eds), pp 1-44, Addison Wesley). All programs may be run using default parameters.

In a specific example, sequence comparisons may be undertaken using the EMBOSS Needle "Pairwise Sequence Alignment" (see https://www.ebi.ac.uk/Tools/psa/emboss_needle/), which determines an optimum alignment (including gaps) of two sequences when considered over their

entire length and provides a percentage identity score. Default parameters for amino acid sequence comparisons ("Protein" option) may be Output format: pair; Matrix: Blosum62; Gap Open: 10; Gap Extend: 0.5; End Gap Penalty: false; End Gap Open: 10; End Gap Extend: 0.5. Default parameters for nucleotide sequence comparisons ("DNA" option) may be Output format: pair; Matrix: DNAfull; Gap Open: 10; Gap Extend: 0.5; End Gap Penalty: false; End Gap Open: 10; End Gap Extend: 0.5.

Sequence comparisons may be performed over the full length of the reference sequence.

Experimental

Particular non-limiting examples of the present invention will now be described with reference to the accompanying figures.

Example 1. Isolation of HCDR3s from Human B cells for the construction of scFv phage display libraries

The isolation of naïve HCDR3s for use in the construction of the "semi-synthetic" scFv antibody libraries was carried out using methods similar to those described previously (see Van Blarcom et al. [2018, supra]; Hoet et al. [2005, supra]; Venet et al. [2012, supra]; Valadon et al. [2019, supra]). Briefly, cDNA was synthesised from RNA isolated from PBMCs of 53 healthy donors, using a Superscript First Strand Synthesis kit. The HCDR3s were amplified from the cDNA using a universal forward primer (HCDR3-univrsl-F) designed on a conserved region in the VH framework 3 (FW3) and an equal mix of three reverse primers that can prime off all six human IGHJ germline genes in the framework 4 (FW4) region (Table 1).

Table 1.

Primer direction	Sequence (5'-3')
Forward	GGACACTGCAGTGTATTACTGTGC (SEQ ID NO: 1)
Reverse	GACACTCGAGACGGTGACCATTGTCC (SEQ ID NO: 2)
Reverse	GACACTCGAGACGGTGACCGTGGTCC (SEQ ID NO: 3)
Reverse	GACACTCGAGACGGTGACCAGGG (SEQ ID NO: 4)

The forward primer contained a PstI restriction enzyme site and the reverse primers a XhoI site that enabled cloning of the amplified fragment into the scFv phagemid display precursor vector pAMP-scFv (see Figure 2). The phagemid vector pAMP-scFv was synthesised by gene synthesis for the display of scFv fragments. Methods for generating scFv and Fab phagemid display vectors are well known in the art since the first scFv and Fab display vectors were described and detailed (McCafferty et al. [1990, Nature 348: 552–554]; Barbas et al. [1991, Proc. Natl Acad. Sci. USA 88: 7978 – 7982]; Hoogenboom et al. [1991, Nucleic Acids Res. 19: 4133–4137]). Most designs are derived from the pHEN1 phagemid vector (Hoogenboom et al. [1991, supra]). In brief, for the pAMP-scFv vector used here, the donor derived HCDR3s/FW4 pools were cloned into PstI/XhoI sites, replacing the tetracycline resistance gene, to generate a scFv which is fused to a 6xHis tag for purification and a HA tag for detection. There is an amber stop codon between the tags and the gene III. In an *E. coli* amber suppressor strain the scFv is fused to the gene III

protein and displayed on the phage. Expression is under control of the LacZ promoter, operator and Cap-binding site. A pelB leader directs expression to the periplasm. The vector has an ampicillin resistance gene and bacterial and phage origins of replication (see Figure 3).

A total of 300 unique scFv phagemid display precursor vectors were constructed by gene synthesis using established procedures. Each of 10 VH germline or germline-like scaffolds were individually combined with 18 VK/JK and 12 Vλ/JL germline or germline like sequences. In some cases, silent mutations were incorporated into the germline nucleic acid sequence. Specifically, silent mutations were incorporated into the VH germline nucleic acid sequences to introduce a PstI restriction site at the corresponding amino acid sequence positions 99-101 (numbering according to IMGT), with the exception of the VH5-51 germline sequence. For the VH5-51 sequence an existing PstI site, found at the corresponding amino acid sequence position 89 and 90, was removed through a silent mutation. Subsequent introduction of the PstI site, corresponding amino acid sequence positions 99-101, resulted in methionine at position 101 being changed to a valine (numbering according to IMGT). Several silent mutations were introduced into the VH3-23 sequence so that it matched the sequence of the universal forward primer listed in Table 1. The VK2-28 germline sequence was modified in CDR1 to remove a sequence liability, asparagine at position 34 was substituted to serine. These 300 H and L chain combinations have the germline or germline like sequence throughout except for the region encompassing the HCDR3 which is replaced by a tetracycline resistance gene. The 10 VH scaffolds used were: VH1-2, VH1-46, VH1-58, VH1-69, VH3-7, VH3-11, VH3-13, VH3-23, VH3-30 and VH5-51. These VH germline scaffolds were reported to have good properties (see Tiller et al., 2013; mAbs 5: 445-470). They also offer structural and sequence variation to improve the chances of binding different epitopes on the target antigen. The L chains used are germline or germline like gene sequences. 9 VK scaffolds combined with either JK2 or JK4 to create a total of 18 different VK genes and 6 Vλ scaffolds combined with either JL1 or JL2 to create a total of 12 different Vλ genes. The H and L chain genes used are summarised in Table 2.

Table 2. H and L chain germline genes used in scFv library construction

VH	**VK**	**JK**	**Vλ**	**JL**
1-2	1-5	J2	2-8	J1
1-46	1-6	J4	2-14	J2
1-58	1-16		2-18	
1-69	1-39		3-27	
3-7	2-28		7-43	
3-11	3-15		7-46	
3-30	3-11			
3-23	3-20			

3-13	3D-7			
5-51				

In all, 180 VH/VK and 120 VH/Vλ precursor vectors were generated. The VH/VK and VH/Vλ pAMP-scFv precursor vectors were then pooled for each of the ten chosen VH germlines. Each vector pool was digested with PstI/XhoI to remove the tetracycline stuffer, and gel purified. The HCDR3s/FW4 were prepared as 5 pools each containing the HCDR3s/FW4 sequences derived from 10 or 11 donors, digested with PstI/XhoI and the fragment column purified. Each of these HCDR3s/FW4 pools were then cloned into the PstI/XhoI sites of each set of VH germline pAMP-scFv precursor vectors (see Figure 3).

As an example, pAMP-scFv containing VH1-46 fragment was paired with 18 VK germline sequences. These vectors were then combined, digested with PstI/XhoI and purified by gel extraction. This vector set was then ligated to the 5 pools of PstI/XhoI HCDR3s/FW4 fragments derived from 10 or 11 donors using T4 DNA ligase, i.e. 5 scFv kappa sub-libraries are generated for each VH germline. The ligation reactions were column purified and then electroporated into TG1 cells. Table 3 gives the library size achieved from using the VH1-46/VK precursor vector set with the 5 pools of the donor derived HCDR3s/FW4 fragments.

Table 3. Library size of the VH1-46/VK vector set with each HCDR3/FW4 donor pool

Library ID	**Library size (cfu)**
VH1-46/VK + HCDR3/FW4 pool 1	5.60E+09
VH1-46/VK + HCDR3/FW4 pool 2	5.40E+09
VH1-46/VK + HCDR3/FW4 pool 3	5.20E+09
VH1-46/VK + HCDR3/FW4 pool 4	5.60E+09
VH1-46/VK + HCDR3/FW4 pool 5	6.40E+09
TOTAL	**2.82E+10**

The transformants from the libraries were rescued from agar plates using 2xYT media supplemented with ampicillin (100 µg/ml) and 17% glycerol to make a library master stock stored at -80 °C. The quality of each library was assessed by Sanger sequencing. The HCDR3 length and IGHJ chain usage was found to mimic the natural human repertoire (Figure 4).

A total of 50 VHVK and VHVλ sub-libraries were generated resulting in a final combined library size of 10^{11}. For selections, phage were produced for all the VHVK sub-libraries. The phage were

then combined to make a master VHVK library, also referred to as the kappa scFv library. Likewise, phage produced from the VHVλ sub-libraries were combined to make a master VHVλ library, also referred to as the lambda scFv library. The final VHVK and VHVλ scFv libraries were kept as separate library pools. Phage from the VHVK and VHVλ scFv libraries were produced by standard procedures, summarised as follows. Library stocks were used to inoculate 2xYT medium supplemented with ampicillin (100 µg/ml) and glucose 2% (w/v) in a sufficient volume to cover the overall library size. The culture was grown at at 37 °C with shaking at 250rpm until OD_{600} was 0.5-0.7. Cells were then infected with M13K07 helper phage at a multiplicity of infection of approximately 10 and incubated for a further 60 mins at 37 °C shaking at 150 rpm. The culture was then centrifuged at 2600g for 10 minutes and the supernatant was completely removed. The cells resuspended in 2xYT media supplemented with ampicillin (100 µg/ml) and kanamycin (50 µg/ml) and grown overnight at 25 °C at 250 rpm. The virions were purified by PEG precipitation.

Example 2. Phage display selection using the scFv libraries

Selections were performed against biotinylated human and cyno PD-1 (Acro Biosystems) by solution phase panning. 10^{12} - 10^{13} phage from each of the VHVK and VHVλ libraries were used in the first round of panning. Phage were blocked with PBS containing 3% (w/v) skimmed milk (MPBS) for one hour at room temperature. Prior to incubation with antigen, the phage were deselected on streptavidin coated magnetic beads (Dynal M-280). The biotinylated human or cyno PD-1 (100 nM) was pre-captured on streptavidin coated beads and washed prior to incubation with the blocked phage library. After an 1 hour incubation at room temperature beads were captured using a magnetic stand and washed with PBS/0.1% Tween 20 followed by PBS. The bound phage were eluted first with triethylamine (TEA) and then with 0.2 M glycine pH2.5. The eluates were neutralized and then used to infect exponentially growing *E. coli* TG1 cells. An aliquot of the infected TG1 was serial diluted to titre the selection output. The remaining infected TG1 were plated on 2xYT agar plates containing 2% glucose and 100 µg/ml ampicillin and incubated overnight. After overnight incubation at 30 °C, the cells were scraped from the surface to generate a master glycerol stock and to produce phage for a second round of selection. Phage were purified essentially as described in Example 1 but scaled down in volume. A second round of selection was performed essentially as described above with the exception that the human and cyno antigen was alternated to enrich for clones specific to both antigens and neutravidin beads were used. Human PD-1 in round 1 followed by cyno PD-1 in round 2 was referred to as selection A and the *vice versa* as selection B. Phage outputs were titrated after each round to identify target specific enrichment (Figure 5).

Example 3. Isolation of HCDR3 from scFv library selection outputs and shuffling with synthetic HCDR1 and HCDR2 to generate a library of diversified VHs

This process takes the HCDR3s from the selection outputs from the scFv libraries as described in the above examples and combines them with fragments containing diversified VH HCDR1 and HCDR2 by splice overlap extension (SOE) PCR. There is an overlap between the two fragments of 23 bp that enabled this. This is referred to as 'HCDR3 shuffling' (see Van Blarcom et al. [2018; supra]), as depicted in Figure 6. The primers used to amplify the HCDR3s from the selection outputs are shown in Table 4.

Table 4. Primers for amplifying HCDR3

Sequence
GACACTGCAGTGTATTACTGTGC (SEQ ID NO: 5)
CCTTGGTCGACGCGCTCGAGACGGTGACC (SEQ ID NO: 6)

The HCDR1 and HCDR2 sequences were designed to reflect natural amino acid abundance and variation found in the respective VH1 and VH3 germline families with sequence liabilities removed (see Tables 5 and 6). The diversity was generated in the HCDR1 and HCDR2 of dsDNA fragments for each of the nominated VH1 and VH3 germline genes up to the start of HCDR3, by gene synthesis. A BglI site was appended to the 5' end of the fragment to allow subsequent cloning into the Fab vectors.

Table 5. HCDR1 and HCDR2 sequences of VH1 and VH3 germline families

Germline family	**HCDR1 sequence** (ORF aa 26-33)	**HCDR2 sequence** (ORF aa 51-58)
1-2	GYTFTGYY (SEQ ID NO: 7)	INPNSGGT (SEQ ID NO: 8)
1-46	GYTFTSYY (SEQ ID NO: 9)	INPSGGST (SEQ ID NO: 10)
1-58	GFTFTSSA (SEQ ID NO: 11)	IVVGSGNT (SEQ ID NO: 12)
1-69	GGTFSSYA (SEQ ID NO: 13)	IIPIFGTA (SEQ ID NO: 14)
3-7	GFTFSSYW (SEQ ID NO: 15)	IKQDGSEK (SEQ ID NO: 16)
3-11	GFTFSDYY (SEQ ID NO: 17)	ISSSGSTI (SEQ ID NO: 18)
3-30	GFTFSSYG (SEQ ID NO: 19)	ISYDGSNK (SEQ ID NO: 20)
3-23	GFTFSSYA (SEQ ID NO: 21)	ISGSGGST (SEQ ID NO: 22)
3-13	GFTFSSYD (SEQ ID NO: 23)	IGTAGDT- (SEQ ID NO: 24)

Table 6. The positions diversified in the HCDR1 and HCDR2 for each of the nominated germlines. The amino acid residues diversified at each position are shown in bold. The corresponding parental germline sequence for HCDR1 and HCDR2 are shaded. Any combinations that give rise to the following sequence liabilities are omitted: Deamidation motif NG and NS; the isomerisation motif DG, DS; the hydrolysis motif DP and DQ; and the N-glycosylation motif NXT and NXS, where X is any amino acid except for proline.

	HCDR1										**HCDR2**							
ORF aa #	26	27	28	29	30	31	32	33			51	52	53	54	55	56	57	58
Germline																		
1-2	G	Y	T	F	T	G	Y	Y			I	N	P	N	S	G	G	T
1-46	G	Y	T	F	T	S	Y	Y			I	N	P	S	G	G	S	T
1-58	G	F	T	F	T	S	S	A			I	V	V	G	S	G	N	T
1-69	G	G	T	F	S	S	Y	A			I	I	P	I	F	G	T	A
	G	**Y**	**T**	**F**	**T**	**G**	**Y**	**Y**			**I**	**S**	**P**	**G**	**S**	**G**	**G**	**T**
		F		**L**	**S**	**S**	**L**	**A**			**F**	**T**	**A**	**Y**	**G**		**E**	**A**
		G				**E**	**R**	**G**			**V**	**V**	**V**	**E**	**F**		**S**	
						Y	**S**	**S**				**I**		**S**			**T**	
														F				
														I				
3-7	G	F	T	F	S	S	Y	W			I	K	Q	D	G	S	E	K
3-11	G	F	T	F	S	D	Y	Y			I	S	S	S	G	S	T	I
3-30	G	F	T	F	S	S	Y	G			I	S	Y	D	G	S	N	K
3-23	G	F	T	F	S	S	Y	A			I	S	G	S	G	G	S	T
	G	**F**	**T**	**F**	**S**	**S**	**Y**	**A**			**I**	**K**	**Q**	**S**	**S**	**S**	**E**	**K**
				V	**G**	**G**	**A**	**Y**				**S**	**S**	**G**	**G**	**G**	**S**	**I**
							H	**G**				**Y**	**G**				**T**	**T**
							S	**S**					**Y**				**Y**	
								T										
3-13	G	F	T	**F**	**S**	**S**	**Y**	**D**			**I**	**G**	**T**	**A**	**G**	**D**	**T**	**-**
	G	**F**	**T**	**F**	**S**	**S**	**Y**	**A**			**I**	**G**	**T**	**A**	**G**	**S**	**T**	
				V	**G**	**G**	**A**	**Y**				**Y**	**S**	**G**				
							H	**G**										
							S	**S**										
								T										

In Example 2 above, the HCDR3 fragments were isolated from the round 2 selection outputs A and B. Briefly, a 100 µl aliquot of the glycerol stock from each output was used. Cells were pelleted and the plasmid purified from them using QIAprep Spin Miniprep Kit according to the manufacturer's protocol. The primers shown in Table 4 were used to amplify the HCDR3s using the plasmid preparation. The gel purified HCDR3 fragments were then combined with the diversified VH HCDR1 and HCDR2 fragments by splice overlap extension (SOE) PCR. The SOE PCR reaction was cleaned up using a Qiagen PCR purification column.

Example 4. Construction of Fab phagemid display vectors for cloning the HCDR3 shuffled fragment

For the Fab phage display library, a pCAT-Fab phagemid display vector was designed and constructed as depicted in Figure 7. The backbone of the vector is similar to the previously described pAMP-scFv vector (see Example 1), but here the displayed antibody is a Fab which consists of separate H and L chains including the CH1 and L chain constant domains. The L chain is preceded by an OmpA leader and the resistance marker is chloramphenicol.

SOE-PCR generated VH fragments from Example 3 were cloned into a pool of linearised pCAT-Fab phagemid display vectors (specifically into the BglI/XhoI site of the pCAT-Fab vector, replacing the tetracycline resistance gene) encoding either kappa or lambda germline L chain genes as used in the initial scFv library. VH fragments from the VHVK library selection outputs were ligated into a pCAT-Fab kappa vector pool and VH fragments from the VHVλ library selection outputs into a pCAT-Fab lambda vector pool, at the BglI/XhoI sites. After clean up, the ligated DNA were electroporated into TG1 cells to generate bacterial libraries. In total, 30 pCAT-Fab phagemid precursor vectors were synthesised. Eighteen of these vectors each contained one of 9 VK germline scaffolds combined with either JK2 or JK4 and twelve with 6 Vλ germline scaffolds combined with either JL1 or JL2. These are the same L chain germline combinations as shown in Table 2 above.

Example 5. Selections and screening against PD-1 using Fab libraries constructed with the shuffled HCDR3.

The assembled VH genes as described in Example 3 were digested with BglI and XhoI and ligated to the respective pool of pCAT-Fab phagemid display vectors encoding either kappa or lambda germline L chain genes. The ligation reactions, after clean-up using a mini-elute column (Qiagen), were electroporated into TG1 cells. Phage from these Fab display libraries were prepared and selected similarly to what was described in Examples 1 and 2 for the scFv libraries but under appropriate antibiotic selection. Three rounds of selections were carried out with decreasing concentration of the biotinylated PD-1 antigen at 100, 50 and 10 nM. The antigen was preloaded onto the beads and the human and cyno form alternated in sequential rounds.

A direct phage ELISA was performed on a small number of clones from chosen rounds of selection that confirmed that the selection had generated specific binders (Figure 8). To perform phage ELISA, PD-1 at 1 µg/ml was coated by passive adsorption onto Maxisorp plates in 1x PBS. Plates were blocked with 3% MPBS. The phage supernatant produced from single colonies was first blocked in 3% MPBS prior to addition to the antigen coated plate. This was subsequently washed three times with PBST, and once with 1x PBS prior to detection with commercially available anti-M13 HRP antibody conjugate (Sino Biological). Colour development was carried out with TMB and after quenching with 0.5 M sulphuric acid the absorbance at 450 nm determined.

A total of 1052 clones were picked, 352 from each round, and expressed soluble Fab in periplasmic extracts tested in direct binding and competition assays. For periplasmic extracts a starter culture for each clone, in 96 well format, was grown overnight in 2xYT supplemented with chloramphenicol 25 µg/ml and 2% glucose. 2 µl from each well was transferred into 2 ml deep well plate containing 900 µl 2xYT supplemented with chloramphenicol 25 µg/ml and 0.1 % glucose. This was incubated at 30 °C at 250 rpm until an OD_{600} between 0.5 -0.8) was reached. At this point soluble Fab expression was induced by the addition of IPTG to a final concentration of 0.5 mM. The plates were then incubated overnight at 30 °C at 250 rpm. Soluble Fab was released from the cell pellets of the overnight cultures by first resuspending the pellet in 120 µl ice cold 1x MES (MOPS, EDTA, sucrose) buffer followed by the addition of 180 µl of 20% ice cold MES buffer. Cells were spun down at x 2800g for 15 min at 4 °C and 180 µl of supernatant containing the soluble expressed Fabs was retained.

The clones were tested in a direct binding TR-FRET assay. 10 µl of diluted periplasmic extracts in 1xPBS/0.1% BSA were added into the wells of a 384 well plate. 5 µl of donor detection mix containing 8 nM human PD-1 Fc (Sino Biological) and 4 nM anti human IgG-cryptate (Cisbio) in 2x KF buffer (0.8 M KF in 1x PBS/ 0.1% BSA) was added into each well. This was followed by the addition of 5 µl of the acceptor mix containing 40 nM Mab anti-HA-D2 (Cisbio) in 2x KF buffer. The plates were protected from light and incubated at 4 °C overnight. The plates were read on a BMG ClarioStar microplate reader using the TR-FRET module. The assay measured the binding of HA and His tagged Fab fragments in crude periplasmic preparations to huFc tagged human or cyno PD-1. In the assay human Fc (IgG1) tagged antigens were detected using an anti-human Fc specific antibody labelled with europium-cryptate. Fabs that bound to the target antigen were detected using a fluorophore labelled secondary antibody specific for the HA or His tag. A FRET complex was formed through the binding interaction of the PD1 with the Fab and the close proximity of donor and acceptor fluorophores lead to an emission at 665 nm. Binding was observed for clones across all 3 rounds of selections for both kappas and lambdas (Figures

9A and 9B). Cross-reactivity (binding to both human and cyno PD1) was also observed (Figures 10A and 10B).

The clones were also tested in a TR-FRET competition assay. Here, Fabs were tested to see if they competed with the PDL1 ligand for binding to PD1. 10 µl of diluted periplasmic extracts in 1xPBS/0.1% BSA were added into the wells of a 384 well plate. 5 µl of donor detection mix containing 8 nM human PD-1 Fc (Sino Biologicals) and 4 nM anti human IgG-cryptate (Cisbio) in 2x KF buffer was added into each well. This was followed by the addition of 5 µl of the acceptor mix containing 100nM PD-L1 twin strep tagged protein (ACRO Biosystems) and 40 nM Strep-Mab Immo-Dy649 (IBA Life Sciences) in 2x KF Buffer. The plates were protected from light and incubated at 4 °C overnight. The plates were read on the BMG ClarioStar microplate reader using the TR-FRET module. A FRET complex was formed through the close proximity binding interaction of the PD-1 with the PD-L1. Specific inhibition of the interaction between PD-1 and PD-L1 was achieved with a number of clones resulting in a reduced fluorescent signal at 665 nm (Figure 11). The number of clones reducing the percentage specific binding increased from selection round 3 onwards for the Lambda library and plateaued after R4 for the Kappa library.

Example 6. Properties of reformatted Fabs as IgG

A subset of the Fab PD-1 inhibitors from Example 5 were produced as IgG in CHO cells using standard methodology. These were tested by ELISA for binding to PD-1 as a titration series. Goat anti-human IgG HRP (Jackson ImmunoResearch) was used as the secondary detection antibody. TMB substrate was used for colorimetric detection and the absorbance at 450 nm measured (Figure 12). The clones were further tested in a PD-1/ PDL-1 Blockade Bioassay (Promega Cat: J1250) according to the manufacturer's instructions. In brief, the PD-1/PD-L1 Blockade Bioassay is a bioluminescent cell-based assay that can measure the potency and stability of antibodies and of biologics designed to block the PD-1/PD-L1 interaction. The bioassay consists of two genetically engineered cell lines: PD-1 effector cells that express human PD-1 and a luciferase reporter driven by an NFAT response element (NFAT-RE); and PD-L1 aAPC/CHO-K1 cells. When co-cultured, the PD-1/PD-L1 interaction inhibits TCR signalling and NFAT-RE mediated luminescence. When the PD-1/PD-L1 interaction is disrupted, for example by an anti-PD-1 antibody, TCR activation induces luminescence that can be detected.

As shown in Figure 13, Antibody 3 gave a response in both assays comparable to the clinically approved anti-PD-1 control antibody (Nivolumab).

Although the present invention has been described with reference to preferred or exemplary embodiments, those skilled in the art will recognise that various modifications and variations to

the same can be accomplished without departing from the spirit and scope of the present invention and that such modifications are clearly contemplated herein. No limitation with respect to the specific embodiments disclosed herein and set forth in the appended claims is intended nor should any be inferred.

All documents cited herein are incorporated by reference in their entirety.

Claims

1. A method of identifying and/or selecting an antibody molecule which binds specifically to a target antigen, the method comprising the steps of:

(1) providing a single-chain variant fragment (scFv) phage display library of a population of engineered scFv nucleic acid constructs encoding scFv molecules, wherein each scFv nucleic acid construct comprises: (a) a first nucleic acid sequence encoding either a human germline VH scaffold amino acid sequence including an HCDR1 and HCDR2, or a modified version thereof having at least 80% sequence identity to the human germline VH scaffold nucleic amino acid sequence; (b) a second nucleic acid sequence encoding either an scFv human germline VL scaffold amino acid sequence including an LCDR1, LCDR2 and LCDR3, or a modified version thereof having at least 80% sequence identity to the human germline VL scaffold nucleic amino acid sequence; and (c) a third nucleic acid sequence encoding either a human B-cell donor-derived amino acid sequence including an HCDR3, or modified version thereof having at least 80% sequence identity to the human B-cell donor-derived amino acid sequence;

(2) screening the scFv phage display library against the target antigen and selecting one or more binding scFv molecules;

(3) identifying an enriched nucleic acid sequence encoding an HCDR3 from the one or more binding scFv molecules;

(4) generating a fragment antigen-binding (Fab) display library of a population of engineered Fab nucleic acid constructs encoding Fab molecules, wherein each Fab nucleic acid construct comprises: (a) a fourth nucleic acid sequence encoding either a VH scaffold amino acid sequence including an HCDR1 and HCDR2, each or both of which are human germline and/or diversified, or a modified version having at least 80% sequence identity thereto; (b) a fifth nucleic acid sequence encoding either a Fab human germline VL scaffold amino acid sequence including an LCDR1, LCDR2 and LCDR3, or a modified version thereof version thereof having at least 80% sequence identity to the Fab human germline VL scaffold nucleic amino acid sequence; and (c) an enriched nucleic acid sequence encoding either an HCDR3 identified in step (3), or a modified version thereof having at least 80% sequence identity to the HCDR3 identified in step (3);

(5) screening the Fab display library against the target antigen and selecting one or more binding Fab molecules; and

(6) optionally, reformatting the one or more binding Fab molecules into an alternative antibody molecule format,

thereby identifying and/or selecting an antibody molecule which binds specifically to the target antigen.

2. The method according to claim 1, wherein the scFv phage display library comprises:

(i) a kappa scFv phage display library in which each second nucleic acid sequence is a kappa ("K") light chain sequence or a modified version thereof; and
(ii) a lambda scFv phage display library in which each second nucleic acid sequence is a lambda ("λ") light chain sequence or a modified version thereof.

3. The method according to claim 2, wherein the kappa scFv phage display library comprises a first set of vectors having first nucleic acid sequences combined with second nucleic acid sequences containing kappa light chain sequences or derivatives thereof, and the lambda scFv phage display library comprises a second set of vectors having first nucleic acid sequences combined with second nucleic acid sequences containing lambda light chain sequences or derivatives thereof.

4. The method according to any preceding claim, wherein the Fab phage display library comprises:
(i) a kappa Fab phage display library in which each fifth nucleic acid sequence is a kappa light chain sequence or a derivative thereof; and
(ii) a lambda Fab phage display library in which each fifth nucleic acid sequence is a lambda light chain sequence of a derivative thereof.

5. The method according to claim 4, wherein the kappa Fab phage display library comprises a third set of vectors having fourth nucleic acid sequences combined with fifth nucleic acid sequences containing kappa light chain sequences or derivatives thereof, and the lambda Fab phage display library comprises a fourth set of vectors having fourth nucleic acid sequences combined with fifth nucleic acid sequences containing lambda light chain sequences or derivatives thereof.

6. The method according to claims 3 and 5, in which the enriched nucleic acid sequences encoding the HCDR3s are combined for the Fab display library with either:
the third set of vectors if the enriched nucleic acid sequences are derived from binding scFv molecules obtained using the first set of vectors;
the fourth set of vectors if the enriched nucleic acid sequences are derived from binding scFv molecules obtained using the second set of vectors.

7. The method according to any preceding claim, in which the population of scFv and/or Fab nucleic acid constructs include a plurality of first nucleic acid sequences and/or fourth nucleic acid sequences which are one or more (for example, at least two, three, four, five, six, seven, eight, nine or ten) of the human germline VH gene segment-derived nucleic acid sequences selected from the group consisting of: VH1-2, VH1-46, VH1-58, VH1-69, VH3-7, VH3-

11, VH3-13, VH3-23, VH3-30, VH5-51, a modified version of any of these segments encoding HCDR1 and/or HCDR2 and having at least 80% sequence identity to the corresponding unmodified segment, and a combination thereof.

8. The method according to any preceding claim, in which the population of scFv and/or Fab nucleic acid constructs include a plurality of second nucleic acid sequences and/or fifth nucleic acid sequences which are: (i) one or more (for example, at least two, three, four, five, six, seven, eight or nine) human kappa L chain gene segment-derived nucleic acid sequences selected from the group consisting of: K1-5, K1-6, K1-16, K1-39, K2-28, K3-15, K3-11, K3-20, K3-D7, a modified version of any of these segments encoding LCDR1, LCDR2 and/or LCDR3 and having at least 80% sequence identity to the corresponding unmodified segment, and a combination thereof, combined with a kappa J gene segment-derived nucleic acid sequence selected from the group consisting of: J2, J4, a modified version of J2 or J4 having at least 80% sequence identity to the corresponding unmodified segment, and a combination thereof; or (ii) one or more (for example, at least two, three, four, five or six) human lambda L chain gene segment-derived nucleic acid sequences selected from the group consisting of: L2-8, L2-14, L2-18, L3-27, L7-43, L4-46 a modified version of any of these segments encoding LCDR1, LCDR2 and/or LCDR3 and having at least 80% sequence identity to the corresponding unmodified segment, and a combination thereof, combined with a lambda J gene segment-derived nucleic acid sequence selected from the group consisting of: J1, J2, a modified version of J1 or J2 having at least 80% sequence identity to the corresponding unmodified segment and a combination thereof; or a combination of (i) and (ii).

9. The method according to any preceding claim, in which the fourth nucleic acid sequence encodes either a diversified HCDR1 and/or diversified HCDR2 modified to reflect natural amino acid abundance and variation in VH1 and VH3 human germline families, optionally excluding HCDR1 and HCDR2 sequences with sequence liabilities, or a further modified version of having at least 80% sequence identity thereto.

10. The method according to claim 9, in which the sequence liabilities are: deamidation motif NA, NG and/or NS; and/or isomerisation motif DG and/or DS; and/or hydrolysis motif DP and/or DQ; and/or unpaired cysteine C; and/or N-glycosylation motif NXT and/or NXS, where X is any amino acid except P.

11. An scFv phage display library obtained or obtainable according to the method of any of claims 1-10.

12. A Fab phage display library obtained or obtainable according to the method of any of claims 1-10.

13. A library of vectors containing the nucleic acid constructs as defined in any of claims 1-10.

14. A host cell or library of host cells containing the library of any of claims 11-13.

15. A kit containing the library as defined in any of claims 11-14.

16. An antibody molecule obtained or obtainable according to the method of any of claims 1-10.

17. The method, library, kit or antibody molecule according to any preceding claim, in which the antibody molecule is a whole antibody, an antibody fragment or a smaller antibody molecule format, for example a single chain antibody molecule, an scFv molecule, a Fab fragment or a bivalent antibody molecule.

Amendments to the claims have been filed as follows

Claims

1. A method of identifying and/or selecting an antibody molecule which binds specifically to a target antigen, the method comprising the steps of:

(1) providing a single-chain variant fragment (scFv) phage display library of a population of engineered scFv nucleic acid constructs encoding scFv molecules, wherein each scFv nucleic acid construct comprises: (a) a first nucleic acid sequence encoding either a human germline VH scaffold amino acid sequence including an HCDR1 and HCDR2, or a modified version thereof having at least 80% sequence identity to the human germline VH scaffold nucleic amino acid sequence; (b) a second nucleic acid sequence encoding either an scFv human germline VL scaffold amino acid sequence including an LCDR1, LCDR2 and LCDR3, or a modified version thereof having at least 80% sequence identity to the human germline VL scaffold nucleic amino acid sequence; and (c) a third nucleic acid sequence encoding either a human B-cell donor-derived amino acid sequence including an HCDR3, or modified version thereof having at least 80% sequence identity to the human B-cell donor-derived amino acid sequence;

(2) screening the scFv phage display library against the target antigen and selecting one or more binding scFv molecules;

(3) identifying an enriched nucleic acid sequence encoding an HCDR3 from the one or more binding scFv molecules;

(4) generating a fragment antigen-binding (Fab) display library of a population of engineered Fab nucleic acid constructs encoding Fab molecules, wherein each Fab nucleic acid construct comprises: (a) a fourth nucleic acid sequence encoding either a VH scaffold amino acid sequence including synthetic HCDR1 and HCDR2which are diversified from human germline VH1 and VH3 sequences to reflect natural amino acid abundance and variation in their respective VH1 and VH3 human germline families, excluding HCDR1 and HCDR2 sequences with sequence liabilities, or a modified version having at least 80% sequence identity thereto; (b) a fifth nucleic acid sequence encoding either a Fab human germline VL scaffold amino acid sequence including an LCDR1, LCDR2 and LCDR3, or a modified version thereof version thereof having at least 80% sequence identity to the Fab human germline VL scaffold nucleic amino acid sequence; and (c) an enriched nucleic acid sequence encoding either an HCDR3 identified in step (3), or a modified version thereof having at least 80% sequence identity to the HCDR3 identified in step (3);

(5) screening the Fab display library against the target antigen and selecting one or more binding Fab molecules; and

(6) optionally, reformatting the one or more binding Fab molecules into an alternative antibody molecule format,

thereby identifying and/or selecting an antibody molecule which binds specifically to the target antigen.

2. The method according to claim 1, wherein the scFv phage display library comprises:
(i) a kappa scFv phage display library in which each second nucleic acid sequence is a kappa ("K") light chain sequence or a modified version thereof; and
(ii) a lambda scFv phage display library in which each second nucleic acid sequence is a lambda ("λ") light chain sequence or a modified version thereof.

3. The method according to claim 2, wherein the kappa scFv phage display library comprises a first set of vectors having first nucleic acid sequences combined with second nucleic acid sequences containing kappa light chain sequences or derivatives thereof, and the lambda scFv phage display library comprises a second set of vectors having first nucleic acid sequences combined with second nucleic acid sequences containing lambda light chain sequences or derivatives thereof.

4. The method according to any preceding claim, wherein the Fab phage display library comprises:
(i) a kappa Fab phage display library in which each fifth nucleic acid sequence is a kappa light chain sequence or a derivative thereof; and
(ii) a lambda Fab phage display library in which each fifth nucleic acid sequence is a lambda light chain sequence of a derivative thereof.

14 06 23

5. The method according to claim 4, wherein the kappa Fab phage display library comprises a third set of vectors having fourth nucleic acid sequences combined with fifth nucleic acid sequences containing kappa light chain sequences or derivatives thereof, and the lambda Fab phage display library comprises a fourth set of vectors having fourth nucleic acid sequences combined with fifth nucleic acid sequences containing lambda light chain sequences or derivatives thereof.

6. The method according to claims 3 and 5, in which the enriched nucleic acid sequences encoding the HCDR3s are combined for the Fab display library with either:

the third set of vectors if the enriched nucleic acid sequences are derived from binding scFv molecules obtained using the first set of vectors;

the fourth set of vectors if the enriched nucleic acid sequences are derived from binding scFv molecules obtained using the second set of vectors.

7. The method according to any preceding claim, in which the population of scFv and/or Fab nucleic acid constructs include a plurality of first nucleic acid sequences and/or fourth nucleic acid sequences which are one or more (for example, at least two, three, four, five, six,

seven, eight, nine or ten) of the human germline VH gene segment-derived nucleic acid sequences selected from the group consisting of: VH1-2, VH1-46, VH1-58, VH1-69, VH3-7, VH3-11, VH3-13, VH3-23, VH3-30, VH5-51, a modified version of any of these segments encoding HCDR1 and/or HCDR2 and having at least 80% sequence identity to the corresponding unmodified segment, and a combination thereof.

8. The method according to any preceding claim, in which the population of scFv and/or Fab nucleic acid constructs include a plurality of second nucleic acid sequences and/or fifth nucleic acid sequences which are: (i) one or more (for example, at least two, three, four, five, six, seven, eight or nine) human kappa L chain gene segment-derived nucleic acid sequences selected from the group consisting of: K1-5, K1-6, K1-16, K1-39, K2-28, K3-15, K3-11, K3-20, K3-D7, a modified version of any of these segments encoding LCDR1, LCDR2 and/or LCDR3 and having at least 80% sequence identity to the corresponding unmodified segment, and a combination thereof, combined with a kappa J gene segment-derived nucleic acid sequence selected from the group consisting of: J2, J4, a modified version of J2 or J4 having at least 80% sequence identity to the corresponding unmodified segment, and a combination thereof; or (ii) one or more (for example, at least two, three, four, five or six) human lambda L chain gene segment-derived nucleic acid sequences selected from the group consisting of: L2-8, L2-14, L2-18, L3-27, L7-43, L4-46 a modified version of any of these segments encoding LCDR1, LCDR2 and/or LCDR3 and having at least 80% sequence identity to the corresponding unmodified segment, and a combination thereof, combined with a lambda J gene segment-derived nucleic acid sequence selected from the group consisting of: J1, J2, a modified version of J1 or J2 having at least 80% sequence identity to the corresponding unmodified segment and a combination thereof; or a combination of (i) and (ii).

9. The method according to any preceding claim, in which the sequence liabilities are: deamidation motif NA, NG and/or NS; and/or isomerisation motif DG and/or DS; and/or hydrolysis motif DP and/or DQ; and/or unpaired cysteine C; and/or N-glycosylation motif NXT and/or NXS, where X is any amino acid except P.

10. An scFv phage display library obtained or obtainable according to the method of any of claims 1-9.

11. A Fab phage display library obtained or obtainable according to the method of any of claims 1-9.

12. A library of vectors containing the nucleic acid constructs as defined in any of claims 1-9.

13. A host cell or library of host cells containing the library of any of claims 10-12.

14. A kit containing the library as defined in any of claims 10-13.

15. An antibody molecule obtained or obtainable according to the method of any of claims 1-9.

16. The method, library, kit or antibody molecule according to any preceding claim, in which the antibody molecule is a whole antibody, an antibody fragment or a smaller antibody molecule format, for example a single chain antibody molecule, an scFv molecule, a Fab fragment or a bivalent antibody molecule.

Application No:	GB2300352.8	**Examiner:**	Dr Jeremy Kaye
Claims searched:	1-17	**Date of search:**	20 February 2023

Patents Act 1977: Search Report under Section 17

Documents considered to be relevant:

Category	Relevant to claims	Identity of document and passage or figure of particular relevance
Y	1-17	WO2014/128628 A1 (AFFINITY BIOSCIENCES PTY LTD.) see esp. Examples 7-9
Y	1-17	mAbs, Vol.1, Gupta, A. et al., "Directed evolution of an...", pp.268-280 see esp. p.278 Methods
Y	1-17	PLoS One, Vol.7, 2012, Venet, S. et al., "Transferring the characteristics...", Article No.: e43471
Y	1-17	mAbs, Vol.11, 2019, Valadon, P. et al., "ALTHEA gold libraries...", pp.516-531
Y	1-17	mAbs, Vol.13, 2021, Teixeira, A.A.R., "Drug-like antibodies with high affinity...", Article No.: 1980942

Categories:

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.

Field of Search:

Search of GB, EP, WO & US patent documents classified in the following areas of the UKC^{X} :

Worldwide search of patent documents classified in the following areas of the IPC

The following online and other databases have been used in the preparation of this search report

WPI, EPODOC, Patent Fulltext, BIOSIS, MEDLINE, XPESP

International Classification:

Subclass	Subgroup	Valid From
None		