CLAIMS

1. A method of identifying a cell which produces a scFv which is a specific binding partner ~~which binds to~~for a target polypeptide, the method comprising the steps:

(a) expressing a library of ~~binding partners~~scFvs in a population of mammalian cells,

~~wherein each binding partner comprises a framework and a plurality of variable regions, each plurality of variable regions endowing that binding partner with a specific binding affinity for a target,~~

wherein each cell in the population of mammalian cells expresses at least one member of the ~~binding partner~~scFv library from one or more retroviral (e.g. lentiviral) vectors which have been integrated into the genome of that cell,

wherein each ~~binding partner~~scFv comprises a first detectable tag,

wherein each ~~binding partner~~scFv is secreted from the cell in the population of cells in which it is produced, ~~and~~

wherein each cell in the population of mammalian cells comprises an expression construct comprising ~~a~~an inducible promoter operably linked to a nucleotide sequence encoding the target polypeptide, wherein the target polypeptide is an integrated membrane protein, wherein the target polypeptide ~~optionally~~ comprises a second detectable tag, and wherein the expression construct is integrated into the genome of each cell;

(b) optionally removing cells from the population of mammalian cells to which scFvs ~~binding partners~~ bind;

(c) ~~expressing or~~ inducing expression of the target polypeptide from the expression construct such that the target polypeptide is displayed on the outer surface of each cell in the population of mammalian cells; and

(d) isolating ~~or identifying~~ cells within the population of mammalian cells to which secreted scFvs which are specific binding partners for the target polypeptide are bound to the target polypeptide, wherein specific binding partners for the target polypeptide are ones which bind to the target polypeptide though at least one antigen recognition site located in a variable region of the scFv,~~,~~

wherein cells to which ~~specific binding partners~~scFvs are bound are isolated ~~or identified~~ using~~:~~

~~(i) flow cytometry, or~~

~~(ii) magnetic sorting~~magnetic activated cell sorting (MACS), ~~and~~or

~~(iii) a micro-fluidics system wherein cells from the population of mammalian cells are contained within each chamber in a plurality of isolated chambers,~~

detecting the first and second detectable tags using independent antibodies to which are attached first and second members of a donor-acceptor FRET pair,

wherein the cells ~~to~~ on which ~~specific binding partners~~scFvs are ~~bound~~ detected are ~~ones~~ cells which produce ~~specific binding partners~~scFvs which bind to the target polypeptide.

~~2. A method as claimed in claim 1, wherein in Step (a), the promoter is an inducible promoter and wherein Step (c) comprises:~~

~~(c) inducing expression of the target polypeptide from the expression construct such that the target polypeptide is displayed on the outer surface of each cell in the population of mammalian cells.~~

~~3. A method as claimed in claim 1 or claim 2, wherein the binding partner is an antibody, antibody fragment or antibody mimetic, preferably an scFv, or a soluble T-cell receptor.~~

~~4.~~2. ~~A~~The method as claimed in ~~any one of the preceding claims~~claim 1, wherein the method additionally comprises, prior to Step (a), the step of:

contacting the population of mammalian cells with a library of retroviral (e.g. lentiviral) particles, each particle comprising a retroviral (e.g. lentiviral) vector encoding a member of a ~~binding partner~~scFv library and a detectable tag, under conditions such that at least one retroviral (e.g. lentiviral) vector is integrated into the genome of each cell (or substantially each cell) in the library.

~~5.~~3. ~~A~~The method as claimed in ~~any one of the preceding claims~~clam 1 or claim 2, wherein the method additionally comprises, prior to Step (a), the step:

integrating, into the genome of each (or substantially each) cell in the population of cells, an expression construct comprising ~~a promoter (preferably~~ an inducible promoter) operably linked to a nucleotide sequence encoding the target polypeptide and wherein the target polypeptide ~~optionally~~ comprises a second detectable tag.

~~6.~~4. ~~A~~The method as claimed in any one of the preceding claims, wherein the detectable tag is HA or a Myc tag.

~~7.~~5. ~~A~~The method as claimed in any one of ~~claims 2 to 6~~the preceding claims, wherein the inducible promoter comprises a plurality of Tet operator sequences to which the Tet repressor protein (TetR) is capable of binding.

~~8.~~6. ~~A~~The method as claimed in any one of the preceding claims, wherein Step (b) and/or Step (c) additionally comprises the step:

contacting all or part of the population of mammalian cells with an excess of capturing cells which express the target polypeptide (and optionally a second detectable tag), but do not express a ~~binding partner~~scFv,

wherein each capturing cell comprises a label (preferably a fluorescence label) which allows the capturing cells to be distinguished from the population of mammalian cells.

~~9. A method as claimed in any one of the preceding claims, wherein, in Step (d), specific binding partners are detected using an antibody against the first detectable tag which is labelled with a fluorophore or a paramagnetic particle.~~

~~10.~~7. ~~A~~The method as claimed in any one of the preceding claims, wherein ~~each binding partner comprises a first detectable tag (preferably HA), wherein the target polypeptide comprises a second detectable tag (preferably Myc epitope), and wherein the first and second detectable tags are detected using independent antibodies to which are attached~~the first and second members of a~~the~~ donor-acceptor FRET pair are ~~(preferably~~ FITC and Alexa Fluor 594~~)~~.

~~11. A method as claimed in any one of the preceding claims, wherein, in Step (d), the flow cytometry is FACS or the magnetic sorting is MACS.~~

~~12. A method as claimed in any one of the preceding claims, wherein, in Step (d), the micro-fluidics system is one wherein cells from the population of mammalian cells are contained within each droplet in a plurality of isolated droplets.~~

~~13. A method as claimed in claim 12, wherein each droplet comprises 30-50 cells.~~

~~14. A method as claimed in claim 12 or claim 13, wherein the droplets have a volume of 1-2000 pL, preferably 100-1000 pL and more preferably about 200 pL.~~

~~15. A method as claimed in any one of the preceding claims, wherein, in Step (d), the micro-fluidics system is one wherein cells from the population of mammalian cells are~~

~~contained within pens which are arranged in an array on a solid substrate (preferably a chip).~~

~~16. A method as claimed in claim 15, wherein a micro-fluidics system is used wherein one or more cells from the population of mammalian cells are contained within each pen in an array of isolated pens; wherein non-target polypeptide displaying cells (preferably CHO cells) are contacted with or juxtaposed against the edges of the isolated pens in the array such that binding partners which are secreted from the cells within the pens are capable of contacting the non-target polypeptide displaying cells;~~
~~and rejecting pens in which cell surface binding of binding partners has been detected on the surface of the non-target polypeptide displaying cells.~~

~~17. A method as claimed in any one of the preceding claims, wherein in Step (d), each chamber comprises one cell which expresses the target polypeptide and a plurality (preferably 1-50) of cells (preferably CHO cells) which do not express the target polypeptide; and wherein chambers in which cell surface binding of binding partners has been detected are rejected if cell surface binding of binding partners is detected on more than one cell per chamber.~~

~~18.~~8. ~~A~~The method as claimed in any one of the preceding claims, wherein the method additionally comprises the step:

(e) sequencing ~~(~~part of all of~~)~~ the nucleotide sequences in the isolated cells or identified cells which encode the ~~specific binding partners~~scFvs which bind to the target polypeptide, preferably using a non-Sanger sequencing method.

~~19. A process for producing a population of mammalian cells, the process comprising the steps:~~

~~(A) integrating, into the genome of each (or substantially each) cell in the population of mammalian cells, an expression construct comprising a promoter (preferably an inducible promoter) operably linked to a nucleotide sequence~~

~~encoding a target polypeptide and wherein the target polypeptide optionally comprises a second detectable tag;~~

~~and~~

~~(B) contacting the population of mammalian cells with a library of retroviral (e.g. lentiviral) particles, each particle comprising a retroviral (preferably lentiviral) vector encoding a member of a binding partner library and a first detectable tag;~~

~~so as to produce a population of mammalian cells wherein each cell (or substantially each cell) in the population of mammalian cells secretes one or more binding partners (preferably antibodies or antibody mimetics), and~~

~~wherein each cell in the population of mammalian cells displays or is capable of displaying (preferably upon induction) the target polypeptide, and optionally a second detectable tag, on the outer surface of the cell.~~