US20100212035 describes methods for generation of rodents capable of expressing exogenous antibody by targeting the immunoglobulin locus of a mammalian embryo with a meganuclease to direct integration of a donor DNA. The potential to create variant libraries of meganucleases to create new DNA cleavage specificities is described but it his does not contemplate the use of meganucleases towards the generation of libraries of binders.

WO 2013/190032 A1 describes integration of genes into a specific locus (Fer1L4) previously modified with exogenous DNA ("a site specific integration" SSI host cell) to incorporate recombinase sites, such as loxP and FRT sites for recombinase-mediated site-specific gene introduction. Nuclease-directed library generation is not described.

WO 2012/167192 A2 describes targetting genes to a locus that can then be selected for amplification. Nuclease-directed methods are employed to target the locus. Nuclease-directed library generation is not described.

US 2009/0263900A1 describes DNA molecules comprising homology arms and their use in methods of homologous recombination. Nuclease-directed library generation is not described.

WO 2011/100058 describes methods for integration of nucleic acid into a genome that avoids the need for long homology arms and instead relies on microhomology or "sticky ends" on the genome and donor to help direct integration. Nuclease-directed library generation is not described.

WO 2011/090804 describes methods for integration of multiple genes or multiple copies of the same gene using different zinc finger nucleases (ZFNs) in sequential rounds. Nuclease-directed library generation is not described.

WO2014/039872 describes methods for engineering plant cells, incorporating a "landing site" into which donor DNA is integrated by homologous recombination or non-homologous end joining using site-directed nucleases. Bacterial artificial chromosome (BAC) libraries are used for initial cloning of donor DNA. Libraries are mentioned in relation to Illumina sequencing methods. Nuclease-directed library generation is not described.

W02007/047859 A2 describes methods for engineering specificity of meganucleases and their used to target genomic loci. Libraries of mutant meganucleases that may contain

Methods of the disclosure thus produce high quality libraries of binders in eukaryotic cells, which can be screened to identify cells encoding and expressing a specific binder for a target of interest.

Also disclosed are new and improved methods of preparing eukaryotic cell libraries, the libraries themselves, isolation of desired binders, encoding nucleic acid and cells from the libraries, and uses of the libraries such as for expression and screening of binding molecules and for screening for the effects of binding molecules. Various methods will be described for producing libraries *in vitro* and using libraries *in vitro* or *in vivo*.

The disclosure provides a method of producing a library of eukaryotic cell clones containing DNA encoding a diverse repertoire of binders, the method comprising using a site-specific nuclease to target cleavage of eukaryotic cell DNA to enhance site-specific integration of binder genes into the cellular DNA through endogenous cellular DNA repair mechanisms.

A method of producing a library of eukaryotic cell clones containing DNA encoding a diverse repertoire of binders may comprise:

providing donor DNA molecules encoding the binders, and eukaryotic cells,

introducing the donor DNA into the cells and providing a site-specific nuclease within the cells, wherein the nuclease cleaves cellular DNA to create an integration site at which the donor DNA becomes integrated into the cellular DNA, integration occurring through DNA repair mechanisms endogenous to the cells.

For multimeric binders comprising at least a first and second subunit (i.e., separate polypeptide chains, such as antibody VH and VL domains presented within a Fab or IgG format), the multiple subunits may be encoded on the same molecule of donor DNA. However, it may be desirable to integrate the different subunits into separate loci, in which case the subunits can be provided on separate donor DNA molecules. These could be integrated within the same cycle of nuclease-directed integration or they may be integrated sequentially using nuclease-directed integration for one or both integration steps.

In one aspect, the present invention provides a method of producing a library of eukaryotic cell clones containing DNA encoding a diverse repertoire of binders that recognise a target, comprising providing donor DNA molecules encoding the binders, and eukaryotic cells, wherein the binders are antibodies, proteins or peptides, introducing the donor DNA into the cells and providing a site-specific nuclease within the cells, wherein the nuclease cleaves a recognition sequence in cellular DNA to create an integration site at which the donor DNA becomes integrated into the cellular DNA, integration occurring through DNA repair mechanisms endogenous to the cells, thereby creating recombinant cells containing donor DNA integrated in the cellular DNA, and culturing the recombinant cells to produce clones,

be cultured to express both subunits. Multimeric binders are obtained by expression and assembly of the separately encoded subunits.

In the above example, nuclease-directed integration is used to integrate DNA encoding a second subunit into cells already containing DNA encoding a first subunit. The first subunit could be previously introduced using the techniques of the present disclosure or any other suitable DNA integration method. An alternative approach is to use nuclease-directed integration in a first cycle of introducing donor DNA, to integrate a first subunit, followed by introducing the second subunit either by the same approach or any other suitable method. If the nuclease-directed approach is used in multiple cycles of integration, different site-specific nucleases may optionally be used to drive nuclease-directed donor DNA integration at different recognition sites. A method of generating the library may comprise:

providing first donor DNA molecules encoding the first subunit, and providing eukaryotic cells, introducing the first donor DNA into the cells and providing a site-specific nuclease within the cells, wherein the nuclease cleaves a recognition sequence in cellular DNA to create an integration site at which the donor DNA becomes integrated into the cellular DNA, integration occurring through DNA repair mechanisms endogenous to the cells, thereby creating a first set of recombinant cells containing first donor DNA integrated in the cellular DNA,

culturing the first set of recombinant cells to produce a first set of clones containing DNA encoding the first subunit,

introducing second donor DNA molecules encoding the second subunit into cells of the first set of clones, wherein the second donor DNA is integrated into cellular DNA of the first set of clones, thereby creating a second set of recombinant cells containing first and second donor DNA integrated into the cellular DNA, and

culturing the second set of recombinant cells to produce a second set of clones, these clones containing DNA encoding the first and second subunits of the multimeric binder,

thereby providing a library of eukaryotic cell clones containing donor DNA encoding the repertoire of multimeric binders.

In another aspect, the present invention provides a method of producing a library of eukaryotic cell clones containing DNA encoding a diverse repertoire of multimeric binders that recognise a target, each binder comprising a first and a second subunit, wherein the method comprises providing eukaryotic cells containing DNA encoding the first subunit and providing donor DNA molecules encoding the second binder subunit, wherein the binders are antibodies, proteins or peptides, introducing the donor DNA into the cells and providing a site-specific nuclease within the cells, wherein the nuclease cleaves a recognition sequence in cellular DNA to create an integration site at which the donor DNA becomes integrated into the cellular DNA, integration occurring through DNA repair mechanisms endogenous to the cells, thereby creating recombinant cells which contain donor DNA integrated in the cellular DNA, and

culturing the recombinant cells to produce clones containing DNA encoding the first and second subunits of the multimeric binder, thereby providing a library of eukaryotic cell clones containing donor DNA encoding the repertoire of multimeric binders.

In yet another aspect, the present invention provides a method of producing a library of eukaryotic cell clones containing DNA encoding a diverse repertoire of multimeric binders that recognise a target, each binder comprising at least a first and a second subunit, wherein the binders are antibodies, proteins or peptides, wherein the method comprises providing first donor DNA molecules encoding the first subunit, and providing eukaryotic cells, introducing the first donor DNA into the cells and providing a site-specific nuclease within the cells, wherein the nuclease cleaves a recognition sequence in cellular DNA to create an integration site at which the donor DNA becomes integrated into the cellular DNA, integration occurring through DNA repair mechanisms endogenous to the cells, thereby creating a first set of recombinant cells containing first donor DNA integrated in the cellular DNA, culturing the first set of recombinant cells to produce a first set of clones containing DNA encoding the first subunit, introducing second donor DNA molecules encoding the second subunit into cells of the first set of clones, wherein the second donor DNA is integrated into cellular DNA of the first set of clones, thereby creating a second set of recombinant cells containing first and second donor DNA integrated into the cellular DNA, and culturing the second set of recombinant cells to produce a second set of clones, these clones containing DNA encoding the first and second subunits of the multimeric binder, thereby providing a library of eukaryotic cell clones containing donor DNA encoding the repertoire of multimeric binders

Site-specific integration of donor DNA into cellular DNA creates recombinant cells, which can be cultured to produce clones. Individual recombinant cells into which the donor DNA has been integrated are thus replicated to generate clonal populations of cells – "clones" – each clone being derived from one original recombinant cell. Thus, the method generates a number of clones corresponding to the number of cells into which the donor DNA was successfully integrated. The collection of clones form a library encoding the repertoire of binders (or, at an intermediate stage where binder subunits are integrated in separate rounds, the clones may

In yet another aspect, the present invention provides a method of screening for a cell of a desired phenotype, wherein the phenotype results from expression of a binder by the cell, the method comprising producing a library by the method disclosed herein, culturing the library cells to express the binders, and detecting whether the desired phenotype is exhibited, and optionally further comprising: recovering cells of a clone that expresses a binder that produces the desired phenotype, and/or: isolating DNA encoding the binder from the recovered clone, thereby obtaining DNA encoding a binder which produces the desired phenotype. In another aspect, the present invention refers to a method of producing a diverse repertoire of binders, comprising producing a library by a method according to the present invention and culturing the library cells to express the binders.

The present disclosure offers advantages over previous approaches for construction of libraries in higher eukaryotes. Some studies have used lentiviral infection to introduce antibody genes into mammalian reporter cells [106]. This has the advantage that large libraries can be generated but there is no control over the site of integration and copy number is controlled by using a low multiplicity of infection (as discussed above). In an alternative approach antibody genes were introduced via homologous recombination, without the benefit of nuclease-directed

Brief Description of the Drawings

Embodiments of the invention will now be described in more detail, with reference to the accompanying drawings, which are as follows:

Figure 1. Vector for expression of IgG formatted antibodies

a. pDUAL D1.3, a dual promoter expression vector for IgG secretion (in in "pCMV/myc/ER" vector backbone)
b. pINT3-D1.3, a dual promoter expression vector for IgG secretion (in "pSF-CMV-f1-Pac1" vector backbone)
c. pCMV/myc/ER vector backbone. ECoR1 site precedes CMV promoter. BstB1 and BstZ171 sites flank the SV40 poly A sites.
d. pSF-CMV-f1-Pac1 vector backbone (Oxford Genetics)
e. synthetic gene with exon encoding PDGFR transmembrane region (TM) and exon causing secretion (sec). Solid arrows represent Rox recombination sites.

Figure 2. Sequence of pD1 (SEQ ID NO: 1, 2, 3, 4 & 5): a dual promoter antibody expression cassette for surface expression.

Features:

pEF promoter 13-1180
BM40 leader 1193-1249
Humanised D1.3 VL 1250-1578
Human C kappa 1577-1891BGH poly A 1916-2130
CMV promoter 2146-2734
Mouse VH leader with intron 32832-3414
Humanised D1.3 VH 3419-3769
Optimised human IgG2 CH1-CH3

Figure 3. Construction of AAVS Donor plasmid (pD2)

a. Representation of the human AAVS locus. Exon 1 and Exon 2 of the AAVS locus (encoding protein phosphatase 1, regulatory subunit 12C, PPP1R12C) are separated by an intron of 4428 bp. Splicing is in frame "0" i.e. splicing occurs between 2 intact codons from each exon. TALENs and CRISPR/Cas9 constructs are available to cleave within this intron. Hatched blocks represent the regions to the left and right of this cleavage site that are

I-Sce1 344-361
Blasticidin resistance 417-818
Poly A 832-1070

Figure 16. Sequence of I-Sce1 meganuclease construct (SEQ ID NO: 22 & 23)

Figure 17. Flow cytometry analysis comparing nuclease-directed integration using I-Sce1 meganuclease with recombinases.
Clone 6F cells were co-transfected with pD5-D1.3 and plasmids encoding the indicated nuclease/recombinase. Cells were selected with blasticidin and analysed 13 days after transfection using biotinylated anti-human Fc antibodies and streptavidin phycoerythrin. Percentage positive cells are indicated (also summarized in Table 5)
a. Non-transfected, b Donor only c. I-Sce1, d, eGFP TALEN, e. Cre, f. Flp recombinase (encoded by pOG44 plasmid).

Figure 18. Nuclease-directed integration drives homologous recombination and "non-homologous end joining" (NHEJ).
a. Representation of structure of plasmid pD5 used to target the multiple "landing site" within the intron of "clone 6F cells showing position of primer J48. The "landing site" in this plasmid incorporates a FRT site, a lox2272 site and an I-Sce1 meganuclease site (but no GFP TALEN site).
b. Representation of integration site within clone 6F (derived from pD4) showing position of primer J44. "Landing site" incorporates elements for directing integration which are FRT, lox 2272, I-Sce1 meganuclease and GFP TALEN.
c. Representation of clone 6F integration site after homologous recombination of pD5, showing position of primers J44 and J48.
d. Representation of integration site of clone 6F after NHEJ or Flp recombination of pD5, showing position of primers J44, J46 and reverse primer J44. The double headed arrow indicates the "extra" plasmid derived DNA incorporated by NHEJ or Flp-directed integration. Note in this example the incoming plasmid DNA (pD5) has homology arms (which direct homologous recombination but are not required for NHEJ). These sequences are retained after integration by NHEJ, causing a duplication of the sequence represented within the homology arms with one pair coming from the plasmid in this case and the other pair representing the endogenous genomic sequences. For simplicity the plasmid encoded homology arms are not shown, just their equivalent sequence within the genome.

a. Single staining FGFR2-488 (sample 1b from Table 7)
b. single staining FGFR1-633 (sample 1b from Table 7)
c. Dual staining FGFR1-633/ FGFR2-488 (sample 1b from Table 7)
d. Dual staining FGFR1-633/ FGFR2-488 (sample 3 from Table 7)

Figure 24. Recovery of antibody genes after flow sorting.
A population of antibodies was selected by one round of phage display and a mammalian display library was created by Flow electroporation using the Maxcyte system. Cells were sorted using either 1nM or 10nM antigen and mRNA isolated directly. The antibody genes were recovered by PCR, cloned into a bacterial expression vector and the proportion of ELISA positives determined ("1nM output", "10nM output"). This was compared with the original round 1 output ("R1 phage output"). Plot shows the profile of ELISA signals obtained with each population.

Figure 25. pINT20 vector for expression of T Cell Receptors.

a. Representation of the dual promoter plasmid pINT20 showing AAVS homology arms, puromycin selectable gene (with region around splice acceptor site shown below). Alpha chain (encompassing variable alpha, mouse alpha constant-CD3ζ) is flanked by Nhe1, Not1 and Acc65I restriction sites and is under the control of pEF promoter. The beta chain (encompassing variable beta, mouse beta constant-CD3ζ) is flanked by Nco1, Xho1 and hind3 sites and is under the control of the CMV promoter.
b. Sequence at the splice acceptor and beginning of puromycin gene (SEQ ID NO: 24 & 25)
c. Sequence of T cell receptor clone c12/c2 alpha chain construct showing Nhe1, Not1 and Acc65I restriction sites (SEQ ID NO: 26 & 27).
d. Sequence of T cell receptor clone c12/c2 beta chain construct showing Nco1. Xho1 and Hind 3 restriction sites (SEQ ID NO: 28 & 29).
e. Sequence of T cell receptor clone 4JFH alpha chain construct showing Nhe1/Not1 restriction sites (SEQ ID NO: 30 & 31).
f. Sequence of T cell receptor clone 4JFH beta chain construct showing Nco1/Xho1restriction sites (SEQ ID NO: 32 & 33).
g. Strategy and primer used to mutate CDR3 of c12/c2 TCR alpha chain (SEQ ID NO: 34, 35, 36 & 37).

45 Orlando, S. J., Santiago, Y., DeKelver, R. C., Freyvert, Y., Boydston, E. A., Moehle, E. A., et al. (2010). Zinc-finger nuclease-driven targeted integration into mammalian genomes using donors with limited chromosomal homology. *Nucleic Acids Research*, *38*(15), e152. doi:10.1093/nar/gkq512

46 Cadinanos, J; Bradley, A. (2007) Nucleic Acids Res. 35(12):e87

47 Zhang, H., Yea, K., Xie, J., Ruiz, D., Wilson, I. A., & Lerner, R. A. (2013). Selecting agonists from single cells infected with combinatorial antibody libraries. Chemistry & Biology, 20(5), 734–741. doi:10.1016/j.chembiol.2013.04.012

48 Porteus, M. H., & Baltimore, D. (2003). Chimeric nucleases stimulate gene targeting in human cells. *Science*, *300*(5620), 763. doi:10.1126/science.1078395

49 Rouet, P., Smih, F., & Jasin, M. (1994). Introduction of double-strand breaks into the genome of mouse cells by expression of a rare-cutting endonuclease. *Molecular and Cellular Biology*, *14*(12), 8096–8106.

50 Jasin, M. (1996). Genetic manipulation of genomes with rare-cutting endonucleases. *Trends in Genetics*, *12*(6), 224–228

51 Davis, L., & Maizels, N. (2011). DNA nicks promote efficient and safe targeted gene correction. *PLoS ONE*, *6*(9), e23981. doi:10.1371/journal.pone.0023981

52 Fujioka, K., Aratani, Y., Kusano, K., & Koyama, H. (1993). Targeted recombination with single-stranded DNA vectors in mammalian cells. *Nucleic Acids Res*, *21*(3), 407–412

53 Khan, I. F., Hirata, R. K., & Russell, D. W. (2011). AAV-mediated gene targeting methods for human cells. *Nat Protoc*, *6*(4), 482–501. doi:10.1038/nprot.2011.301

54 Deyle, D. R., & Russell, D. W. (2009). Adeno-associated virus vector integration. *Current Opinion in Molecular Therapeutics*, *11*(4), 442–447

55 Benatuil, L., Perez, J. M., Belk, J., & Hsieh, C. M. (2010). An improved yeast transformation method for the generation of very large human antibody libraries. *Protein Engineering, Design and Selection*, *23*(4), 155–159.

56 Feldhaus, M. J., Siegel, R. W., Opresko, L. K., Coleman, J. R., Feldhaus, J. M., Yeung, Y. A., et al. (2003). Flow-cytometric isolation of human antibodies from a nonimmune Saccharomyces cerevisiae surface display library. *Nat Biotechnol*.

57 Zhao, A., Nunez-Cruz, S., Li, C., Coukos, G., Siegel, D. L., & Scholler, N. (2011). Rapid isolation of high-affinity human antibodies against the tumor vascular marker Endosialin/TEM1, using a paired yeast-display/secretory scFv library platform. Journal of Immunological Methods, 363(2), 221–232. doi:10.1016/j.jim.2010.09.001

117 Renaut, L., Monnet, C., Dubreuil, O., Zaki, O., Crozet, F., Bouayadi, K., et al. (2012). *Affinity maturation of antibodies: Optimized methods to generate high-quality scfv libraries and isolate igg candidates by high-throughput screening. Methods in Molecular Biology* (Vol. 907, pp. 451–461)

118 Dyson, M. R., Zheng, Y., Zhang, C., Colwill, K., Pershad, K., Kay, B. K., et al. (2011). Mapping protein interactions by combining antibody affinity maturation and mass spectrometry. *Anal Biochem*, *417*(1), 25–35. doi:10.1016/j.ab.2011.05.005

119 de Felipe P (2002) Polycistronic viral vectors. Curr Gene Ther 2: 355–378. doi: 10.2174/1566523023347742.

120 Foote, J., & Winter, G. (1992). Antibody framework residues affecting the conformation of the hypervariable loops. *J Mol Biol*, *224*(2), 487–499.

121 Massie, B., Dionne, J., Lamarche, N., Fleurent, J., & Langelier, Y. (1995). Improved adenovirus vector provides herpes simplex virus ribonucleotide reductase R1 and R2 subunits very efficiently. *Nature Biotechnology*, *13*(6), 602–608.

122 Kim, D. W., Uetsuki, T., Kaziro, Y., Yamaguchi, N., & Sugano, S. (1990). Use of the human elongation factor 1 alpha promoter as a versatile and efficient expression system. *Gene*, *91*(2), 217–223.

123 Holden, P., Keene, D. R., Lunstrum, G. P., Bächinger, H. P., & Horton, W. A. (2005). Secretion of cartilage oligomeric matrix protein is affected by the signal peptide. *J Biol Chem*, *280*(17), 17172–17179.

124 Sadelain, M., Papapetrou, E. P., & Bushman, F. D. (2011). Safe harbours for the integration of new DNA in the human genome. *Nature Reviews Cancer*, *12*(1), 51–58.

125 Sanjana, N. E., Cong, L., Zhou, Y., Cunniff, M. M., Feng, G., & Zhang, F. (2012). A transcription activator-like effector toolbox for genome engineering. *Nat Protoc*, *7*(1), 171–192. doi:10.1038/nprot.2011.431

126 Falk, R., Falk, A., Dyson, M. R., Melidoni, A. N., Parthiban, K., Young, J. L., et al. (2012). Generation of anti-Notch antibodies and their application in blocking Notch signalling in neural stem cells. *Methods*, *58*(1), 69–78. doi:10.1016/j.ymeth.2012.07.008

127 Martin, C. D., Rojas, G., Mitchell, J. N., Vincent, K. J., Wu, J., McCafferty, J., & Schofield, D. J. (2006). A simple vector system to improve performance and utilisation of recombinant antibodies. *BMC Biotechnology*, *6*, 46.

128 Reyon, D., Tsai, S. Q., Khgayter, C., Foden, J. A., Sander, J. D., & Joung, J. K. (2012). FLASH assembly of TALENs for high-throughput genome editing. *Nature Biotechnology*, *30*(5), 460–465. doi:10.1038/nbt.2170

Claims

What is claimed is:

1. A method of producing a library of eukaryotic cell clones containing DNA encoding a diverse repertoire of binders that recognise a target, comprising

providing donor DNA molecules encoding the binders, and eukaryotic cells, wherein the binders are antibodies, proteins or peptides,

introducing the donor DNA into the cells and providing a site-specific nuclease within the cells, wherein the nuclease cleaves a recognition sequence in cellular DNA to create an integration site at which the donor DNA becomes integrated into the cellular DNA, integration occurring through DNA repair mechanisms endogenous to the cells, thereby creating recombinant cells containing donor DNA integrated in the cellular DNA, and

culturing the recombinant cells to produce clones,

thereby providing a library of eukaryotic cell clones containing donor DNA encoding the repertoire of binders.

2. The method according to claim 1, wherein the binders are antibody molecules; T cell receptors; and/or wherein the binders are multimeric, comprising at least a first and a second subunit.

3. The method according to claim 2, wherein the binders are antibody molecules and the antibody molecules are full length immunoglobulins, IgG, Fab, scFv-Fc or scFv.

4. A method of producing a library of eukaryotic cell clones containing DNA encoding a diverse repertoire of multimeric binders that recognise a target, each binder comprising a first and a second subunit, wherein the method comprises

providing eukaryotic cells containing DNA encoding the first subunit and providing donor DNA molecules encoding the second binder subunit, wherein the binders are antibodies, proteins or peptides,

introducing the donor DNA into the cells and providing a site-specific nuclease within the cells, wherein the nuclease cleaves a recognition sequence in cellular DNA to create an integration site at which the donor DNA becomes integrated into the cellular DNA, integration occurring through DNA repair mechanisms endogenous to the cells, thereby creating recombinant cells which contain donor DNA integrated in the cellular DNA, and

culturing the recombinant cells to produce clones containing DNA encoding the first and second subunits of the multimeric binder,

thereby providing a library of eukaryotic cell clones containing donor DNA encoding the repertoire of multimeric binders.

5. A method of producing a library of eukaryotic cell clones containing DNA encoding a diverse repertoire of multimeric binders that recognise a target, each binder comprising at least a first and a second subunit, wherein the binders are antibodies, proteins or peptides, wherein the method comprises

providing first donor DNA molecules encoding the first subunit, and providing eukaryotic cells,

introducing the first donor DNA into the cells and providing a site-specific nuclease within the cells, wherein the nuclease cleaves a recognition sequence in cellular DNA to create an integration site at which the donor DNA becomes integrated into the cellular DNA, integration occurring through DNA repair mechanisms endogenous to the cells, thereby creating a first set of recombinant cells containing first donor DNA integrated in the cellular DNA,

culturing the first set of recombinant cells to produce a first set of clones containing DNA encoding the first subunit,

introducing second donor DNA molecules encoding the second subunit into cells of the first set of clones, wherein the second donor DNA is integrated into cellular DNA of the first set of clones, thereby creating a second set of recombinant cells containing first and second donor DNA integrated into the cellular DNA, and

culturing the second set of recombinant cells to produce a second set of clones, these clones containing DNA encoding the first and second subunits of the multimeric binder,

thereby providing a library of eukaryotic cell clones containing donor DNA encoding the repertoire of multimeric binders.

6. The method according to claim 5, wherein the second donor DNA molecules are integrated by a method comprising providing a site-specific nuclease within the cells, wherein the nuclease cleaves a recognition sequence in cellular DNA to create an integration site at which the donor DNA becomes integrated into the cellular DNA, integration occurring through DNA repair mechanisms endogenous to the cells.

7. The method according to any of claims 3 to 6 wherein the multimeric binders are antibody molecules comprising a heavy chain variable (VH) domain and a light chain variable (VL) domain as separate subunits.

8. The method according to claim 7, wherein the multimeric binders are whole immunoglobulins.

9. The method according to claim 7 or 8 wherein the antibody molecule further comprises one or more additional subunits, which may be introduced on the same donor DNA as the first or second subunit or which may be integrated at separate sites in the cellular DNA.

10. The method according to any of the preceding claims, wherein the cells are higher eukaryotic cells with a genome size of greater than 2×10^7 base pairs.

11. The method according to any of the preceding claims, wherein the cells are mammalian.

12. The method according to claim 11, wherein the cells are HEK293 cells, Chinese hamster ovary (CHO) cells, T lymphocyte lineage cells or B lymphocyte lineage cells or any of the cell lines listed in the "Cancer Cell Line Encyclopedia" or "COSMIC catalogue of somatic mutations in cancer".

13. The method according to any of the preceding claims, wherein the recognition sequence is in genomic DNA of the cells or wherein the recognition sequence is in episomal DNA within the cells.

14. The method according to any of the preceding claims, wherein the recognition sequence for the site-specific nuclease occurs only once or twice in the cellular DNA.

15. The method according to any of the preceding claims, wherein the site-specific nuclease cleaves cellular DNA to create a double strand break serving as an integration site.

16. The method according to any of the preceding claims, wherein the nuclease is a meganuclease, a zinc finger nuclease (ZFN), a TALE nuclease, or wherein DNA cleavage is directed by the CRISPR/Cas system.

17. The method according to any of the preceding claims, wherein the donor DNA comprises a genetic element for selection of cells into which the donor DNA is integrated.

18. The method according to any of the preceding claims, wherein integration of the donor DNA into the cellular DNA places expression of the binder and/or expression of a genetic selection element under control of a promoter present within the cellular DNA.

19. The method according to any of the preceding claims, wherein the library contains at least 100, 10^3, 10^4, 10^5 or 10^6 clones, each clone being derived from an individual recombinant cell produced by integration of donor DNA.

20. The method according to any of the preceding claims, wherein the library encodes at least 100, 10^3, 10^4, 10^5 or 10^6 different binders.

21. The method according to any of the preceding claims, wherein each clone contains integrated donor DNA encoding only one or two members of the repertoire of binders, or wherein each clone contains integrated donor DNA encoding a single member of the repertoire of binders.

22. The method according to any of the preceding claims, wherein the eukaryotic cells are diploid and contain a recognition sequence for the site-specific nuclease at duplicate fixed loci in the cellular DNA.

23. The method according to any of the preceding claims, wherein the donor DNA molecules each encode a single binder or binder subunit.

24. The method according to any of the preceding claims, wherein the binders are displayed on the cell surface.

25. The method according to any of the preceding claims, wherein the binders are secreted from the cells.

26. The method according to any of the preceding claims, wherein the donor DNA is sourced from rounds of phage display selection.

27. The method according to any of the preceding claims, further comprising:

culturing the library to express the binders,

recovering one or more clones expressing a binder of interest, and

generating a derivative library from the one or more recovered clones, wherein the derivative library contains DNA encoding a second repertoire of binders optionally wherein generating the derivative library comprises isolating donor DNA from the one or more recovered clones, introducing mutation into the DNA to provide a derivative population of donor DNA molecules encoding a second repertoire of binders, and introducing the derivative population of donor DNA molecules into cells to create a derivative library of cells containing DNA encoding the second repertoire of binders or wherein generating the derivative library comprises introducing mutation into the donor DNA in the one or more recovered clones by inducing mutation of the DNA within the clones.

28. A method of producing a diverse repertoire of binders, comprising producing a library by a method according to any of claims 1 to 27 and culturing the library cells to express the binders.

29. A method of screening for a binder that recognises a target, comprising:

producing a library by the method of any of claims 1 to 27,

culturing cells of the library to express the binders,

exposing the binders to the target, allowing recognition of the target by one or more cognate binders, if present, and

detecting whether the target is recognised by a cognate binder and optionally;

further comprising recovering cells of a clone containing DNA encoding the cognate binder.

30. A method of screening for a cell of a desired phenotype, wherein the phenotype results from expression of a binder by the cell, the method comprising

producing a library by the method of any of claims 1 to 27,

culturing the library cells to express the binders, and

detecting whether the desired phenotype is exhibited,

and optionally further comprising:

recovering cells of a clone that expresses a binder that produces the desired phenotype, and/or:

isolating DNA encoding the binder from the recovered clone, thereby obtaining DNA encoding a binder which produces the desired phenotype.

31. The method according to claim 29 or 30, wherein the binders are antibody molecules and the target is an antigen, or wherein the binders are TCRs and the target is an MHC:peptide complex.

32. The method according to claim 31, further comprising isolating DNA encoding the binder from the recovered clone, thereby obtaining DNA encoding a binder that recognises the target

33. The method according to claim 30 or 32, comprising introducing mutation or converting the DNA to modified DNA encoding a restructured binder.

34. The method according to claim 33, wherein the binder is a scFv and the method comprises converting DNA encoding the scFv to DNA encoding an Ig or fragment thereof while maintaining the original variable VH and VL chain pairings.

35. The method according to claim 29, 30 and 32-35, further comprising introducing the DNA into a host cell, and optionally further comprising culturing the cells and concentrating the cells to provide a cell pellet or concentrated cell suspension.

36. The method according to claim 29, 30 or 35, further comprising culturing the cells to express the binder, and purifying the binder.

37. Use of a site-specific nuclease for targeted cleavage of cellular DNA in the construction of a library of eukaryotic cells containing DNA encoding a repertoire of binders by a method according to any one of claims 1 to 36, wherein nuclease-mediated DNA cleavage enhances site-specific integration of binder genes through endogenous cellular DNA repair mechanisms.

38. Use of the library produced by a method according to any of claims 1 to 27 as a display library for selecting binders to a desired target or for screening for cells displaying a desired cellular phenotype, wherein the phenotype results from expression of a binder by a cell.