Mammalian Cell Display System: Construction of a Naïve Library by Insertion of Human Immunoglobulin Germline Sequences Using TARGATT™ Technology

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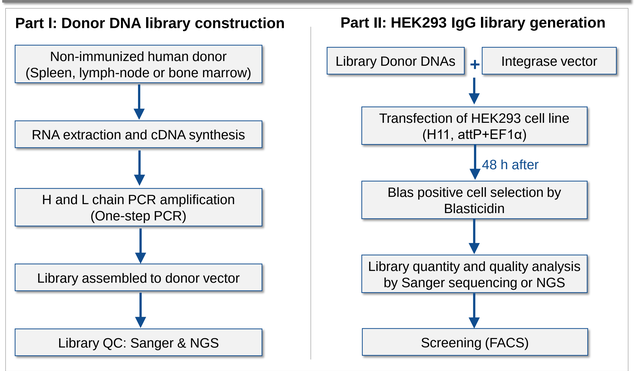
Abstract: We aim to establish a first-in-class human naïve antibody screening system using our proprietary TARGATT™ integrase gene knockin technology. Current antibody screening systems have their drawbacks. For example, the phage display system lacks posttranslational modification that are required for antibody function. While the mammalian display system can provide full length IgG, it displays multiple copies of the antibodies with different specificities on a single cell surface. Thus, making it difficult to identify and isolate antibodies with a desired property. Some of the existing mammalian systems do provide site-specific, single copy transgene insertion, but the insertion efficiency is very low. Here, we are using the TARGATT™ integrase in HEK293 cells to build a display platform to overcome the above drawbacks. Our antibody library will be displaying a uniform, single copy, full IgG length and a population of naïve, germline encoded heavy and light Immunoglobulin chains - which can be used to screen against any antigens, therefore with no limitation for therapeutic applications. We expect that our TARGATT™ mammalian naïve antibody library system will become a powerful display platform for antibody discovery/screening.

Materials & Methods:



The HEK293 cell line was originally from ATCC and has been adapted to suspension cells, in-house. The TARGATT™ integrase vector and the donor vector backbone containing the constant domains of the light chain and the heavy chain, and the transmembrane domain (TM) were prepared previously in-house. We constructed high-quality human naïve IgG antibody libraries using RNA isolated from several non-immunized human donors, followed by cDNA synthesis, PCR amplification, Golden Gate assembly of VH and VL repertoires into the donor DNA library backbone. After the DNA library is constructed, transfection will be performed together with the TARGATT™ integrase in the TARGATT™ HEK293 Master Cell Line. After transfection, the culture medium will be selected by Blasticidin. Only cells integrated with antibody genes and those which express Blasticidin would survive under selection. The stable cell pool will serve as the final antibody library for FACS analysis and screening against antigens.

Display System Using the TARGATT™ Integrase:

Site specific recognition through two attachment sites:

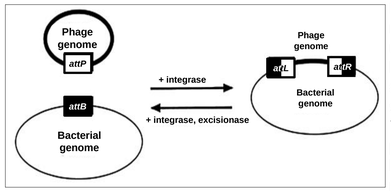


Fig 1. Serine integrases are recombinase enzymes that drive unidirectional recombination between a phage *att* site (*attP*) and bacterial *att* site (*attB*). In this study we have opted to utilize a TARGATT™ integrase, which permits both high efficiency and high-fidelity recombination in mammalian cell.

Creating Naïve Antibody Library Using TARGATT™:

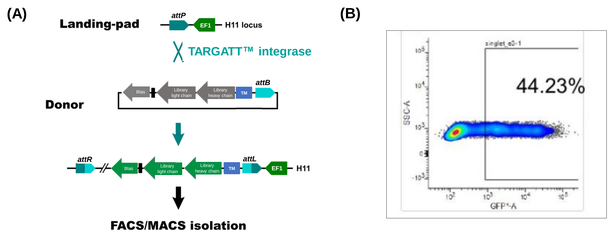


Fig 2. (A) The landing pad was placed in the H11 locus, as it enables efficient gene knockin and higher level of expression. (B) With our improved expression vector, we are able to achieve TARGATT™ integrase-mediated plasmid integration with an efficiency of 44% as assessed through a split-cassette GFP assay.

Library QC by Sanger Sequencing:

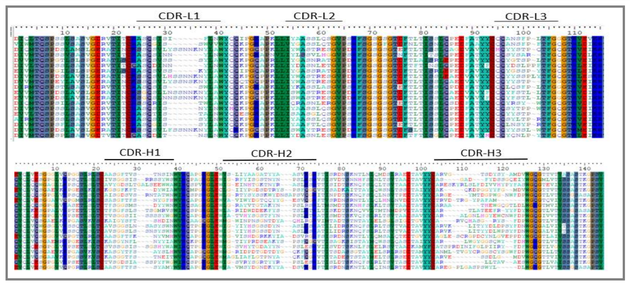


Fig 3. Sanger Sequencing to confirm that both the light and heavy chains of the library show good diversity with ~ 90% inserted ratio and no repeat sequences.

Advantages of Using TARGATT™ Mammalian Display Compared to Other Widely Used Display Systems:

	Phage Display (Yeast, Bacteria)	Mammalian Display (Virus)	TARGATT™
Antibody Format	scFv or Fab	scFv, Fab, & IgG	scFV, Fab, & IgG
Post-translational Modification	No	Yes	Yes
Library Size	10^9 - 10^{10}	10^3 - 10^6	10^7 - 10^9
Site-specific Gene Insertion	No	No	Yes
Protein Level	Vary	Vary	Consistent
Size of Insertion	Limited	Limited	Less Limited
Cost	Low	High	Low
Time	Short	Long	Short
Copy No. of Inserted Genome	Multiple	Vary	Single Copy
Specificity per Cell	Single	Vary	Single

Table 1. The TARGATT™ integrase system is capable of site-specific insertion of a single copy of an antibody into the human genome with uniform and stable expression. It also provides post-translational modification, ability to express full IgG format as well as low cost for high-throughput antibody screening.

Advantages of Using TARGATT™ Comparing to Other Mammalian Systems:

	Flp-In™	Iontas	TARGATT™
Cell Line	HEK293/ CHO	HEK293	HEK293
Modified Genetic Locus	Unknown	AAVS1	H11
Recombinase or Nuclease	Flp recombinase	TALE nucleases/ CRISPR	Serine integrase
Reversibility of Gene Recombination	Reversible	Irreversible	Irreversible
Efficiency of Plasmid Integration/ Recombination (% Starting Cells)	0.015%	0.5 – 5.1%	~ 44%
Split Cassette System	No	No	Yes
Library Size	10^6	10^6	10^7 – 10^9 (Estimated)

Table 2. The TARGATT™ technology has a knockin efficiency that is 2-3 orders of magnitude higher than that observed with the other recombinase-based insertion systems. The irreversible integrase-mediated recombination also enables a stable integration. This provides the foundation for a very efficient mammalian cell screening platform to build large antibody libraries.

Conclusions:

1. The TARGATT™ platform offers several advantages over existing antibody screening technologies: (a) A codon-optimized integrase enzyme that allows for knockin efficiencies up to 44%; (b) Site-specificity potentially eliminates off-target and/or random integration events; (c) Single copy display of antibody on each individual cells; (d) H11 safe harbor genomic locus enables stable gene knockin and expression at high levels.
2. In this study, the DNA library construction has been completed by PCR amplification and golden gate assembly. The transfection of the DNA library into the TARGATT™ HEK293 master cell line will be performed followed by NGS to QC the final cell library. This TARGATT™ HEK293 cell library will provide an efficient and novel tool for high-throughput antibody discovery and screening.

References:

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- Chi X, Zheng Q, Jiang R, Chen-Tsai RY, Kong LJ. A system for site-specific integration of transgenes in mammalian cells. PLoSOne. (2019) 14(7),e0219842.