APEX™ Antibody Discovery and Production Technology

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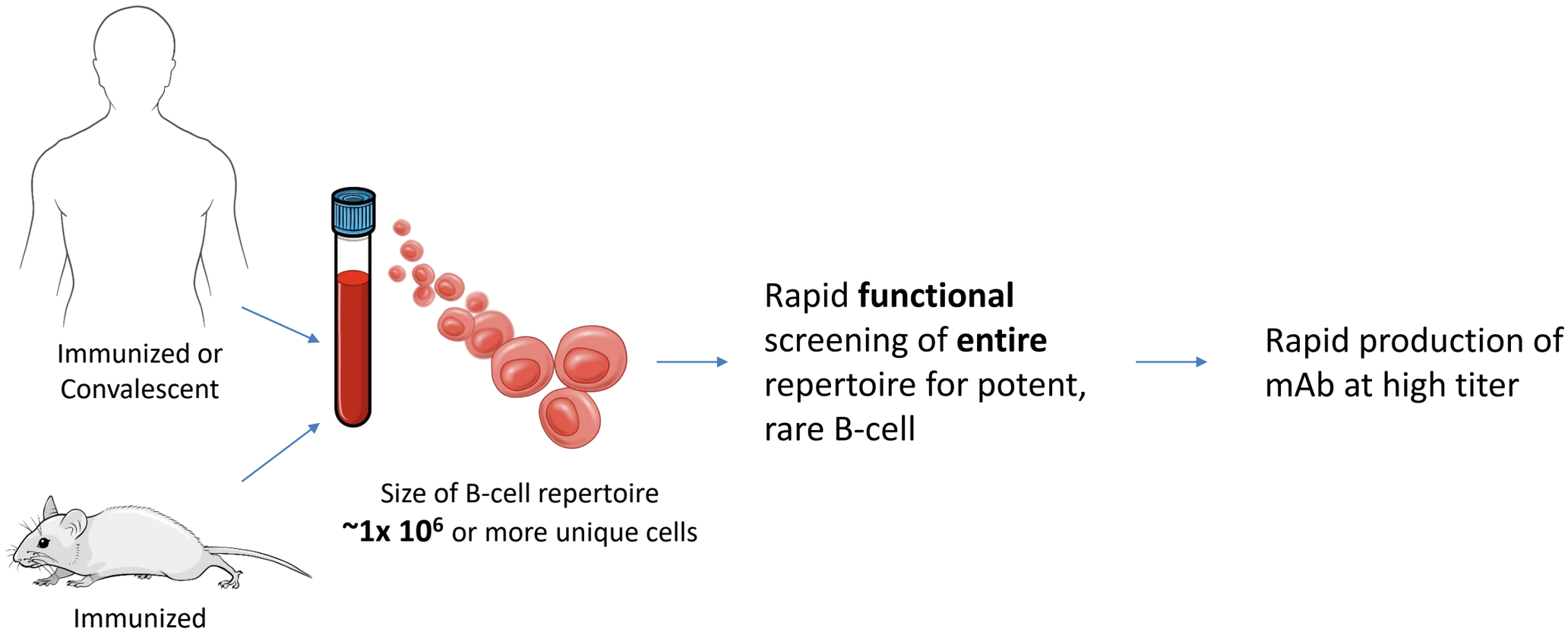
Outline

- Single cell cloning & mAb discovery
- B-cell immortalization using cell fusion
- Enhanced mAb production of established cell lines
    - CRISPR-enabled activation of transcription regulatory genes
- mAb production CHO cell line technology

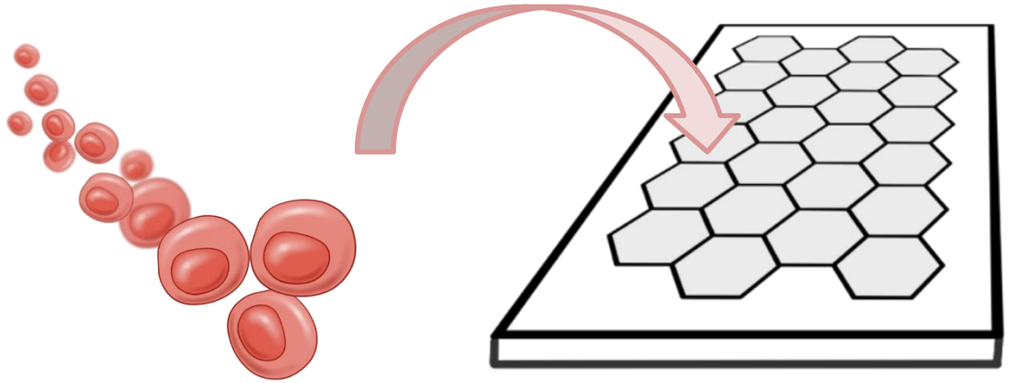
Outline

- **Single cell cloning & mAb discovery**
- B-cell immortalization using cell fusion
- Enhanced mAb production of established cell lines
  - CRISPR-enabled activation of transcription regulatory genes
- mAb production CHO cell line technology
- COMING SOON: Transposons-mediated gene amplification

Antibody Discovery and Production Challenges



Single Cell Screening & Clonal Selection Using NanoArrays

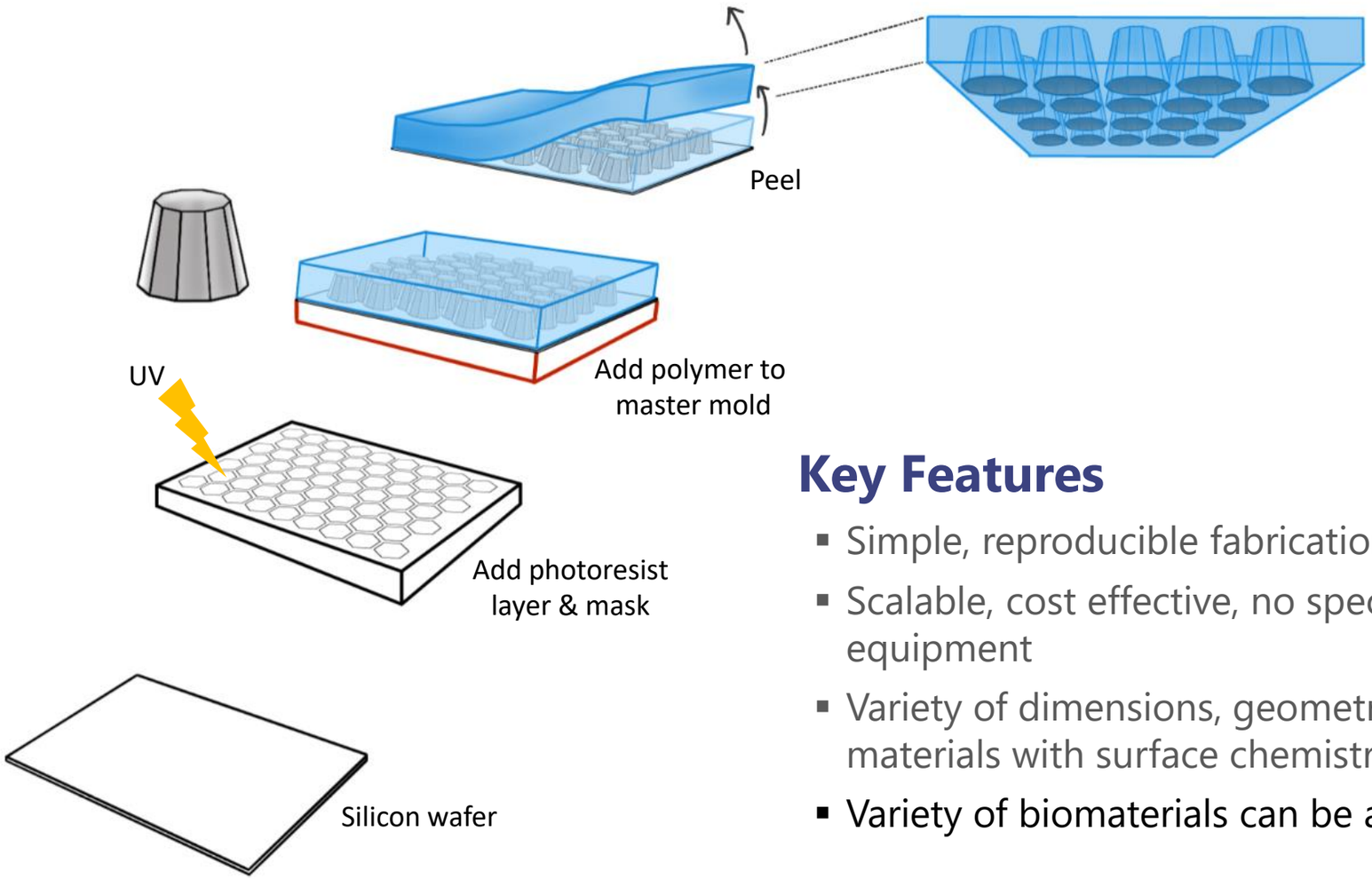


APEX™
Nanoculture Arrays

Key Features

- Enables comprehensive, **functional screening at single cell level** of 10^6-10^7 cells simultaneously
- **~100,000x minimization** of tissue culture wells. **0.5 to 3 million wells** can be accommodated in a 96-well plate surface area
- **Increase protein concentration** by ~5-orders of magnitude enables sub-picogram mAb detection
- Enables simultaneous **single cell cloning and plucking** by microneedles for expansion
- Proteins, viruses, bacteria can be adsorbed as antigen for detection of mAb
- **Highest binders can be identified in ~1-2 days**

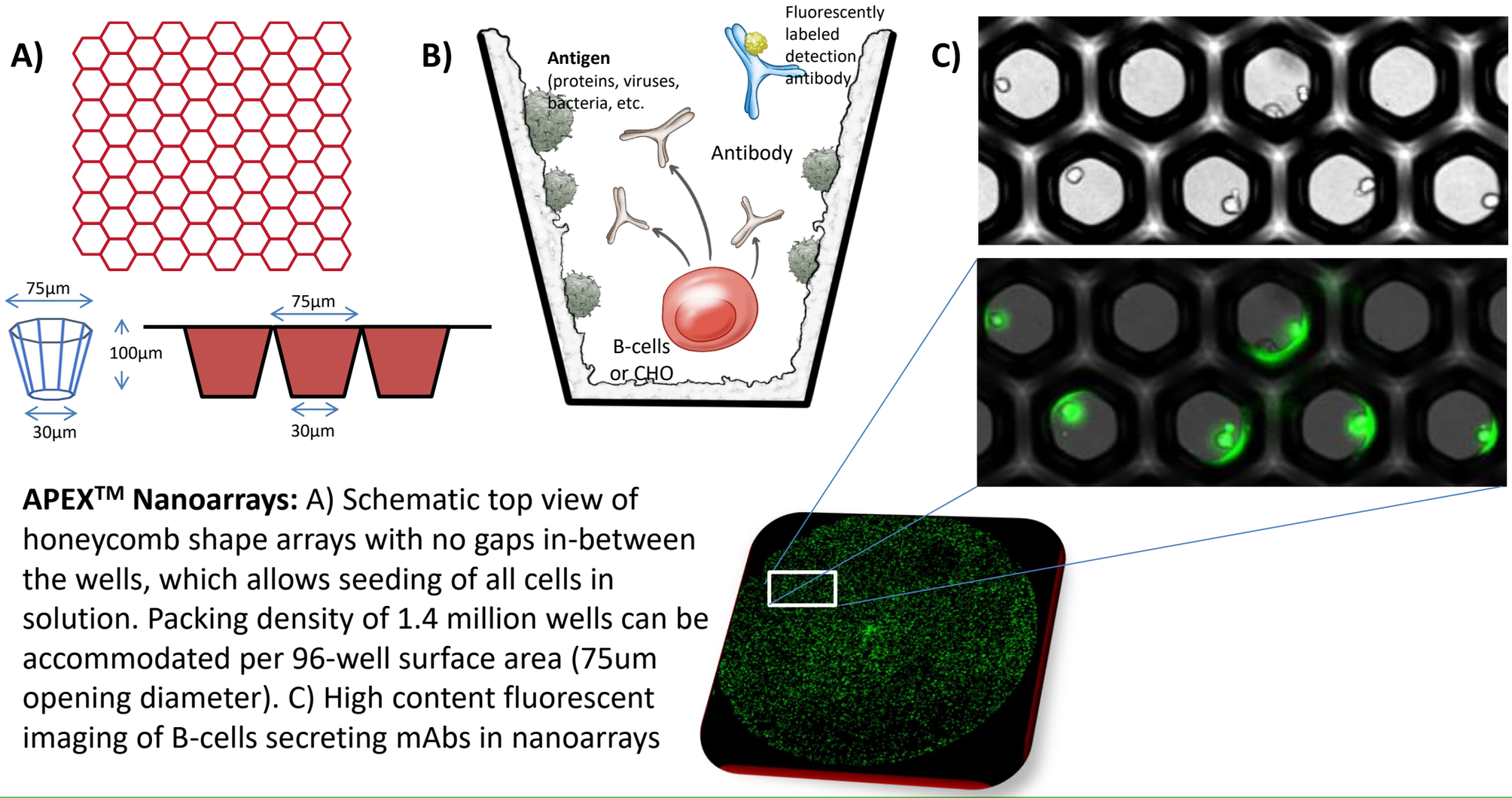
Fabrication of Nanoculture Arrays



Key Features

- Simple, reproducible fabrication process
- Scalable, cost effective, no specialized companion equipment
- Variety of dimensions, geometries, biocompatible polymer materials with surface chemistries
- Variety of biomaterials can be adsorbed

B-cell Repertoire Screening, Single Cell Analysis & Single Cell Cloning with APEX™ Nanoarrays

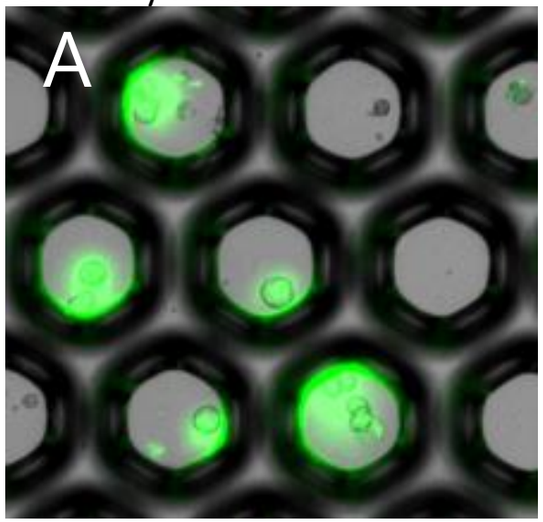


APEX™ Nanoarrays: A) Schematic top view of honeycomb shape arrays with no gaps in-between the wells, which allows seeding of all cells in solution. Packing density of 1.4 million wells can be accommodated per 96-well surface area (75um opening diameter). C) High content fluorescent imaging of B-cells secreting mAbs in nanoarrays

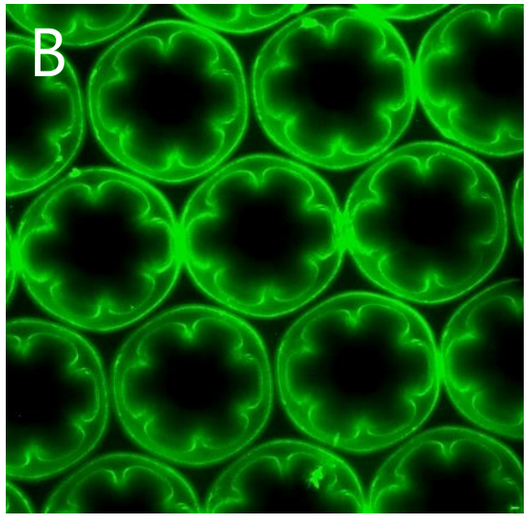
Versatile On-chip Assay Format for Single Cell Analysis

Top view of Nanoculture wells (A-D). Wells are embedded with antigen as indicated, followed by detection with fluorescence labeled secondary mAb

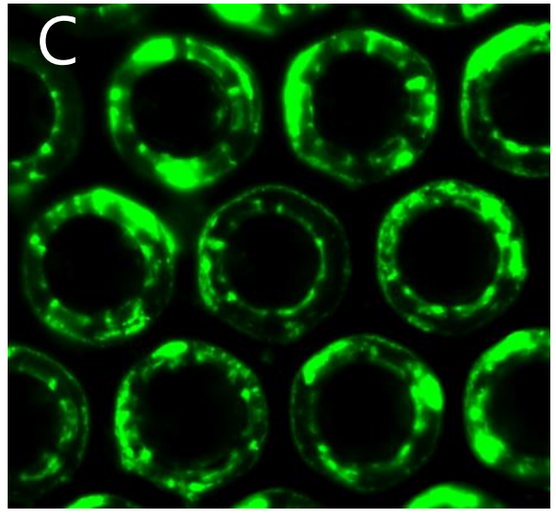
Anti-IgG



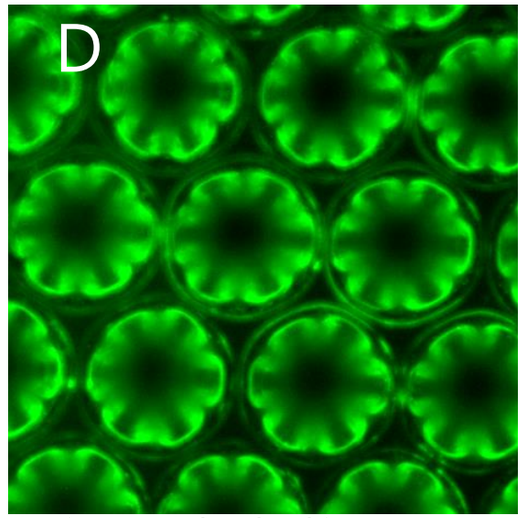
Live B-cell secreting Anti-RSV F-protein IgG (=antigen)



Antigen= Human AR-105 mAb

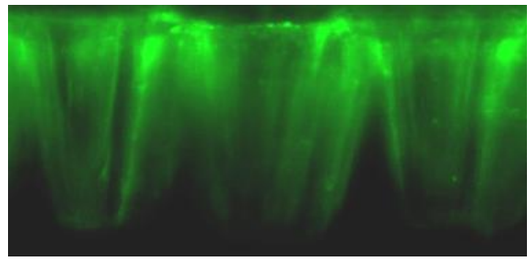


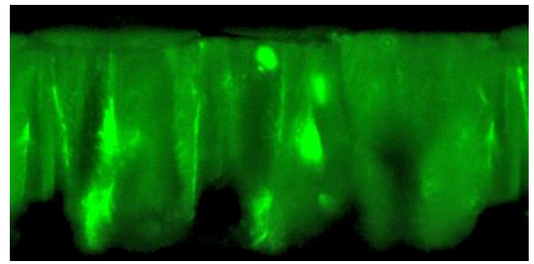
Antigen= adsorbed whole cell *P. aeruginosa* bacteria expressing alginate (detected w/ anti-alginate mAb)



Antigen= Adsorbed intact rotavirus expressing G1 protein (detected with anti-G1 mAb)

Sideview of Nanoculture wells

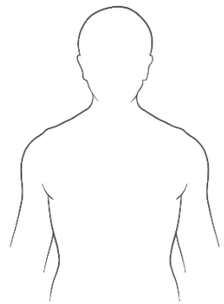


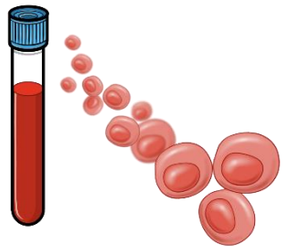


APEX™ Technology Suite

- Single cell cloning & mAb discovery
- **B-cell immortalization using cell fusion**
- Enhanced mAb production of established cell lines
  - CRISPR-enabled activation of transcription regulatory genes
- mAb production CHO cell line technology

Current Practice: Recombinant approaches to mAb discovery & production requires 1.5 - 2 years from discovery to drug in vials

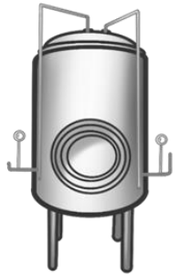




Cloned CDRs or H&L chains of IgG's

Expression Vector Construction, Clonal Selection

Upstream & Downstream Process Development, Scale Up



GMP production

Mouse immunization

Mouse B-cell screening

Recombinant DNA cloning, humanization, transfection, single cell cloning

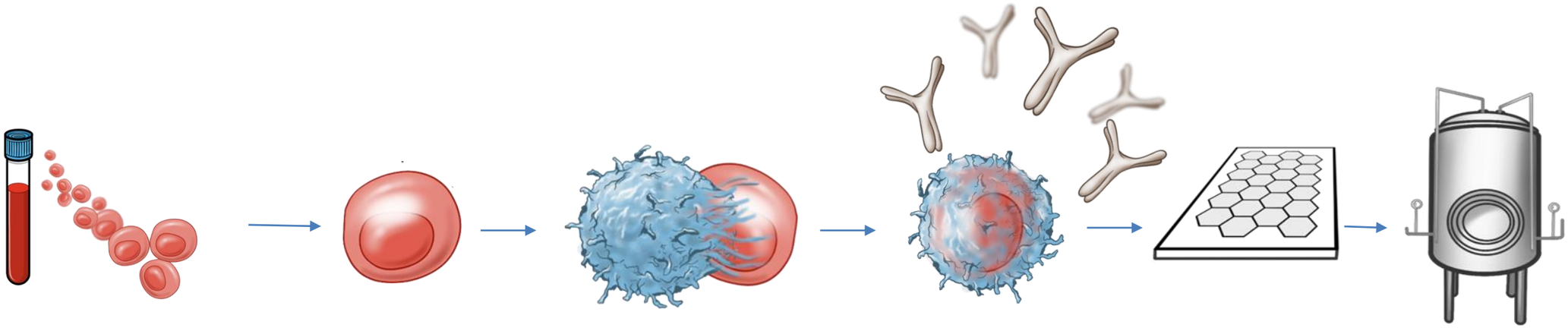
Cell line characterization & process development

GMP Manufacturing

Months

0 3 6 9 12 15 18 21 24 27

Bypassing Recombinant Approaches Using B-cell Fusion & Immortalization



Selected B-cell

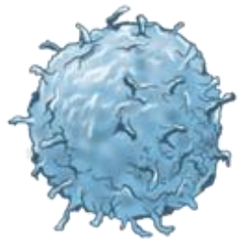
Fusion with Fusion Partner

Immortalized Hybridoma

Nanoculture Clonal Select

GMP production

Engineering of the Fusion Partner Cell Line



Genetically engineered, non-secreting IgG mouse heteromyeloma cell line developed with high cell fusion efficiency

Genetic modifications introduced to **activate master transcription factors** associated with increased transcription and translation of H & L chain genes of monoclonal antibodies. Exhibits

- Immortality
- CHO-level productivity
- Stability

Key Feature

Contains stable integrated CRISPR machinery

- Cas enzyme, accessory proteins, and guided RNAs necessary to activate endogenous master transcription factors

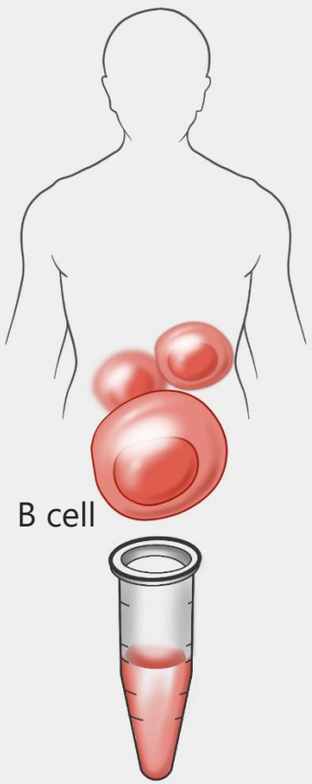
B-cell Screening & Cell Fusion Proof-of-Concept Study: RSV mAb Discovery

RSV-F antigen used for PoC study

- Availability of primary RSV-F+ B-cells from biobanks
- Well described structure of RSV-F protein
- Antigen specific tools, assays and reagents are available

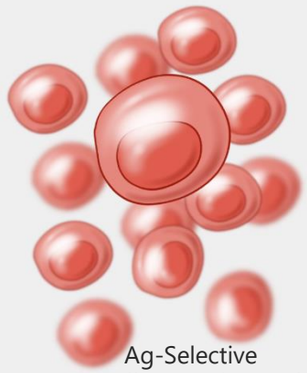
Repertoire Screening & Cell Fusion Work Flow

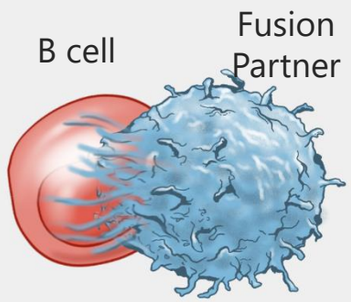
ISOLATE PBMCS



PBMC isolation from convalescent patients

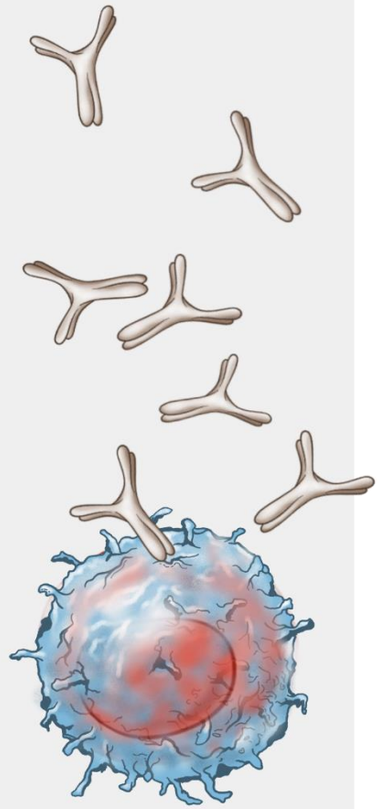
B-CELL EXPANSION





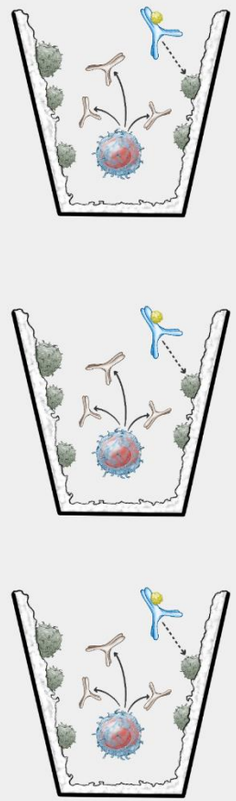
B Cell Fusion with Partner Cell Line

IMMORTALIZED MAB SECRETING PROGENY



B Cell fused with Partner Cell Line

CLONAL SELECTION



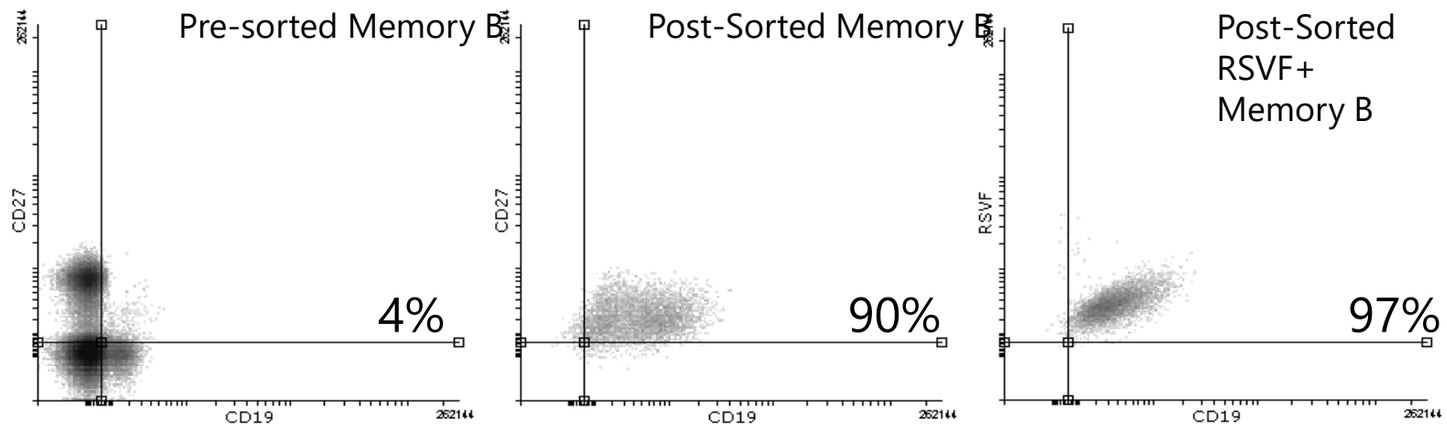
Nanoculture Screening

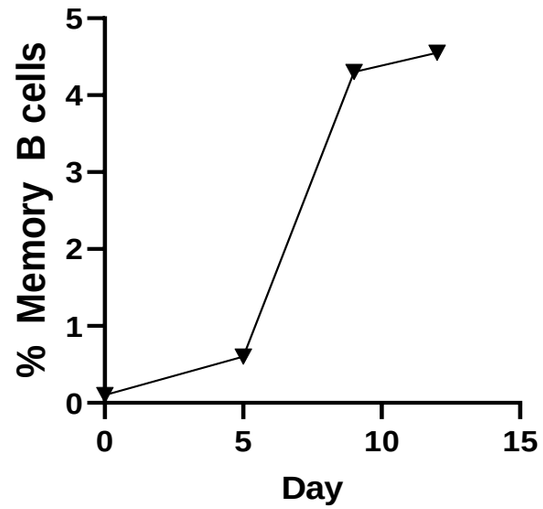
PRODUCTION

Stable & high titer mAb production

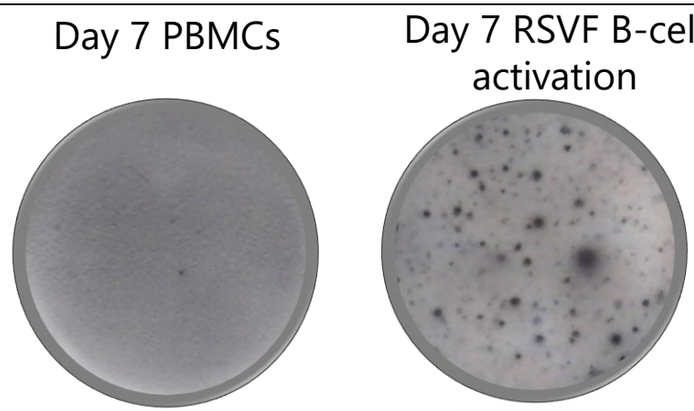
RSV-F Ag-specific Human B Cell Activation

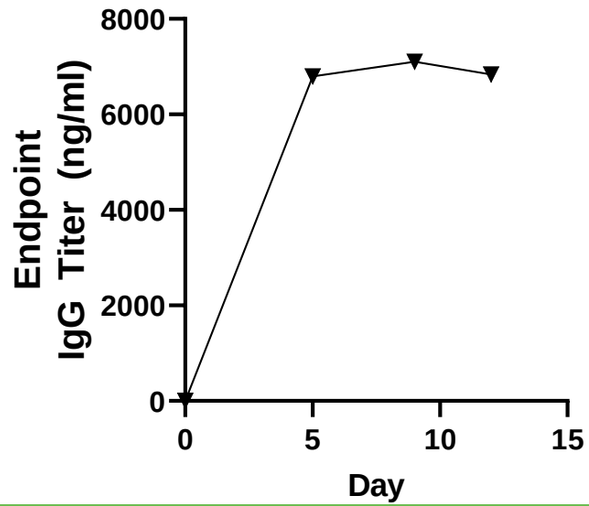
Day 7 Ag-specific Human B-cell Activation



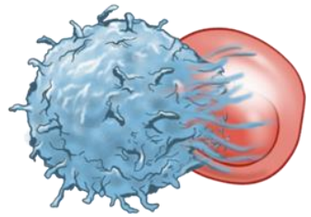


RSV-F memory B-cell secretion



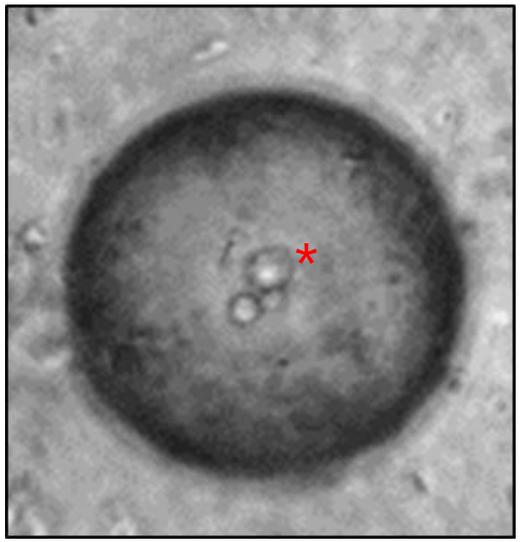


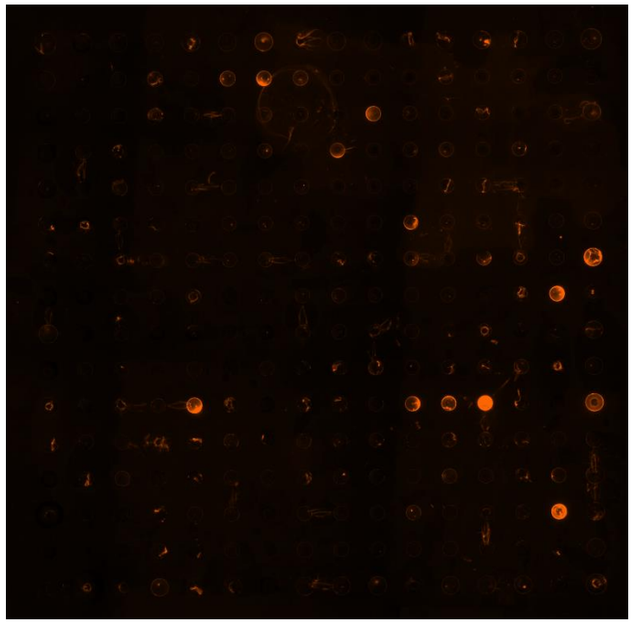
Engineered Fusion Partner Exhibits High Fusion Efficiency

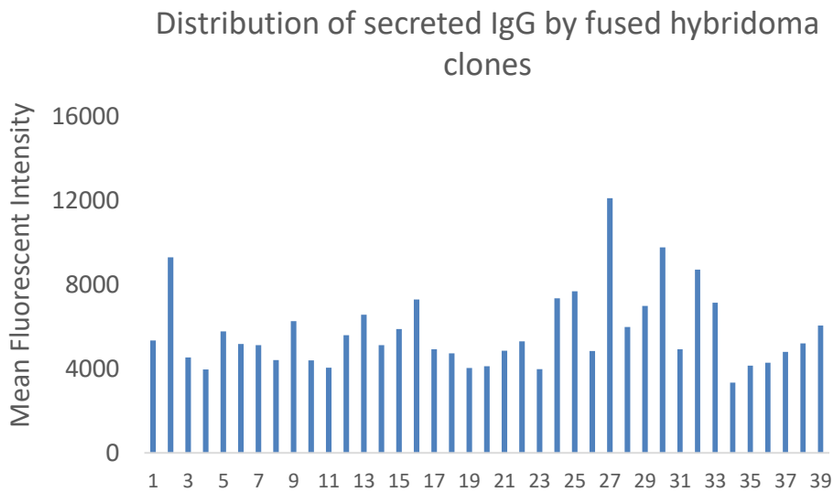


On-chip Single Cell Fusion: 10%-20% Fusion Efficiency [i.e. 10-20% of progenies that fused, survived, and are producing mAb]

After Fusion



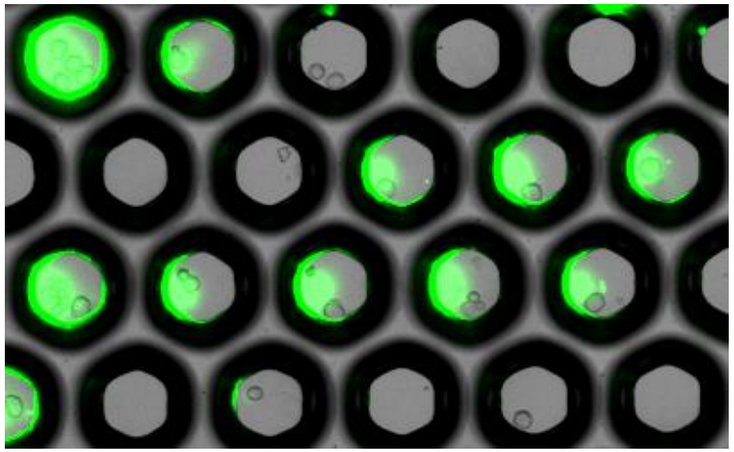




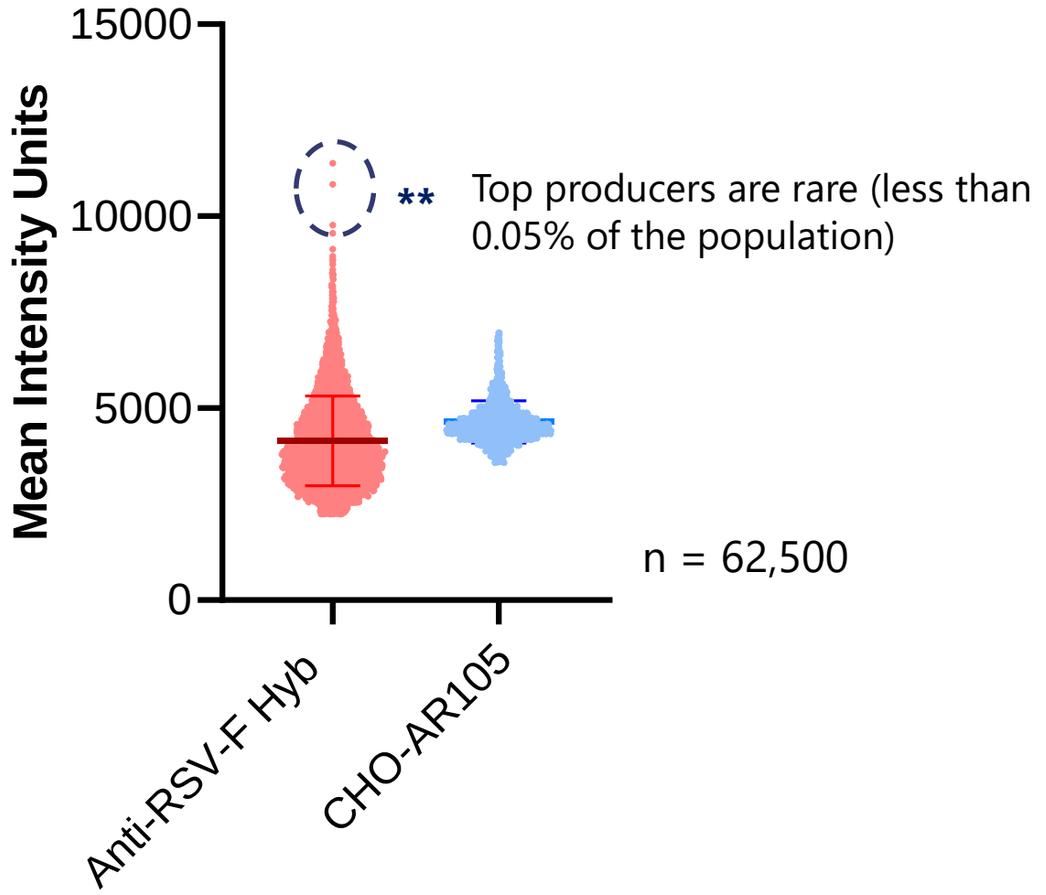
Left: 16x16 array of fused hybridoma at Day 27 post-fusion; Right: distribution of hybridoma clones that are producing IgG. Fluorescence intensity is used as a metric of IgG secretion level

Rapid identification of high-producing RSV-F specific hybridoma

A

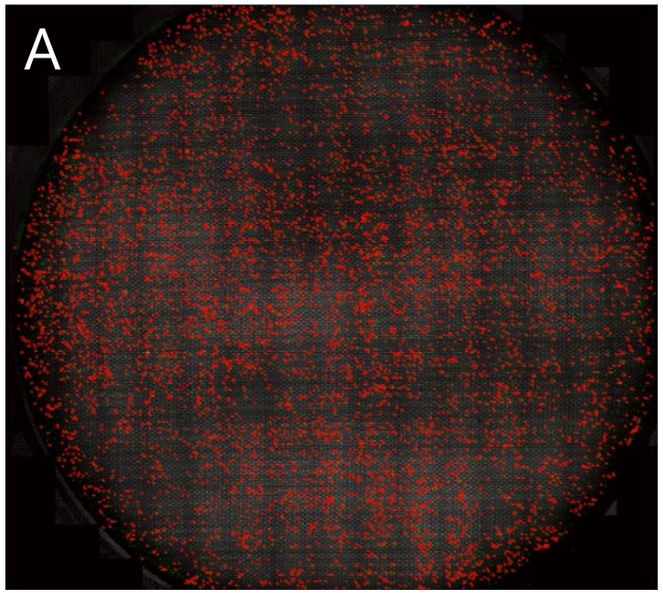


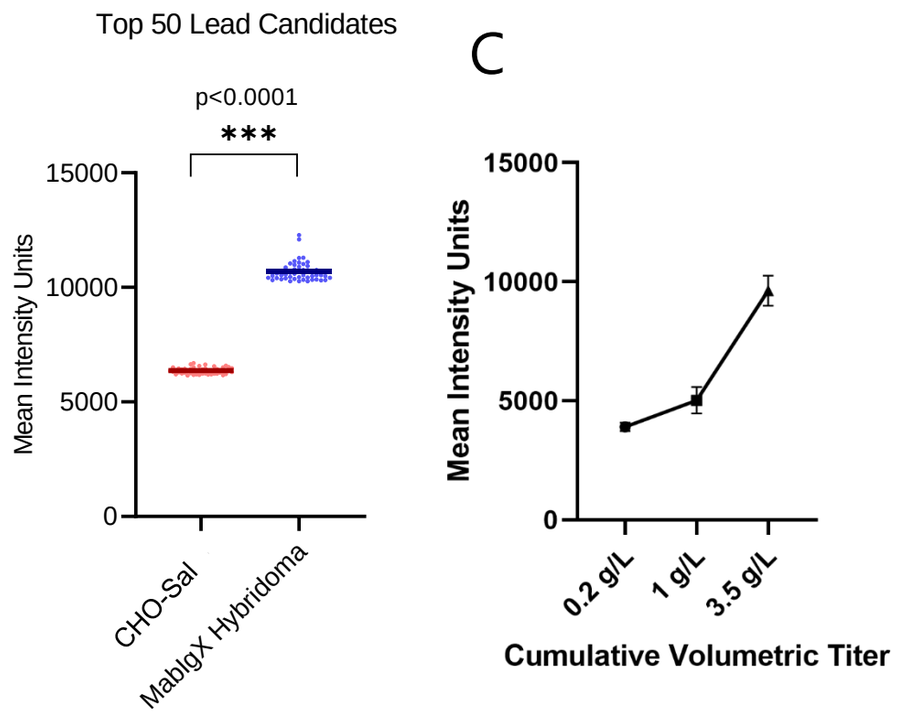
B

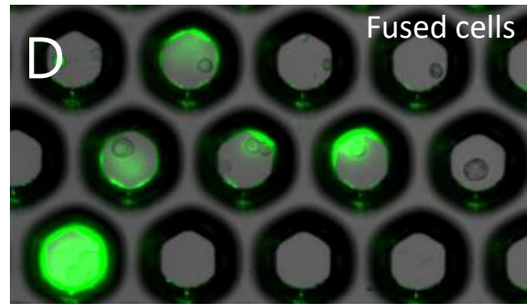


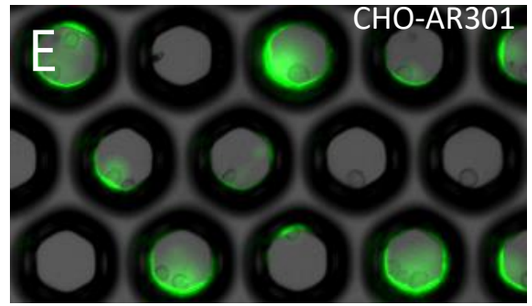
A. Panel of nanowells of hybridoma producing Human anti-RSVF mAb. **B.** Distribution of fluorescent ring intensity of anti-RSVF production compared to CHO-Aer. ** Denotes production levels higher than CHO-Aer producing at 3.5 g/L.

High content screening allows for rapid identification of rare high-producing hybridoma clones







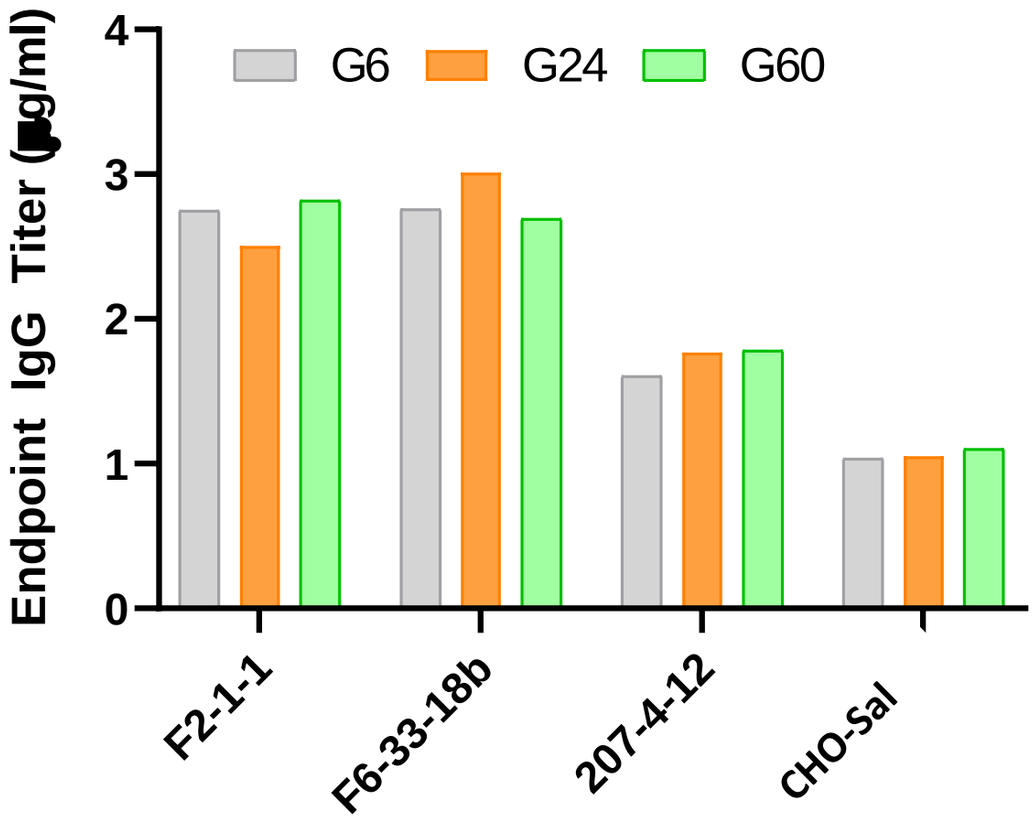


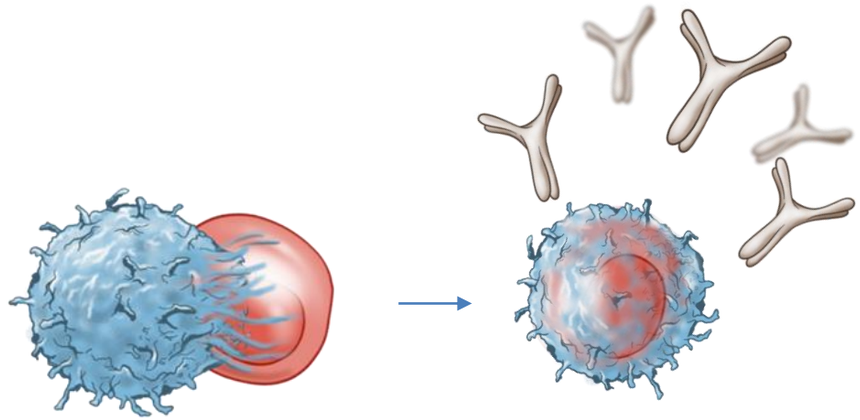
(A) Hybridomas generated from cell fusion compared to CHO-Sal were screened for mAb secretion on nanocultures. ~24,000 positives identified for MabIgX ® hybridomas/ ~ 33,000 positives identified for CHO-Sal.

(B) Mean fluorescence intensity of the (B) 50 highest producing clones and **(C)** Correlation of mean intensity units to cumulative volumetric titer of cell lines with experimentally-determined productivity (*** $p<0.0001$ by Student's t-test, n=50.

(D-E) Representative panel showing single cell IgG production of **(D)** Hybridoma and **(E)** CHO-Sal (1 g/L volumetric titer)

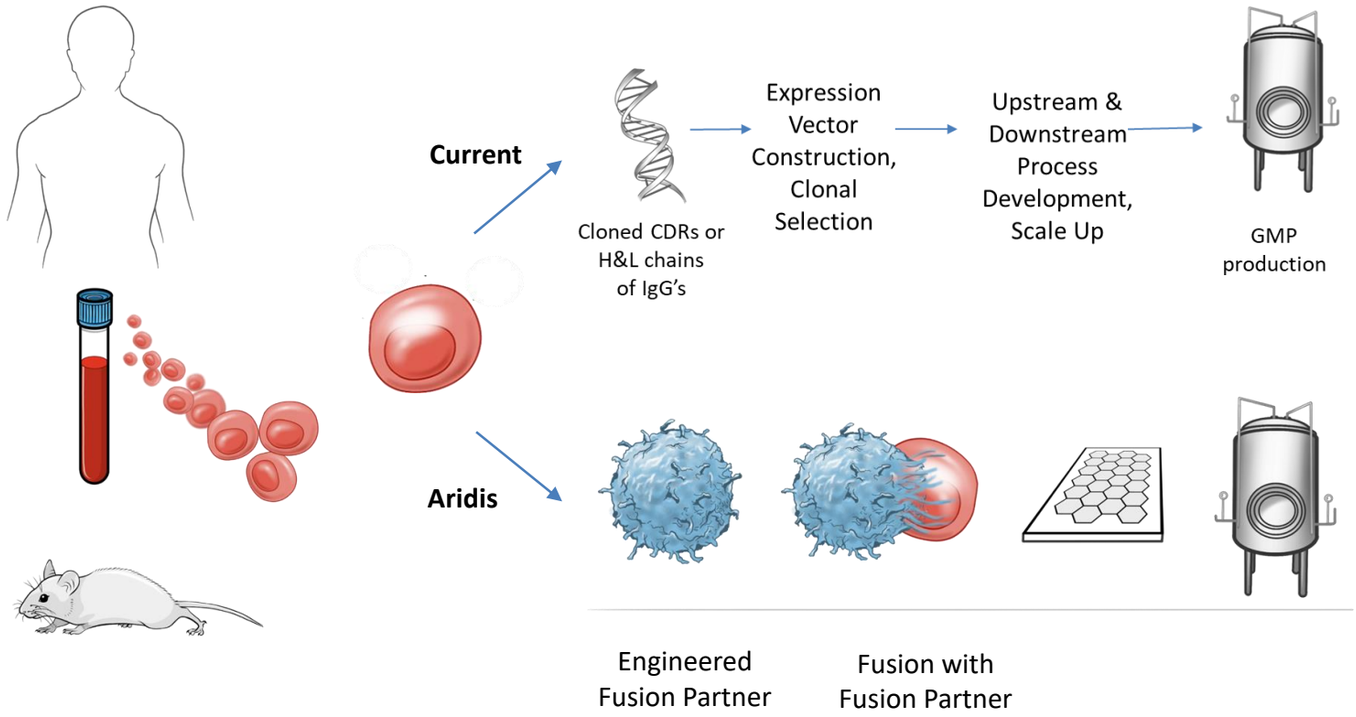
Stable Hybridoma Productivity Comparable to CHO over 60-Generations





- Endpoint titer of 3 hybridoma clones producing human IgG demonstrates consistent productivity at each time point
- Titer at generations 6, 24, and 60 shown
- CHO-Sal producing at 1 g/L was used as a comparator for all stability studies

Cell Fusion to Create Hybridoma: Advantages & Disadvantages



Advantages:

- Well established process

Disadvantages:

- Up to ~1 year longer than the hybridoma approach

Advantages:

- Convenient B-cell starting point
- Bypasses recombinant steps
- Little to no process development needed
- Fastest to clinical manufacturing

Disadvantages:

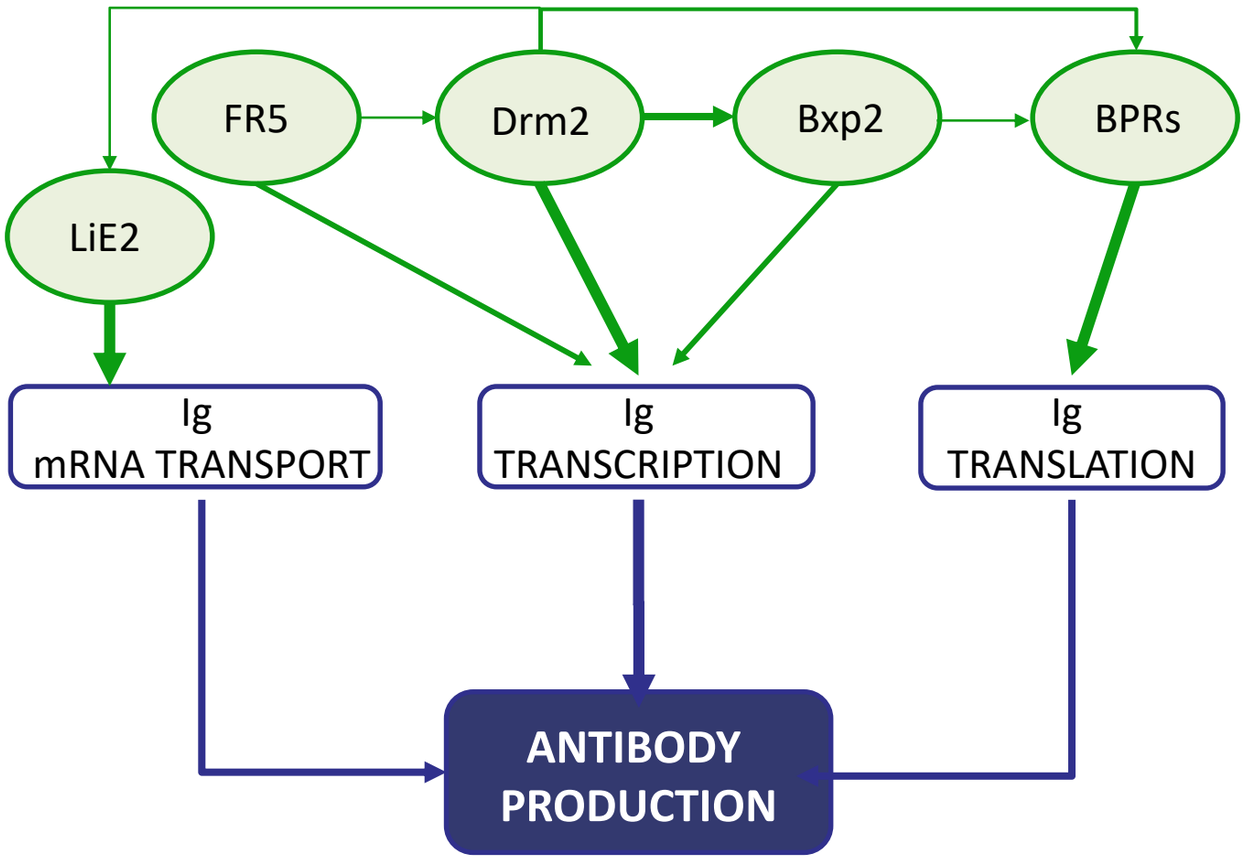
- Involves hybridoma*

*Can derive CHO while in Phase 1-2 clinical studies

Outline

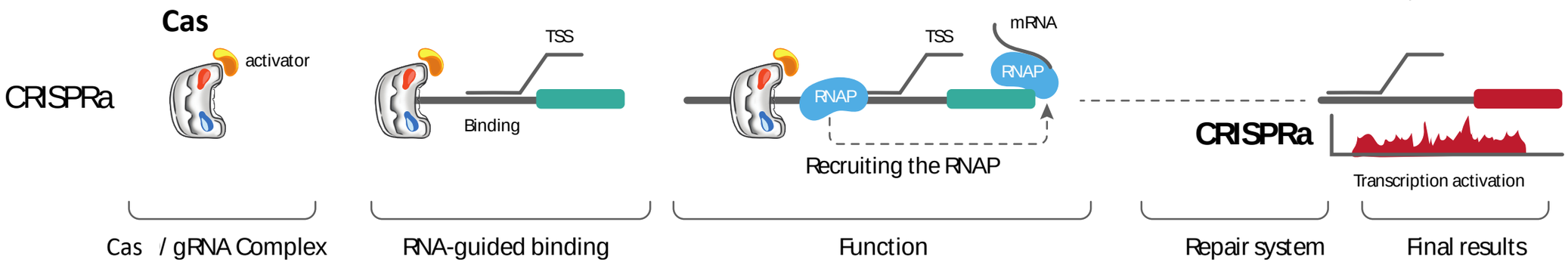
- Single cell cloning & mAb discovery
- B-cell immortalization using cell fusion
- **Enhanced mAb production of established cell lines**
  - **CRISPR-enabled activation of transcription regulatory genes**
- mAb production CHO cell line technology

Key Master Transcription Factor Genes of Interests



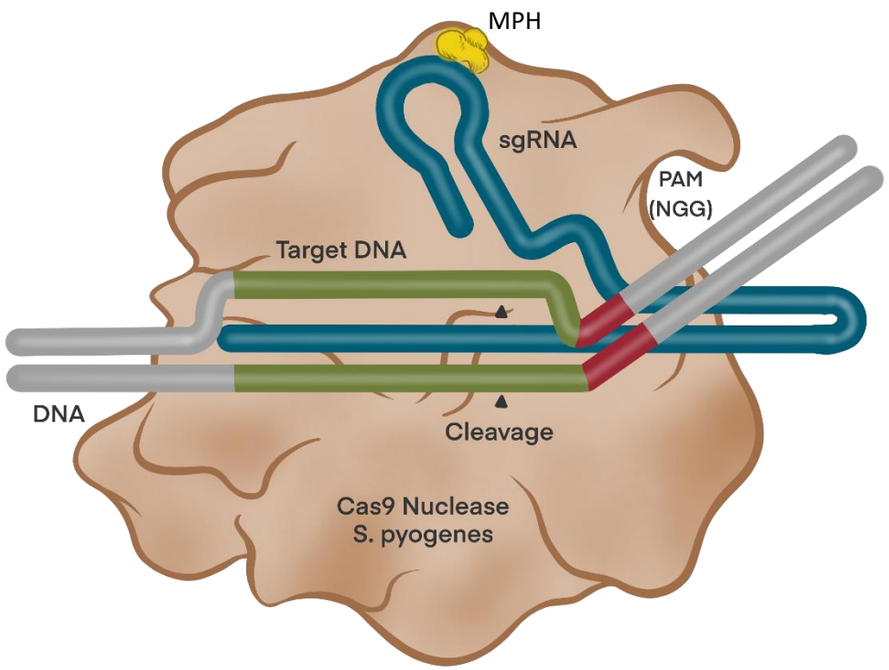
- **Transcription factor genes selected for CRISPR mediated addition & activation**
  - Drm2
  - Fr5
  - Bxp2
- **Promotor activation is universal and can be applied to any host cell irrespective of B cell lineage**

CRISPR-Cas Targeted Gene Activation



*Transcriptional state site (TSS), homology-directed repair (HDR), non-homologous end joining (**NHEJ**)

Multiplex CRISPR Guided Activation (CRISPRa)



1. In traditional activation, open reading frames (ORF) of genes need to cloned under a ubiquitous promoter. The size of ORF is a rate-limiting step as only 1-2 genes can be activated

2. Short gRNA (20 nt) for transcriptional activation allows for multiple concomitant activation of genes
   - Potent activation of genes from 20-100 fold

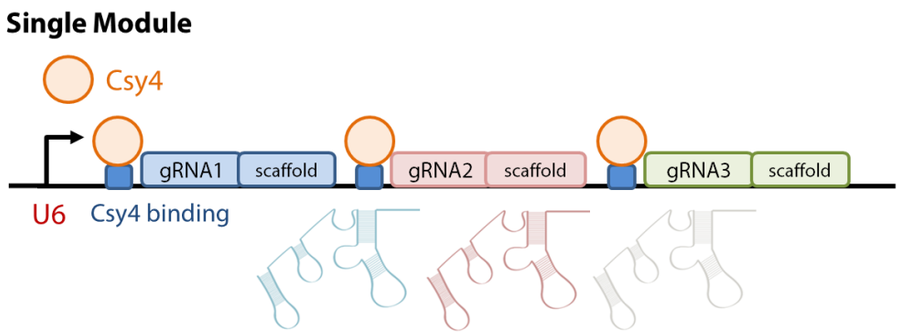
3. Stable expression of dCas-VP64 recognizes canonical protospacer motif (PAM) on gRNA targeting regions within 200bp of the transcriptional start site (TSS) of the plasma cell master regulators: Drm2, Fr5 and Bxp2.

4. Activation of the three transcription factors: Drm2, Fr5 and Bxp2 trigger a cascade of events to enhance antibody production:

Multiplex TF Activation by CRISPRa

Current Challenges

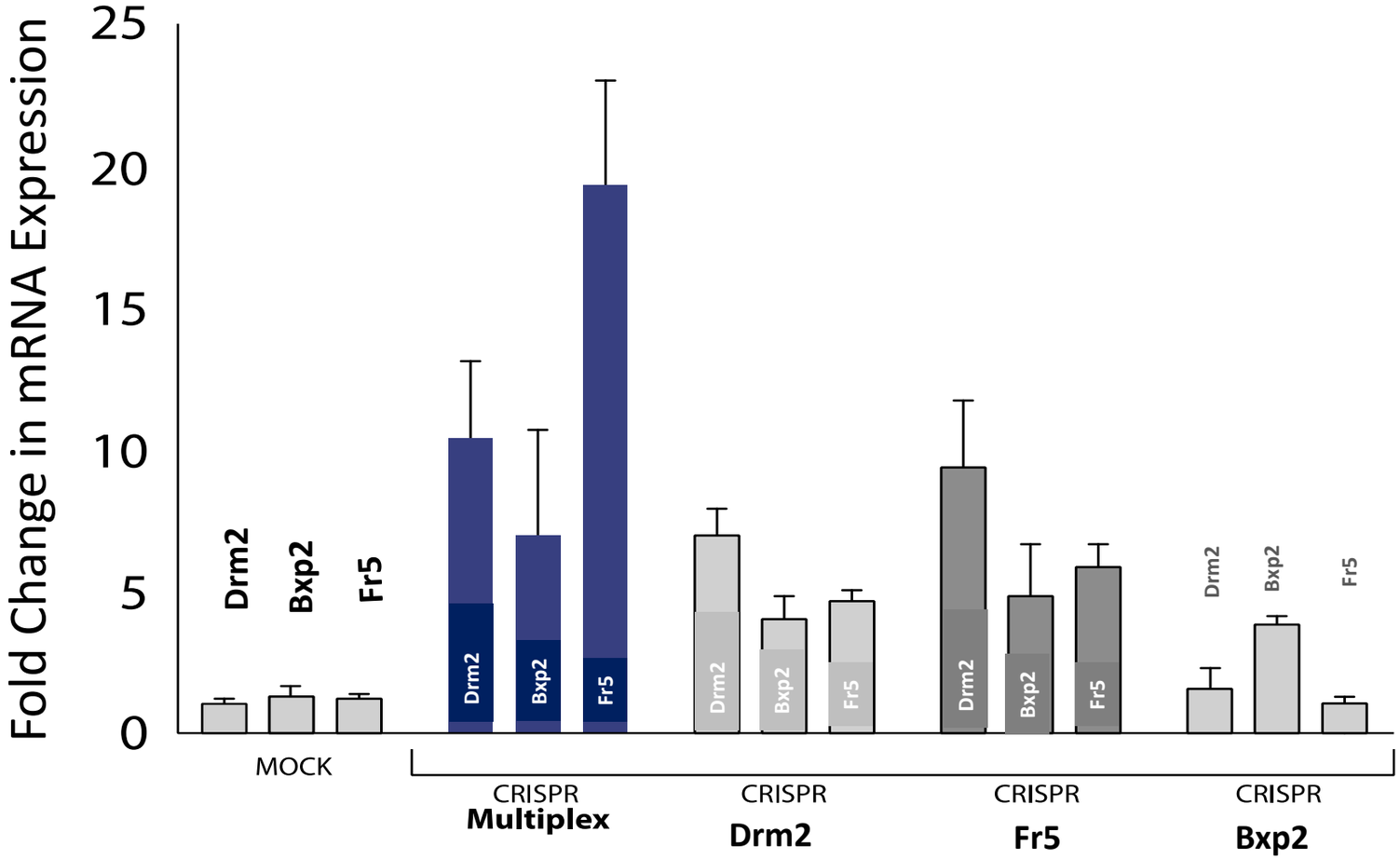
- gRNA that allow for potent transcriptional activation needs to be empirically be determined
- Not all gRNAs will activate gene transcription
- Multiple transfection and transduction are required for a single gene
- Current CRISPRa technologies at best can only activate two gRNAs at a time



Aridis Multiplex CRISPRa

- Utilizes a unique and modular gRNA scaffold design for multiple processing of gRNAs in a single vector
- Synergistic activation of multiple genes in the sample molecular pathway superior to a single gRNA activation

Potent synergistic activation of transcription factors in Fusion Partner by multiplex guided RNAs



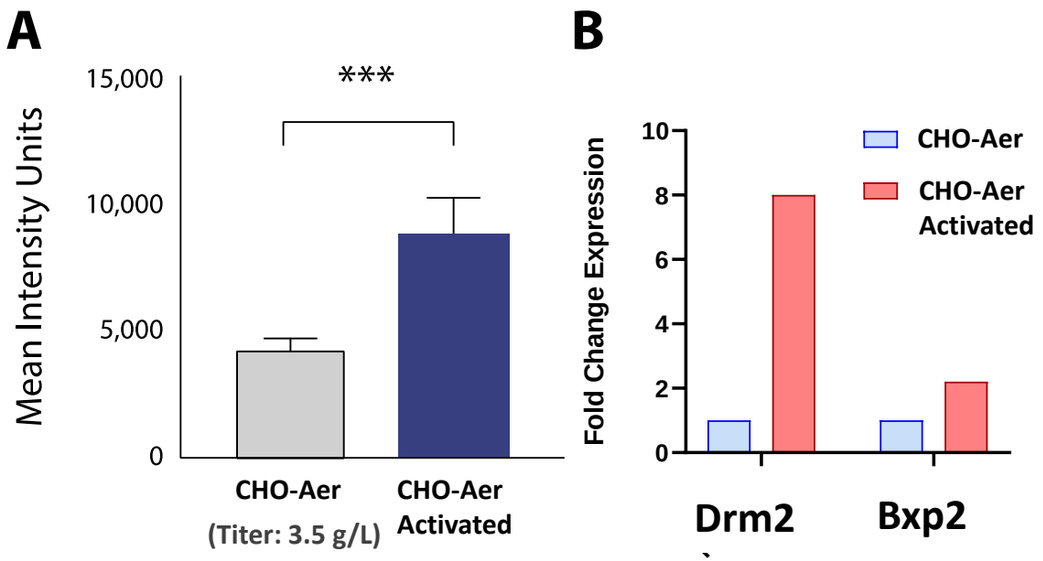
Synergistic activation of master transcription factor genes

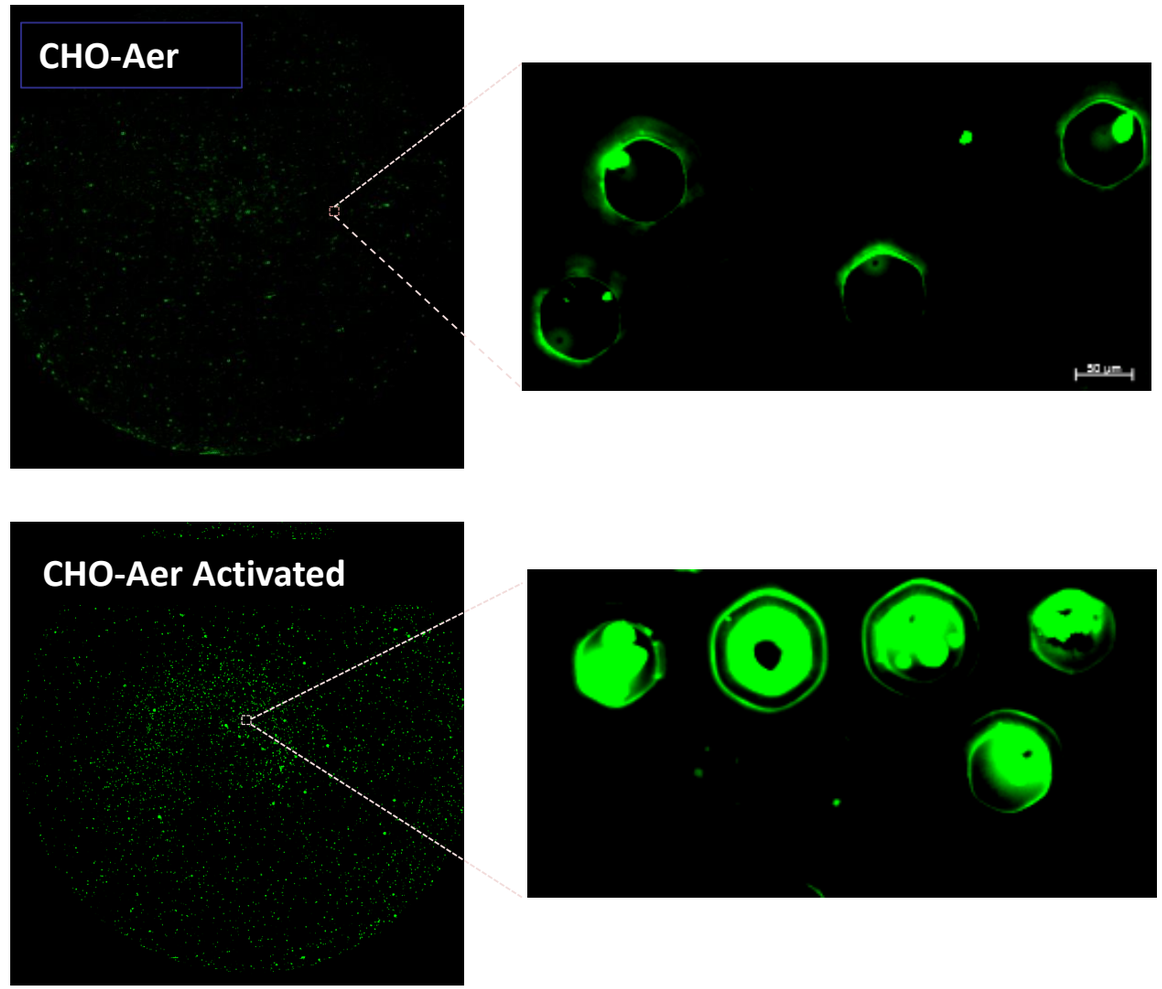
- Predicted synergistic activation of targeted transcription factors with single gRNA
- Multiplex approach showed higher transcriptional activation transcription factors compared to single gRNA activation

Hypothesis: ***CHO production cell lines have similar master transcription regulatory elements, but are not comparably activated or utilized as in B-cells***

Question: ***Can B-cell MTREs Enhance Productivity of Existing mAb Production CHO Cell Lines?***

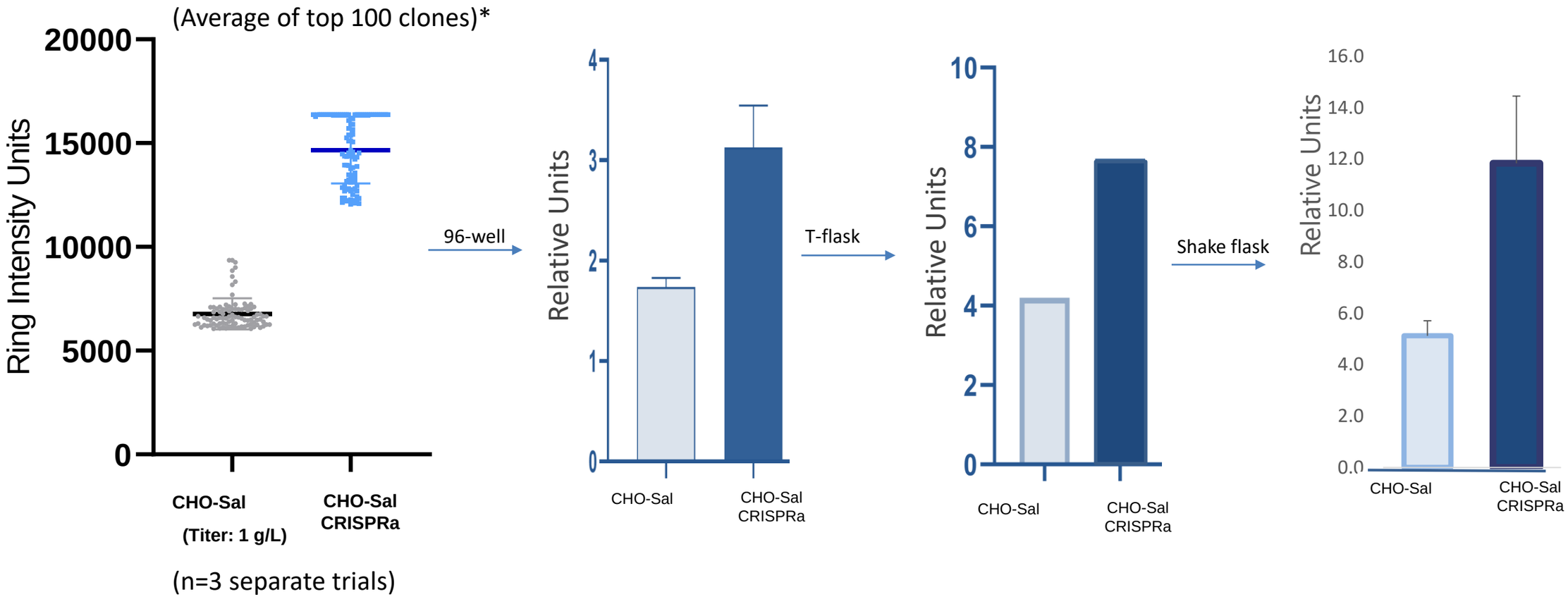
CRISPR-Activation of Endogenous Master TF Regulatory Elements Increases Productivity a CHO Production Cell Line





A-B. Significant increase in single cell IgG production measured using nanoculture arrays. B. Expression of Drm2 & Bxp2 in CRISPRa CHO **C.** Representative panel. Right panels show an inset of single well containing 100,000 nanowells. Data represents average titer over 100,000 wells. *** $p<0.0001$ by Student's t-test, n = 100,000.

Case Study #2: CRISPR-Activation of Endogenous Master Transcription Regulatory Elements of CHO-Sal Cell Line

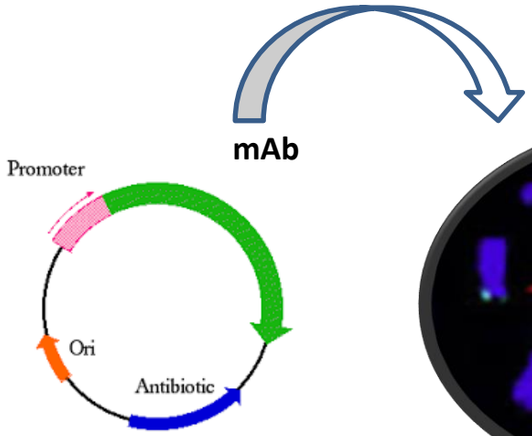


*Pools of APEX CHO-Sal after 3 weeks selection in antibiotics were seeded on nanowells and top 50 clones were isolated using micromanipulator. E2 clone was further subcloned and expanded for 3 weeks in CHO CD medium. Following expansion, CHO-Sal and APEX CHO-Sal (E2) clone was plated on nanowells for IgG Diffusion Assay. Figure on left shows top 100 lead clones ring intensity distribution after 1 day for original CHO-Sal (volumetric titer: 1 g/L) and stable APEX CHO-Sal (E2 clone) with stable MTRE

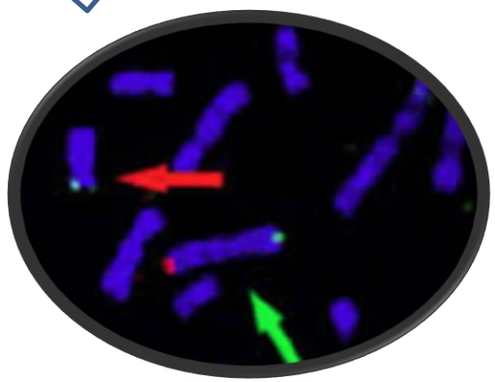
Outline

- Single cell cloning & mAb discovery
- B-cell immortalization using cell fusion
- Enhanced mAb production of established cell lines
  - CRISPR-enabled activation of transcription regulatory genes
- **mAb production CHO cell line technology**

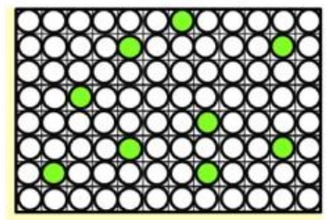
Current State of the Art in mAb Manufacturing



mAb gene expression vector



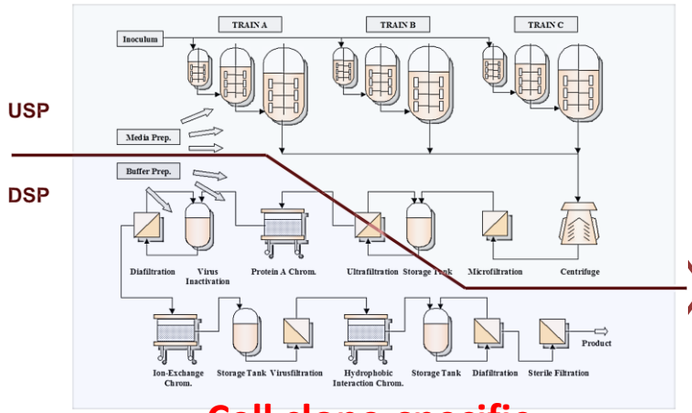
Random integration into genome



Single cell screening & Clonal selection

5-8 months

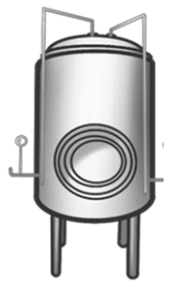
~$0.5m



Cell clone-specific manufacturing process development

8-10 months

~$1m



GMP Production

4-6 months

$2m-$4m

Months

0 3 6 9 12 15 18 21 24

Product Concept:

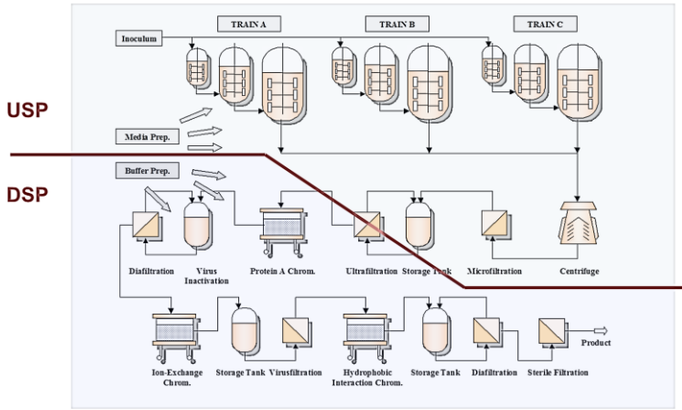
- ***Engineered CHO cell lines with CRISPR-mediated transcription activation harboring a full length mAb H/L genes that is designed to be swapped out for the CDR or H/L of interest***
  - ***Integration sites and H/L gene copy numbers are known and unchanged***

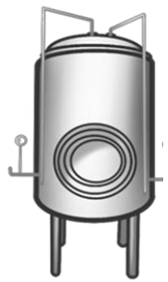
- ***CDR or H/L can be switched from the cell line without significant modification of established upstream & downstream manufacturing processes***

APEX's BREATH™ CHO Master Cell Line:

Development of a Manufacturing Process Template

Master Cell Line
Harboring IgG_1





Ready for IgG_1 Gene Swap

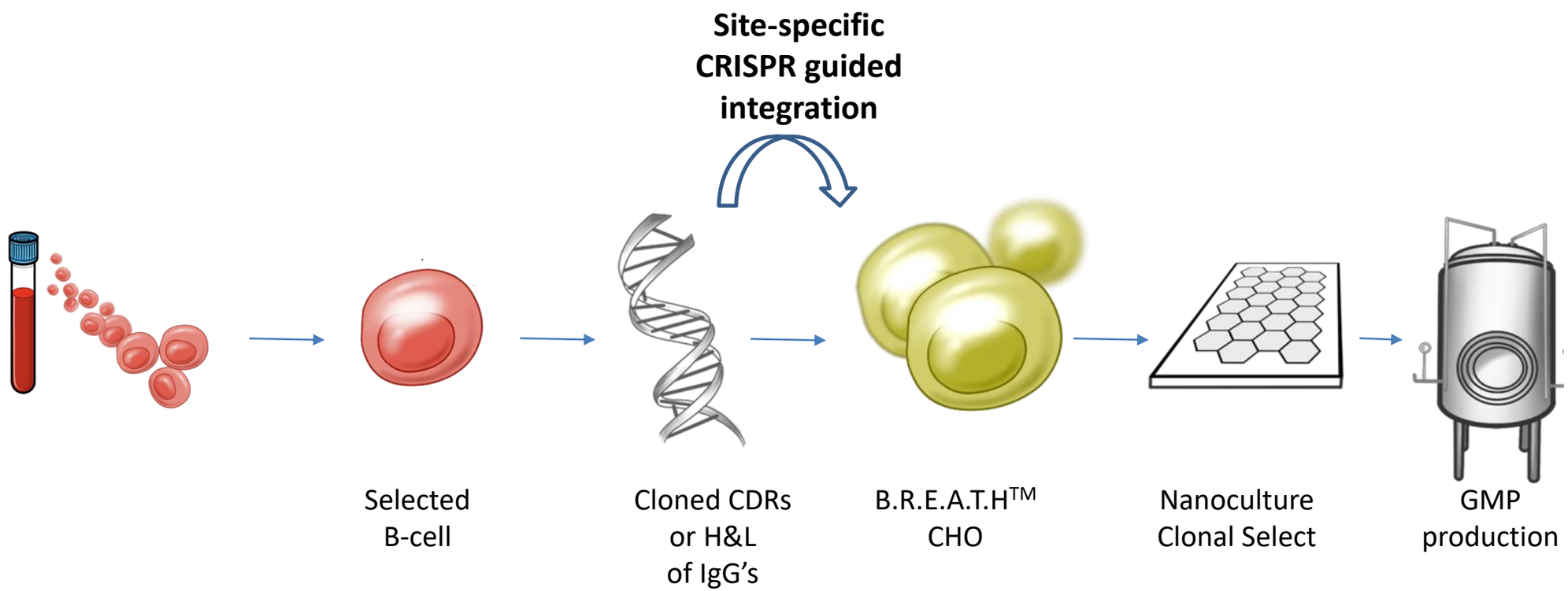
√ MTRE activated
By CRISPR

√ Manufacturing Process
to be Developed

√ Scalability to
GMP Manufacturing to be
demonstrated

B.R.E.A.T.H™ B-cell Regulatory Elements Assisted Transcription Host

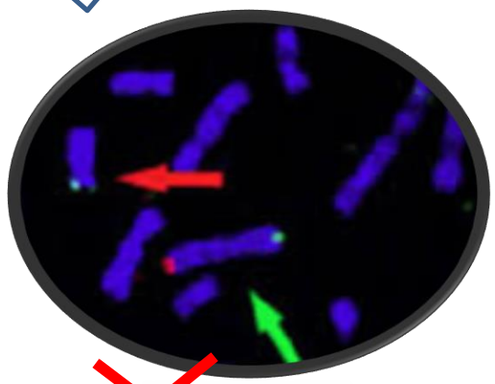
APEX™ CHO Cell Line: CDR or H/L Chain Swappable CHO Host



B.R.E.A.T.H™ B-cell Regulatory Elements Assisted Transcription Host

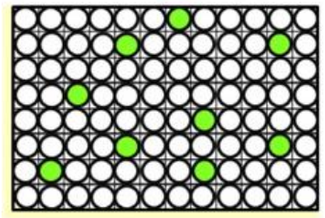
APEX™ Technology Solutions

mAb gene expression vector

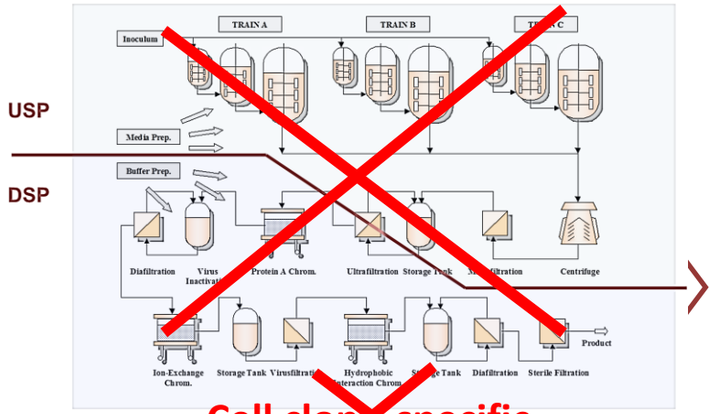


Random integration into genome

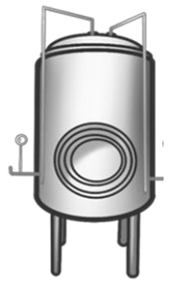
APEX Nanoarrays



Single cell screening & Clonal selection



Cell clone-specific manufacturing process development



GMP Production

APEX: Discovery, Development, and Manuf.

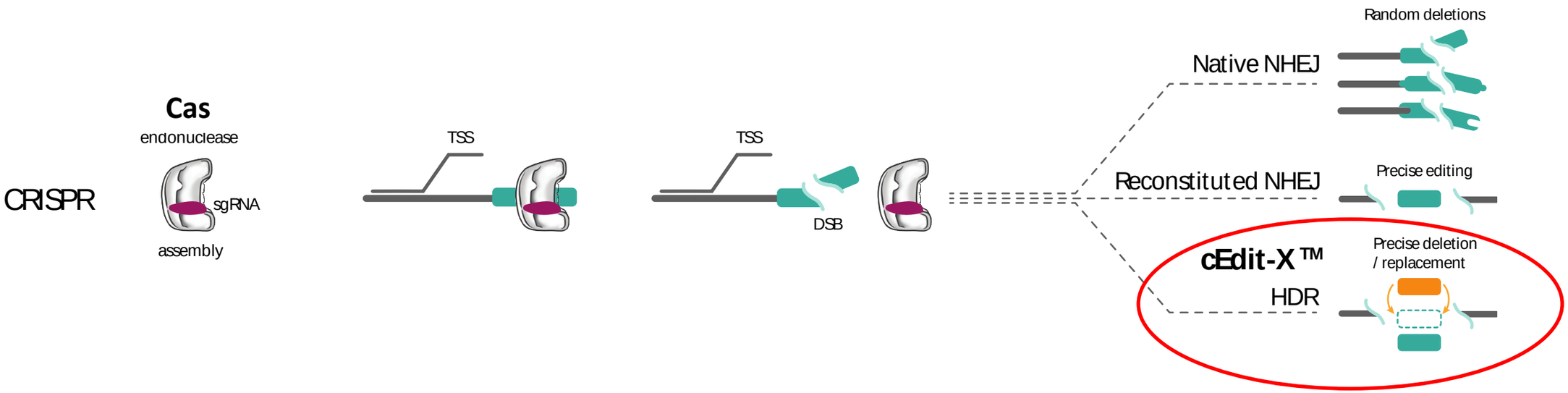
~12-15 months time saving

~$0.8m - $1.2m cost saving

Months

0 3 6 9 12 15 18 21 24

CRISPR-Cas Targeted Gene Replacement



*Transcriptional state site (TSS), homology-directed repair (HDR), non-homologous end joining (**NHEJ**)

Engineering of CHO with B-cell Master Transcription Factors (B.R.E.A.T.H™ CHO)

Genetically engineered transfection-ready production CHO cell line harboring H &L chains of a human IgG1 and B-cell master transcription factor regulatory elements

Proprietary CRISPR-Mediated Activation

Genetic modifications introduced to activate transcription factors to promote productivity and stability

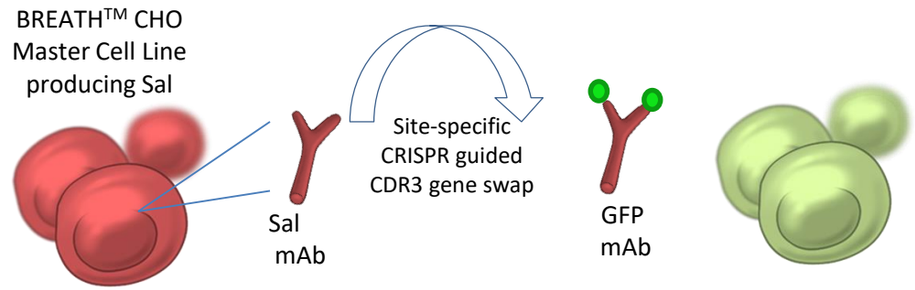
- High productivity
- Stability

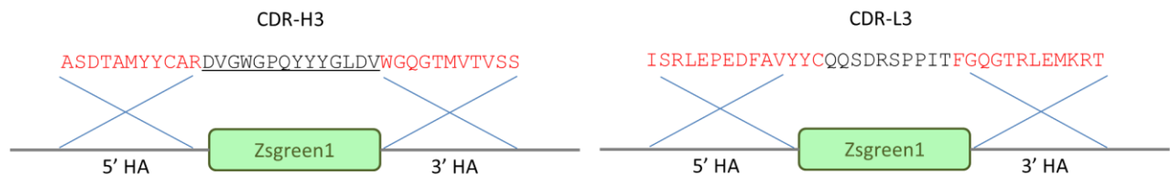
Engineered for Productivity

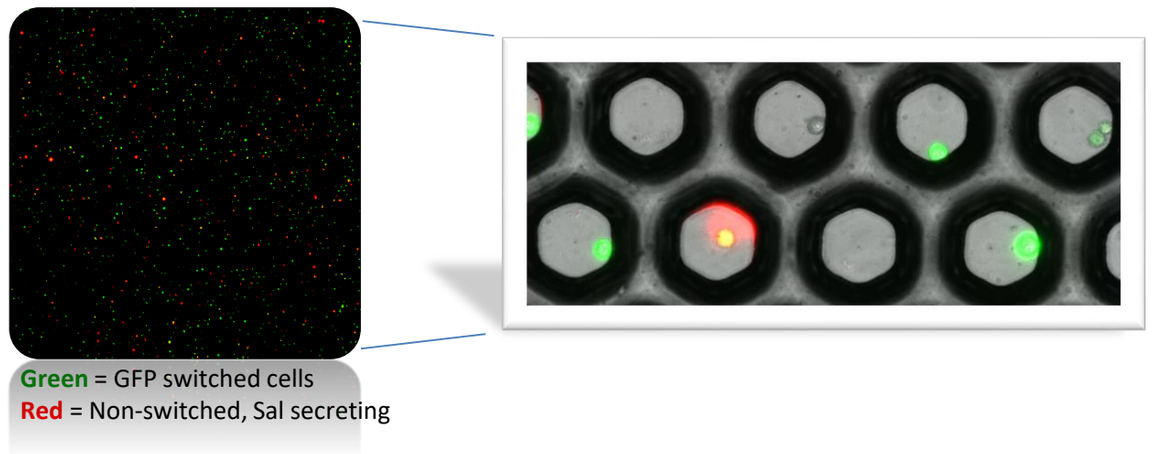
- Engineered as a host cell line to accommodate cloning of human H & L chain CDRs or full-length H & L chains by homologous recombination
- Proprietary vector system allows for dual promoter, high-copy amplification of plasmid DNA

Target Specific Insertion of Heavy and Light Chain CDR3 Genes

Aridis' approach technology utilizes CRISPR/Cas to achieve site specific integration of antibody heavy and light chain genes for maximum productivity







APEX™ Technology Suite

B-cell Repertoire Screening: APEX™ NanoArrays

B-cell immortalization using cell fusion

mAb Productivity Enhancement: APEX™ CRISPR-assisted TF activation

BREATH™ CHO Master cell line designed for CDR swapping