

SAFCB Development Services: Single Cell Clone Generation



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Senior Research Scientist
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Overview

- **Development Services Capabilities**
- **High-throughput Cloning**
- **CellXpress Assisted Clone Generation vs. Limiting Dilution**
- **SAFCB CD media and feed performance**

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Development Services

Integrated Cell Culture Improvement

- **Cell line optimization**
 - Upstream clone selection services
 - Selection of clones based upon productivity, cell growth or protein quality
- **Medium optimization**
 - Selection of formulation to improve net productivity, protein quality or stability
 - Screening, media mixing and component optimization
- **Process optimization**
 - Feed development to further improve net productivity or protein quality
 - Bioreactor parameter optimization
- **Integration of all optimization capabilities**

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SAFCB Cell Line Development

- Expression Feasibility
 - Transfection efficiency evaluation
 - Manufacturing assessment
 - Stable transfectant pool generation
 - Transfectant pool evaluation
- Clone Selection
 - Single cell cloning
 - Clone expansion
 - Clone characterization
 - Media Screen

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Clone Selection Facility

LEAP instrument



Room dedicated to client projects.

Quality systems in place.



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Cell Xpress

High Throughput Clone Selection



Two key components

1. High-throughput cell imaging
2. Laser-mediated cell manipulation

Capabilities

1. Can image up to 10,000 cells per minute
2. Can target / process up to 1000 cells per minute

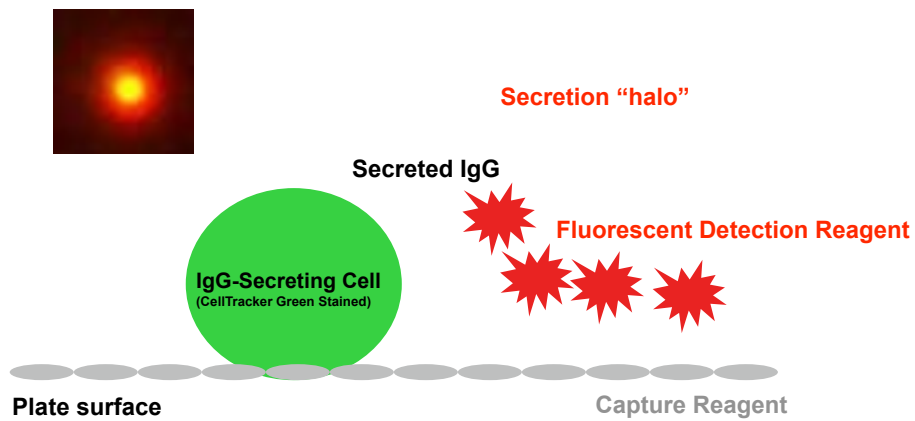
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Cell Xpress™

In Situ Detection of Secreted Antibody

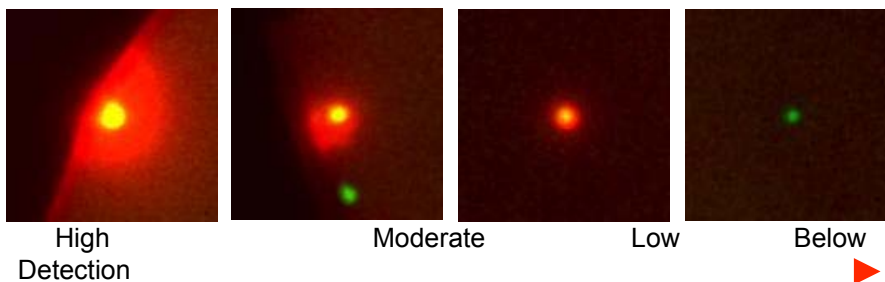


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Cell Xpress™ Enables Tracking of Single Cell Antibody Production



Decreasing level of IgG secretion

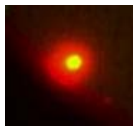
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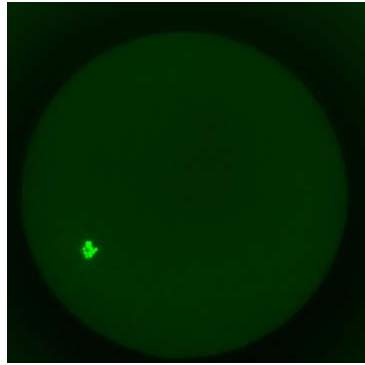
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Cell Xpress Single Cell Clone Expansion

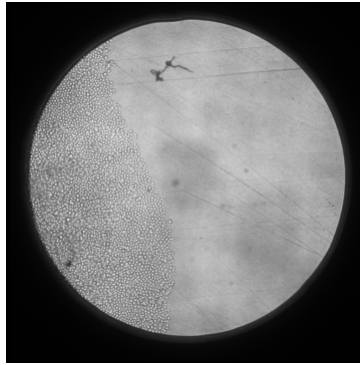
Image History Provided From Single Cell Stage



d0 single cell
secretion



d6 clonality
confirmation,
live cell stain



d12
outgrowth,
bright field

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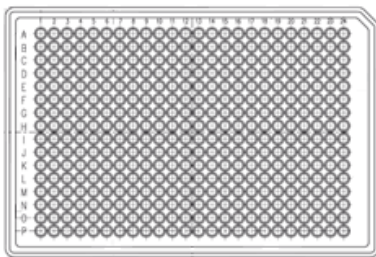
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Secretion Heterogeneity Analysis

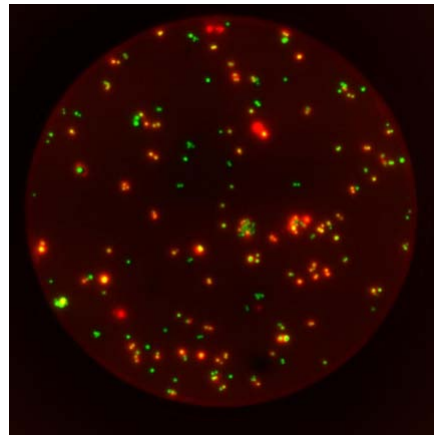
IgG Transfected Pool in CHOK1SV

384 well plate

~32,000 cells analyzed per plate



- High-throughput cell imaging (3-20X mag.)
- Imaging of green (cells) & red (captured antibody)
- Automated image segmentation to associate secretion with each cell



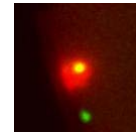
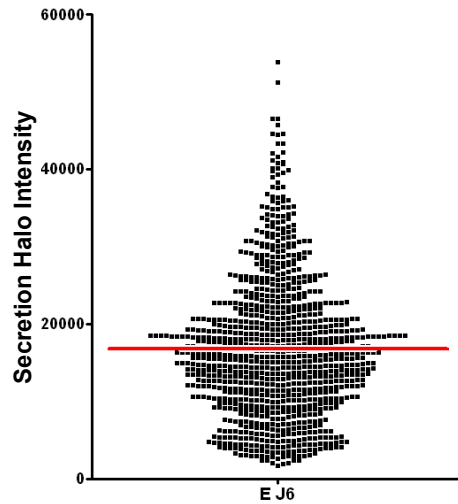
- CHOK1SV cells and vector systems licensed from Lonza Biologics

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Cell Xpress Secretion Heterogeneity

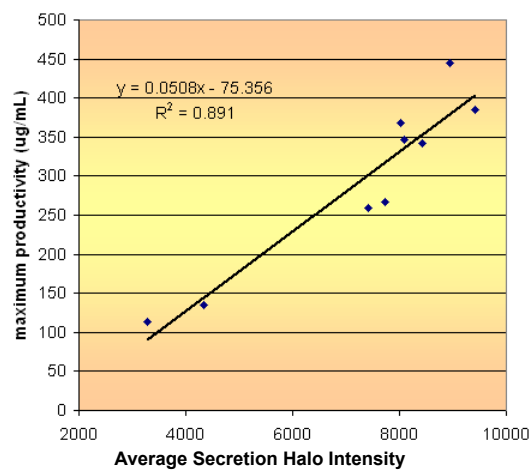


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Cell Xpress Secretion Correlates with Shake Flask Productivity



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Experiment Workflow

CellXpress Assisted Clone Generation vs Limiting Dilution

Clone Pool

CellXpress
Assisted
Cloning

Limiting
Dilution

Clone
Characterization

Clone
Characterization

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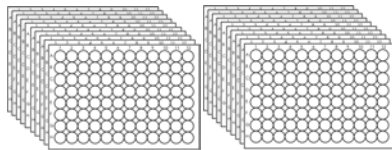
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Limiting Dilution vs. Cell Xpress Single Cell Cloning

A comparison of methods

Limiting Dilution

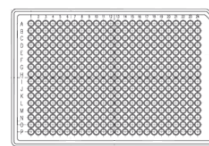


(twenty 96-well plates)

~1000 cells analyzed

limiting dilution cloning
expansion
secretion assay (e.g. ELISA)

Cell Xpress



(one 384-well plate)

~32000 cells analyzed

Cell Xpress™ secretion assay
and enrichment
Cell Xpress™ secretion assay
and single cell cloning
expansion

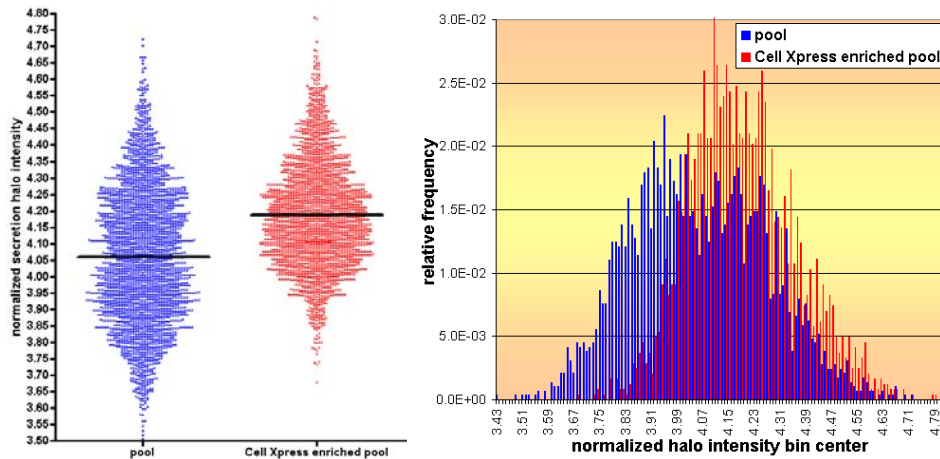
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Laser Enrichment of Transfected Pool

Comparison of original pool and Cell Xpress enriched pool



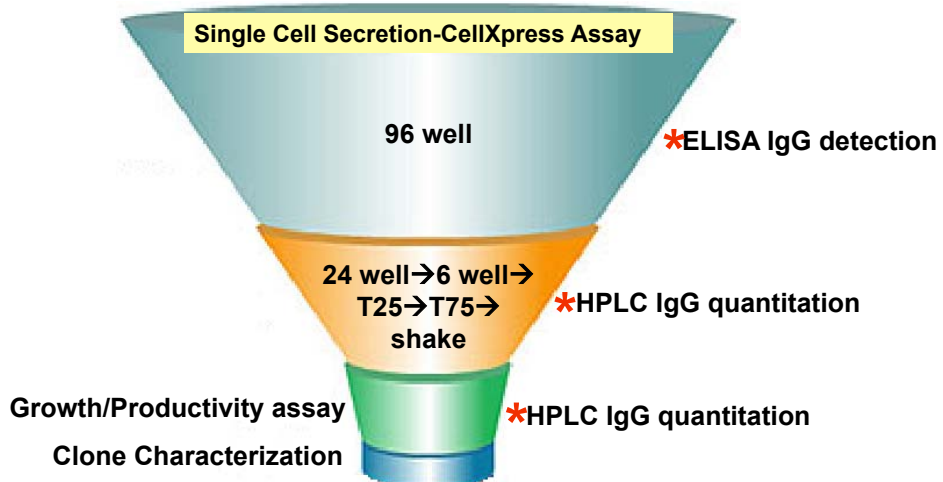
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Scale-up of Clones from a Transfected Pool

Selection parameters



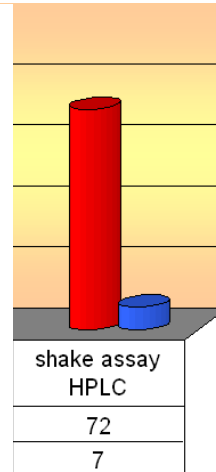
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Clone Selection During Scale-Up Transfected Pool

- CD media
- Non-fed batch
- Spin-tube

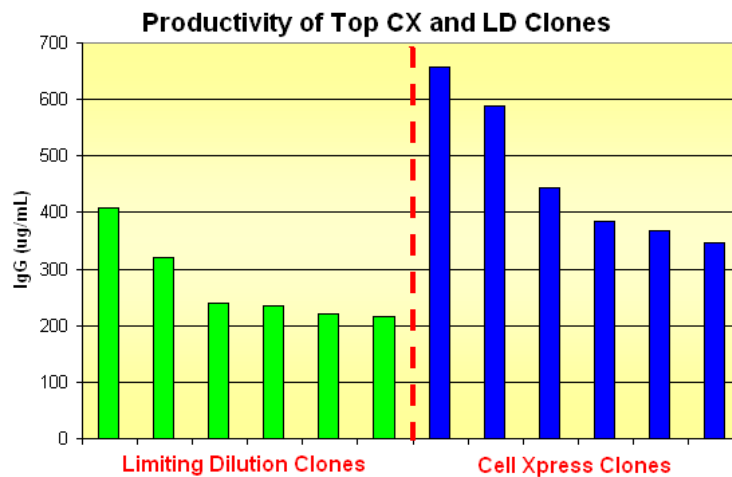


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Productivity of Top Clones CX vs LD

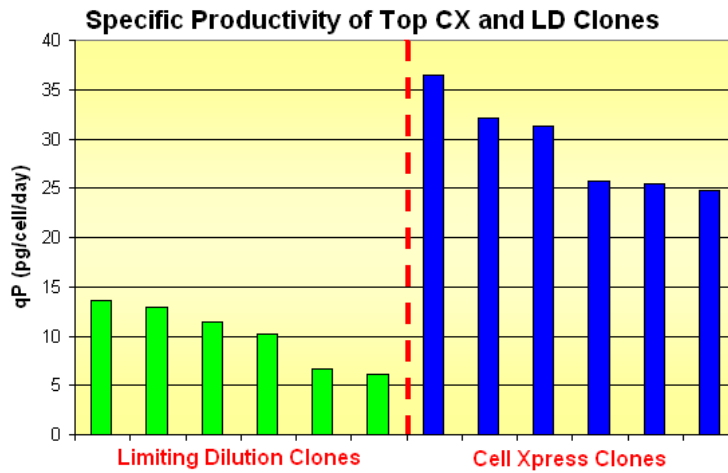


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Specific Productivity of Top Clones CX vs LD



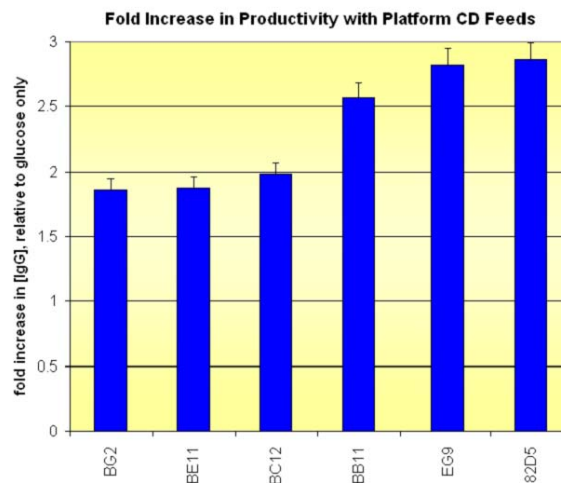
• Evaluation of top candidate clones in a CD fed batch system

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Productivity Improvement with CD Feeds CellXpress Clones



1.6g/L

- CD media and feeds
- Unoptimized process

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Development Services

Single Cell Cloning

- **SAFCB's Single Cell Cloning process**
 - Comparable timelines with limiting dilution, but initial productivity assessment is performed much earlier
 - Provides high-throughput clone screening, using Cytellect's CellXpress technology
 - 40% higher productivity
 - >2 fold improvement in specific productivity
 - Allows full image documentation of early clone expansion
- **SAFC Biosciences' clone generation can deliver more high producing clones for downstream optimization**

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Typical Clone Generation project

16- 24 weeks

Transfected Pool Evaluation



Single Cell Cloning



Clone
Expansion and
Banking



Media
formulation
screen

• Comparable timeline for limiting dilution and CellXpress assisted clone generation

• Higher productivity and specific productivity of clones using CellXpress assisted clone generation

▶ **Media and Feed Optimization**

▶ **Stability Studies**

(Optional)

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Acknowledgements

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–Nan Lin

–Kathy Roeder

Vectors and CHOK1SV
cells were licensed from
Lonza Biologics

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