Transient Gene Expression or what Hi5 insect cells can mean for you

RG Recombinant Protein Expression and Protein sample production facility (PSPF)

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Recombinant Protein Expression
Recombinant Protein Expression Activities

- Department of Structure and Function of Proteins at Helmholtz Centre of Infection Research
- Helmholtz Protein Sample Production Facility (www.pspf.de)
- Instruct-ERIC - Integrated Structural Biology Infrastructure for Europe Centre for Protein Production (www.structuralbiology.eu)
- Protein Production and Purification Partnership in Europe Network, Training, Exchange, Symposia
- NEW!!! CSSB Hamburg at DESY Campus (PP@CSSB)
Platform Support: What do we offer?

- Scientific Cooperation

- Support to get protein samples for your project
  - Advice and Materials for do-it yourself cloning

- Protein sample production
  - Entry requirement – Expression clone
  - Insect cell expression (BEVS)
  - Mammalian cell expression (CHO, HEK 293-6E)
  - Optionally Yeast (Pichia pastoris)

- High Throughput Cloning by SLIC technology (KBU, DAG, WBL)
Production of soluble mammalian protein and protein complexes

- Structural analysis of receptor-ligand complexes
- Host-Pathogen interaction
- Functional proteomic studies
- Complex biologics
- Drug target protein
- Proteins for new strategies in vaccinology
I. Development of a FACS-based selection method to isolate high producer mammalian cell lines for structural biology

FACS-sorting of GFP-transfected cells

Avoids antibiotic selection

Selection for strong, stable GFP expression

Long-time Stability of selected expression cell lines

Stable Cell Line Development using Recombinase-mediated cassette exchange (RMCE)

From master to producer cell line in less than 2 months

Essentials for Multi RMCE Cell Line Development

**Genomic Locus 1: eGFP**

- New Fluorescent gene: tdTomato
- New Set of FRT sites: F13 - F14
- New Exchange Vector: F13 – F14 Polylinker
- New Selection trap: Δpuro
- New Cell Line: Multi RMCE
Binary-RMCE Cell Lines (SMT_dneo(2)_24 tdTomato)

High tdTomato Fluorescence

Lower tdTomato Fluorescence
Binary cell lines tdTomato and double tdTomato

CHO Lec3.2.8.1

SW13_26

- BBA10-tdTomato

SMT_dneo(2)_24

- SMT_dneo(2)_24-Tomato

TE3-B4-H1

- TE3-B4-H1-tdTomato/tdTomato

TE3-B4-L1.1

- TE3-B4-L1.1-tdTomato/tdTomato
Expression of tdTomato is cumulative
Conclusion:

Stable binary RMCE Master cell lines

Long term stable expression without selection pressure

Expression level specific for each locus

Problem:

Still Time Consuming - Not suitable for Construct Screening

How do we create a compatible HTP expression screen?
Construct screening in insect cell lines

Baculoviral Expression

Plasmid based Expression

4 weeks

72 h

µg eGFP yield/10⁶ cells

476.2

24.8

BEVS

Plasmid Transfection
The BioLector microcultivation system for HTP expression screening

- 48 well with max. 2 mL cell culture
- Shaking (400 – 1500 rpm; 3 mm orbital)
- Temperature (20°C - 50°C)
- Humidity (no/>80%)
- Online Biomass measurement (scattered light)
- Online GFP measurement (486 nm/510 nm)
Split GFP Transient Transfection screen

GFP11 (= 15AA)

Insoluble construct => NBD GFP

Soluble construct => GFP
Experimental set up for target NOD2

- Coexpression of GFP1-10 and GFP11-NOD2 construct

- Transfection:
  - 0.5*10^6 cells/mL, Hi5-cells, 2 mL
  - 2 µg DNA/well
  - 1:2 DNA/Lipofectin Ratio

- Cultivation at 900 rpm, 27°C, 96 h
NOD2 constructs

Nucleotide-binding oligomerization domain-containing protein 2 (NOD2) also known as caspase recruitment domain-containing protein 15 (CARD15)
Correlation of Fluorescence to expression

Purification of 9 different Strep tagged NOD constructs in BEVS (15*10⁶ cells)

Purification of 9 different Strep tagged NOD constructs in BEVS (15*10⁶ cells)
Result:

Transient Gene Expression in Insect Cells (TGE)

Split GFP allows detection of Expression

Biolector System for HTP expression screen

Option 1:
Direct transfer into Baculo Viral Expression System

Option 2:
Generate RMCE Production Cell Line with optimized Construct

Option 3:
Scale up of TGE in Hi5
Expression level of the viral OpiE2 promoter in Sf21 compared to Hi5 cells

Max eGFP Yield for OpiE2 promoter in Hi5 50 times higher than Sf21
Comparison to the BEVS system

- Volumetric yield of plasmid based expression ~50% of BEVS in Hi5
Comparison of the Hi5 to the HEK293-6E transient expression

Max. eGFP yield in Hi5 cells 55% of max. eGFP yield in HEK293-6E cells
Optimized and Simple Protocol for Transient Gene Expression in Hi5

- Harvest exponentially growing High Five cells
- Resuspend at 5 x 10^6 cells/ml
- Add 1 µg pOplE2-eGFP plasmid for each 10^6 cells. Mix
- Directly add PEI in a ratio of 4:1 to DNA. Mix and incubate for 3 hr (160 rpm, 27°C)
- Dilute with fresh medium to 1 x 10^6 cells/ml and incubate (130 rpm, 27°C)
- Follow Expression by flow cytometry
Growth rate after adaptation for at least 3 months to different media
Doubling time varies from 15 to 17 hours
The pOpIE2-eGFP expression vector was used to analysis various parameter on transfection efficiency in High Five cells
After adaptation for at least 3 months High Five cells performed best in Excel 405 > Express Five > Sf900II
Effect of Bacviral DNA Sequences on Transient Gene Expression in Hi5

eGFP-HA,
eGFP-HA Δ 603
eGFP-HA Δ 1629
eGFP-HA Δ 603 Δ 1629
**Project 53 Intracellular P58 protein**

**SDS-gel**

594

PSPF-Nr. HZI 53

**P58 1-488_c-term Strep / OP 228**

ÄKTA-FPLC / 1ml StrepTactin Superflow Cartridge (IBA)

**TGE/ Hi5 /1L Culture / asa**

15,8g Pellet / 80% Transfectionsefficiency (eGFP Reporter)

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**Lane** | **Sample** | **Amount**
---|---|---
1 | Precision Plus – unstained- (Biorad) | 10 µl
2 | Lysat | 15 µl
3 | DL | 15 µl
4 | A1 | 15 µl
5 | B8 | 15 µl
6 | B9 | 15 µl
7 | B10 | 15 µl
8 | B11 | 15 µl
9 | B12 | 15 µl
10 | C1 | 15 µl

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**SDS-gel / 12 %**

**Instant Blue**

size of the protein: 57,8 kD

**Intracellular protein**

Sample: 4µl 4xLLPP + 15µl Probe

cell lysis: 20mM HEPES pH 8.0; 500mM NaCl; 1mM EDTA;
10% v/v Glycerin; 2mM TCEP; cOmplete-Mini;
Benzonase; 0,5% NP40

**Remark:** → Pool: B9 – C1   
→ Dialyse / Waschpuffer pH 7,4 / 6-8000MWCO / 5L/ 4° C
Project 41 Intracellular P35 protein

SDS-gel

606
PSPF-Nr. 41 int

**P35_NStrep / OP 276**
1,5ml StrepTactin Superflow (IBA)
MobiCol / Batch 500ml Lysat / ON
TGE / Hi5 / 1L Culture / asa
25g Pellet / 56% Transfectionsefficiency (eGFP Reporter)

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<thead>
<tr>
<th>Lane</th>
<th>Sample</th>
<th>Amount</th>
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<tbody>
<tr>
<td>1</td>
<td>Precision Plus - unstained- (Biorad)</td>
<td>10µl</td>
</tr>
<tr>
<td>2</td>
<td>DL</td>
<td>10µl</td>
</tr>
<tr>
<td>3</td>
<td>Wasch 3</td>
<td>10µl</td>
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<tr>
<td>4</td>
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<tr>
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<tr>
<td>9</td>
<td>Elu 7</td>
<td>10µl</td>
</tr>
</tbody>
</table>

SDS-gel / 12 %

Instant Blue

dimension of the protein: 62 kD

**Intracellular protein**

sample: 4µl LLPP + 15µl sample

cell lysis: 0,5% IgEpal

remark: Pool E1 – E7 (1,0mg/mlkorr.) in ca. 11ml [11,1mg total]

→ Dialyse (50mM Tris/HCl pH 8,0; 500mM NaCl;
10% v/v Glycerol; 2mM TCEP)

5L / 4°C / ON MWCO 6-8000
→ Superdex 200 26/60

P35
A Versatile Integrated Recombinant Expression Platform

- First: Transient Transfection Screen with Split GFP Analysis

- Second: Large scale Production
  Stable cell line (CHO)
  Baculo Viral Expression (Hi5)

- NEW: Scale-Up TGE in Hi5 Insect cells in shake flask up to 1L

- Further optimization of TGE in Hi5 for secreted protein is ongoing
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