VBCF – Protein Technologies	**Standard Operation Procedure** **Transient Transfection of EXPRES2 cells in 24- well plates**

1. **Aim:**
   Transiently transfect Expres2 cells in 24-well format for production of secreted recombinant proteins
2. **Applicability:**
   Sequence-verified expression constructs in Expres2 vector for expression in S2 cells
3. **Responsibilities:**
   Main Responsible: Christian Aigner
   Substitute: Orla Dunne
4. **Required materials:**
   Chemically competent E. coli NEB10β cells (self-made)
   LB, SOC media (media kitchen)
   EX-CELL® 420 Serum-Free Medium for Insect Cells (Sigma 14420C)
   24 well block (Qiagen 19583)
   DNA purification mini-prep kit (AnalytikJena 845-KS-1041250)
   0.2 µM syringe filter (VWR 514-0073)
   Corning 250 ml Flask vented cap (Corning 431406)
   ExpreS2 Insect – transfection reagent TRx5 (Expres2ionbio S2-55A-001)
   500mM CuSO4

5. **Procedure:**

A. DNA Preparation

Prior to transfection, sufficient amounts of plasmid DNA need to be prepared (7.5 µg), this can be prepared in advance and stored at -20° C.

Step 1

Transform plasmids into NEB10β cells (200 µl of cells split into 4) using the standard procedure. Reconstitute cells in 300 µl of SOC media for 1 h at 37°C and split into 2 x 5 ml LB+Antibiotic (mini A and mini B) and place at 37° C overnight (hands on time for 24 transformations: 1.5 h)

Step 2

Mini prep one 5 ml culture per plasmid (mini A) using the standard procedure, however, for the last wash and elution steps do this using the bunsen burner (2.5 h).

Step3

Check the concentration, and if required mini-prep mini B. Mini A and B can be pooled.

B. Transfection

Day 0 (preferably Wednesday, 15min)

Split the cells by centrifugation (450 g, 3 min.) and resuspend the cells to $8x10^6$ cells/ml in EX-CELL® 420 Serum-Free Medium for Insect Cells in shake flasks and incubate at 25° C at 110 rpm.

Day 1 (Thursday, 45min)

- Count the cell stock (usually 1:3 dilution necessary)
- Prepare ~80ml with 8*106 cells/ml in EX-CELL® 420 Serum-Free Medium (72ml needed) – centrifuge the cells (450 g, 3 min) and resuspend in 80 ml media.

- Transfer 3ml of the cells for each transfection into a well of the 24-well plate (use Multipette – 10 ml Tip)
- Add 7.5 µg DNA to each transfection
- Add 37.5 µl ExpreS2 Insect – TR 5x to each transfection (use Multipette – 0.5 ml Tip)
- Gently shake the plate to mix the transfection Reaction
- Incubate 5 minutes at room temperature
- Incubate the 24-well plate at 200 rpm at 25 °C for 24 h

Day 2 (Friday, 5min)

Induce each well with 3 µl 500mM CuSO4 and incubate at 25° C at 110 rpm for 3 days.

Day 5 (Monday, 1h)

- Harvest the cultures by centrifugation 20 min at 4000 g at 4 °C.
- Using a syringe with a needle, remove the supernatant from the 24-well plate and filter (0.2 µM syringe filter) it into a 15ml Falcon tube which you can store at 4 °C.

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