ESACT-UK Bursary Winner: Saba Hussain (King's College London)

Round: February 2023

Conference attended: 6th Annual Bioprocessing Summit Europe, Barcelona (15th-16th of March 2023)

Saba Hussain's Conference Report

I attended the Cambridge Health Tech Institute's 6th Annual Bioprocessing Summit Europe on 15th – 16th of March 2023 in Barcelona, thanks to the generous funding provided by ESACT-UK.

Attending this conference allowed me to improve understanding of the bioprocessing industry, gain insight into what a career in this area would entail and present a research poster of my current project findings.

In this conference, there were multiple streams. I attended the most relevant stream to me, which was *Cell Line Development*. There were many interesting talks, some of which I have summarised below. In general, many of the talks in this stream were focused on improving the selection of high expressing clones and many discussed the use of transposons to increase the number of high expressors. Attending these has enabled me to understand the "bigger picture" behind the context of my research and has given me interesting directions that I would like to explore.

As well as attending talks, I have connected with many researchers and industry experts in the field. It was good to meet people in person and improve my networking skills – which have suffered under the COVID19 pandemic! Additionally, I was able to talk to various traders and vendors, which was a good way to keep up with developments in the machinery involved in bioprocessing. I was also able to gain some free samples and receive advice about some cell culture problems I have been facing.

I was also able to present my research poster titled: "Optimising cell-line development by defining Ubiquitous Chromatin Opening Elements mechanisms of action". Here, I was able to explain my research to other researchers and gain their views and opinions on how to improve my work. This will be especially useful as I move into the latter stages of my project.

One of the talks presented was from ATUM, which described the use of a leap-in transposon system a "Cut and paste" system. The transposase enzyme is used to move the target DNA (the transposon) from the expression vector which is flanked by inverted terminal repeats and tetra nucleotide sequence. The transposon is moved into the CHO chromosomal target site scarlessly, which is engineered to have the correct flanking sites, using transiently expressed transposase. This leads to stable long-term integration of the target gene. They claim it's superior to random integration, due to the random concatamerization of the target genes that can occur from random integration. The

main benefit of this system is faster cell line development as they have a higher number of high expressing clones, meaning they can generate bulk pools instead of mini-pools. They stated some very impressive timelines with bispecific antibodies.

Another talk was given by Katarzyna Sobkowiak who works in the Cell Line Technologies group at Merck Serono. They focussed on the creating of a new CHO cell line that contains a reporter for fast screening. They did this so they were free from licence with a secreted reporter in particular. They used a tripartite split-GFP complementation assay, which uses a GFP fragment with 2 small tags fused. When the 2 proteins interact, the GFP can fluoresce which can be detected usual. They fused one tag each to the heavy and light chain, and when the chains assemble, the tags come together and GFP will fluoresce.

In order to enrich the GFP signal, they used beads which bind to the antibody with one of the tags, which results in fluorescence. They saw around 20% GFP + cells after 2 month selection and growth. Their final cell line was created with the Berkley Beacon and showed a small increase in titre.

There was a talk from a professor from KAIST, who gave a good overview of past and future advancements in therapeutic protein production. He described the development of the DHFR and GS-based selection system. He also describes vector rearrangement that can occur during random integration, then follows with the use of RMCE based targeted integration to produce high expressing clones. There was also talk of the downstream processes, such a protein glycosylation. Addition he described recent advancements in trying to engineer CHO cells to be more like plasma cells, using the master TF: BLIMP1. In summary, he described the main issues in cell line development being: time, quantity and quality.

Another talk was given by Colin Clarke who described using single-cell 'omics to investigate recombinant protein production. They investigated an unstable producing cell line using sc and bulk RNA-seq and were able to track the heterogeneity as the cell line evolved.

Second day followed with a talk by the leader of a CLD team from Novartis, who discussed the systems they used to create cell lines and recent developments to improve the quality of their titre. She discussed their DNA vector technologies to improve productivity. She also described their leaky stop codon technology to aid in single cell cloning for high expressors. In this system there is a leaky stop codon after the heavy chain which enables around 5% of a molecule to be expressed on the cell surface, which will bind to secreted IgG. This display can be used during flow cytometry to identity high produces using a FITC-labelled antic Fc antibody. They also identified up and down regulated genes between high and low producers, all of which were located at the telomeric region of ChR8.

Next, there was a talk by Fay Saunders from Fujifilm, who described the ApolloX expression system, which is used to produce complex bi-specific antibodies. This system is based on DHFR selection in CHO DG44 cells and uses random integration. Using this system, they were able to produce cell lines expressing 3-5g/L

BsAb. They also saw a positive result for Fc-fusion molecules.

There was another talk on the use of transposases for CLD, by a rep from Boehringer Ingelheim. They used a semi-targeted integration (STI) technology to generate a therapeutic antibody at 2,000L scale in less than 3 months and scale up to 12000L bioreactor in 9 months. The use of this technology has meant that their transfected cell recover faster and the pools have increased homogeneity as well as higher productivity. They also found after sc cloning, that al the clones were highly representative of the original pool. Interestingly, they claim that using too much transposase enzyme leads to fragmentations and random integration events.

Finally, there was interesting talk by Advanced Instruments who described their use of a AI neural network to identify clonality. They specify it's use with their Solentim single cell seeding machine. They first prepared data, by taking many images of pools and single cells. Then, after developing the network, they trained the network on what a single cell should look like. This eliminates the problem of a scientist being unable to identify which well contains the single cell.

Overall, I found the conference very interesting and very beneficial for me to attend. Once again thanks to ESACT-UK for their contribution.

POSTER presented:

Expediting cell line development by defining Ubiquitous Chromatin Opening Elements (UCOE®) molecular mechanisms of action

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Background

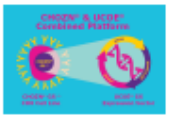
What are UCOE® Elements?

- Elements derived from housekeeping genes
- Span at least one transcriptional start site
- Contains a methylation-free CpG island
- Have seen commercial success
- Promote an open chromatin structure
- Able to negate transgene silencing

UCOE® Applications

Biomanufacturing

- Maintenance and increased expression



Gene therapy

- Stability and high expression in viral vectors

Aim

Improve our understanding of UCOE® molecular mechanisms of action

- Identify functionally relevant regions within mouse Rps3 UCOE® using deletion series mutants and functionally analysing in CHOZN-K1 cells

Methodology

1. Construct 5 minimised Rps3 UCOE ® fragments

- Using hCMV promoter and UCOE® fragment driving expression of secreted embryonic alkaline phosphatase (SEAP)

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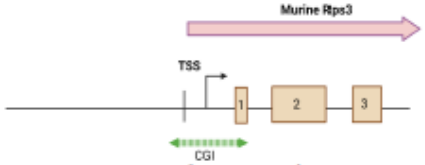

Figure 1: Rps3 UCOE® genomic location

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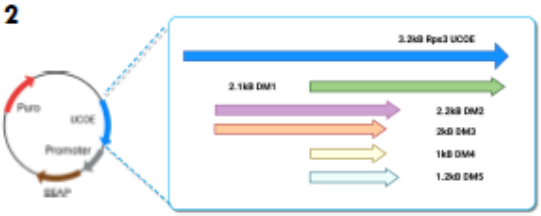

Figure 2: Summary of Rps3 UCOE® deletion series.

2. Functional analysis of minimised Rps3 UCOE® fragments

- Constructs stably transfected into CHOZN-K1 cells; selected with puromycin
- SEAP levels in media assayed
- Tested in bulk pools initially, then in minipools

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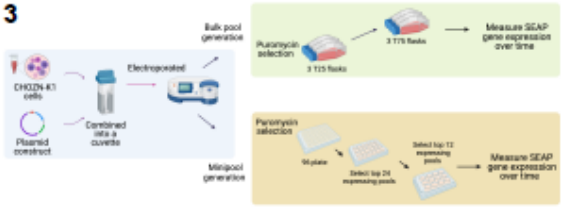

Figure 3: Electroporation and selection procedure for CHOZN-K1 transfection into either bulk pools or minipools

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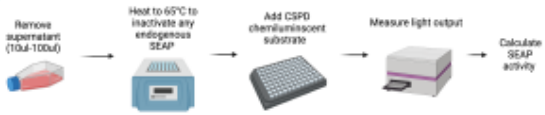

Figure 4: Workflow for the detection of SEAP in tissue culture medium.

Results

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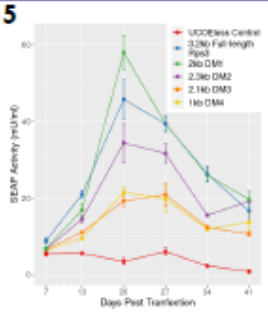

Figure 5: SEAP activity in media of stably transfected CHOZN-K1 cell pools harbouring the SEAP reporter gene constructs. All results shown are from Day 7-42 post-transfection. All results are corrected for cell number. N=3, error bars represent mean ±SD.

6

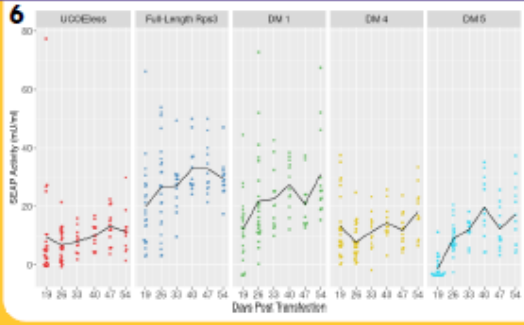

Figure 6: SEAP activity in media of stably transfected CHOZN-K1 minipools harbouring the SEAP reporter gene constructs. Puromycin selection was removed on day-26. All results shown are from Day 19-54 post-transfection. All results are corrected for cell number. N=24 and 12, black line represent mean.

Conclusions

- We can remove 1kb from the 5' end of Rps3 UCOE® with no loss of function

Future work

- 3' end of Rps3 UCOE® to be dissected further but identifying elements and transcriptional factors crucial for UCOE function

References

Neville JJ, Orlando J, Mann K, McCloskey B, Antoniou MN (2017) Ubiquitous Chromatin-opening Elements (UCOEs): Applications in biomanufacturing and gene therapy. *Biotechnol Adv*. **35**: 557-564.

Acknowledgments

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