

Engineering Conferences International ECI Digital Archives

Cell Culture Engineering XV

Proceedings

Spring 5-9-2016

Establishing a robust two-step cloning strategy for the generation of cell lines with a high probability of monoclonality

Alison Young

Fujifilm Diosynth Biotechnologies

Claire Lovelady

Fujifilm Diosynth Biotechnologies

Mahesh Shivare

Fujifilm Diosynth Biotechnologies

Jarka Glassey

ISRU

Shirley Coleman

ISRU

See next page for additional authors

Follow this and additional works at: http://dc.engconfintl.org/cellculture_xv

 Part of the [Biomedical Engineering and Bioengineering Commons](http://dc.engconfintl.org/cellculture_xv)

Recommended Citation

Alison Young, Claire Lovelady, Mahesh Shivare, Jarka Glassey, Shirley Coleman, and Alison Porter, "Establishing a robust two-step cloning strategy for the generation of cell lines with a high probability of monoclonality" in "Cell Culture Engineering XV", Robert Kiss, Genentech Sarah Harcum, Clemson University Jeff Chalmers, Ohio State University Eds, ECI Symposium Series, (2016). http://dc.engconfintl.org/cellculture_xv/58

This Abstract is brought to you for free and open access by the Proceedings at ECI Digital Archives. It has been accepted for inclusion in Cell Culture Engineering XV by an authorized administrator of ECI Digital Archives. For more information, please contact franco@bepress.com.

Authors

Alison Young, Claire Lovelady, Mahesh Shivare, Jarka Glassey, Shirley Coleman, and Alison Porter

Establishing a robust two-step cloning strategy for the generation of cell lines with a high probability of monoclonality

Alison Young¹, Clare Lovelady¹, Mahesh Shivare¹, Jarka Glassey², Shirley Coleman², Alison Porter¹

1 Fujifilm Diosynth Biotechnologies, Billingham, UK

2 ISRU, Newcastle University, Newcastle UK

A regulatory requirement for the production of therapeutic proteins from mammalian cells is that the production cell line is clonal, that is, derived from a single progenitor cell. It is therefore standard procedure to include at least one cloning step during the development of a recombinant cell line for therapeutic protein production. Numerous techniques can be employed for cloning cell lines, but regardless of the cloning method used there should be appropriate evidence to support that the method is fit for purpose. A point highlighted by the increasing interest from regulatory bodies regarding the cloning method used and the probability of monoclonality ($P(\text{monoclonality})$) achieved during cell line development (CLD).

FUJIFILM Diosynth Biotechnologies have thoroughly considered the cloning approach used during CLD: A two-step cloning strategy employed which combines the ClonePix™ as a cloning and screening tool followed by a second cloning step using the industrially accepted method of limiting dilution cloning will be discussed. A collaboration with statisticians led to the development of a method to estimate the resulting $P(\text{monoclonality})$ of cell lines generated using the ClonePix™ and experimental data to support this statistical method was generated, thereby ensuring that the ClonePix™ cloning step is robust. We will highlight the challenges of using the ClonePix™ for a single round of cloning and the advantages of combining it with a second cloning step. We will demonstrate how we achieve a minimum probability of monoclonality of $\geq 99.78\%$ and typically achieve a $P(\text{monoclonality})$ of 99.9% using a two-step cloning strategy.