Simplifying Progress

Strategies to Assess Heterogeneity in CHO Cell Lines

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Introduction

Genome editing tools and several engineering strategies are increasingly applied during cell line development to optimize growth, gene expression, protein folding, and glycoengineering of cell lines. The heterogeneity of the edited cell population should be well understood to evaluate the efficiency and reproducibility of different genome editing tools and engineering strategies. This poster focuses on the assessment of heterogeneity in a CHO DG44 host cell line under batch and fed-batch conditions.

1. Methods

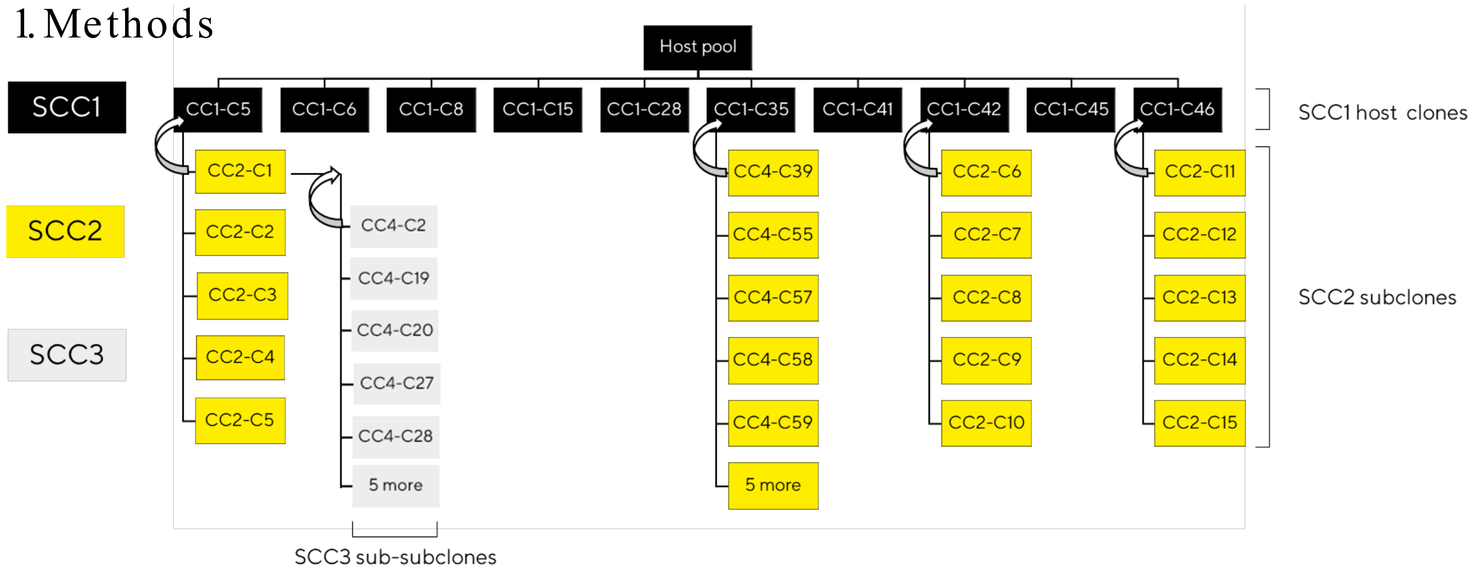

Figure 1: Descendant Tree of Clones and Subclones. Arrows Refer to the Parental Host Pool or Host Clone.

A CHO DG44 host cell line adapted to suspension growth was subjected to multiple rounds of subcloning using the ALS CellCelector (SCC1 and SCC3) and Limiting Dilution (SCC2). After stable growth in shake flasks, clones were transfected with the gene of interest (GOI) and evaluated under batch and fed-batch conditions (Fig. 1).

While the population of SCC1 host clones represents the heterogeneity of the parental host pool, the population of SCC2 subclones and SCC3 sub-subclones populations represent the heterogeneity of the parental host clone population (SCC1 host clones or SCC2 subclones).

2. Results

Evaluation of the batch and fed-batch data by principal component analysis (PCA) suggested that heterogeneity was present in both the host pool (Fig. 2A) and the host clone populations (Fig. 2B and C) but appears to be reduced in the host clone populations (Fig. 2B and C).

Figure 2: Principal Component Analysis (PCA) of the Fed-Batch Endpoint data of Host Clones vs. Host Pool or Parental Host Clone.

3. Assessment Strategies

Several strategies can be applied to assess heterogeneity. Here, we decided on 1) Mean Distance Analysis (Fig. 3) and 2) Sum of Variance of All Variables (Fig. 4). Therefore, host clone data was normalized to the host pool data and subclone data to the respective parental host clone data (Fig. 5).

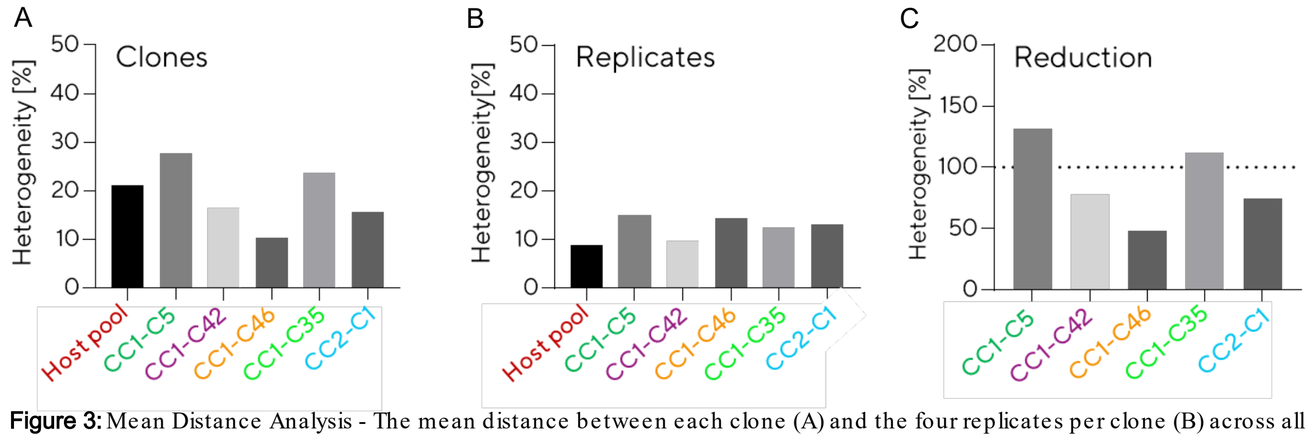

Figure 3: Mean Distance Analysis - The mean distance between each clone (A) and the four replicates per clone (B) across all process indicators (growth, productivity, process time Fig. 5) was divided by the host pool data or respective host clone data.

Technical variability is lower than biological variability (Fig. 3A and B) indicating that the heterogeneity is driven biologically. SCC leads to the reduction of heterogeneity in two out of four host clone populations, CC1-C42 excluded N = 2 (Fig. 3C). Moreover, subpopulation CC2-C1 showed lower heterogeneity than its host clone population CC1-C5 indicating that subcloning seems to further reduce heterogeneity (Fig. 3C).

	N-1	S2	F	P-value
Host pool	39	170589		
CC1-C5	11	69088	2.47	0.05
CC1-C42	7	57465	2.14	0.068
CC1-C46	18	11860	14.38	9.2e-08
CC1-C35	14	97569	1.75	0.13
CC2-C1	14	25152	6.78	0.0002

Figure 4: Sum of Variance of All Variables Mean-centered (F-Test and One-Way ANOVA, $\alpha = 0.05$).

Assessment of heterogeneity using the Sum of Variance of All Variables confirmed the results of strategy 1) Mean Distance Analysis. Two out of four host clone populations showed a reduction of heterogeneity, CC1-C42 excluded N = 2 (Fig. 4). The heterogeneity of the subpopulation CC2-C1 was lower than the heterogeneity of the host clone population CC1-C5 (Fig. 4).

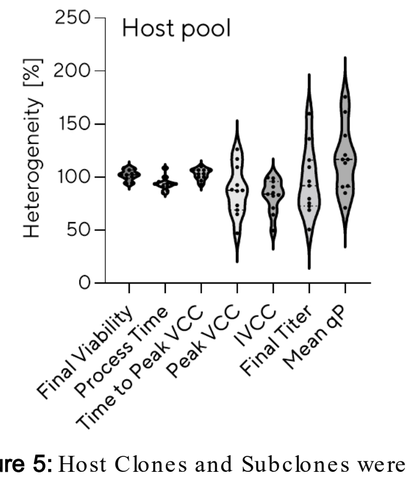

Figure 5: Host Clones and Subclones were Normalized to the Parental Pool/Clone.

Normalized process indicators show that main contributors to the cell populations' heterogeneity are mean qP and final titer (Fig. 5).

4. Conclusion

The CHO DG44 cell line is a heterogeneous cell population which can be reduced by subcloning.
The data here indicates that, instead of the heterogeneous CHO DG44 pool, CHO DG44 clones with limited heterogeneity should be used as starting material to assess genome editing tools and engineering strategies. More data has to be evaluated to make a statement about the reliability and reproducibility of the reduction of heterogeneity.