SELEXIS SURE*scan*™: De-risking Cell Bank Generation with Comprehensive Genomic Analysis

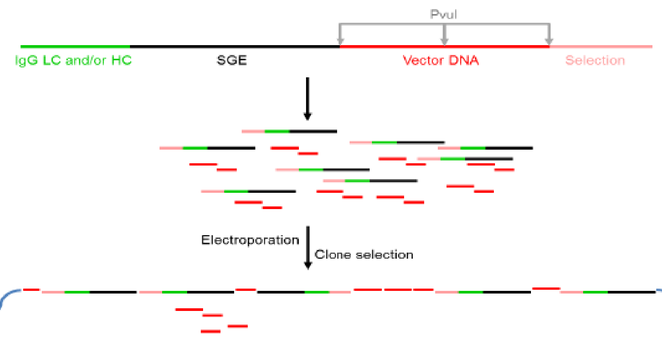
PIERRE-ALAIN GIROD,
SELEXIS SA, 18 CHEMIN DES AULX, 1228 PLAN-LES-OUATES, SWITZERLAND,
pierre-alain.girod@selexis.com

Introduction

Recombinant therapeutic protein production processes must guarantee a sufficiently small variability in the product quality. To keep this variability low, it is critical to run the process in a totally reproducible way. This requires controlling all cultivation parameters. The use of new analyzers, generating new data sets, besides cultivation parameters (e.g. viable cell density, metabolite concentrations) is a desirable way to innovate bioprocesses. The advent of Next-generation Sequencing (NGS) has led to the ability of using genome information to find reasons for variability. Research Cell Banks (RCBs) are not necessarily cell populations with identical genomes or single integration sites even though they arise from a single isolated cell. These mixed populations can lead to unacceptable manufacturing variability. Selexis' SUREscan™, consisting of the detailed CHO-M genomic map and propri-etary bioinformatics tools, decreases manufacturing risks by ensuring transgene in-tegrity in RCBs and by surveying for the emergence of deleterious mutations either in the transgene sequence or in genes that are important for cell survival at a yet unknown resolution.

Configuration of host/construct insertion

Transfection with inearized expression construct:



Structure at insertion site:

Regulatory expectations

Support for Clonality

- Genetic stability = clonality
- Sequencing of gene of interest = clonality
- Integration sites provide better information regarding clonality, if integration is not site-specific.
- Methods used for supporting studies need to be qualified to demonstrate sufficient sensitivity.

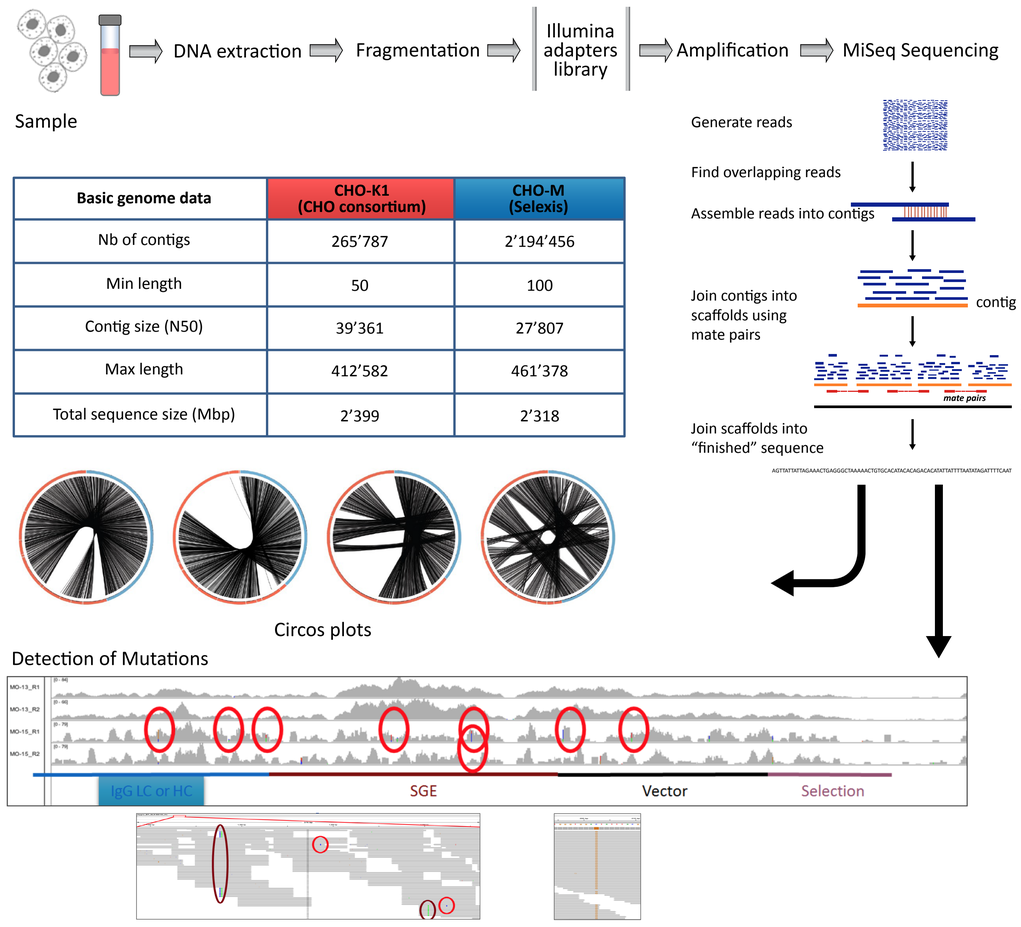
If a cell bank is subcloned for the purpose of supporting clonality:

- A sufficient number of subclones need to be analyzed.
- Subcloning and testing should be performed on cells as close to the original cloning as possible, to ensure that one line does not out-compete another slower growing line(s).

Next-generation Sequencing applications

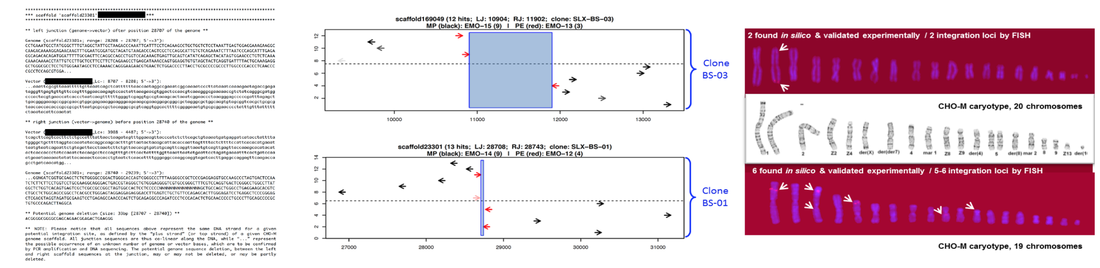
- Determine the integrity of transgene sequence
- Identify the site(s) of transgene insertion
- Determine the sequences flanking the transgene insertion site(s)
- Determine the copy number of inserted transgene
- Ensure the RCB/MCB cell population is clonal
- Ensure barcode tracking of the clone
  (Progeny descending from a parental cell can thus be readily identified by the same DNA barcode)

Plateform for Next-generation Sequencing

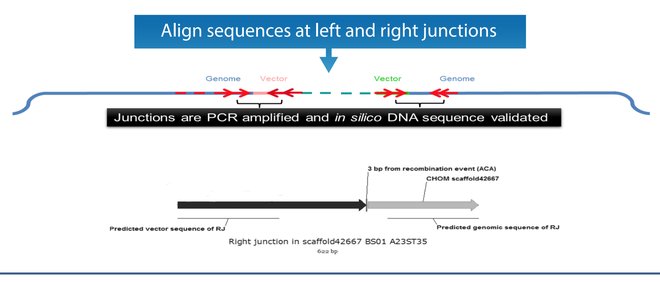


Basic genome data	CHO-K1 (CHO consortium)	CHO-M (Selexis)
Nb of contigs	265'787	2'194'456
Min length	50	100
Contig size (N50)	39'361	27'807
Max length	412'582	461'378
Total sequence size (Mbp)	2'399	2'318

Identification of junctions of transgene insertion

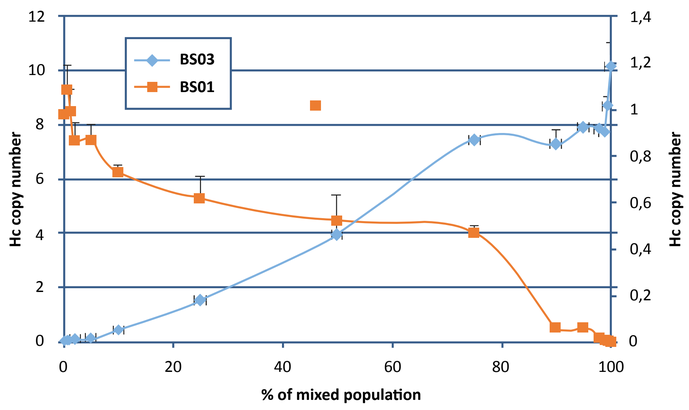


Confirmation of junctions by PCR



Gene copy Nb	BS-01	BS-03
Heavy chain	10	1
Light chain	15	1

Assessment of clonality



- Detection sensitivity is dependent on transgene copy number
- Clonality assessment by NGS enables detection sensitivity of 0,5-1% of contaminant population

Summary

- Clonal expansion results in overrepresentation of a sequence
- Use of MCB in production requires clonality assessment
- NGS-based methods enable identification of the exact clonal sequence and its relative abundance in a given population
- Gene rearrangement, transgene insertion, SNPs, adventitious viruses are unique "fingerprint" for a particular clone
- NGS-based methods allow the tracking of specific clones (barcode)

www.selexis.com