

# WHOLE GENOME SEQUENCING TO SURVEY GENETIC CHANGES IN STABLE CHO CELL LINES

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**1** Chinese hamster ovary (CHO) cells represent the most frequently applied host cell system for industrial manufacturing of recombinant protein therapeutics. Generating and identifying high producing clones in a fast and efficient way such that they do not lose their expression capability over time has been a major focus of the industry. Using cytogenetic analysis combined with Next-Generation Sequencing (NGS) technology and proprietary bioinformatic tools called SUREscan™, provide us with a unique ability to quickly analyze the whole genome of any generated cell line. Our data show that phenotypic changes in growth behavior and metabolism typically caused by cellular stress such as adaptation to a different media are associated with a rise in single nucleotide polymorphisms (SNPs). However, karyotype analysis of a large number of RCBs revealed that our CHO lineage is chromosomally stable indicating that the critical stages of a cell line production platform do not induce chromosomal changes. This contrasts with previous studies that have shown large chromosomal rearrangements in CHO cell lines [1, 2].

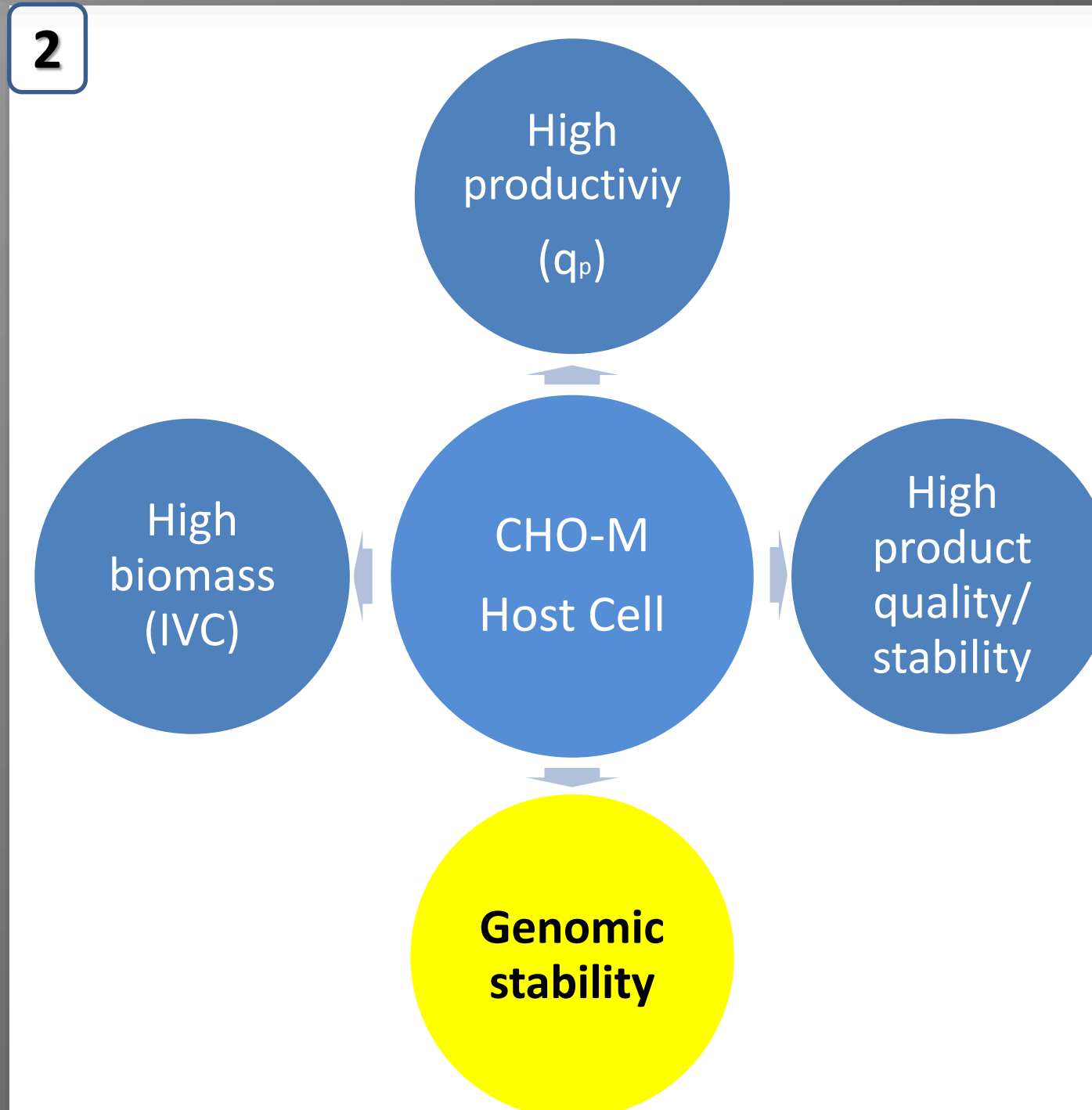
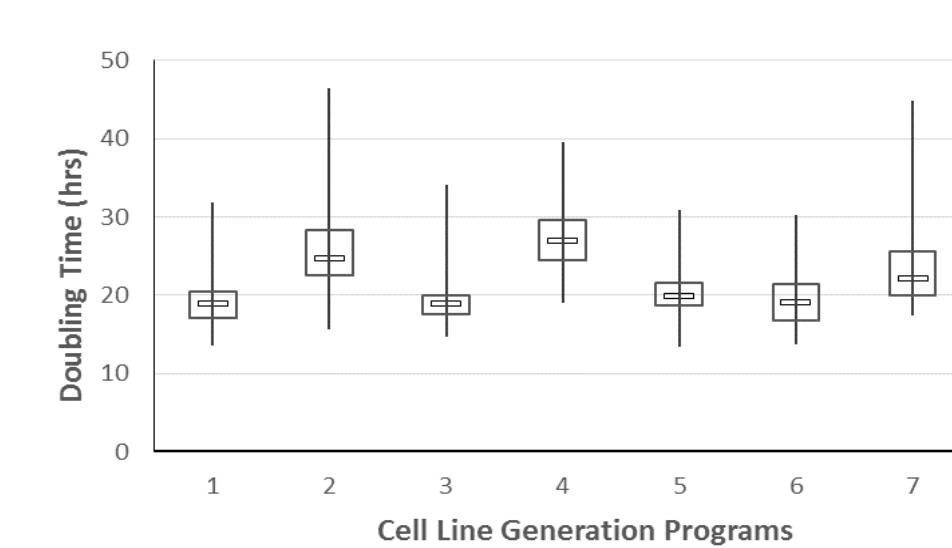
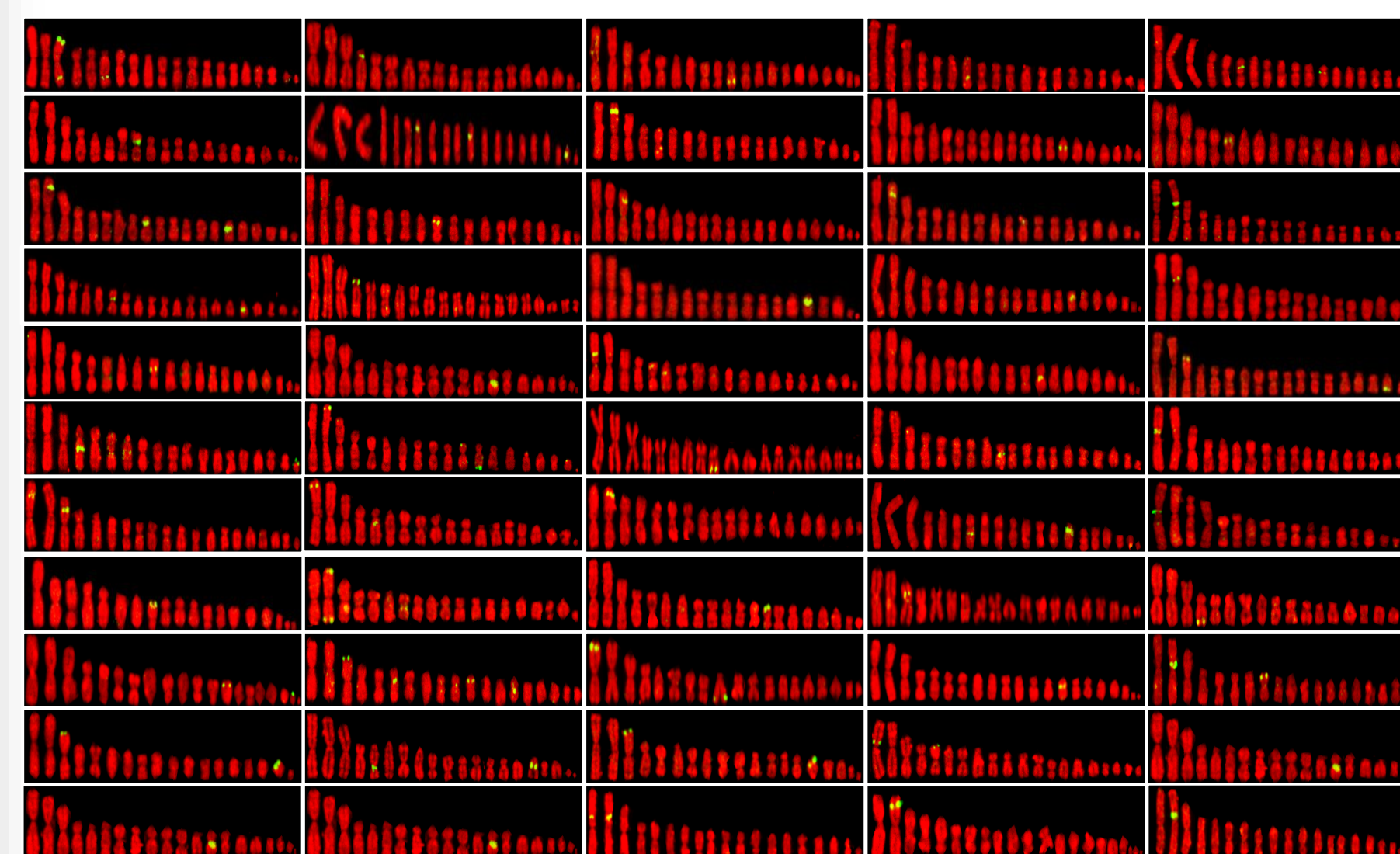


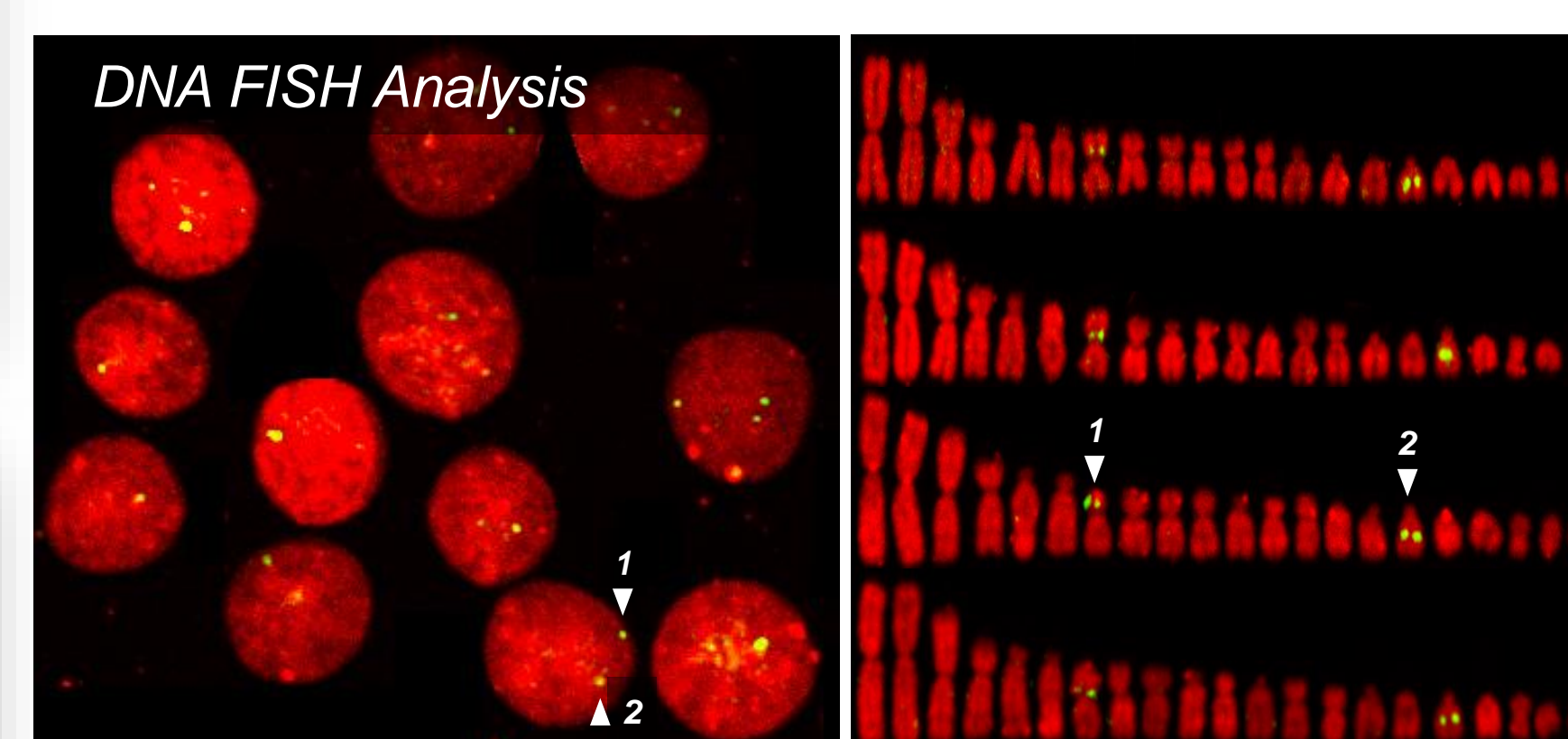
Diagram of CHO-M host cell characteristics: generation of high producing clones requires a host cell line that is genetically stable, exhibit rapid growth and is capable of producing a molecule both at high amounts and with a proper folding and correct glycosylation pattern.

## 5 Cytogenetic Analysis of over 100 RCBs Shows Karyotype Stability

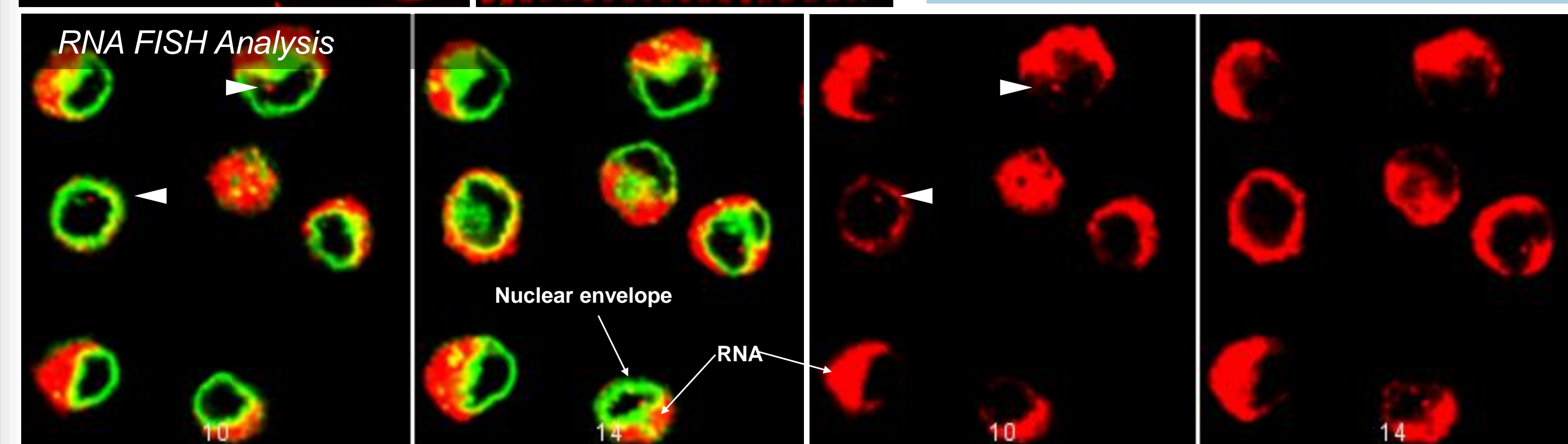


All Research Cell Banks (RCBs) display similar chromosome pattern indicating that the critical stages of a cell line development platform do not induce chromosomal alterations. Chromosome stability is also strengthened by comparable cell doubling time.

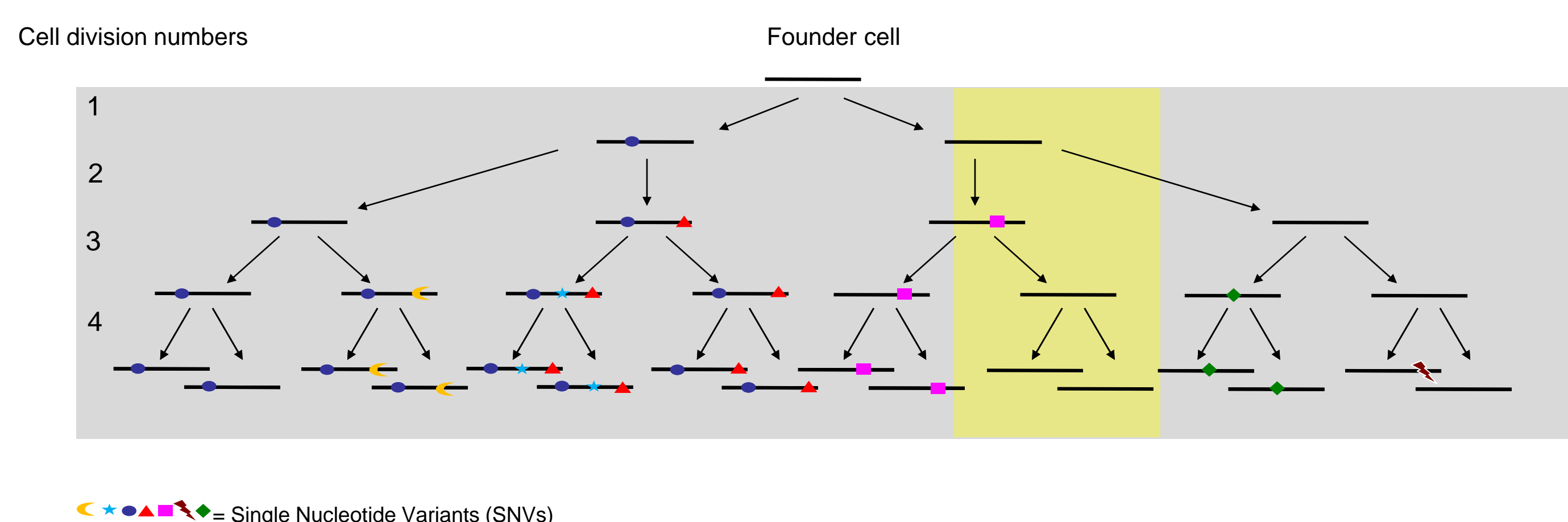
## 6 Case Study1: Clonal stability of a recombinant IgG4-producing cell line



The genetic stability of CHO-M cells is essential for maintaining a constant production. Each Selexis RCB used for recombinant protein production is clonally derived. (Cells overgo two successive rounds of subcloning procedure). All cells within a given clonal cell population are genetically (See DNA FISH), phenotypically (See RNA FISH) and morphologically identical.



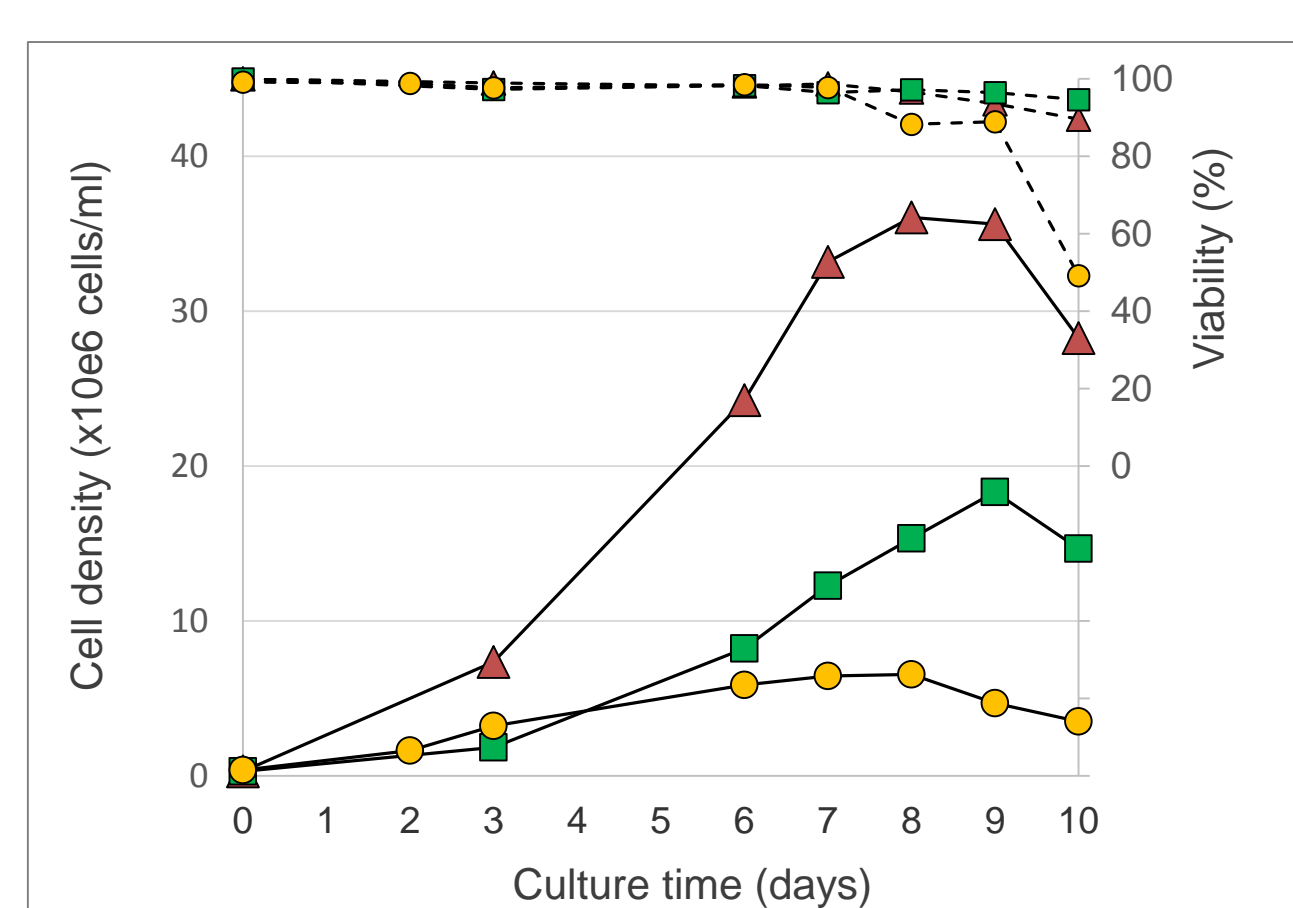
## 3 Genetic plasticity of Selexis CHO-M Host Cell Line



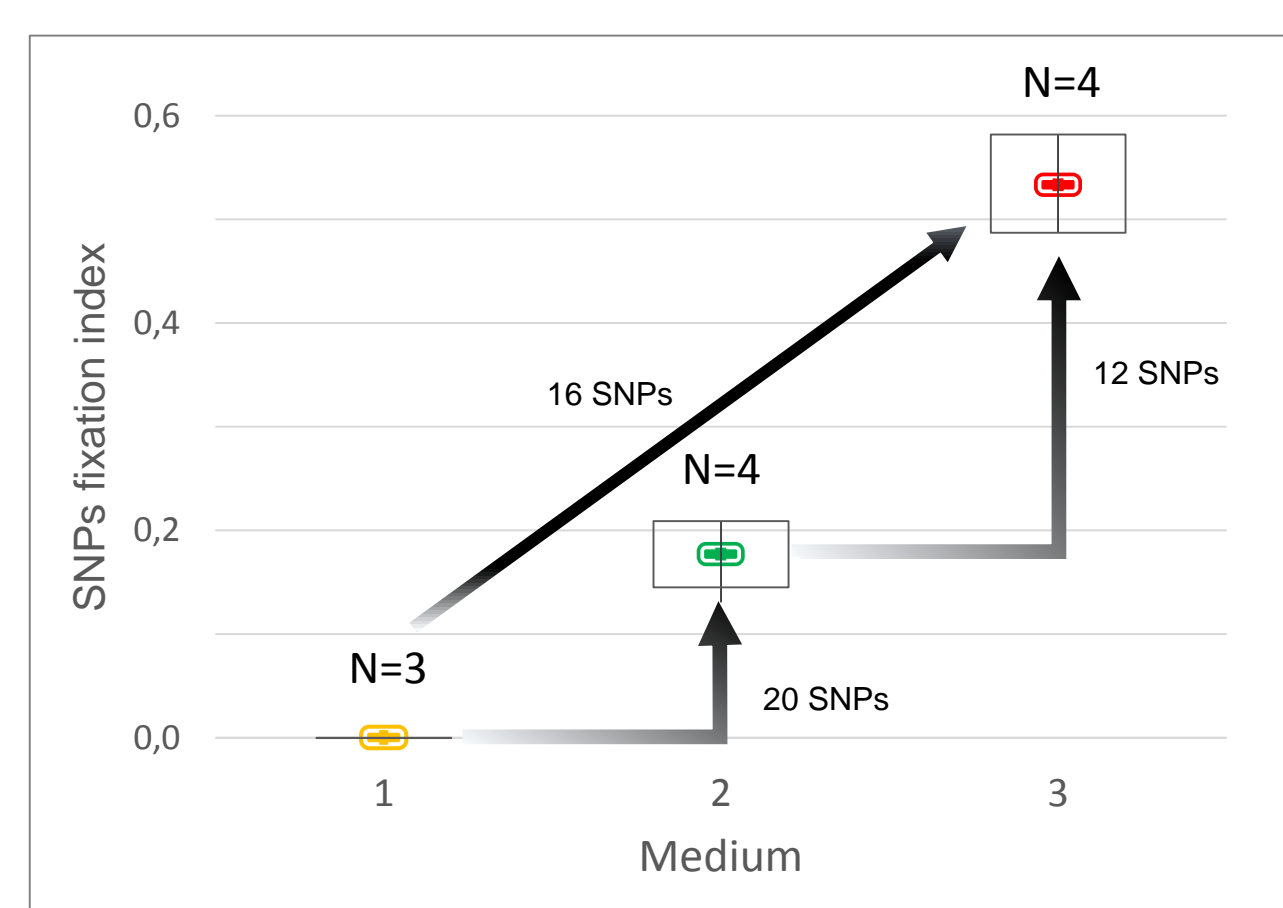
Single nucleotide variants (SNVs) arise constantly across the CHO genome. During cell division, SNVs can be either repaired (in yellow) or fixed as single variant polymorphisms (SNPs) (in gray) depending on the environmental factors. Thus, the presence of SNVs can confer a selective advantage for cell adaptation to a new environment.

## 4 Genetic stability of Selexis CHO-M Host Cell Line

### A. Cell fitness improvement through sequential media adaptation



### Genome-wide SNPs Analysis

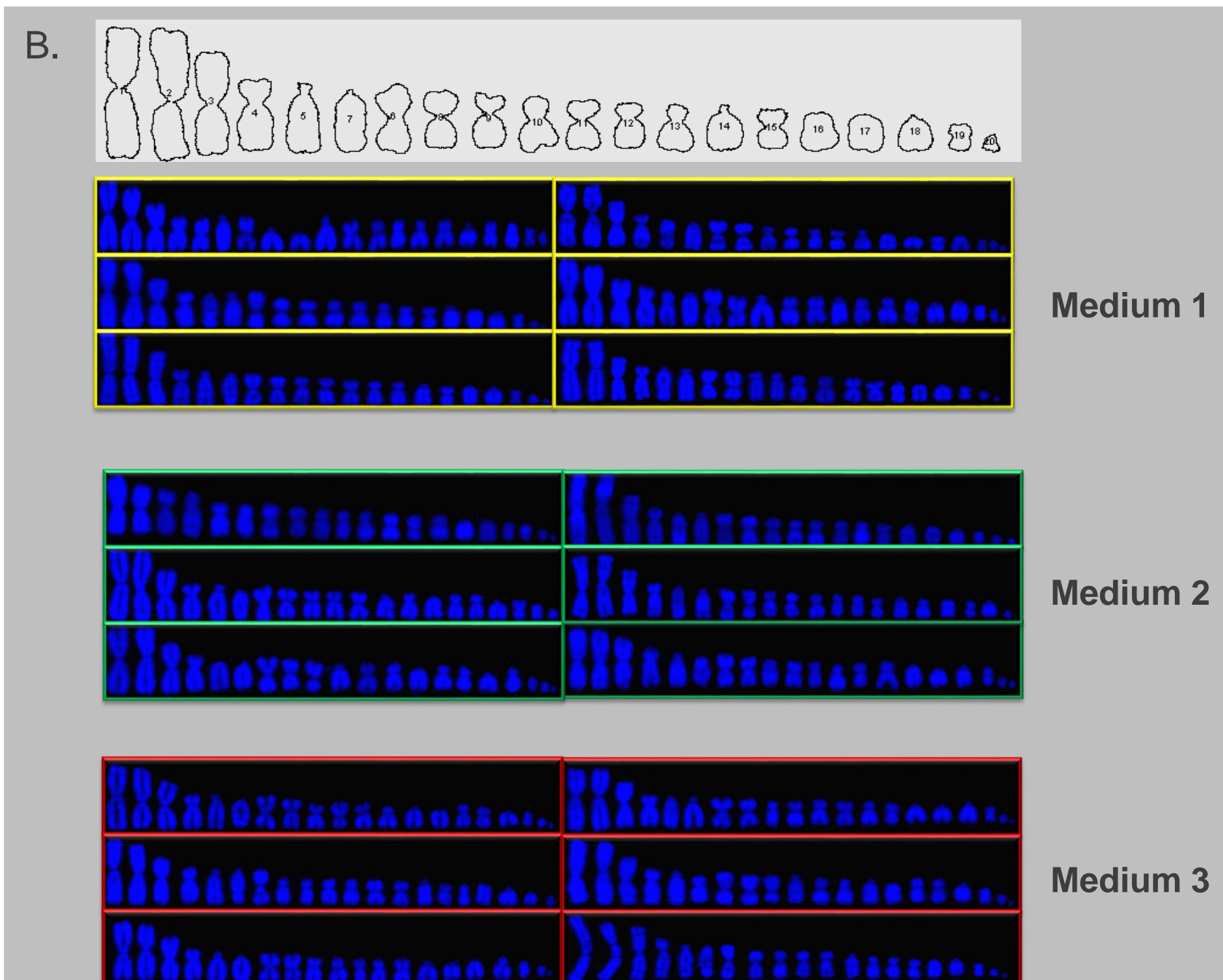


A. Efficient production of biotherapeutics requires an increase of cell number that is achieved by Host Cell culture media optimisation. Adaptation to new media is linked to an important SNVs fixation.

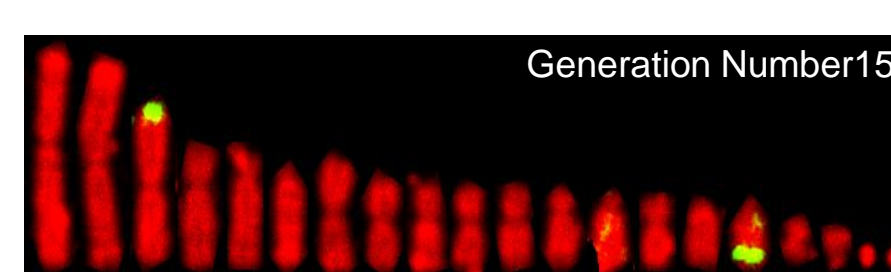
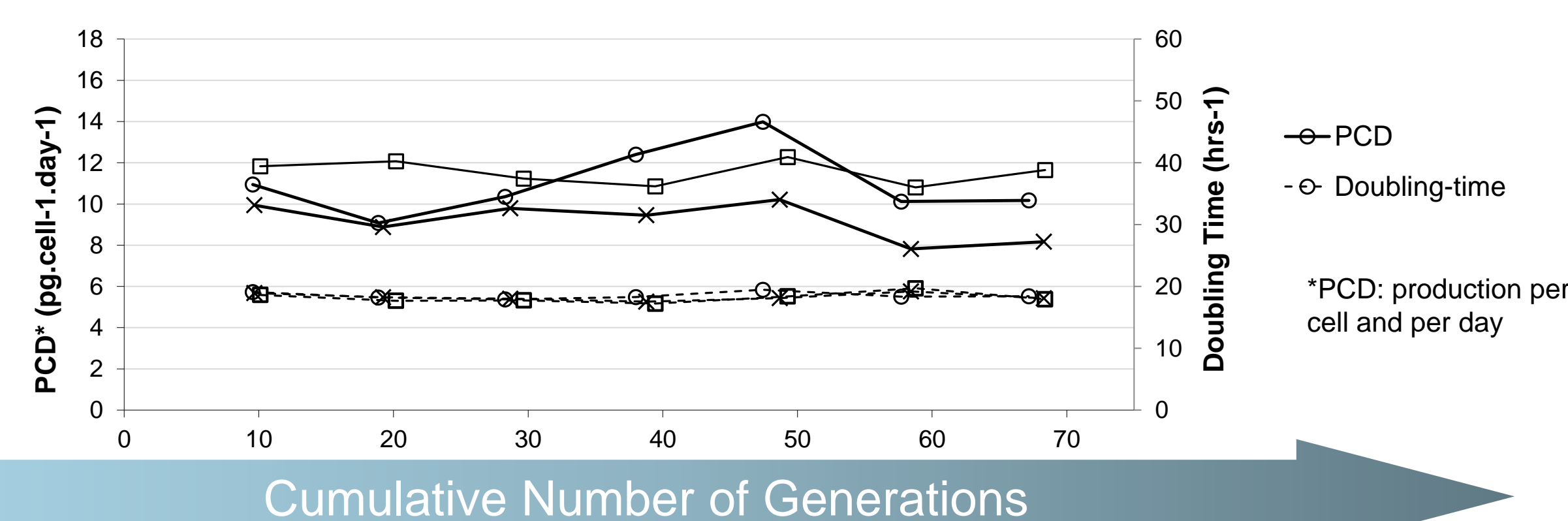
B. CHO-M cell cytogenetic analysis reveals:

- No variation in chromosome number
- Reproductive chromosome shape

Over 300 generations in various subcultivation media, CHO-M depicted high cell fitness by maintaining karyotype similarity.

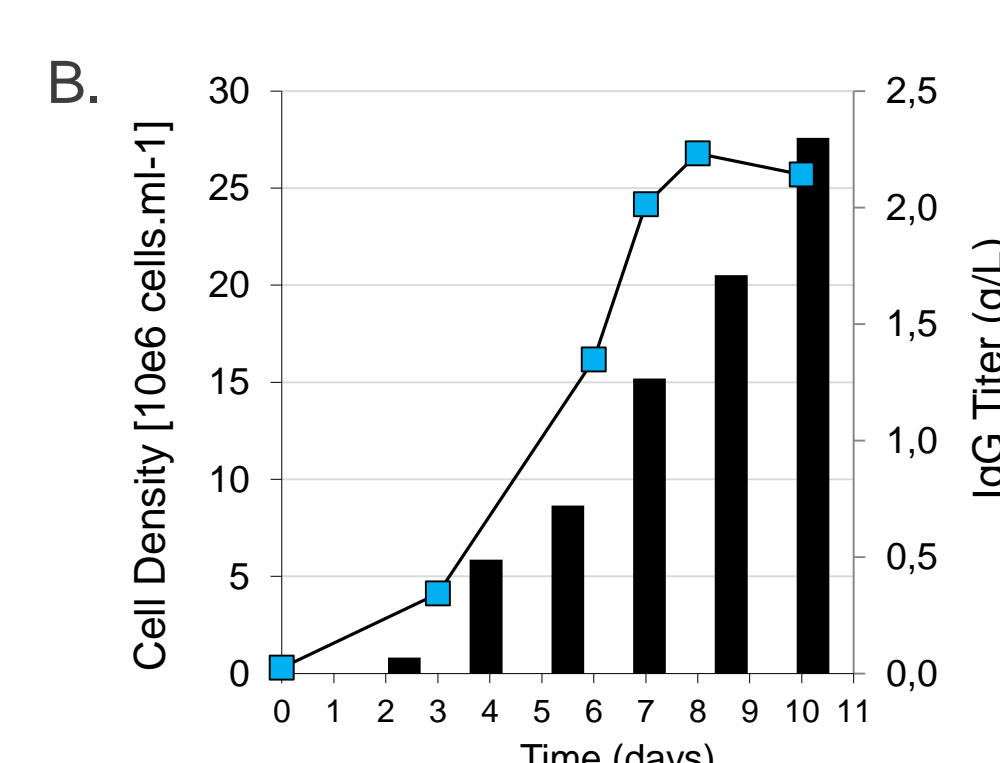
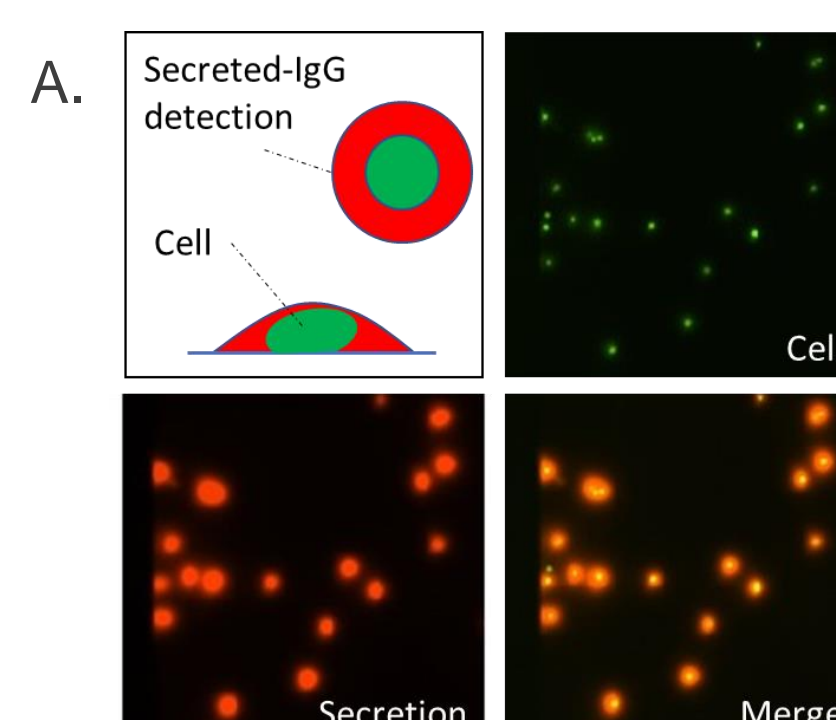


## 7 Case Study 2: long term cultivation stability of a bispecific antibody producing cell line



Constant production levels as well as doubling times are observed over more than 60 generations in absence of selective pressure.

## 8 Highly qualified Host Cell Line allows generation of high producing clones



Both Cell Secretion Assay (A) and Enzyme-Linked Immunosorbent Assay (ELISA) titration of Fed-Batch production (B) reveal a homogenous population of highly secreting clones. This data matches perfectly with CHO-M genetic stability

## Conclusion

We found that phenotypic changes in growth behavior and metabolism that are typically caused by cellular stress such as adaptation to a different media are associated with a rise in single nucleotide polymorphisms (SNPs) detected using a single SNP regression approach. In light of the FDA and EMA recent concerns regarding establishment of clonality for IND and BLA submissions, these SNPs could be used to assess monoclonality. SUREscan™ can be used to improving traceability of RCBs, MCBs and WCBs. Singularly, karyotype analysis of over 100 RCBs revealed that our CHO lineage is chromosomally stable. Thus, none critical stage of the SUREtechnology Platform™ (transfection, selection and expansion) induce chromosomal changes.

### Acknowledgements

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### References

- 1-Derouazi, M., et al., *Genetic characterization of CHO production host DG44 and derivative recombinant cell lines*. Biochem Biophys Res Commun, 2006. **340**(4): p. 1069-77.
- 2-Baik, J.Y. and K.H. Lee, *A framework to quantify karyotype variation associated with CHO production instability*. Biotechnol Bioeng, 2016.