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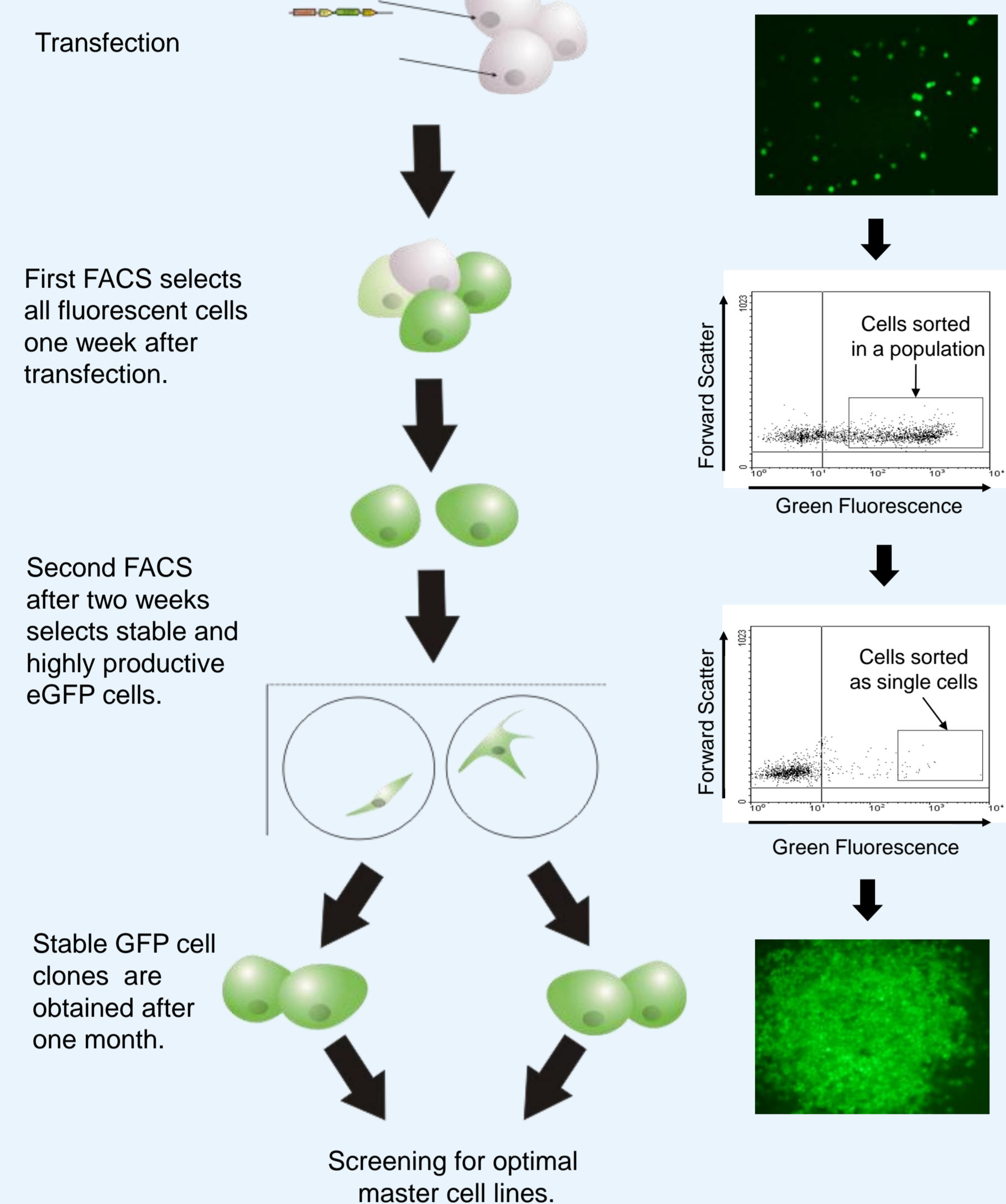
## Introduction

High-level production of high-quality recombinant proteins is a prerequisite for crystallographic studies. In particular, when expressing challenging mammalian proteins, the widely used bacterial, yeast and insect cell systems can fail. In this case, mammalian cells are required, since they ensure the correct post-translational modifications and folding of the desired protein. Due to the time and effort of establishing stable mammalian production cell lines, new strategies involving fluorescence activated cell sorting (FACS) (reviewed in Browne *et al.*,

2007) and site-specific recombination systems are of great interest (reviewed in Oumard *et al.*, 2006). Therefore, the aim of this study was the application of FACS and Flp recombination systems to create CHO Lec3.2.8.1 cell lines producing proteins that are suitable for crystallisation. We have established production cell lines for human hepatocyte growth factor with mutations preventing the naturally occurring cleavage into two chains (single chain HGF, scHGF). scHGF will allow studying the structural consequences of HGF cleavage into two chains.

## Establishing stable cell lines by FACS

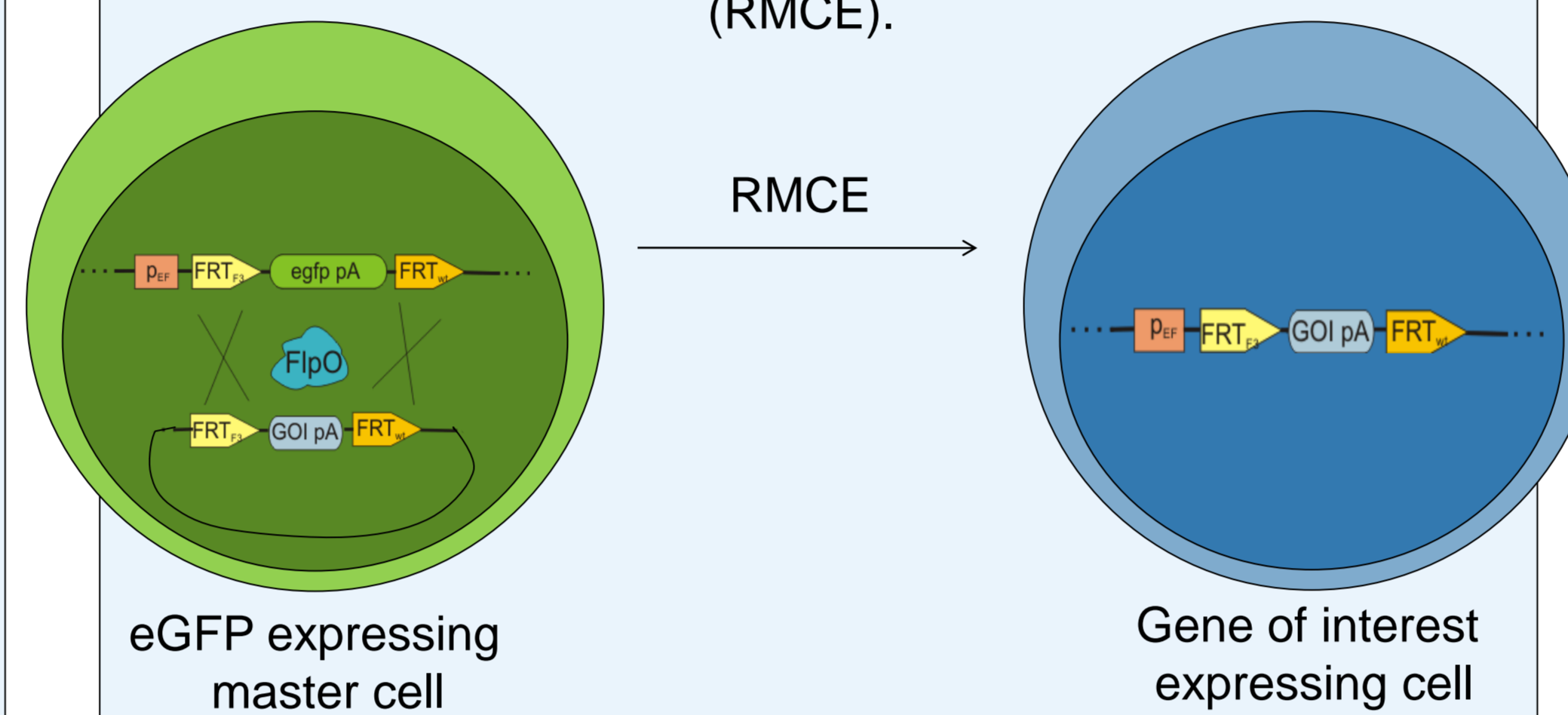
Two rounds of cell sorting established stable cell lines that uniformly expressed eGFP as a reporter protein and hence did not require antibiotic selection.



Depending on the cell-clone intracellular eGFP levels between 10 and 30 mg/l were available in one month. The combination of this cell sorting technique and a site-specific recombination system is a powerful tool to express predictable levels of any desired protein.

## Recombinase-mediated cassette exchange

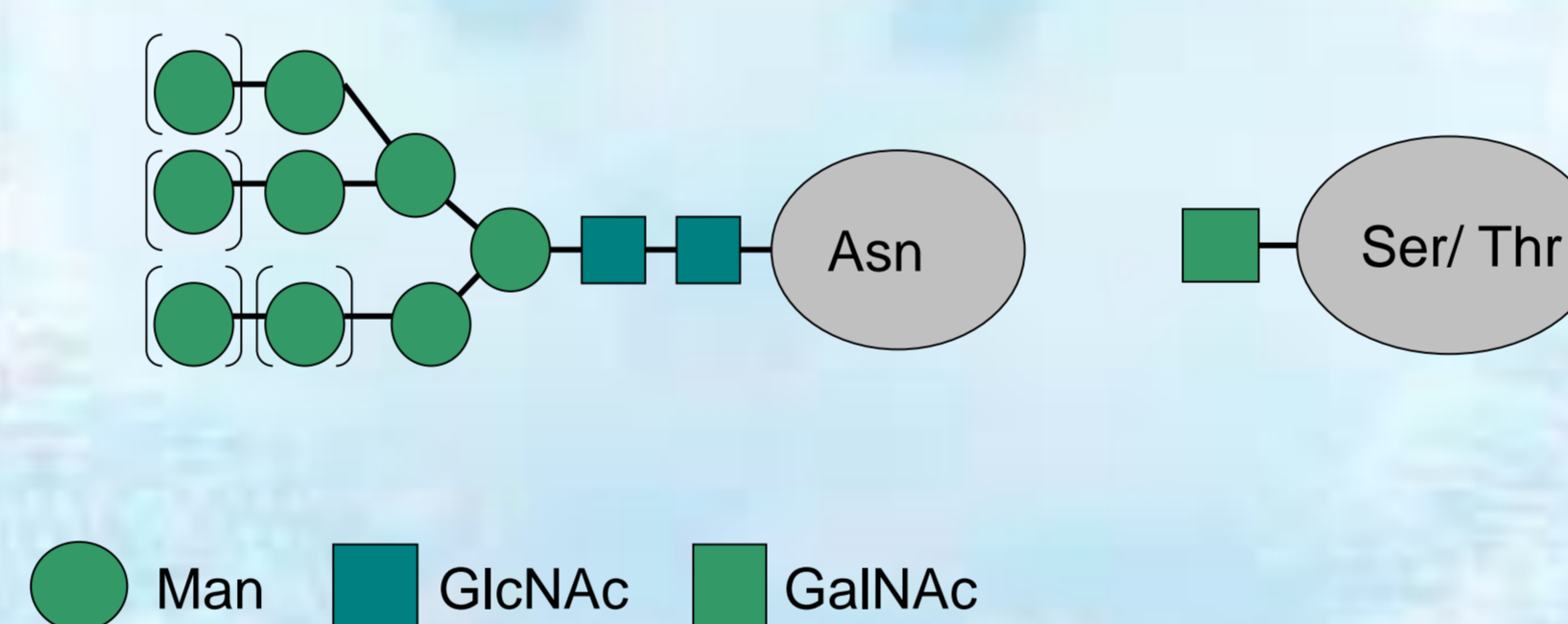
A single locus in the host cell genome is tagged with an eGFP marker for selection of robust protein expressing cells. Resulting master cell clones are co-transfected with an exchange vector bearing the gene of interest and a Flp expression vector. The *egfp* reporter is exchanged against the gene of interest by recombinase-mediated cassette exchange (RMCE).



## CHO Lec3.2.8.1 cell line

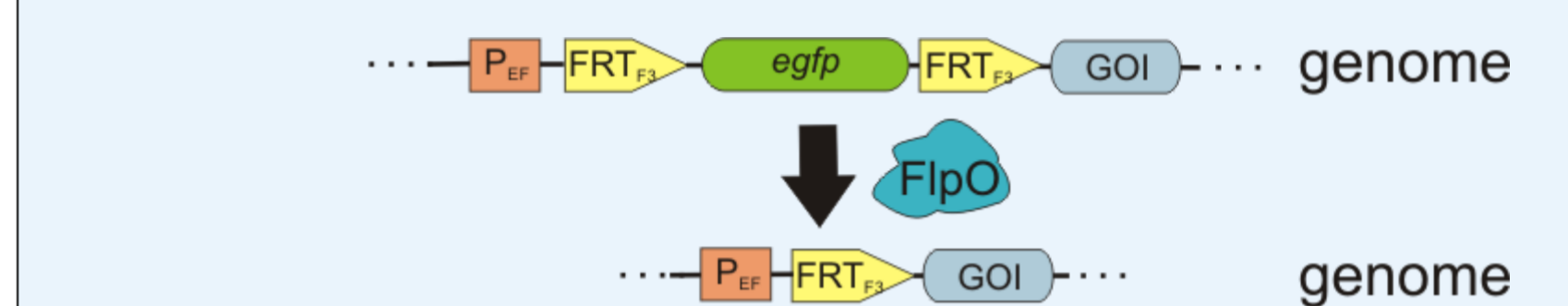
The glycosylation deficient CHO Lec3.2.8.1 cell line is a good producer for structural biology. The higher homogeneity of the glycoproteins has a beneficial effect on the formation of well diffracting crystals.

Types of protein glycosylation:

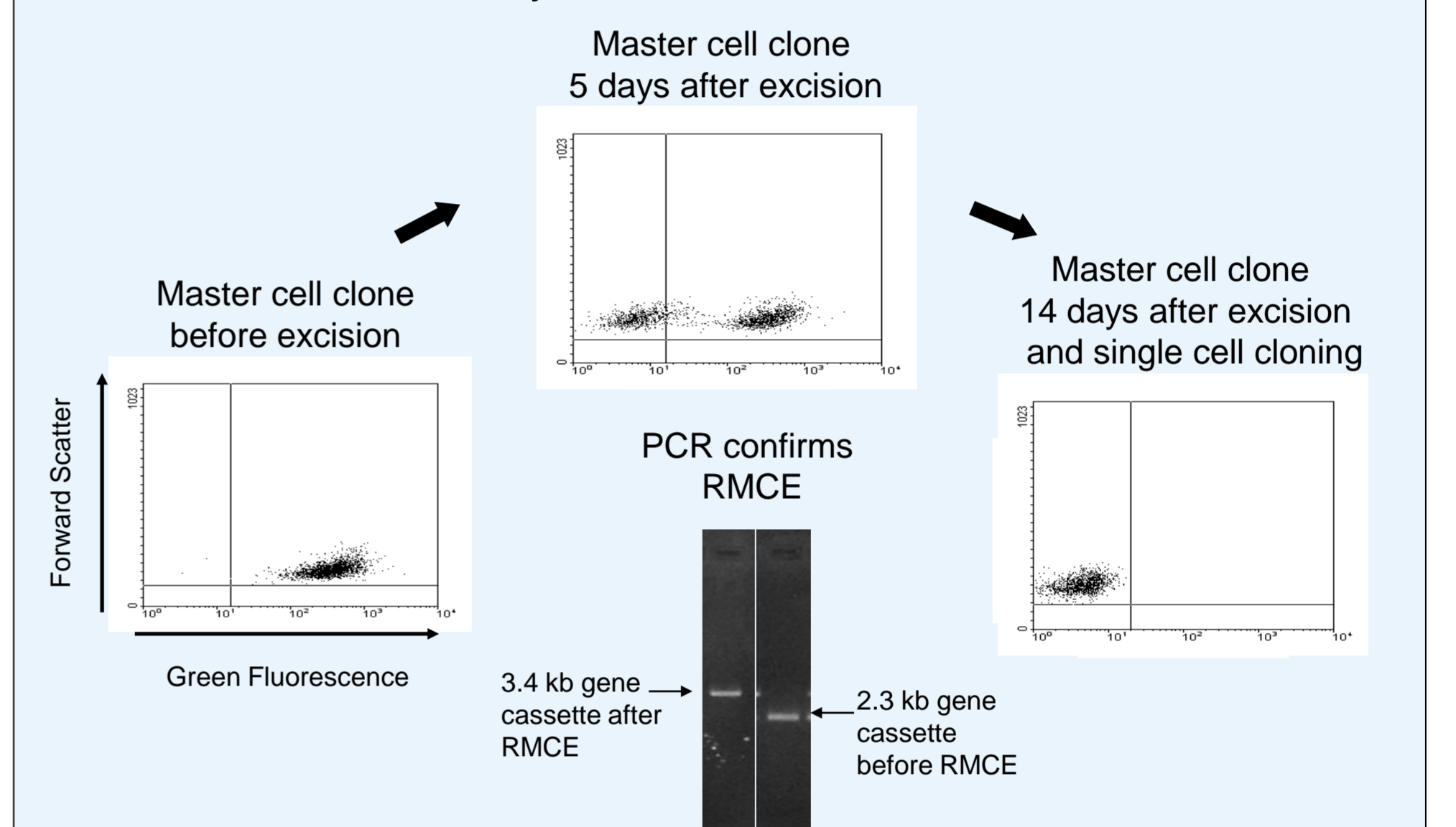


## Reporter gene excision

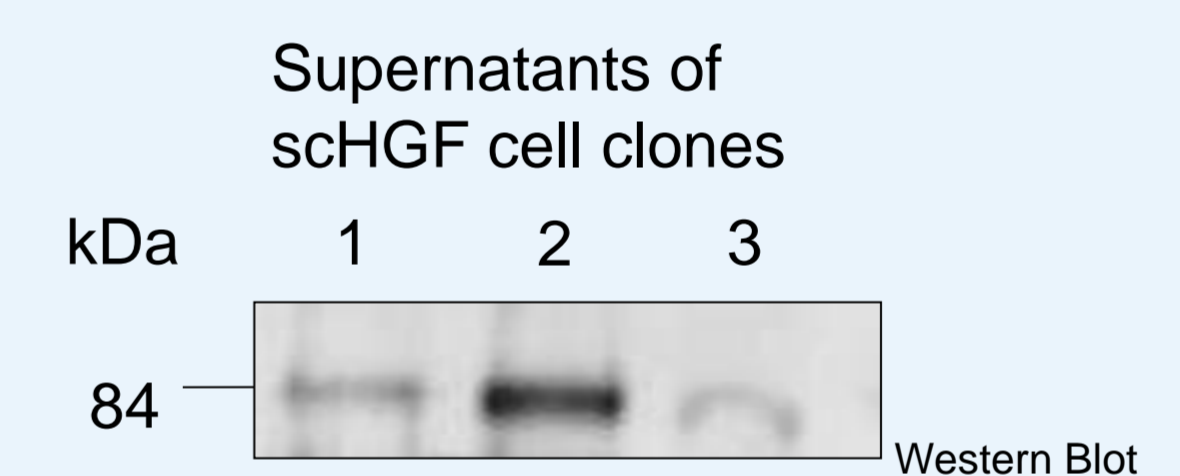
The 'flirting' (Flp/FRT) principle is applied to mediate a switch of transgene expression in master cell lines:



Master cell clones were transfected with a Flp expression vector. After excision of the *egfp* reporter gene the non fluorescent cells were cloned without any antibiotic selection.

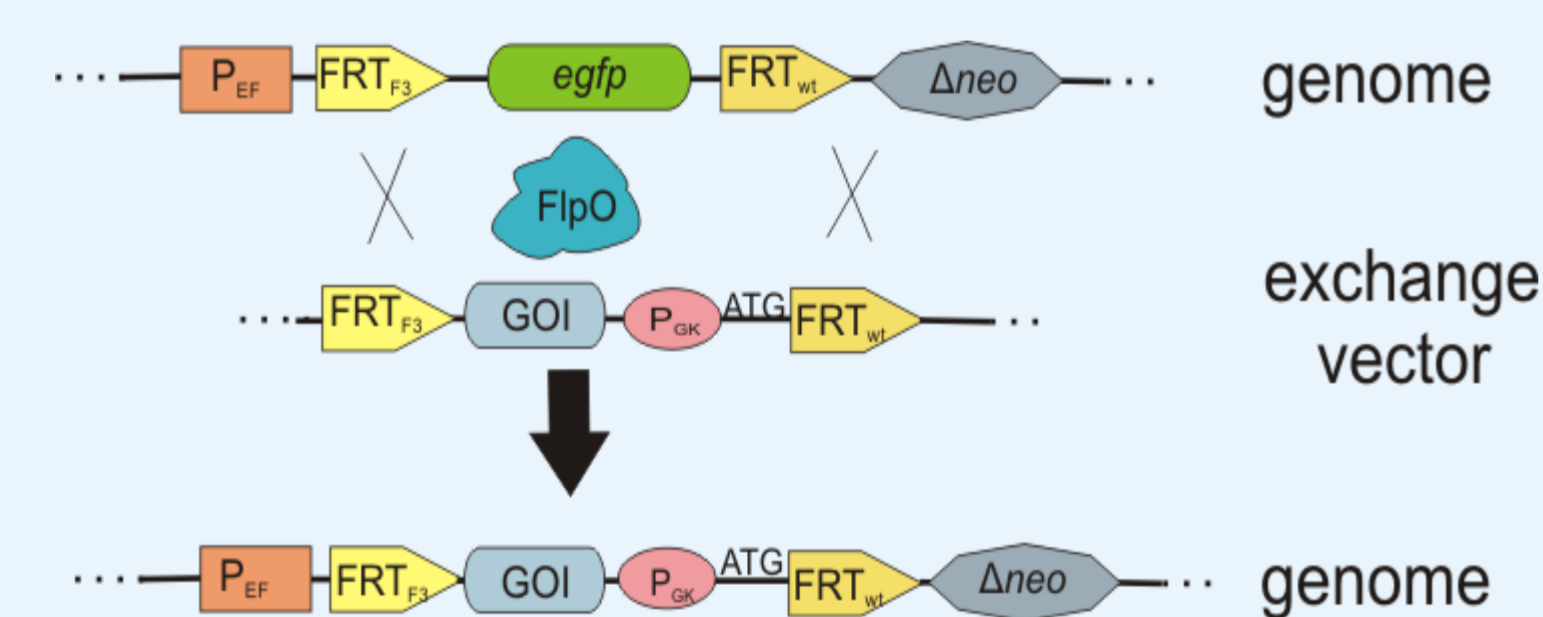


Expression test of scHGF by three cell clones after excision of the *egfp* gene.



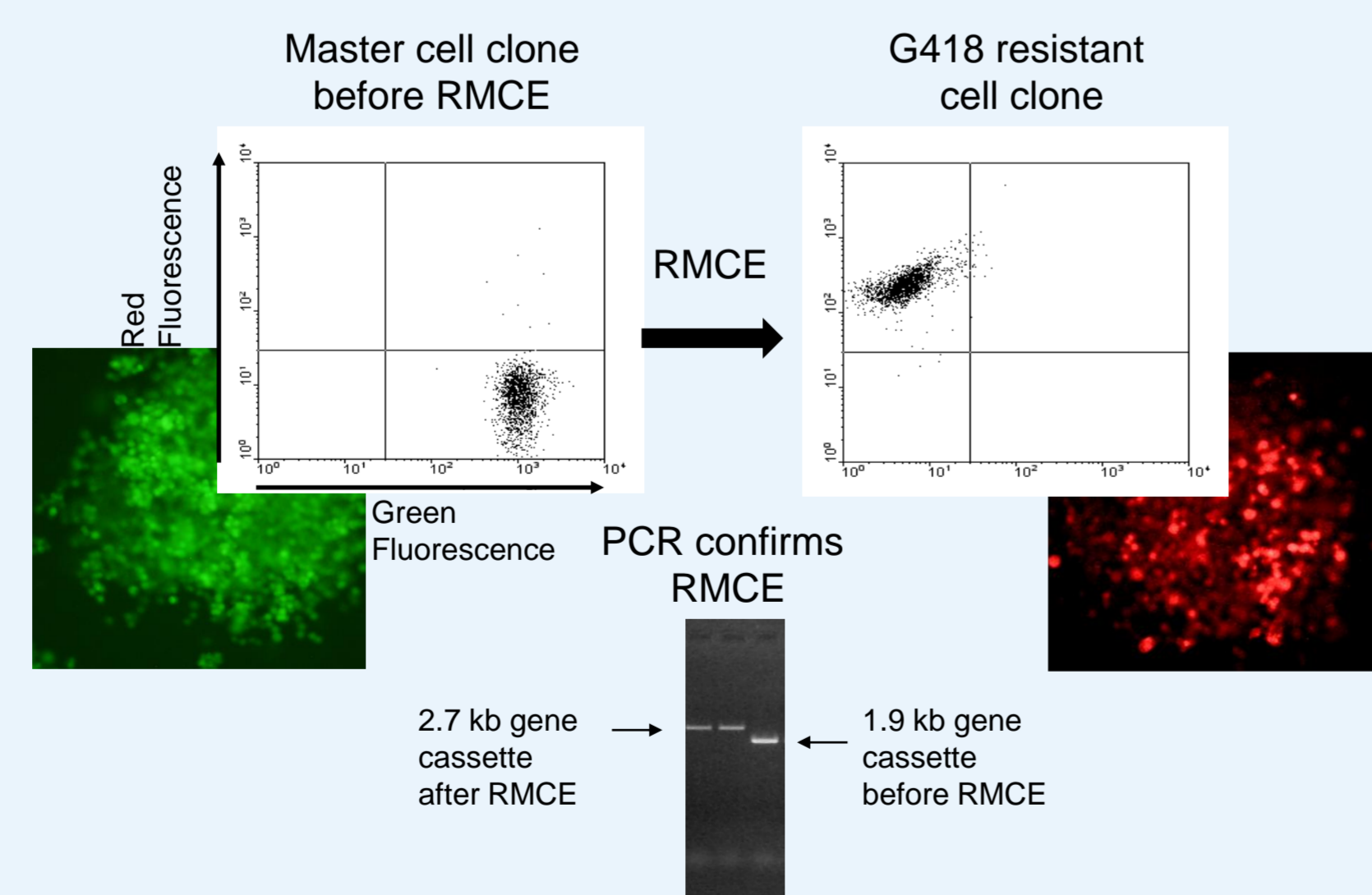
A small-scale expression test determined the highest producing scHGF cell clone. In a 25 litre perfusion fermentation process about 2-3 mg/litre protein were produced.

## RMCE with selection trap



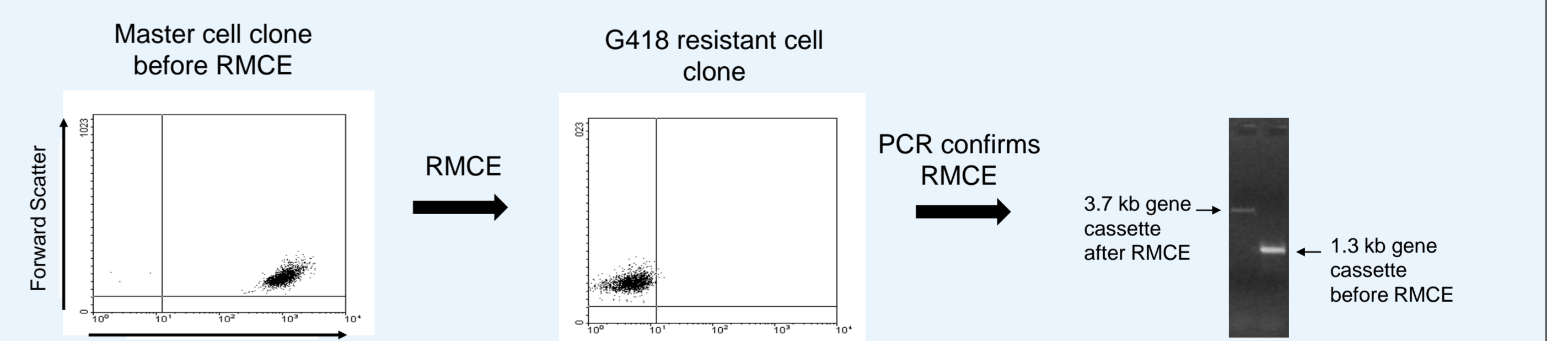
A selection trap allows to select for rare RMCE events: a neomycin resistance gene lacking the start codon and a promoter is localised downstream of the FRT cassette. Upon exchange the missing genetic elements are added and the selection marker is expressed (Schucht *et al.*, 2006).

- Master cell clones were transfected with a *rfp* exchange vector and a Flp expression vector.
- After G418 selection all resulting cell colonies were RFP<sup>+</sup>/eGFP<sup>+</sup>.
- The exchange of *egfp* against *rfp* was confirmed by flow cytometry on the protein level and PCR on the genomic level. The cell clones were stable for more than six weeks.



## Expression of scHGF

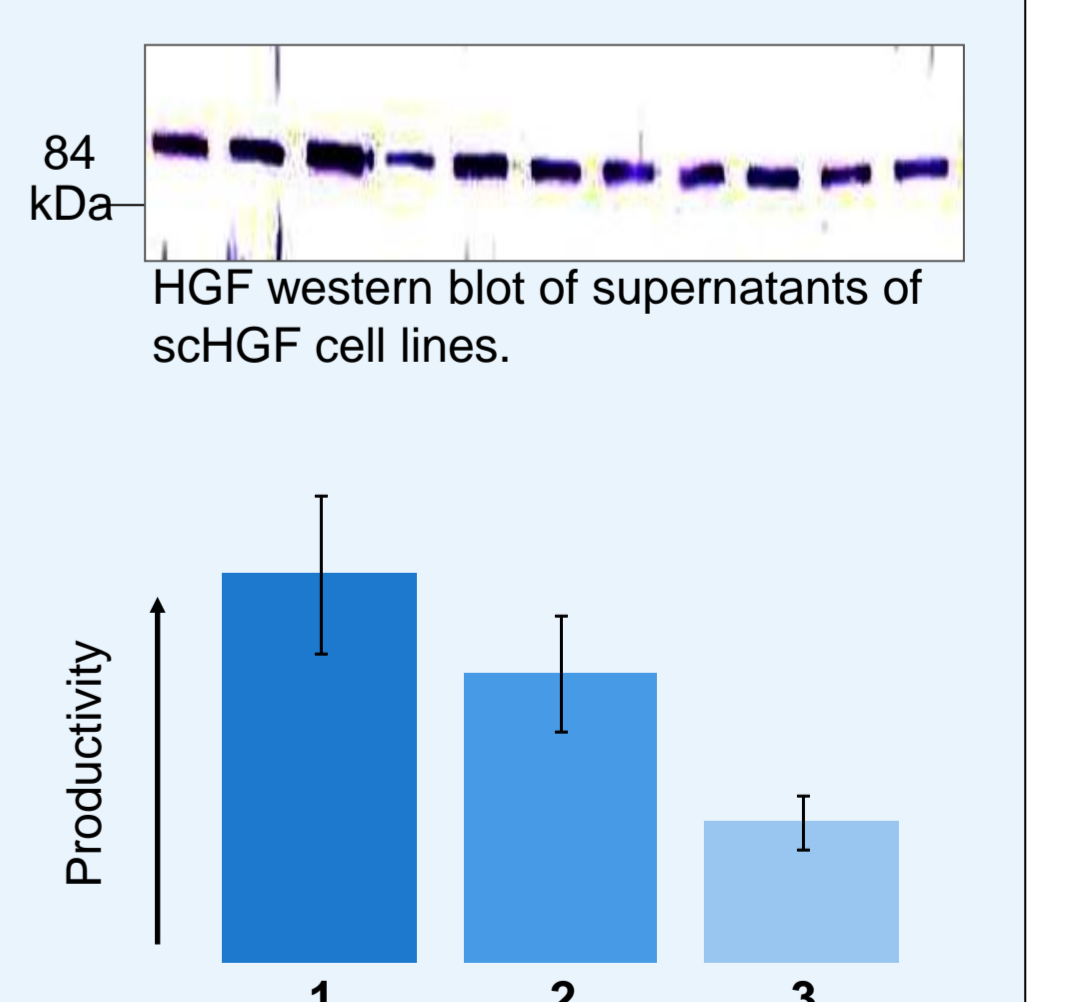
- A stable and good production master cell clone from the RMCE system was chosen to generate cell clones for scHGF in about one month.



- The master cell line was transfected with a scHGF exchange vector and a Flp expression vector.
- Western Blot confirmed similar scHGF expression levels by eleven clonal cell lines.

### Comparison of specific scHGF productivity by ELISA

- RMCE system, 1.3 pg cell<sup>-1</sup> day<sup>-1</sup>
- Flirting/excision system, 1 pg cell<sup>-1</sup> day<sup>-1</sup>
- Conventionally generated HGF cell line, 0.5 pg cell<sup>-1</sup> day<sup>-1</sup>



## Conclusion

Stable CHO Lec3.2.8.1 cell lines were generated for two recombination systems. The combination of FACS and site-specific recombination reduced the time for cloning of production cell lines considerably and lead to completely stable and well-producing cell lines. RMCE with selection trap is superior to reporter excision, since RMCE master cell lines are universal and

only have to be established once. Further evaluation of the recombination systems as well as establishment of production cell lines expressing interesting proteins for structural biology are in progress.

## Acknowledgement

The vectors ptagSVHTGdneoC and pPGKMFGeGFP1 were provided by Dagmar Wirth (HZI, Braunschweig) and were used for cloning the tagging vector pEF-FS-eGFP-dneo and the targeting vector pFS-RFP-PGK.

## References

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