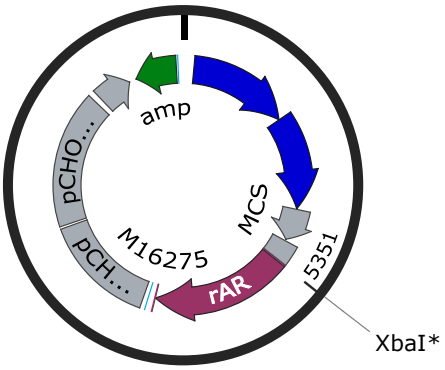
University of Helsinki: Doctoral Programme In Biomedicine

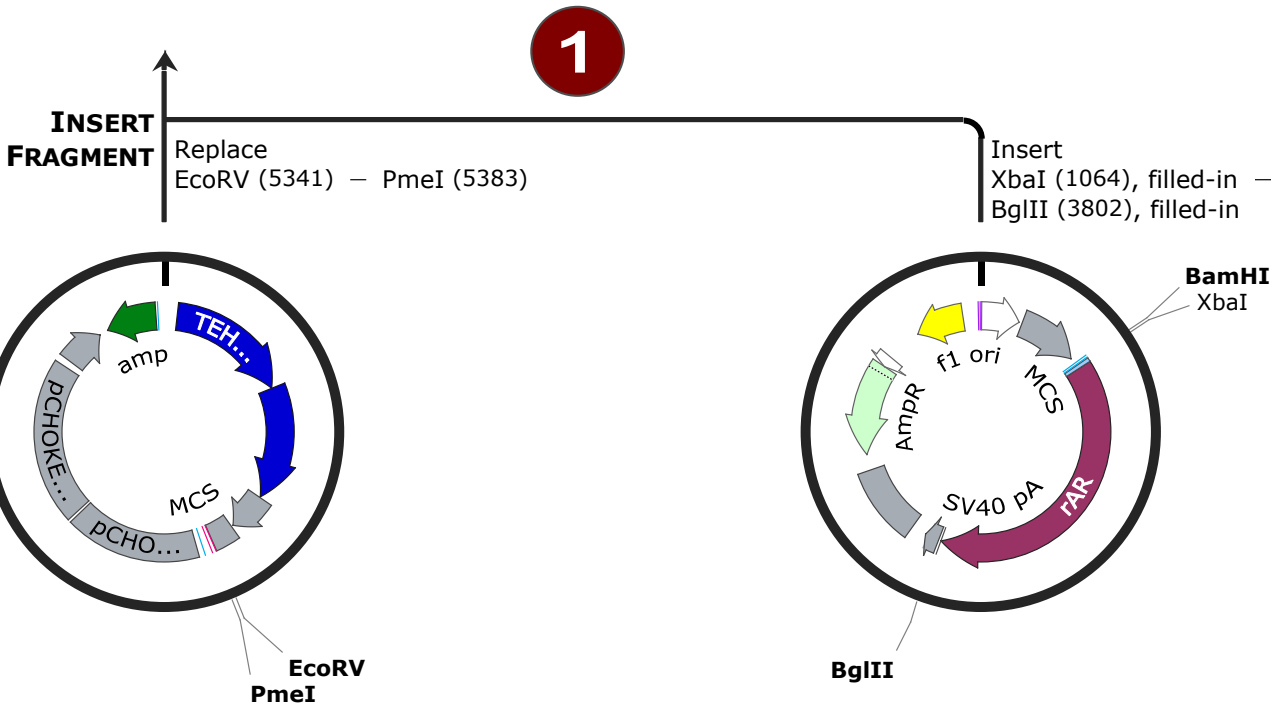
Practical Molecular Biology and Genetic Engineering Course (Practical Part)

Project 2: Expression of rat androgen receptor in mammalian cells (page 1 of 1)



pCHOKE-A-rAR
14 987 bp

In this project, we originally wanted to generate a vector for transient and stable expression of human androgen receptor (hAR) in mammalian cells.
However, GE Healthcare/Dharmacon did not manage to timely deliver the hAR cDNA and therefore we needed to quickly come up with a backup plan. We managed to get rat androgen receptor cDNA from Päivi Pihlajamaa, which we used as a surrogate.
We are using the pCHOKE-A expression vector, which has a double selection marker (zeocin and dhfr). The expression of the zeocin resistance marker and rAR are both driven by the same promoter using a TTG-initated zeocin resistance open reading frame and an ATG-initiated rAR open reading frame. This ensures that stable cells achieve significant zeocin-resistance only by integration of many copies or gene amplification, which results in high expression of rAR. This vector was originally developed for stable CHO expression, but it works very well in transient expression in a variety of cell lines like 293T or PC3.



pCHOKE-A
12 287 bp

pSG5-rAR
6823 bp

The cloning is single-step and straightforward, but both vector and insert are large and the cloning is a non-directed blunt-end cloning. Therefore, we did expect a low cloning efficiency in addition to the fact, that on average 50% of the inserts would have the wrong orientation. Because our schedule does not allow us to fail, we decided to pick 20 clones from the plate. That appeared too much as most clones did have inserts and about 50% of those did have the correct orientation. When we did the isolation of the insert, the size difference between the insert and the backbone was not as large as we would have liked (2738bp versus 3931bp). Consequently, the background that we could see resulted (based on the "insert only" control) mostly from vector backbone that had copurified together with the insert due to the similarity in size.