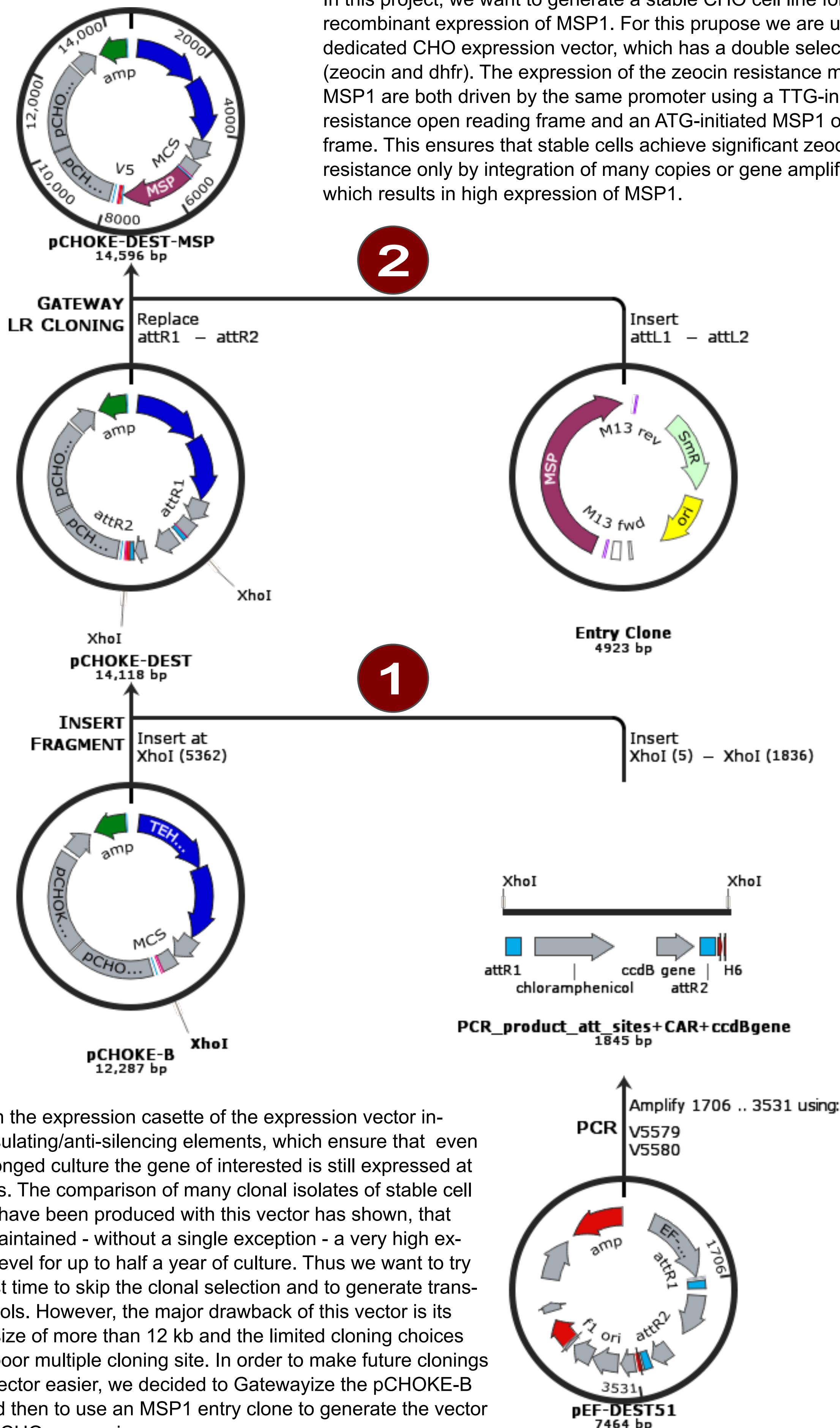
University of Helsinki: Doctoral Programme In Biomedicine

Practical Molecular Biology and Genetic Engineering Course (Practical Part)

Project 1: Expression of MSP1 in CHO cells (page 1 of 2)

In this project, we want to generate a stable CHO cell line for the recombinant expression of MSP1. For this prupose we are using a dedicated CHO expression vector, which has a double selection marker (zeocin and dhfr). The expression of the zeocin resistance marker and MSP1 are both driven by the same promoter using a TTG-initated zeocin resistance open reading frame and an ATG-initiated MSP1 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 MSP1.



In addition the expression casette of the expression vector includes insulating/anti-silencing elements, which ensure that even after prolonged culture the gene of interested is still expressed at high levels. The comparison of many clonal isolates of stable cell lines that have been produced with this vector has shown, that they all maintained - without a single exception - a very high expression level for up to half a year of culture. Thus we want to try for the first time to skip the clonal selection and to generate transfectant pools. However, the major drawback of this vector is its massive size of more than 12 kb and the limited cloning choices due to a poor multiple cloning site. In order to make future clonings into this vector easier, we decided to Gatewayize the pCHOKE-B vector and then to use an MSP1 entry clone to generate the vector for stable CHO expression.

Cloning steps:

1. We PCR-amplify the gateway casette (which contains the chloramphenicol resistance gene, the ccdb ("kill") gene and the att homologies for the recombination)
2. The PCR product is cleaved by XhoI and inserted into the XhoI-opened pCHOKE-B vector.
3. Good clones are selected on ampicillin/chloramphenicol plates
4. Because the insertion is not directed, we need to identify a clone with the correct orientation by restriction analysis
5. A Gateway LR reaction is done with the Gatewayized pCHOKE-B (called now pCHOKE-DEST) and pENTR223-MSP1