

### **Inserts amplification for knockout cassette construction.**

Objectives: Identification of optimal amplification conditions for upstream and downstream fragments of O-acetylhomoserine sulphydrylases encoding gene *MET15*, homoserine O-acetyltransferases encoding gene *MET2* and cystathionine- $\gamma$ -synthases encoding gene *STR2* from *C. albicans*.

Methods: Amplification of *MET15*, *MET2* and *STR2* upstream and downstream fragments of genes were performed using properly designed primers, genomic DNA from *C. albicans* SC5314 and Phusion polymerase. The amplified sequences were modified by introducing a cleavage site for restriction enzymes *SacI*, *SacII*, *ApaI*, *XhoI*, thanks to which cloning into a plasmid containing elements of the knockout cassette were possible. The prepared samples (2  $\mu$ L) were loaded with loading dye on the 1% agarose gel and electrophoresis was conducted for 1 h, 10 V  $\text{cm}^{-1}$ .

Results:

All products were amplified using mentioned primers. All amplified products will be digest using *SacI*, *SacII*, *ApaI*, *XhoI* restriction enzymes and will be used for preparing gene knockout cassettes.