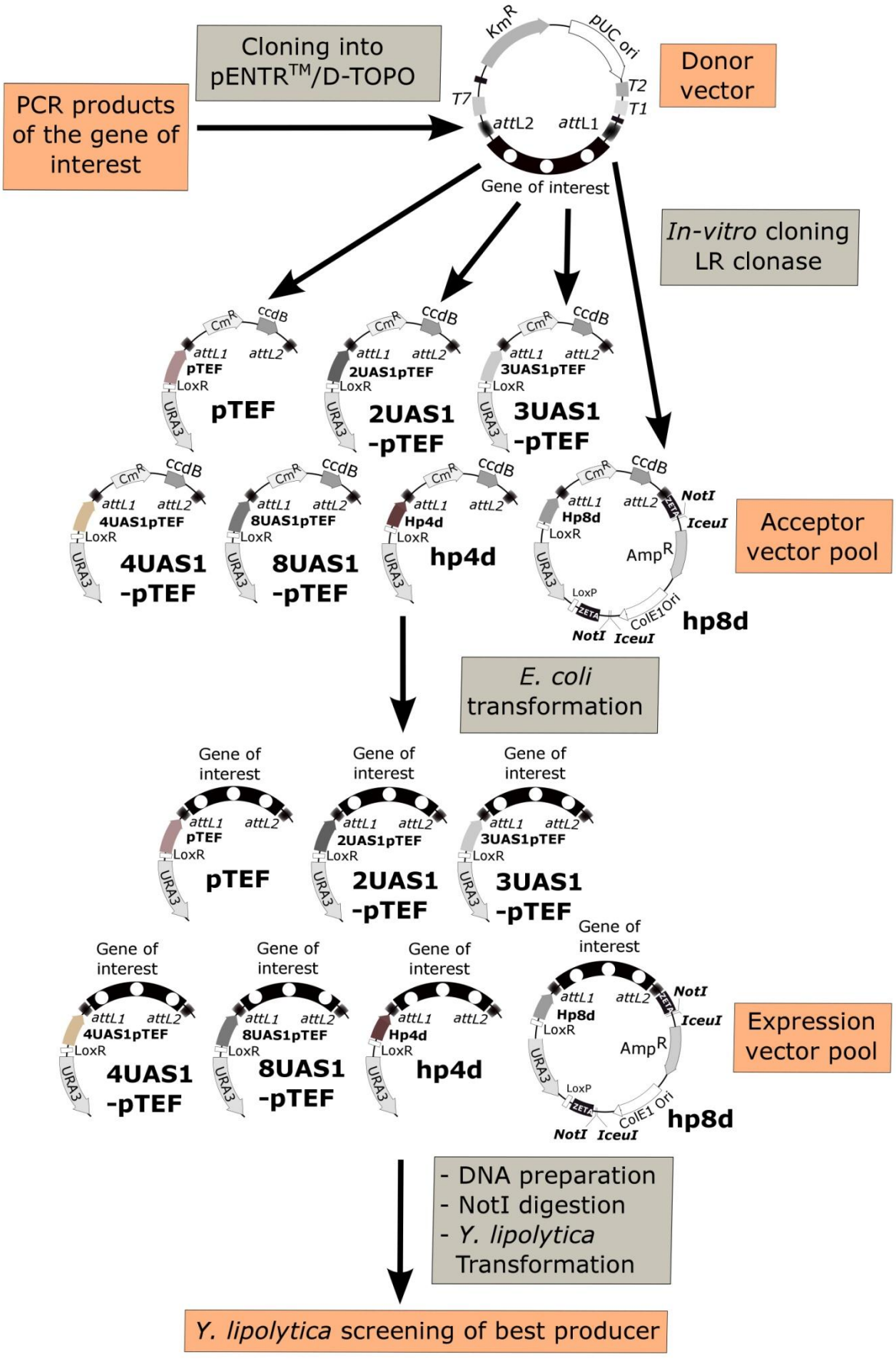
Supplementary Figure 3: Schematic representation of the construction of the vector pool. The gene of interest was cloned into the donor vector pENTR™/D-TOPO®, which was then transferred into the gateway pool of recipient vectors (i.e., consisting of JMP62-derived plasmids containing different vectors) using LR Clonase®. The LR reaction was used to transform *E. coli*. DNA was then extracted from the *E. coli* pool and digested by *Not*I. The resulting pool of expression cassettes was used to transform *Y. lipolytica*.


PCR products of the gene of interest
Cloning into pENTR™/D-TOPO
KmR
pUC ori
T2
T1
T7
attL2
attL1
Gene of interest
Donor vector
In-vitro cloning LR clonase
CmR
ccdB
attL1
attL2
pTEF
LoxR
URA3
pTEF
2UAS1pTEF
2UAS1 -pTEF
3UAS1pTEF
3UAS1 -pTEF
4UAS1pTEF
4UAS1 -pTEF
8UAS1pTEF
8UAS1 -pTEF
Hp4d
hp4d
Hp8d
NotI
IceuI
AmpR
LoxP
ZETA
ColE1Ori
hp8d
Acceptor vector pool
E. coli transformation
Gene of interest
Expression vector pool
- DNA preparation
- NotI digestion
- Y. lipolytica Transformation
Y. lipolytica screening of best producer