Targeting CRISPR/Cas9 system to AMN1 gene

The pRCC-K plasmid was used as vehicle to deliver CRISPR/Cas9 machinery in yeast. To specify the Cas9 break to AMN1, a sgRNA was designed using gRNA Design from ATUM web site (https://www.atum.bio/) and inserted into gRNA scaffold region of pRCC-K using the NEBuilder HiFi DNA Assembly Cloning kit (NEB).

Donor (Wt)/Hom restriction analysis. The looking of donor site verified the presence of gRNA in pEC vector derived from pRCC-K.

The vector-based delivered of CRISPR/Cas9 pRCC-K (4 alleles) developed by Generoso et al. (2016) and elements of gRNA region.

Donor DNA for Homology-Directed Repair (HDR) to AMN1 locus

To ensure the repairing of the Cas9 DNA double strand break by homologous recombination, a double strand DNA (donor) was co-transformed with the CRISPR/Cas9 plasmid in yeast cells. The donor DNA was composed of the resistance gene for nourseothricin (NatMX6) and the eGFP gene amplified with chimeric primers.

Cloning of sgRNA in pRCC-lead the new pCE plasmid which is able to target a DNA cleavage on AMN1 by the Cas9 endonuclease

CRISPR engineering of Saccharomyces cerevisiae Lalvin EC1118® strain

CRISPR/Cas9 construction elements

CRISPR engineering targeted to AMN1 by co-transformation pEC vector and donor DNA in the diploid EC1118® strain.

WT

AMN1::GFP

The CRISPR/Cas9 system successfully GFP-tagged the commercial EC1118® strain by insertion of a GFP gene into AMN1 (C3). Nevertheless off-target effect was detected (C1) suggesting a non-optimal sgRNA design to target AMN1.

Oenological characterization of CRISPRRed yeast

No modification of oenological capability was detected between CRISPRRed strain and parental strain EC1118. CRISPRRed yeast C3 presents a stable GFP signal during fermentation.

Conclusions

In this successful experiment, we used the single plasmid pRCC-K, expressing Cas9 and guide-RNA (Generoso et al., 2016) to insert GFP gene into the Lalvin EC1118® commercial yeast. CRISPRRed yeast didn’t present any modification of oenological characteristics (i.e. fermentation rate, total alcohol production) compare to parental strain, as expected from this chosen target gene. More optimization, however, is required to obtain better transformation frequencies particularly if this system has to be used with industrial Saccharomyces and non-Saccharomyces strains. Nevertheless, the EC1118 AMN1::GFP C3 strain could be used in future experiments to test yeast-yeast interactions in oenological conditions.