Using CRISPR/Cas platform for Genetic Modification of Commercial Saccharomyces cerevisiae strains

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Background: Traditional wine fermentation is a complex microbial process initiated by various yeast species classified as Saccharomyces and non-Saccharomyces species. To better understand the different interactions occurring within wine fermentations and track a specific yeast population, we wish to obtain GFP tagged yeast cells that stably express fluorescence signal without compromising the fermentative capability of the strain. To this end, the CRISPR/Cas9 system was investigated to genetically modify the commercial Saccharomyces cerevisiae diploid strain Lalvin EC1118® (LALLEMAND EUROPE).

The emergence of the CRISPR/Cas9 system has revolutionized genome-editing technologies. Recently a set of vectors for simultaneous expression of Cas9 with gRNA cassette enabling simple and fast deletions of genes have been developed (Generoso et al., 2016). To modify genetically commercial yeast, the CRISPR/Cas9 machinery, along with a homologous recombination sequence, were undertaken to insert eGFP gene into the EC1118® AMN1 gene. The AMN1 gene, which encodes a protein required for daughter cell separation, has already been deleted in haploid cells without modify fermentation properties (Marsit et al., 2015).

CRISPR/Cas9 construction elements

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).

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

Donor/Vector restriction analysis. The lacking of donor site verified the presence of gRNA in pEC vector derived from pRCC-K.

Donor site restriction analysis. The lacking of donor site verified the presence of gRNA in pEC vector derived from pRCC-K.

CRISPR engineering of Saccharomyces cerevisiae Lalvin EC1118® strain

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

Donor/Vector restriction analysis. The lacking of donor site verified the presence of gRNA in pEC vector derived from pRCC-K.

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CRISPR engineering of Lalvin EC1118® strain

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.

Homologous repair was identified by the absence of NatMX6, eGFP and the presence of eGFP expression in AMN1::GFP strain

Cloning of an AMN1::GFP strain was successfully achieved by expressing Cas9 and guide-RNA (Generoso et al., 2016) to insert GFP gene into the Lalvin EC1118® commercial yeast. CRISPRCas9 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.

REFERENCES

