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 ENOLOGY). 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 repair 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 pRECC-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 pRECC-K using the NEBuilder HiFi DNA Assembly Cloning kit (NEB).

The vector-based delivered of CRISPR/Cas9 pRECC-K (4 mutations) 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 pRECC lead the new pEC plasmid which is able to target a DNA cleavage on AMN1 by the Cas9 endonuclease**

**CRISPR engineering of Saccharomyces cerevisiae Lalvin EC1118® strain**

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 pRECC-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.

**REFERENCES**