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# Deliverable D3.3

## Metabolic engineering of oleaginous yeasts for efficient acetate fermentation and production of medium and long chain TAGs

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# Abbreviations

| | |
|---|---|
| DGA | Diacylglyceride acyl-transferase |
| FAME | Fatty Acid Methyl Ester |
| FFA | Free Fatty Acid |
| GC | Gas Chromatography |
| OD | Optical Density |
| PEG | Polyethylene Glycol |
| TAG | Triacylglyceride |
| YNB | Yeast Nitrogen Base medium |
| YPD | Yeast Peptone Dextrose medium |

# Contents

# Executive Summary

The main objective of Work Package 3 (WP3) is to develop at lab scale a two-stage biological gas-to-liquid process for the conversion of $CO_2$/CO/$H_2$ (syngas) produced by gasification into triacylglycerides (TAGs) that will be upgraded to biofuels.

The second stage of the biological process involves the production of TAGs from acetate fermentation using oleaginous yeasts. To fulfil this aim D3.3 has covered two major goals:

a.- To identify the main genes and metabolic routes of oleaginous yeasts involved in acetate tolerance/high TAGs production

b.- To genetically modify oleaginous yeasts in order to use the acetate and convert it into C14 and C16-18 TAGs.

We have tested several *Yarrowia lipolytica* strains to select the best strain that will be used to produce TAGs from acetate. The selected strain was the wild type strain *Y. lipolytica* W29.

Using the *Y. lipolytic*a Po1d (W29. Leu$^-$, Ura$^-$, Xpr2$^-$) strain, a mutant of the wild type *Y. lipolytica* W29 strain, as a host we have constructed a recombinant strain named *Y. lipolytica* DGA (W29. Leu$^-$, Xpr2$^-$, MFE1$^-$:DGA1$_{rt}$:DGA2$_{cp}$) that overexpresses two heterologous diacylglyceride acyl-transferase enzymes (DGA), *i.e.*, DGA1 from *Rhodosporidium toruloides* and DGA2 from *Claviceps purpurea*, and has a MFE1 deletion producing, thus, 40% more lipids in flask shake cultures than the wild type strain *Y. lipolytica* W29.

On the other hand, we have constructed a *Y. lipolytica* W29 recombinant strain with higher intracellular lipase activity which could be useful in the transformation of TAGs into free fatty acids (FFAs) for the final biofuel processing.

New obese strains combining the heterologous overexpression of DGA1 and DGA2, the MFE1 deletion, and the random overexpression of the 3 acyl-transferases genes LRO1, SCT1, SLC1 from *Y. lipolytica* has been designed.

# 1. Introduction

The second stage of the biological process that has been proposed to be developed in WP3 involves the production of triacylglycerides (TAGs) from acetate fermentation using oleaginous yeasts. To fulfil this aim D3.3 considers two major goals:

a.- To identify the main genes and metabolic routes of oleaginous yeasts involved in acetate tolerance/high TAGs production

b.- To genetically modify oleaginous yeasts in order to use the acetate and convert it into C14 and C16-18 TAGs.

Concerning the second fermentation stage, the production of TAGs from acetate is a high yield process when acetate is efficiently transported and converted to acetyl-CoA by the acetyl-CoA synthetase. So far, the most efficient microorganisms in carrying out this conversion are the so-called oleaginous yeasts, such as *Yarrowia lipolytica*. Although *Cutaneotrichosporon oleaginosus* previously known as Trichosporon oleaginosus, *Cryptococcus curvatus, Apiotrichum curvatum* or *Candida curvata* D have been also proposed at a putative chassis to produce TAGs, *Y. lipolytica* is the most-well studied oleaginous yeast, has a well-primed metabolism for the biosynthesis of TAGs when grown in nutrient-limited conditions, and has a high potential for industrial applications that has been exploited since more than 70 years using wild-type isolates or improved strains (Madzak, 2021). Moreover, *Y. lipolytica* has also been shown to be rather robust and able to grow on a variety of substrates and there is availability of genetic tools. Compared with *Y. lipolytica*, the metabolic engineering of other oleaginous yeasts is still limited due to the lack of genetic tools and generally insufficient knowledge of cellular genetics (Shi and Zhao, 2017). In addition, *Y. lipolytica* is generally regarded-as-safe oleaginous yeast, which is very important to develop large scale industrial processes. Considering all these arguments *Y. lipolytica* was selected as the target strain to produce TAGs from acetate.

In order to obtain improved strains that exhibit high lipid concentration, and a high yield from acetate conversion, a metabolic engineering strategy of *Y. lipolytica* can be adopted, considering, also that genetic engineering tools and metabolic models have

already been developed for this yeast. In *Y. lipolytica* the lipid metabolism is well known and a robust metabolic model has been developed (iYL_2.0 containing 1471 reactions, Wei et al., 2017). A metabolic scheme of the production of TAGs from acetate is shown in Figure 1.

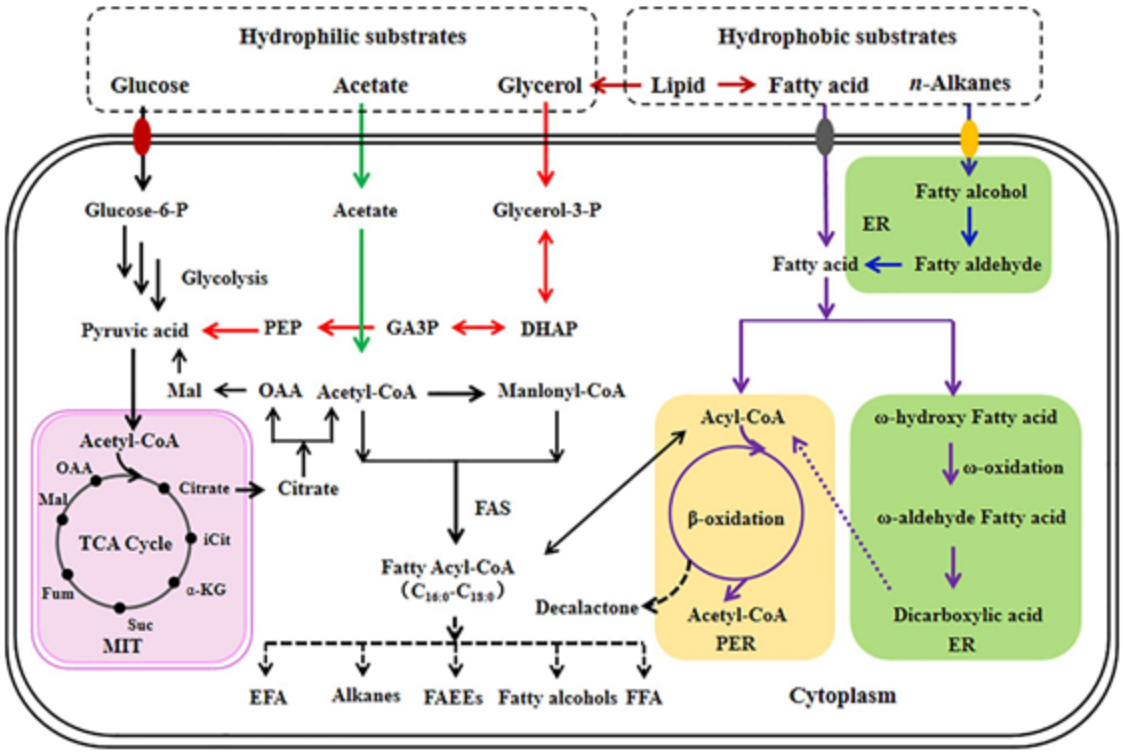


**Figure 1**. Metabolic pathway for TAGs production through acetate fermentation (Liu et al. 2021).

Several mutant strains have been already generated to improve lipid production in *Y. lipolytica* (Friedlander et al., 2016; Silverman et al, 2016; Lazar et al., 2018; Shi et al., 2021). For instance, it has been shown that a simultaneous overexpression of the stearoyl-CoA desaturase (SCD), the acetyl-CoA carboxylase (ACC1), and the diacylglyceride acyl-transferase (DGA1) generates a fast cell growth with a lipid overproduction and a high carbon to lipid conversion yield. This strain was able to grow in media with high acetic acid concentration (30% v/v) to produce lipids with a titer of 51 g/L and 61% lipid content. Other strains have been engineered by overexpressing the acylglycerol-phosphate acyltransferase (SLC1), achieving a lipid content higher than 90%. Aside from genes encoding enzymes that directly catalyse the reactions of lipid synthesis, other ways in which lipogenesis has been increased include

overexpressing the glycerol-3-phosphate dehydrogenase (GPD1) to increase production of glycerol head groups, and overexpressing the 6-phosphogluconolactonase (SOL3) from the oxidative pentose phosphate pathway to increase NADPH availability for fatty acid synthesis. Nevertheless, up to now, metabolic engineering strategies have not been applied to improve the utilization of acetate in oleaginous yeasts. Such strategies will include increased acetate tolerance and improved acetate uptake.

In the framework of activities on Task 3.2 (subtask 3.2.2) systems metabolic engineering has been used to improve selected yeast strains. We have modified the oleaginous yeast strains to express an intracellular lipase, or immobilized in the cell wall, that should facilitate the downstream process.

## 2. Identification of the best strains of *Yarrowia lipolytica*

In this task we have worked to identify the best strains of *Y. lipolytica* able to produce TAGs using acetate as a carbon source. The *Y. lipolytica* strains that we have tested are:

1. *Y. lipolytic*a Po1d (W29. Leu$^{-}$, Ura$^{-}$, Xpr2$^{-}$)
2. *Y. lipolytica* W29 (WT strain)
3. *Y. lipolytica* Po1 (W29, Xpr2$^{-}$)
4. *Y. lipolytica* YB-392 (WT strain)
5. *Y. lipolytica* INAG 35667 (Lys$^{-}$, Ura$^{-}$, Leu$^{-}$)
6. *Y. lipolytica* Po1a (W29. Leu$^{-}$, Ura$^{-}$)
7. *Y. lipolytica* H222-S4 (H222. Ura$^{-}$)

These strains have been selected from our yeast collection to be screened as putative chassis since their genomes have been sequenced and have been previously engineered. These strains have a different genetic background that may confer advantages in the acetate fermentation. Moreover, these strains have a number of mutations that can facilitate genetic manipulations and they have been already used for industrial purposes.

Figure 2 shows the appearance of some *Y. lipolytica* strains growing on agar plates.

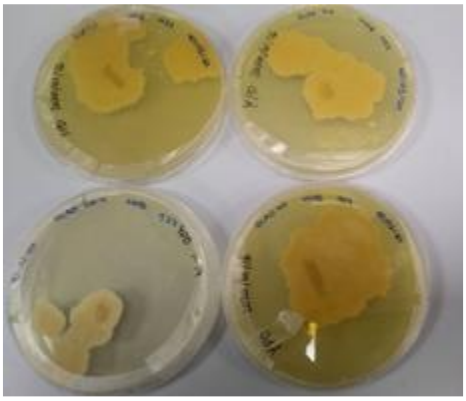

**Figure 2**. Example of *Y. lipolytica* strains growing on YPD agar plates.

The growth of *Y. lipolytica* strains was tested in rich liquid medium (YPD), with 20% glucose as carbon source, in comparison with minimal medium (YNB) with acetate at 7% as carbon source. An Optical Density (OD) at 600 nm of 40-58 was reached in YPD, while in YNB all strains reached only an $OD_{600}$ between 6-8, due to the basification of the medium. The results of these test can be observed in Figure 3.

The strains derived from W29, YB-392 or H222 were able to grow well in the YNB medium containing acetate as a carbon source. However, the INAG 35667 strain grew very poorly and can be discarded as a chassis.

It is worth mentioning that when yeast cells grow using acetate a dramatic pH shift from 7.0 to 9.0 stops the growth of the cells after 40 h of culture. Because of that the biomass obtained with glucose was about 10-times higher than the biomass obtained with acetate. This means that the cultures using acetate as carbon source will require a fine control of pH in the bioreactors to reach high amounts of biomass.

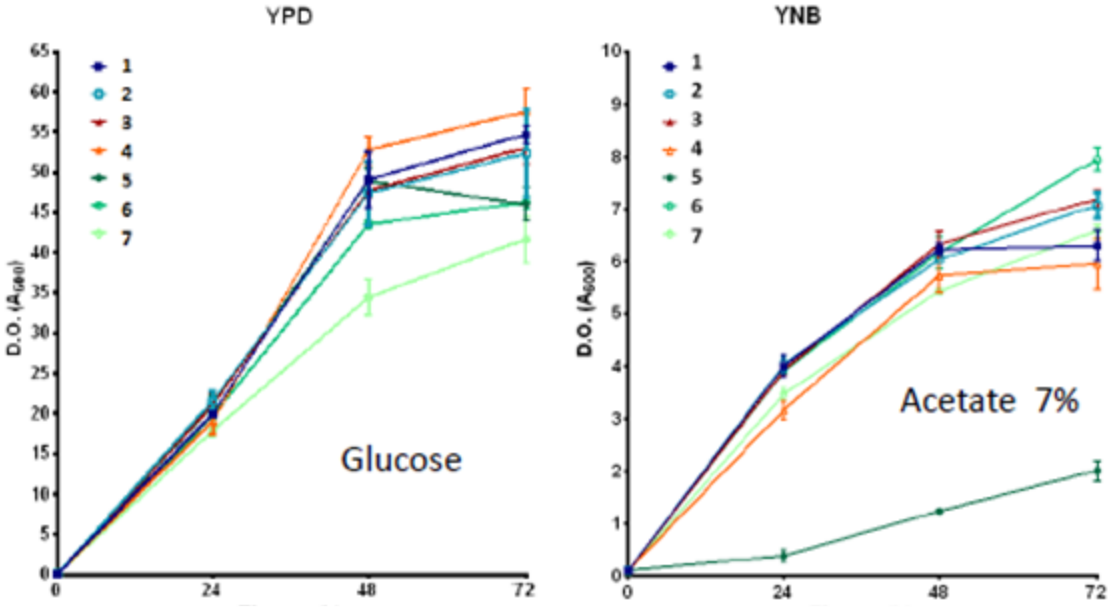


**Figure 3**. Growth curves of different strains of *Y. lipolytica* cultured in YPD medium with 20% glucose or YNB medium containing 7% acetate as carbon sources. 1. *Y. lipolytic*a Po1d (W29. Leu$^{-}$, Ura$^{-}$, Xpr2$^{-}$). 2. *Y. lipolytica* W29 (WT strain). 3. *Y. lipolytica* Po1 (W29, Xpr2$^{-}$). 4. *Y. lipolytica* YB-392 (WT strain). 5. *Y. lipolytica* INAG 35667 (Lys$^{-}$, Ura$^{-}$, Leu$^{-}$). 6. *Y. lipolytica* Po1a (W29. Leu$^{-}$, Ura$^{-}$). 7. *Y. lipolytica* H222-S4 (H222. Ura$^{-}$)

We have determined the total lipid content of the different *Y. lipolytica* strains growing on YPD medium or acetate by gravimetry (Table1). Total lipid extractions were obtained from 100 mg samples (cell dry weight) of cultures of the *Y. lipolytica* strains. *Y. lipolytica* cells were centrifuged, washed with water, freeze dried, and then resuspended in a 2:1 chloroform/methanol solution and vortexed with glass beads for 20 min. The organic solution was extracted and washed with 0.4 ml of 0.9% NaCl solution before being dried at 60 ºC overnight and weighed to quantify lipid production

**Table 1.** Biomass and lipid productions of the *Y. lipolytica* strains growing on YPD medium containing glucose or minimal medium containing acetate as a sole carbon and energy source.

| *Y. lipolytica* Strain / medium | Biomass (g/L) | TAGs (g/L) | lipids/biomass (%) |
|---|---|---|---|
| Po1d / YPD | 15.51 | 0.80 | 5.18 |
| Po1d / acetate | 2.84 | 0.25 | 8.83 |
| W29 / YPD | 15.64 | 0.94 | 6.00 |

| | | | |
|---|---|---|---|
| W29 / acetate | 1.12 | 0.08 | 7.16 |
| H222-S4 / YPD | 13.61 | 0.77 | 5.69 |
| H222-S4 / acetate | 1.02 | 0.03 | 3.22 |
| YB-392 / YPD | 15.77 | 1.01 | 6.43 |
| YB-392 / acetate | 1.28 | 0.12 | 9.66 |
| INAG 35667 / YPD | 10.63 | 0.62 | 5.80 |
| INAG 35667 / acetate | 1.28 | 0.07 | 5.49 |

All the strains with the exception of INAG 35667 grew well using acetate as carbon source and produced a significant amount of TAGs both from glucose or acetate.

Considering that W29 strain is a model chassis previously used for lipid production (Niehus et al., 2018), that grows well in acetate and that we have several available mutants in our collection useful for genetic engineering, this strain was selected as our target to be further improved for the production of TAGs.

## 3. Improving selected strains using metabolic engineering to increase TAGs production.

The genetic engineering improvement of *Y. lipolytica* W29 was performed in two consecutive steps (with the over expression and deletion of different genes) in order to reduce the risk of creating an unstable strain by the introductions of too many modifications at the same time. Figure 4 shows the main metabolic pathways involved in the synthesis of TAGs in *Y. lipolytica*.

**Step 1**

According to the data obtained in the previous task we have decided to use *Y. lipolytica* W29 as the model strain to improve the production of TAGs by metabolic engineering. Nevertheless, to carry out the genetic manipulation we have used the strain *Y. lipolytic*a Po1d (W29. Leu$^{-}$, Ura$^{-}$, Xpr2$^{-}$) which is a mutant derived from strain W29, but prepared to carry out the selection procedures required to perform the genetic modifications. The initial objective was to obtain an obese strain, i.e., a strain that accumulates high

amounts of TAGs, by deleting the MFE1 protein that is involved in the fatty β-oxidation in the peroxisome, at the same time that we overexpress two diacylglyceride acyl-transferases DGA1 and DGA2. The *dga1* gene from *Rhodosporidium toruloides* and the *dga2* from *Claviceps purpurea* were codon optimized and synthesized by GeneScript. These specific genes were selected based on previous works (Friedlander et al., 2016).

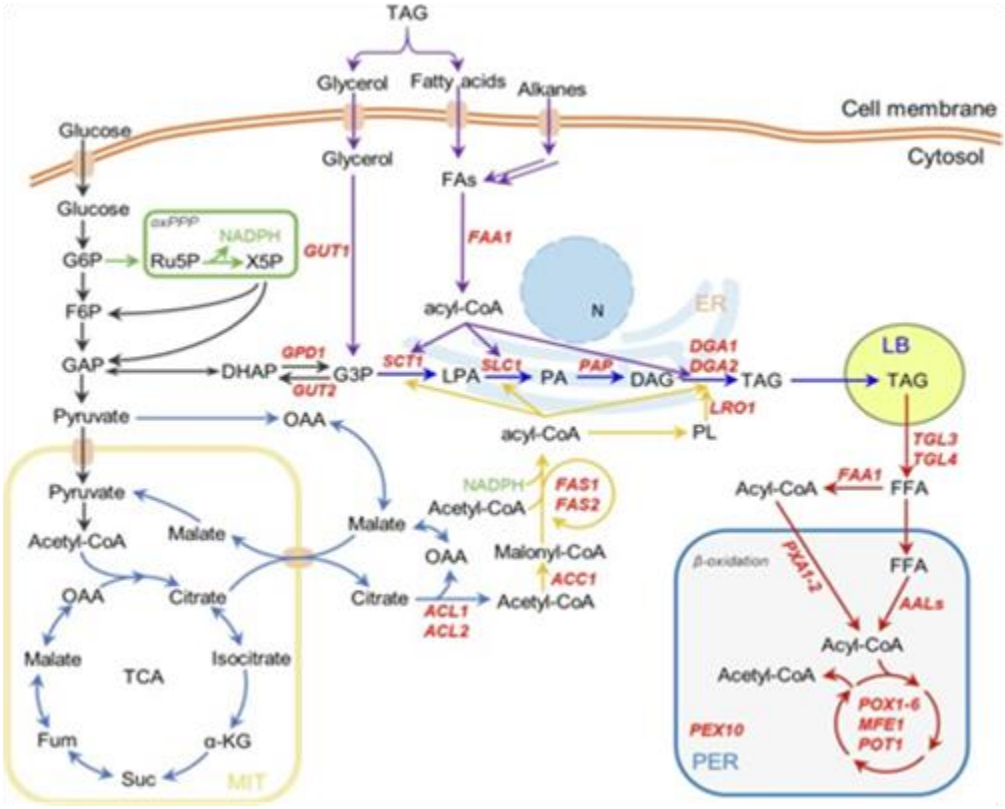


**Figure 4.** TGA metabolism of *Y. lipolytica* (Wang et al., 2020). The *MFE1*, *DGA1*, *DGA2*, *SCT1*, *SCL1* and *LRO1* genes used in this work are indicated in this scheme.

After the transformation, we have isolated a recombinant obese strain named *Y. lipolytica* DGA (W29. Leu$^{-}$, Xpr2$^{-}$, MFE1$^{-}$:DGA1$_{rt}$:DGA2$_{cp}$). We have determined by GC the fatty acid methyl esters profile (FAMEs profile) of the TAGs produced by the cells growing on YPD medium or acetate containing medium. The results of these experiments are shown in Table 2.

**Table 2**. FAMEs profiles of TAGs produced by *Y. lipolytica* wild type and obese DGA strain cultured on acetate or YPD media.

| **Media** | **ACETATE** | | **YPD** | |
|---|---|---|---|---|
| **FFA (area)** | ***Y. lipolytica WT*** | ***Y. lipolytica DGA*** | ***Y. lipolytica WT*** | ***Y. lipolytica DGA*** |
| **Tetradecanoate** | 134341 | 823783 | 213042 | 728572 |
| **Pentadecanoate** | 187339 | 376852 | 421143 | 1428552 |
| **Hexadecenoate** | 6293568 | 9295441 | 15884721 | 20809255 |
| **Hexadecanoate** | 5235278 | 7155305 | 4397696 | 8967605 |
| **Heptadecenoate** | 41319 | 39814 | 396855 | 532881 |
| **Heptadecanoate** | 347823 | 259093 | 334071 | 1084176 |
| **Octadecadienoate** | 2233081 | 2125180 | 3568332 | 3810702 |
| **Octadecenoate** | 50830513 | 67980132 | 92070239 | 145991713 |
| **Octadecanoate** | 5604491 | 6171567 | 906239 | 3814950 |
| **Total FFA (area/sample)** | 70907753 | 94227167 | 118192338 | 189247575 |
| **Sample biomass (mg)** | 23.9 | 23.9 | 23.4 | 24.0 |
| **Total biomass (g/L)** | 3.0 | 4.1 | 16.5 | 14.4 |
| **Total FFA (area/L)** | 0.90E+10 | 1.60E+10 | 8.33E+10 | 11.35E+10 |

The results of GC analysis showed that the FAMEs profiles of wild type and obese strains are not very different, as expected, since the mutations introduced in the obese strain are not created to change the fatty acid profile, but to increase the TAG yield. The main fatty acids detected were C18:1, C16:1 and C16:0. The results of these experiments indicate that the obese strain produced 40-80% more TAGs than the wild type strain depending on the culture media. In addition, these results agree with our objetive of obtaining TAGs with high content in C16 and C18 FFA.

**Step 2**

In order to improve the production of TAGs in the obese strain *Y. lipolytica* DGA, the strain has been engineered to overexpress other genes involved in the synthetic pathway of TAGs from glycerol-3-phosphate such as the LRO1, SCT1, SLC1 fatty acyl-transferases (Figure 4). SCT1 transfers a fatty acid from fatty acyl-CoA to the sn-1 position of glycerol-3-phosphate to produce lysophosphatidic acid. SLC1 is an acyltransferase that catalyses the sn-2-specific, acyl-CoA-dependent acylation of lysophosphatidic acid to phosphatidic acid. LRO1 specifically transfers acyl groups

from the sn-2 position of a phospholipid to a diacylglycerol, thus forming an sn-1-lysophospholipid and a TAG.

The three *lro1, sct1, slc1* genes encoding the acyl-transferases from *Y. lipolytica* were cloned into the pJMP62-LEU plasmid independently and in all possible combinations. The main idea was to design 7 different recombinant *Y. lipolytica* strains by using 7 different constructions (the maps of the plasmids are shown in Annexes). These recombinants will allow us to learn which gene combination could be more effective to improve the production of TAGs in our DGA obese strain.

## 4. Overexpression of lipases in *Yarrowia lipolytica.*

The aim of this task was to express intracellularly or immobilized in the cell surface of *Y. lipolytica* (cell-surface display) a lipase, with the aim of breaking down the TAGs produced by the yeast into fatty acids when the cells are lysed improving the downstream processing of TAGs. In this sense, two well-known lipases have been overexpressed intracellularly or anchored to the cell wall, the Lip2 lipase from *Y. lipolytica* and the OPE lipase from *Ophiostoma piceae*. A scheme of a surface display is described in Figure 5.

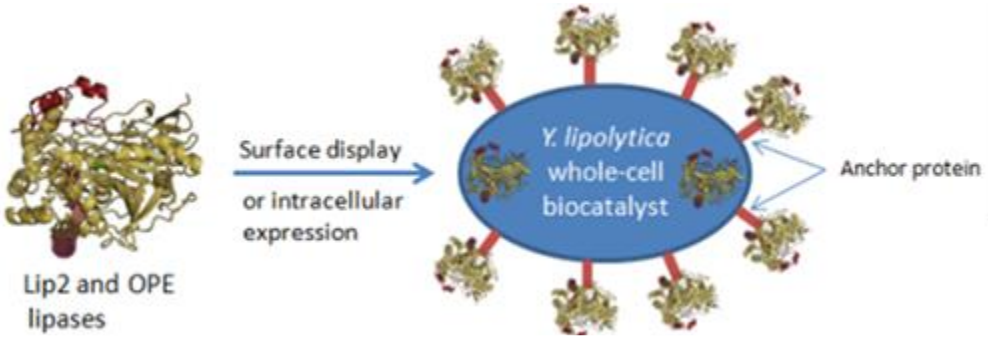


**Figure 5**. Overexpression of lipases in *Y. lipolytica* anchored to the cell surface or intracellular.

We have constructed 4 different plasmids to express the lipases. Two constructions contain the lipases fused to a signal peptide and a cell binding domain to be secreted and anchored to the membrane. Two other constructions contain the lipases devoid of a signal peptide and a cell binding domain to be expressed intracellularly (see annexes).

We have been able to express the lipases only in the cytoplasm since the construction used to anchor the enzymes to the surface were not expressed at significant levels.

The results of these experiments carried out with the intracellular constructions are shown in Figure 6. According to these results we can conclude that the Lip2 lipase is expressed efficiently in the recombinant cells, but the OPE lipase did not show a significant activity. Thus, the recombinant cells that overproduce Lip2 enzyme inside the yeast can be used to hydrolyse the TAGs after disruption of the cells to facilitate the recovery of FFAs.

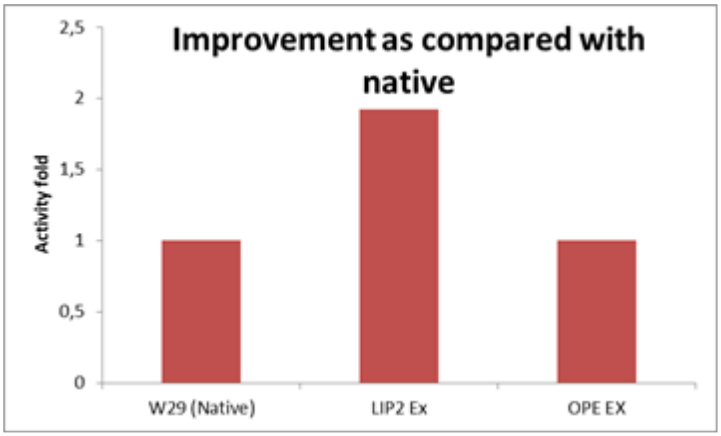


**Figure 6**. Lipase activity in the extracts of *Y. lipolytica* W29 cells transformed with the genes encoding the Lip2 lipase from *Y. lipolytica*, and the OPE lipase from *O. piceae*.

## 5. Conclusions

1. We have tested several *Y. lipolytica* strains to select the best strain that will be used to produce TAGs from acetate. The strain selected was the wild type strain *Y. lipolytica* W29.
2. We have constructed a *Y. lipolytica* W29 recombinant obese strain named *Y. lipolytica* DGA (W29. Leu$^{-}$, Xpr2$^{-}$, MFE1$^{-}$:DGA1$_{rt}$:DGA2$_{cp}$) that overexpresses the diacylglyceride acyl-transferase enzymes DGA1 and DGA2, and has a MFE1 deletion. The obese strain produces 40-80% more TAGs when cultured in shacked flasks than the wild type *Y. lipolytica* W29 strain.

3. We have constructed a *Y. lipolytica* W29 recombinant strain with higher intracellular Lip2 lipase activity which could be useful in the transformation of TAGs into FFAs for the final biofuel processing.
4. We have designed a collection of 7 new obese strains using as host the obese DGA strain by combining the overexpression of three genes encoding the acyl-transferases LRO1, SCT1, or SLC1.

## 6. Future actions

To further improve the *Y. lipolytica* obese strains that we have developed we propose the following actions that will be reported as an updated version of D3.3 or in other deliverables of WP3 such as D3.5 “Lab scale downstream processing for TAGs recovery and purification using conventional and novel strategies” and D.3.6 "Optimization of acetate fermentation process parameters for C14 and C16-18 TAGs production (2nd stage)":

1- To test the obese recombinants of *Y. lipolytica* generated by the combinations of the genes encoding the LRO1, SCT1 and SLC1 acyl-transferases and determine if one specific combination produces more TAGs or TAGs with a different profile.

2.- To transform the best *Y. lipolytica* obese strain with the *lip2* gene encoding the lipase to facilitate the extraction of FFAs.

3.- To improve the uptake of acetate by the obese recombinants of *Y. lipolytica* we will study the possibility of cloning specific acetate transporter genes.

4.- To improve the production of TAGs with a higher content in C:14 FA we will follow an approach similar to that developed by Rigouin et al. (2018) using our best obese *Y. lipolytica* strain as chassis. We will create a *α*FAS-I1220W (Fatty Acid Synthase) mutant that displays an Ile-Trp mutation in the *αFAS* gene at position 1220, in the ketoacyl synthase domain and an ELO1 mutant that reduces the elongation activity of FA.

5.- TAG hydrolysis tests will be carried out with the extracts of wild type *Y. lipolytica* W29 strain and the recombinant obese strains expressing the intracellular Lip2 lipase to determine the improvements on FFA release after cell lysis.

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# Annexes

## Analysis of fatty acids by Gas Chromatography (GC)

Preparation of yeast cells:

- Grow yeast cultures overnight for a specified amount of time (normally 3 days for lipids accumulation).
- Centrifuge 5000 rpm for 5 min to pellet the cells. Remove supernatant.
- Put the cells at -80 ºC for 30 min to 1 h.
- Freeze-dry the cells.

Esterification or FAME process (directly from yeast cells or TAG silica spots):

- Weigh around 20 - 30 mg dry yeast cells (freeze-dried or dried by nitrogen gas) in test tube, record exact weight.
- Add 10-20 µl (10 mg/ml) of heptadecanoic (C17:0) or tridecanoic (C13:0) acids (dissolved in hexane) as internal standard. (Adding either 10 or 20 µl does not matter as long as it is recorded for future reference when normalising data).
- Add 2 ml Magic methanol (methanol/hydrochloric acid/chloroform (10:1:1), seal test tube, gentle shake the test tube (avoid the cell pellets stick to the test tube wall).
- Heating at 90 °C for 60 min for normal lipids, convert lipids to fatty acid methyl esters (FAME).
- Cool the test tubes to room temperature (could use running cooling water or ice batch to speed up the process).
- Add 1 mL saline (0.9 % NaCl in water).
- Add ~2 ml hexane, vortex for 10 s.
- Centrifuge 3000 rpm, 3 min to allow the samples to separate to two layers, and the upper hexane layer contain FAME.
- The hexane layer used for GC analysis, samples may need to be diluted to fall within the calibration curve. Therefore, you can make several dilutions to ensure (such as 10-fold, 900 µl Hexane + 100 µl sample) before transfer to a GC vial for FAME analysis.

## Protocol to transform *Y. lipolytica*

Preparation of competent cells:

- Spread the strain that you want to transform on a YPD plate (28°C during 16-24 h).
- Put a loop of cells into 1 ml of TE in sterile Eppendorf.
- Centrifuge 1 min at 10 000 rpm and remove supernatant.
- Resuspend cell gently in 600 µl of lithium acetate 0.1 M pH 6.0.
- Put 1 h at 28°C in a water bath without agitation.

- Centrifuge 2 min at 3 000 rpm and remove supernatant.
- Resuspend gently cells in 60 µl of lithium acetate 0.1 M pH 6.0.

Transformation:

- In a new eppendorf tube, mix slightly together with pipette:
  - 3-5 µl of carrier DNA
  - 2-5 µl cassette DNA
  - 40 µl competent cells
- Incubate 15 min at 28°C in a water bath without agitation.
- Add 350 µl of PEG solution.
- Incubate 1 h at 28°C in a water bath without agitation.
- Do the thermal shock 10 min at 39°C.
- Add 600 µl lithium acetate (0.1 M pH 6.0).
- Spread 200 µl (or more) per plate containing the adequate selection medium (without uracil or leucine).

**YPD medium**

For the routine growth of *Saccharomyces cerevisiae* for molecular biology studies, Sherman (2002).

Composition:

- Glucose, 20 g/l
- Peptone, 20 g/l
- Yeast extract, 10 g/l

**YNB medium**

Yeast Nitrogen Base is considered ideal for culturing wild type yeast. The presence of amino acid is known to prevent selectable marker utilization. Hence, this media is formulated without amino acids and is useful for cloning and manipulation of yeast artificial chromosome.

Yeast Nitrogen Base is a highly-referenced growth medium used for the cultivation of yeast. This nutrient-rich microbial broth contains nitrogen, vitamins, trace elements and salts.

Composition:

Nitrogen Sources:

Ammonium sulphate, 5.0 g/l

Vitamins:

Biotin, 2.0 µg/l

Calcium pantothenate, 400 µg/l
Folic acid, 2.0 µg/l
Inositol, 2.0 mg/l
Nicotinic acid, 400 µg/l
*p*-Aminobenzoic acid, 200 µg/l
Pyridoxine HCl, 400 µg/l
Riboflavin, 200 µg/l
Thiamine HCl, 400 µg/l
Trace Elements:
Boric acid, 500 µg/l
Copper sulfate, 40 µg/l
Potassium iodide, 100 µg/l
Ferric chloride, 200 µg/l
Manganese sulfate, 400 µg/l
Sodium molybdate, 200 µg/l
Zinc sulfate, 400 µg/l
Salts:
Potassium phosphate monobasic, 1.0 g/l
Magnesium sulfate, 0.5 g/l
Sodium chloride, 0.1 g/l
Calcium chloride, 0.1 g/l

**Sequence of *dga1* gene from *Rhodosporidium toruloides* (codon optimized for *Y. lipolytica*) expressed from TEF1 constitutive promoter**

ATGGGACAGCAGGCTACCCCCGAGGAGCTCTACACCCGATCCGAGATTTCTAAGATTAAGTT
CGCCCCTTTTGGAGTGCCCCGATCCCGACGACTCCAGACCTTCTCCGTTTTTGCCTGGACCA
CTGCTCTGCCCATTCTGCTCGGCGTCTTCTTTCTGCTCTGCTCTTTCCCCCCTCTCTGGCCC
GCCGTCATCGCTTACCTGACCTGGGTGTTCTTTATCGACCAGGCCCCTATTCACGGCGGTCG
AGCTCAGTCCTGGCTGCGAAAGTCTCGAATTTGGGTTTGGTTCGCCGGTTACTACCCCGTCT
CTCTCATCAAGTCGGCTGACCTGCCCCCTGATCGAAAGTACGTGTTCGGCTACCACCCTCAT
GGTGTTATCGGTATGGGAGCCATTGCTAACTTTGCCACCGATGCTACTGGTTTCTCCACCCT
CTTTCCCGGACTGAACCCTCACCTGCTCACTCTCCAGTCTAACTTCAAGCTCCCCCTGTACC
GAGAGCTGCTCCTGGCCCTGGGTATCTGCTCCGTCTCTATGAAGTCTTGTCAGAACATTCTC
CGACAGGGACCTGGTTCGGCTCTGACCATCGTCGTGGGAGGAGCTGCTGAGTCGCTCTCCGC
CCATCCTGGAACCGCTGACCTCACTCTGAAGCGACGAAAGGGCTTCATCAAGCTCGCCATTC
GACAGGGTGCTGACCTGGTGCCCGTTTTCTCCTTTGGAGAGAACGATATTTTCGGCCAGCTG
CGAAACGAGCGAGGAACCCGACTCTACAAGCTGCAGAAGCGATTTCAGGGTGTGTTCGGCTT

CACCCTCCCTCTGTTCTACGGACGAGGCCTCTTTAACTACAACGTTGGACTGATGCCCTACC
GACACCCTATCGTCTCGGTTGTCGGCCGACCCATTTCCGTGGAGCAGAAGGACCATCCTACC
ACTGCCGATCTCGAGGAGGTGCAGGCCCGATACATCGCTGAGCTGAAGCGAATTTGGGAGGA
GTACAAGGACGCCTACGCTAAGTCTCGAACCCGAGAGCTGAACATCATTGCCTAA

**Sequence of *dga2* gene from *Claviceps purpurea* (codon optimized for *Y. lipolytica*) expressed from TEF1 constitutive promoter**

ATGTCCGCCACGGGCGTTGATGTGGCCAACGGCCGCAGCGGCGCGCGACGACGCAACGATAC
TGCCGTCGACGAGACTATATCCGCCGTCACGGCCGAGATGCGTTCCTCGTCGCATCCAACAT
ACCGCCATGTGTCTGCTGTGCACTCCACGAGCCGGCCCTCGTGTCTGAGCCATGATTCTGAC
GCTGCGCCGAGCTTCATTGGCTTTCGAAATCTCATGGTCATTGTTCTGGTCGTTGGCAATGT
TCGATTAATGATTGAAAATCTAAAAAAGTACGGCGTACTGATATGCCTCCGATGTCACTCGT
ATAAAAACGAAGACATCATTATCGGCGGACTGCTCTACTTCCTGATCCCCTGCCACTTGCTT
GTCGCCTACGGAATCGAGTTAGCCGCCGCCAGACAAGCACGCGAATCTCGAACTCGTCCACC
AGGCCAGTCCGACACGGCGTCGAAATCAACAGAAGATGACAACAAGCACTTCCACTCAACAT
GGGTGCTCGCTGCCTGGGCACACATCATCAACATGACACTTTCCTTCATCCTCACCACCTTC
GTCGTCTACTACTACGTGCACCATCCCCTCGTCGGCACCCTGACCGAGATGCACGCCGTCAT
CGTCTCTCTCAAAACAGCTTCCTACGCATTCACCAACCGAGATCTTCGCCACGCATACCTCC
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ACCTTTGGCAATCTCGTGTATTTCTGGTGGGCCCCCACGCTGGTATACCAGCCCGTGTATCC
GCGCACCGACAAGATCAGATGGGTTTTTGTTTTTTAAAAGACTAGGCGAAGTCTGCTGTCTCA
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TCTCGTCATCTGGCTCGCCGGCTTCTTCGCCCTGTTCCAGTCTTTCCTGAATGCCCTGGCTG
AGGTATTGCGCTTTGGGGACCGGTGCTTCTACGACGATTGGTGGAATAGCGAGAGTCTGGGG
GCGTATTGGAGGACGTGGAACAGGCCTGTGTATACCTACTTCAAGCGCCATGTGTATGTGCC
CATGATTGGGAGGGGATGGAGTCCCTGGACTGCTAGTTGTACTGTTTTTTTTGTGTCGGCGG
TGCTGCACGAGGTTCTTGTTGGGGTGCCCACCCACAATATCATTGGTGTCGCCTTTGTGGGC
ATGTTTCTGCAGCTTCCCCTAATAGCCCTCACCGCTCCCATGGAAAAGAAGAAATGGGGCCA
CACCGGCCGTGTGATGGGCAATGTTATTTTCTGGGTGTCCTTTACAATCTTTGGGCAGCCCT
TTGCAGCGCTCATGTACTTTTATGCCTGGCAGGCCAAGTACGGGAGCGTGAGTCGGCAAATT
GTGCTGGTGAATCCGGTGGAGGAGGCGTCTTGA

**Sequence of *slc1* gene from *Y. lipolytica* expressed from TEF1 constitutive promoter**

ATGAACAGTTGGATATATGTCGCTGTGATTGCAGTTGCTGCCGTTCTCATTGCCCGAATGTC
CGTTGCATCCAAGCTCGTCTTCTACGTCCGCGCCGCCATCGCCGTGGTCATCTTTGCCGCCT

GTGCCACCTACGGCGTGCTGGCGTCCACCATTCTCACCGCCATCGGCAAGCAGGGCCTGGCC
CAATGGACCGTTGCCAGAGCCTTCTACTACTCGGTGCGCATCTTCCTGGGTATCAGCATCAA
GCTGCGTAGCCGGCAGGTGACCGGAACCGCCGGTCTGGATGCCTCCAAGATCCAGGTCGCCA
ACACCACCAAGCCCATTGACGACATCACCAAACACCTGCCCCGACCATGCATTCTGATTTCC
AACCACCAGAACGAAATGGACATTCTGGTGCTCGGTCGCATCTTCCCCCAGTACTGCTCCGT
CACCGCCAAAAAGGCCCTCAAGTGGTACCCTCTGCTGGGCCAGTTCATGGCGCTGTCCGGCA
CCATCTTCCTGGACCGAAAGGACCGAACCAAGTCCGTGCAGACCCTCGGCGGCGCCGTCAAG
ACCATCCAGAGCGGCAACGGAGGCAAGGGCCAGAGCGTCTTCATGTTCCCCGAGGGAACCCG
ATCCTACTCCAAGGACGTCGGCATCATGCCCTTCAAGAAGGGCTGTTTCCACCTGGCGGTCC
AGTCGGGCGCTCCCATTGTCCCCGTGGTGGTCCAGAACACCTCCCGAATGTTTTCTTTCGGC
CGAGGCAAGCTGGACGCCGGAGAGATCCTTGTCGACGTCCTGAGCCCCATTGAGACCAAGGG
TCTGGACGCCAGCAACGTCGACGCTCTCATGGCCACCACTTATAAGGCCATGTGCGAGACTG
CCGACCAGATTGGCTACGCTGGCCAGAAGACTCAGTAG

## Sequence of *sct1* gene from *Y. lipolytica* expressed from TEF1 constitutive promoter

ATGTCCGAAACCGACCATCTGCTGGCCGCCGAGCCCGTGGCTGAGTACCCCCAGTACACGCC
TTGGCCCAACTCCCGAAAATCAGTGGACACGGAGTTTTCCGCAACCTCGTGGATTTACGACT
TGGTTCTGTGGATTTTCACGGCTTGCTTTGACATTTTTTTCAGAGAAATCCGGCCACGTGGT
GCCTTCCGAATCCCCAGAAAGGGCCCCGTGCTGTTCGTGGCTGCCCCCCACGCAAACCAGTT
TGTGGACCCCGTCATCCTCATGAACCAGGTCAAACAGGAGGCCGGACGACGAATCTCCTTCC
TTGTGGCCGAGAAGTCCATGCGACGAGCTGCAGTCGGACGAATGGCCCGAAGCATGAACTCA
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GCCTCGTGGTCTGCTCGTCCTCTGTCGGCTCAATTGACGTGGCTGAGATTGTGTCCGACACT
CTGCTCATTGCAAGAAAGGAATTCAAGGGCCCCAAAGCCAAGGAGGCTCTCAAGGAATCCAA
CGGAGGAATCACATACAAGTACGCCGACTACGTCAACCAGGCCACAGTCTACCGATCCGTAT
TCGACAAATTGCACCATGGAGGCTGTGTGGGTATCTTCCCAGAGGGAGGATCTCATGACCGA
ACCGAACTGCTGCCCCTTAAGGCCGGTGTTGCTATCATGGCTCTGGGGGCTCTCGCAGAGGA
CCCCTCTTGTGGTGTGCGAATCGTCCCCTGTGGTCTCAACTACTTCCACGCCCACAAGTTCC
GATCTCGGGCCGTGGTGGAGTTTGGCTCTCCTATTGCCATTCCTCCGGATCTCGTGGAGAAG
TACAAGGCAGGAGGAGAGGCCAAGCGGGAGGCTGTCAAGACCGTTCTAGACATTACTGCCGC
TGGTCTCAAGTCTGTGACTGTTCAGGTGCAGGATTTCGACACCCTGATGCTGATCCAGGCCA
TTCGACGACTCTACCGACCTCCCGGAAAGAAGATTCCTCTGCCCATGGTTGTAGAGCTCAAC
CGTCGACTTGTATACGCCTACAACCACTACAAGGACGATCCCCGTATCGAGGAGATGAAGCA
GGAGATTCGAAAGTACAACAAGTTCCTGCAGGCCATGGGTCTCAAGGACCATCAGGTAGAGA
AGGCCCGAATCTCCAAGATTGAGATTCTGGGCCGGCTTCTGTACCGGTCCATCAAGCTTGTG

TTCTTGTCCATTGGCTGTCTCCCCGGTCTGCTTTTGTTTTCTCCCATCTTCATCATTTCTAA
GTCCATTTCCAAAACCAAGGCCAAGGAGGCTCTCAAGGCCTCCAGTGTCAAAATCAAGGCTA
ACGATGTGGTTGCCACTTGGAAGGTGCTGGTTGCAATGGGTCTGACCCCAGTTCTTTACATT
CTCTATTCACTGGTTGGATCTGTGGTGATTCGAAAGCTCGATCTCATCTCCTGGTTCCCCAC
AATTCTTCTTCCCGGCCTCGTTTTAAGCATCATCATCACAACCTCATACGCCGCCCTGGCTA
TGGGAGAGGCCGGTATGGACATTTTCAAGTCTCTTCGACCACTTGCATTGGCTCTCAACCCT
TCCACCAAAAACTCTCTGCTCAAGCTGCAAAATGAACGAAAGCGACTTGTGCTCAAGTCTTC
CGAGCTCGTTACCTCTTTGGGCCCTGAGCTGTTCCCCGACTTCCCCGAGAACTCCATTCTGC
AGGGAAGCGATAAGTTTGAGGACGAGGAGAACTACGAAAACGAGAAGCGATCGCATTCCAGA
TCCACTTCTGCCACTTCTCTATCTGCCATGAGCGAGGGAGACGGTGATGAGCTTGTTCGGGA
GGTCCGAAAGGGTGCTAGCTACTTCCCTGTGAGTACCATTTCTGAGGACGAAGACCAAGCCA
TCTCGCGAGTGGGCTCTGAGGCATCTCTTGCTGACATTCCTCTGTTTGGTATGTCCCGATCA
CAATCTGGAGCTTCTCTTTCGGAAGCCTCCACACACGGCTCTTCTACTGGAGCTGATGCCGA
GGAGGCTAAGACGGAGGTGACTCGCAGAATTGCATTGGCGATGGAGGAAAAACGACGAGAGC
AGGATGAGGAATAA

## Sequence of *lro1* gene from *Y. lipolytica* expressed from TEF1 constitutive promoter

ATGACACAACCTGTGAATCGGAAGGCGACTGTCGAGCGGGTCGAGCCAGCAGTGGAGGTGGC
TGACTCCGAGTCCGAGGCCAAGACCGACGTCCACGTTCACCACCATCATCACCACCACAAGC
GAAAATCCGTCAAGGGCAAGATTCTCAACTTCTTCACCCGAAGTCGACGTATCACCTTCGTC
CTCGGCGCCGTGGTCGGTGTGATAGCCGCGGGATACTACGCTGCGCCACCGGAGCTCAGCAT
TGATATCGATGCTCTTCTCGGCGACTTGCCCTCGTTCGACTTTGACGCTCTATCTCTCGACA
ACTTGTCCATGGACAGTGTGTCGGACTTTGTACAAGACATGAAATCGCGGTTTCCGACCAAG
ATTCTGCAGGAGGCGGCCAAGATCGAGAAGCACCAGAAAAGCGAACAGAAGGCTGCCCCTTT
TGCTGTGGGCAAGGCTATGAAGAGCGAGGGACTCAACGCCAAGTACCCGGTGGTGCTGGTGC
CCGGCGTCATCTCCACGGGACTGGAGAGCTGGTCCCTGGAGGGAACCGAGGAGTGTCCCACC
GAGTCGCACTTCAGAAAGCGAATGTGGGGCTCCTGGTACATGATCCGAGTCATGCTGCTGGA
CAAGTACTGCTGGCTGCAGAACCTGATGCTGGACACAGAGACCGGTCTAGACCCTCCCCATT
TCAAGCTGCGAGCCGCCCAGGGATTTGCCTCCGCCGACTTCTTTATGGCAGGCTACTGGCTG
TGGAACAAGCTGCTCGAGAACCTGGCTGTTATTGGATACGATACGGATACAATGTCTGCTGC
GGCGTACGACTGGAGACTGTCCTACCCTGATTTGGAGCACCGAGACGGATACTTCTCCAAGC
TCAAAGCTTCAATCGAAGAGACTAAGCGTATGACAGGTGAGAAGACAGTTCTGACGGGCCAT
TCCATGGGCTCCCAGGTCATCTTCTACTTCATGAAGTGGGCTGAGGCCGAGGGATATGGAGG
AGGAGGTCCCAACTGGGTCAATGACCATATTGAATCCTTTGTCGACATTTCCGGCTCCATGC
TGGGTACTCCCAAGACCCTGGTTGCTCTTCTGTCTGGAGAAATGAAGGATACCGTGCAGCTG
AACGCGATGGCTGTGTATGGACTGGAGCAGTTCTTCTCTCGACGAGAGCGAGCCGATCTGCT

GCGAACATGGGGAGGAATTGCTTCCATGATTCCCAAGGGTGGTAAGGCTATCTGGGGTGATC
ATTCTGGAGCCCCTGATGACGAGCCCGGCCAGAATGTCACCTTTGGCAACTTCATCAAGTTC
AAGGAGTCCTTGACCGAGTACTCTGCTAAGAACCTCACCATGGATGAAACCGTTGACTTCCT
GTATTCTCAGTCTCCCGAGTGGTTTGTGAACCGAACCGAGGGTGCTTACTCCTTTGGAATTG
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GTATCGCTGGAAACGACCCTGATGGTGTGCTTATGGGTCAGGGCGATGGAACCGTCTCCCTT
GTGACCCATACCATGTGTCACCGATGGAAGGACGAGAATTCCAAGTTCAACCCTGGTAACGC
CCAGGTCAAGGTTGTGGAGATGTTGCACCAGCCTGATCGACTTGATATTCGAGGCGGTGCTC
AGACTGCCGAGCATGTGGACATTCTGGGGCGTTCTGAGTTGAACGAGATGGTTCTGAAGGTG
GCTAGTGGAAAGGGAAATGAGATTGAAGAGAGAGTCATCTCCAACATTGATGAGTGGGTGTG
GAAGATTGATCTCGGCAGCAATTAG

**Sequence of Lip2 lipase from *Y. lipolytica* expressed from TEF1 constitutive promoter**

ATGAAACTTTCCACCATCCTCTTCACAGCCTGCGCTACCCTGGCTGCCGCCCTCCCTTCCCC
CATCACTCCTTCTGAGGCCGCAGTTCTCCAGAAGCGAGTGTACACCTCTACCGAGACCTCTC
ACATTGACCAGGAGTCCTACAACTTCTTTGAGAAGTACGCCCGACTCGCAAACATTGGATAT
TGTGTTGGTCCCGGCACCAAGATCTTCAAGCCCTTCAACTGTGGCCTGCAATGTGCCCACTT
CCCCAACGTTGAGCTCATCGAGGAGTTCCACGACCCCCGTCTCATCTTTGATGTTTCTGGTT
ACCTCGCTGTTGATCATGCCTCCAAGCAGATCTACCTTGTTATTCGAGGAACCCACTCTCTG
GAGGACGTCATAACCGACATCCGAATCATGCAGGCTCCTCTGACGAACTTTGATCTTGCTGC
TAACATCTCTTCTACTGCTACTTGTGATGACTGTCTTGTCCACAATGGCTTCATCCAGTCCT
ACAACAACACCTACAATCAGATCGGCCCCAAGCTCGACTCTGTGATTGAGCAGTATCCCGAC
TACCAGATTGCTGTCACCGGTCACTCTCTCGGAGGAGCTGCAGCCCTTCTGTTCGGAATCAA
CCTCAAGGTTAACGGCCACGATCCCCTCGTTGTTACTCTTGGTCAGCCCATTGTCGGTAACG
CTGGCTTTGCTAACTGGGTCGATAAACTCTTCTTTGGCCAGGAGAACCCCGATGTCTCCAAG
GTGTCCAAAGACCGAAAGCTCTACCGAATCACCCACCGAGGAGATATCGTCCCTCAAGTGCC
CTTCTGGGACGGTTACCAGCACTGCTCTGGTGAGGTCTTTATTGACTGGCCCCTGATCCACC
CTCCTCTCTCCAACGTTGTCATGTGCCAGGGCCAGAGCAATAAACAGTGCTCTGCCGGTAAC
ACTCTGCTCCAGCAGGTCAATGTGATTGGAAACCATCTGCAGTACTTCGTCACCGAGGGTGT
CTGTGGTATC

**Sequence of OPE lipase from *Ophiostoma piceae* (codon optimized for *Y. lipolytica*) expressed from TEF1 constitutive promoter**

ATGGAGGCATACGTCGAATTTACCACTGTTAATGTCAACTATCCTGAGGGCGAAGTGGTTGG
AGTGTCCGTGCTCGGCATCGAGTCTTTTCGGGGAGTTCCTTTCGCTCAACCTCCTGTCGGCA
ACCTCAGACTCAAGCCTCCTGTCCGTTATACAGAGAATATCGGCACTAAGGATACAACAGGA
ATTGGTCCCTCGTGCCCTCAAATGTATCTGTCaACTGGAAATGGCGAACTTCTGTTTCAACT
GGTGGGAAATCTCATCAACATTCCCCTGTTCCAGACAGCCACTCTTTCCAGCGAGGACTGCC
TGACCCTCAATATCCAACGGCCCGCTGGTACTACCTCTAATTCCTCGCTGCCCGTCCTGTTT
TGGATTTTCGGCGGTGGTTTCGAGCTTGGAACCAACCAATATTACGACGGAATCGATCTTCT
TACTGAAGGTATCTCTCTCGGCGAGCCTTTTATCTTTGTGGCCATTAACTATCGGGTCGGTG
GATTTGGTTTCCTCGGTGGAAAAGAGATCAAGGCTGACGGCTCaAGCAATCTTGGCCTGCTG
GACCAAAGAATCGCTCTCGAATGGGTTGCCGATAACATCGCATCGTTCGGTGGAGATCCCTC
TAAAGTGACTATTTGGGGTGAGAGCGCAGGcTCCATTTCTGTTTTTGACCAGATGGCACTGT
ACGGTGGCAACAACAAGTACAAAGGTAAGGCCCTCTTTCGTGGTGGTATTATGAACTCCGGT
AGCGTCGTGCCCGCTGCCCCTGTTGACGGCGTTAAAGCCCAGGCCATCTATGATCACGTCGT
GTCTGAAGCAGGATGTGCTGGCACTAGCGACACGCTTGCATGTCTGCGGACGGTGGACTACA
CCAAGTTTCTCACTGCTGTGAACAGCGTCCCCGGCATCGTTTCGTATTCGTCGATTGCTCTT
TCTTACCTTCCCCGACCTGACGGCGTTGTTCTGATCGATTCTCCCGAAGAGATCGTGAAGAA
TAAGCAATACGCAGCAGTTCCTATGATCATCGGAGATCAAGAAGATGAGGGAACCCTGTTCG
CCGTCCTGCCCAATAACATCACTTCCACTGCTAAGATCGTTCAATATTTCCAGGACCTCTAT
TTCTATAATGCCACAAAGGAACAGCTCACCGCCTTCGTTAATACCTATCCCACGGACATTAC
TGCTGGAAGCCCTTTCAATACAGGCATTTTCAATGAaCTCTATCCTGGTTTTAAGCGACTCG
CAGCTATCCTCGGAGATATGACGTTCACGCTCGCCCGAAGAGCCTTTCTTCAGCTCTGCTCC
GAGGTGAATCCCGATGTCCCTTCTTGGTCTTATCTCGCATCTTATGATTACGGATTCCCTTT
TCTGGGAACATTTCACGCCACAGATATTCTCCAAGTTTTCTATGGAGTGCTGCCCAACTACG
CTTCCGGTTCGATTCAGAAATACTACATCAATTTCGTTACTACCGGAGACCCTAATAAAGGC
GCTGCCGTgGACATTCAATGGCCTCAGTGGTCGGCAAAGAAGAACATCCTTCAaATCTACGC
TACAAAGGCAGTCATTGTTGCTGACAACTTCCGGGCTAAGTCCTATGAGTATCTTTACAACA
ACATCGGCATCTTCCGGATC

## Sequence of lip2 signal peptide

ATGAAACTTTCCACCATCCTCTTCACAGCCTGCGCTACCCTGGCTGCCGCCCTCCCTTCCCC
CATCACTCCTTCTGAGGCCGCAGTTCTCCAGAAGCGAG

## Sequence of membrane binding domain

GGTGGGGGTTCGGGCGGCGGCGGCAGCGGTGGCGGCGGCTCCCAGAAGTTCTCCCTGGTCGC
CATCCGATCCGGCTCCGACATCCAGAACGACGCCATCACCTCCGACGGCAAGTCCCTGGTCC
TGAACGGCAACACCCCCACCACCTACGAGGTCATCGGCCGATCCCTGTACGCCAACGGCAAG
CCCGTCCAGTTCGGCGACAACGCCACCATCGTCGCCACCGACGGCCAGGCCTCCAAGGACAT

CATCGTCAACGGCGACAACCACCTGTACGTCCCCAACTTCGACTTCACCGCCTGCCCCAACG
GCAAGAACGGCTACGACATCGTCAACAACCAGTTCTGCAAGTCCGGCGGCCTGGGCTTCGCC
GCCCGAGCCGTCTTCGAGGGCGGCTCCTCCTCCGCCGCCGCCCCCACCTCCTCCTCCGCCGC
CTCCCACGCCGCCTCCTCCGCCGCCGCCTCCGCCTCCCACGCCGCCTCCTCCGCCGCCGCCT
CCAAGGCCTCCTCCGCCGCCGCCTCCAAGGCCTCCTCCGCCGTCGCCGCCCCCAAGTCCGAG
GCCGCCGCCGGCGCCCACGCCACCGCCGGCGCCATCGTCTCCCAGATCAACGACGGCCAGAT
CCAGGCCCCCCACTCCACCGGCCCCGCCCAGGCCCCCAAGGCCTCCGCCCCCCCCGCCCAGG
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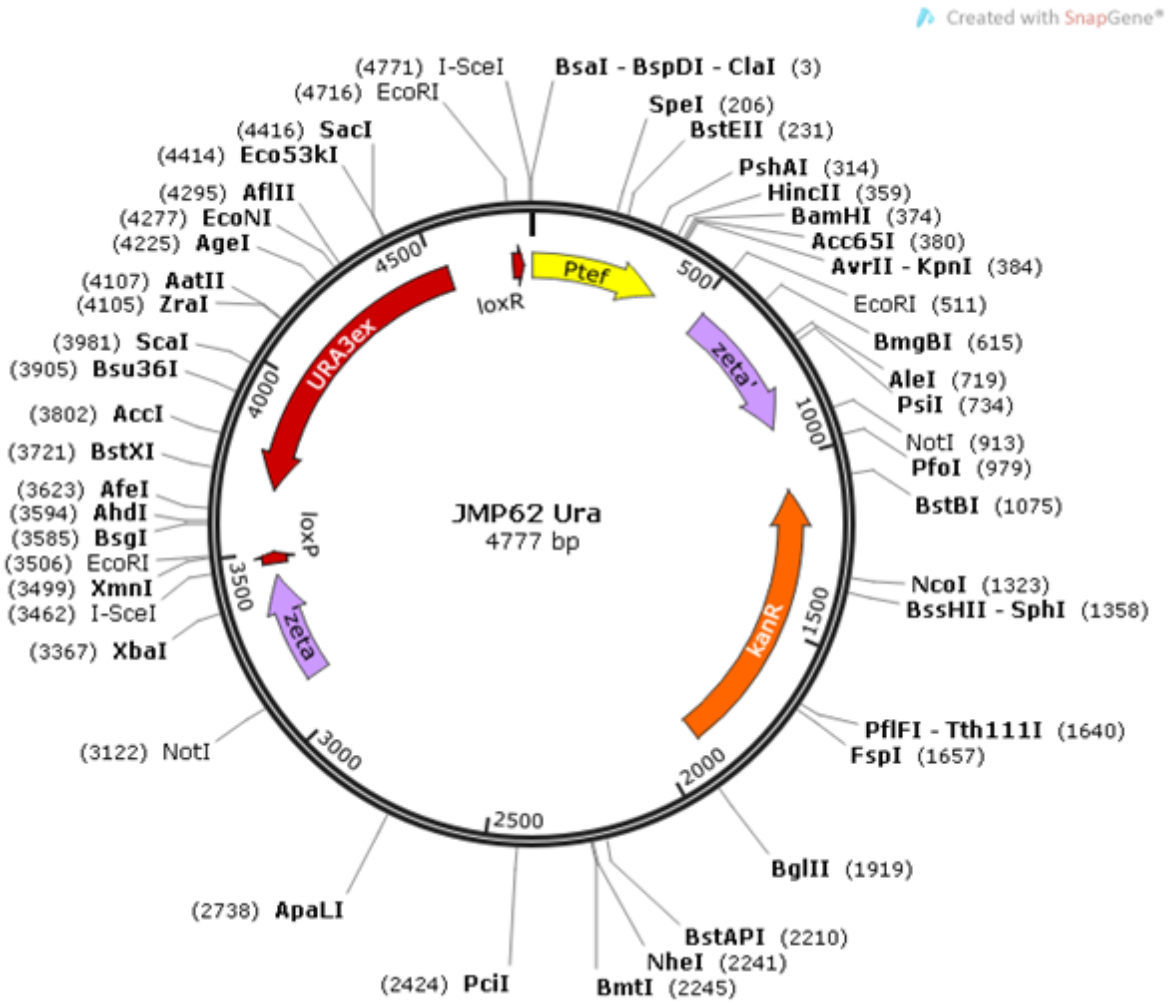
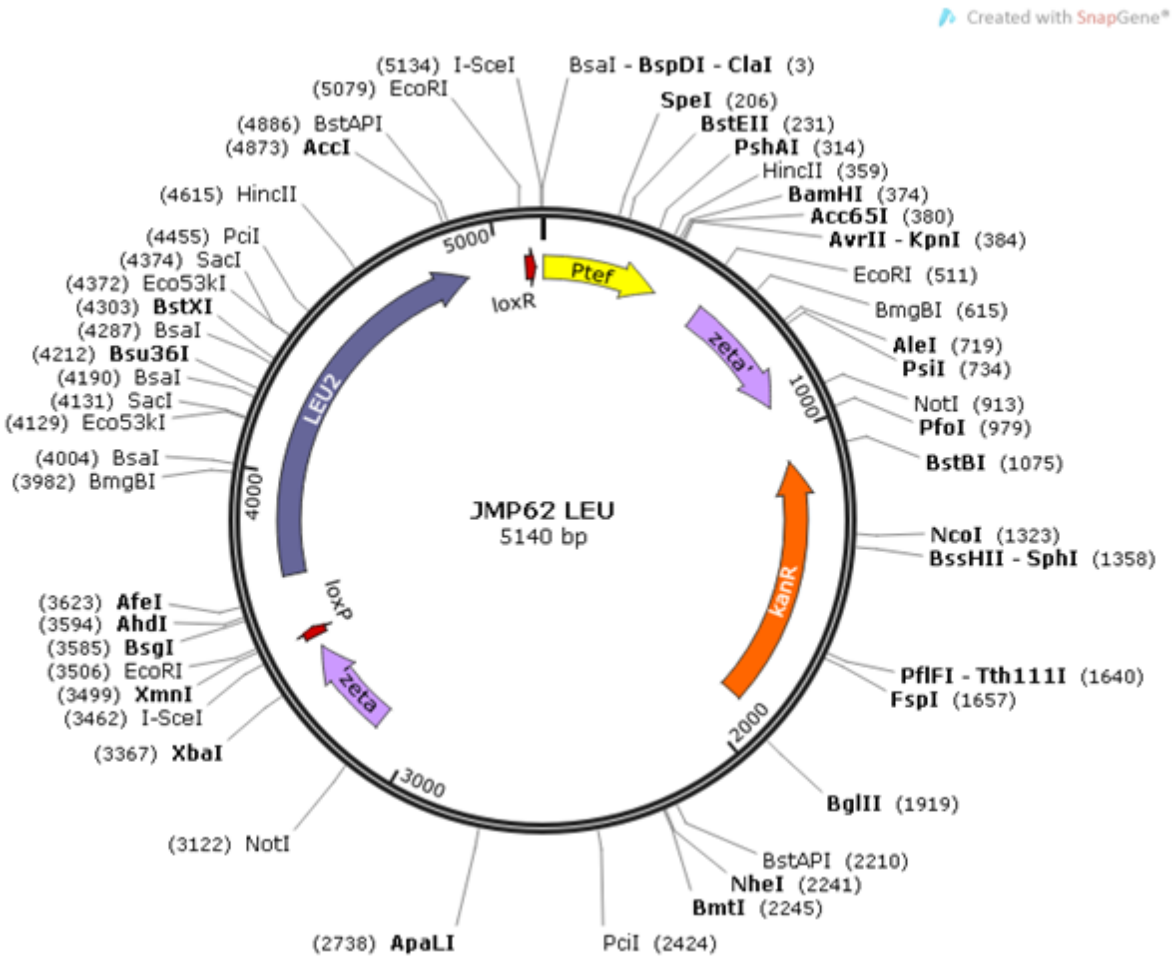
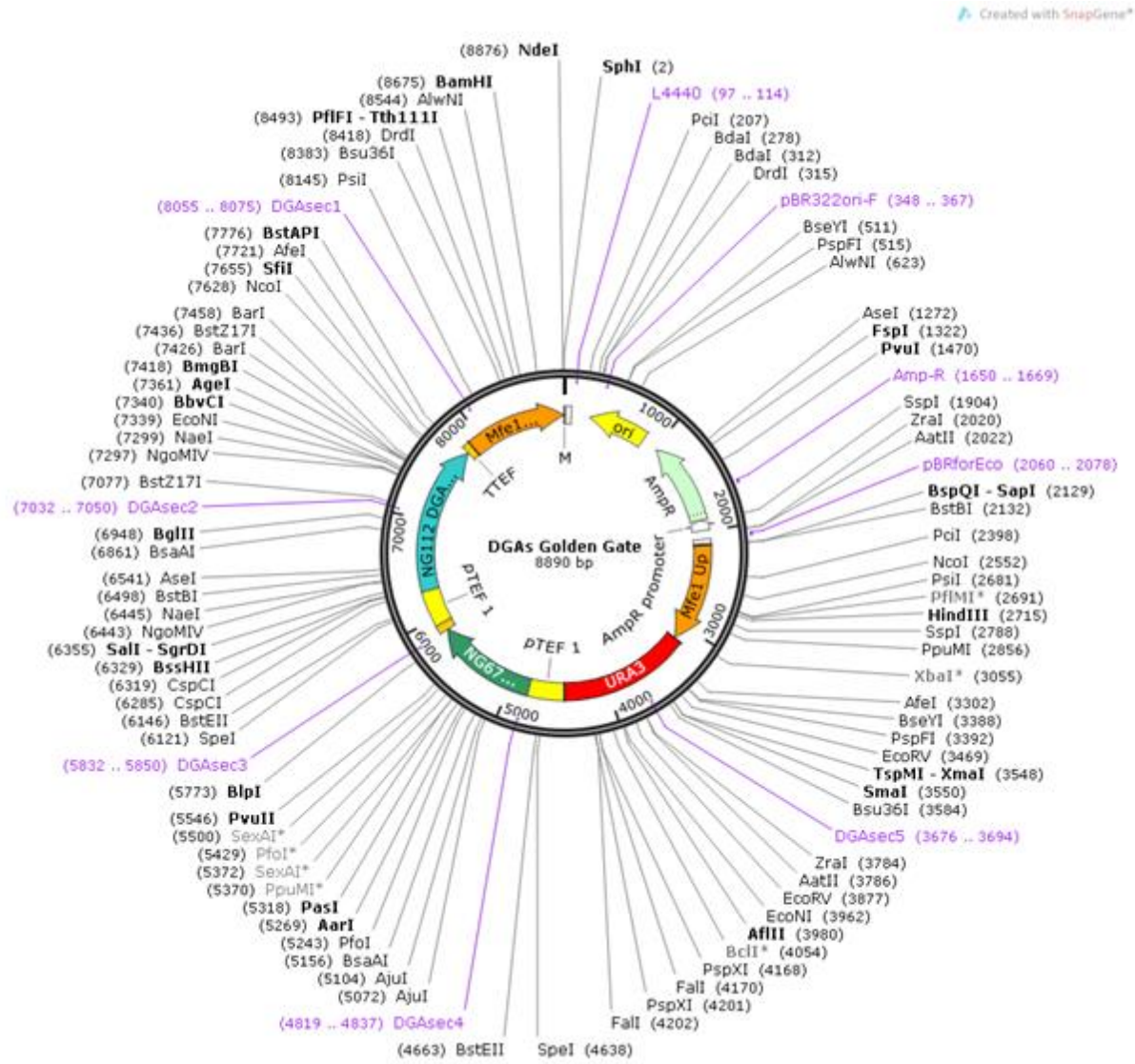
## Map of plasmid JMP62 Ura



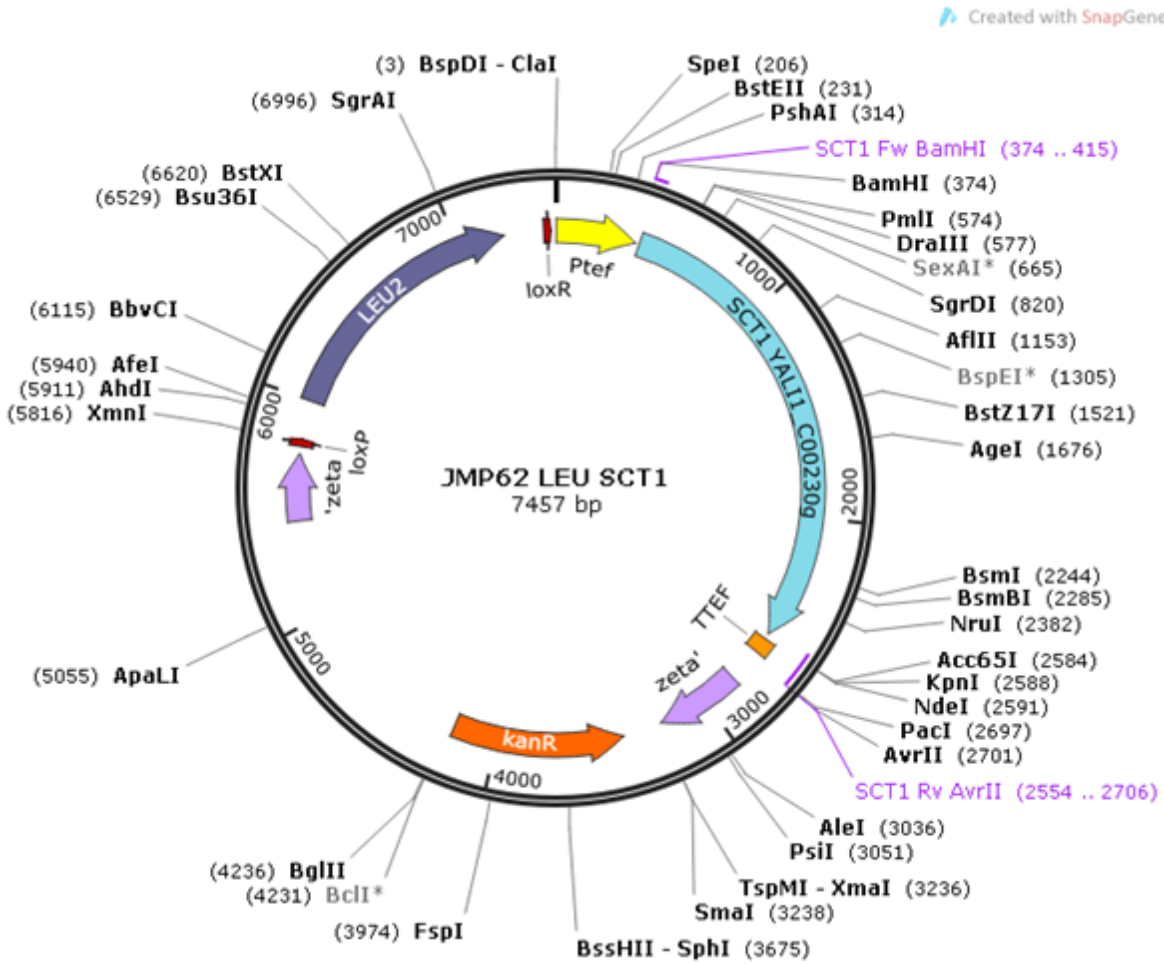
## Map of plasmid JMP62 LEU



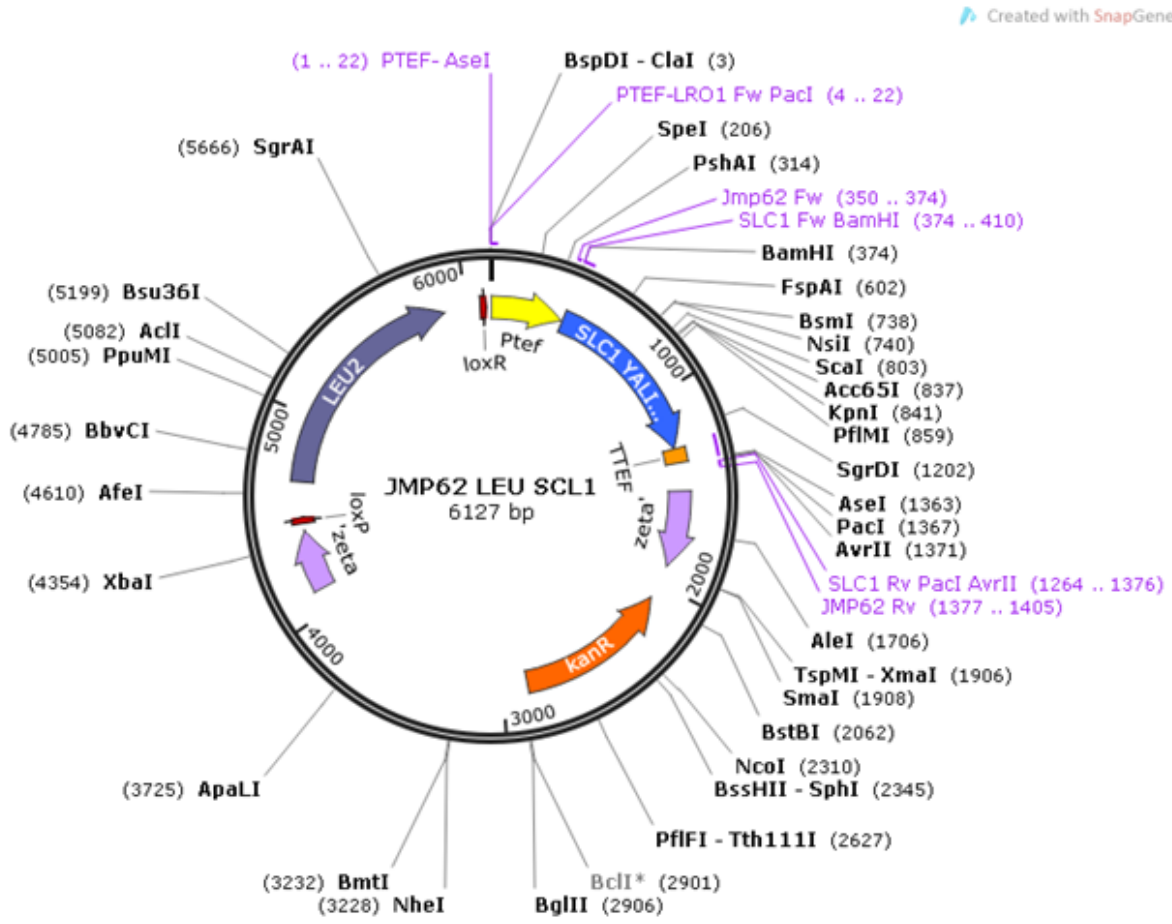
## Map of plasmid DGAs Golden Gate



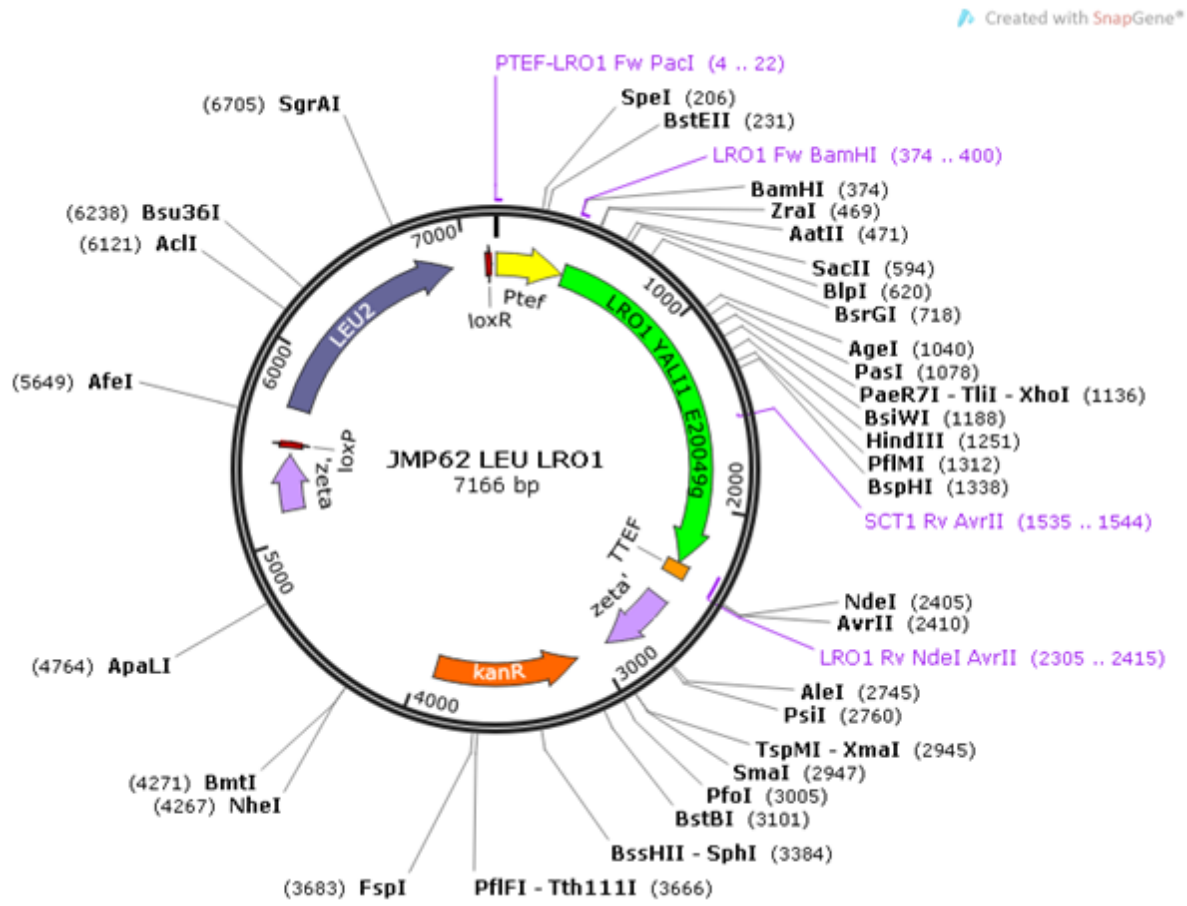
## Map of plasmid JMP62 LEU SCT1



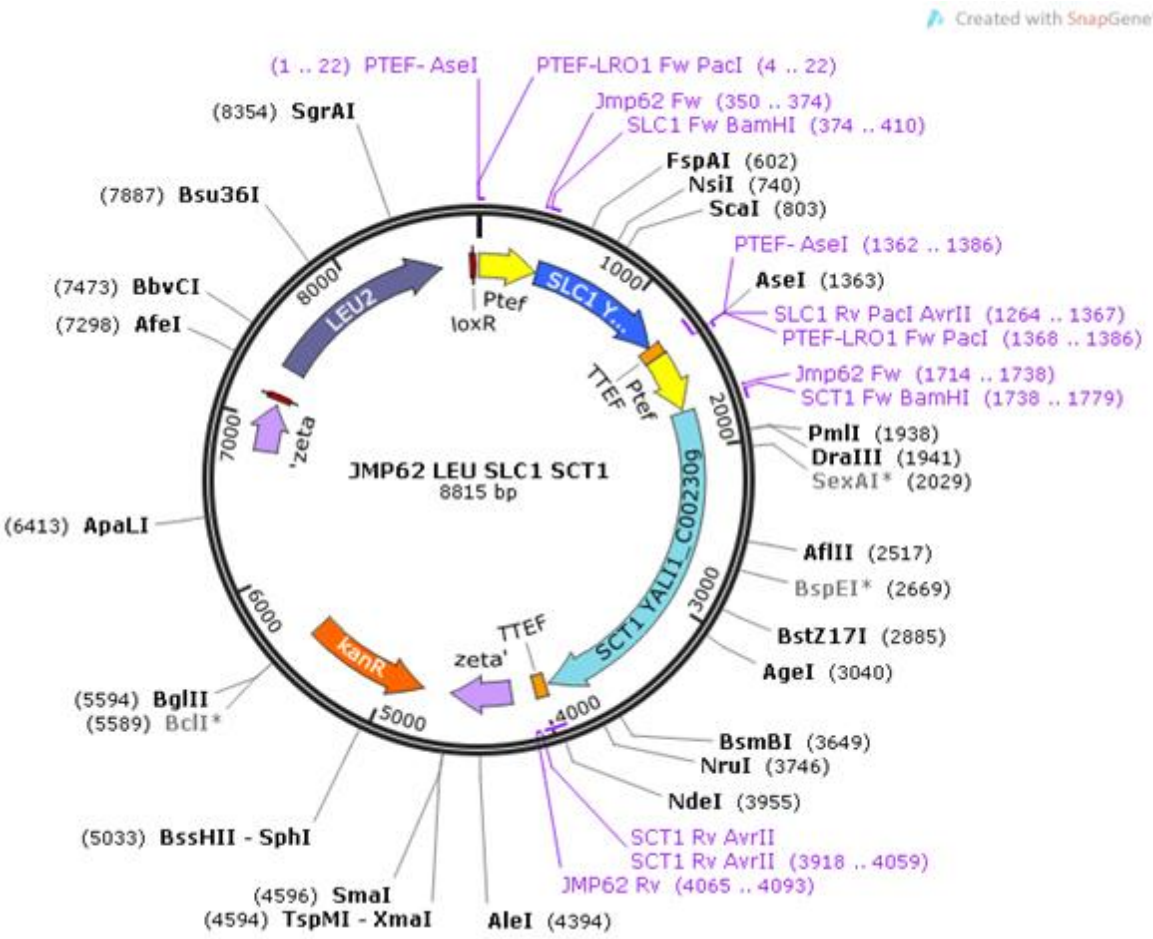
## Map of plasmid JMP62 LEU SCL1



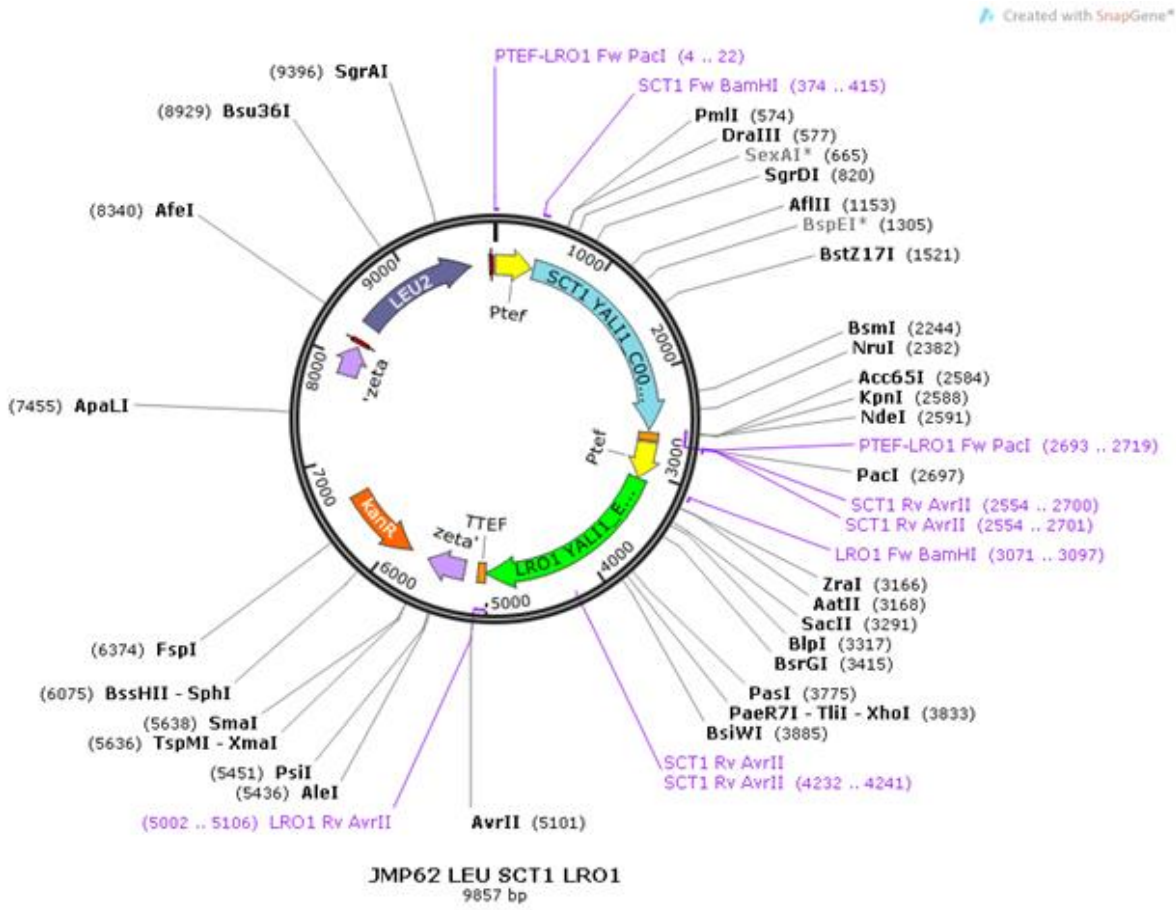
## Map of plasmid JMP62 LEU LRO1



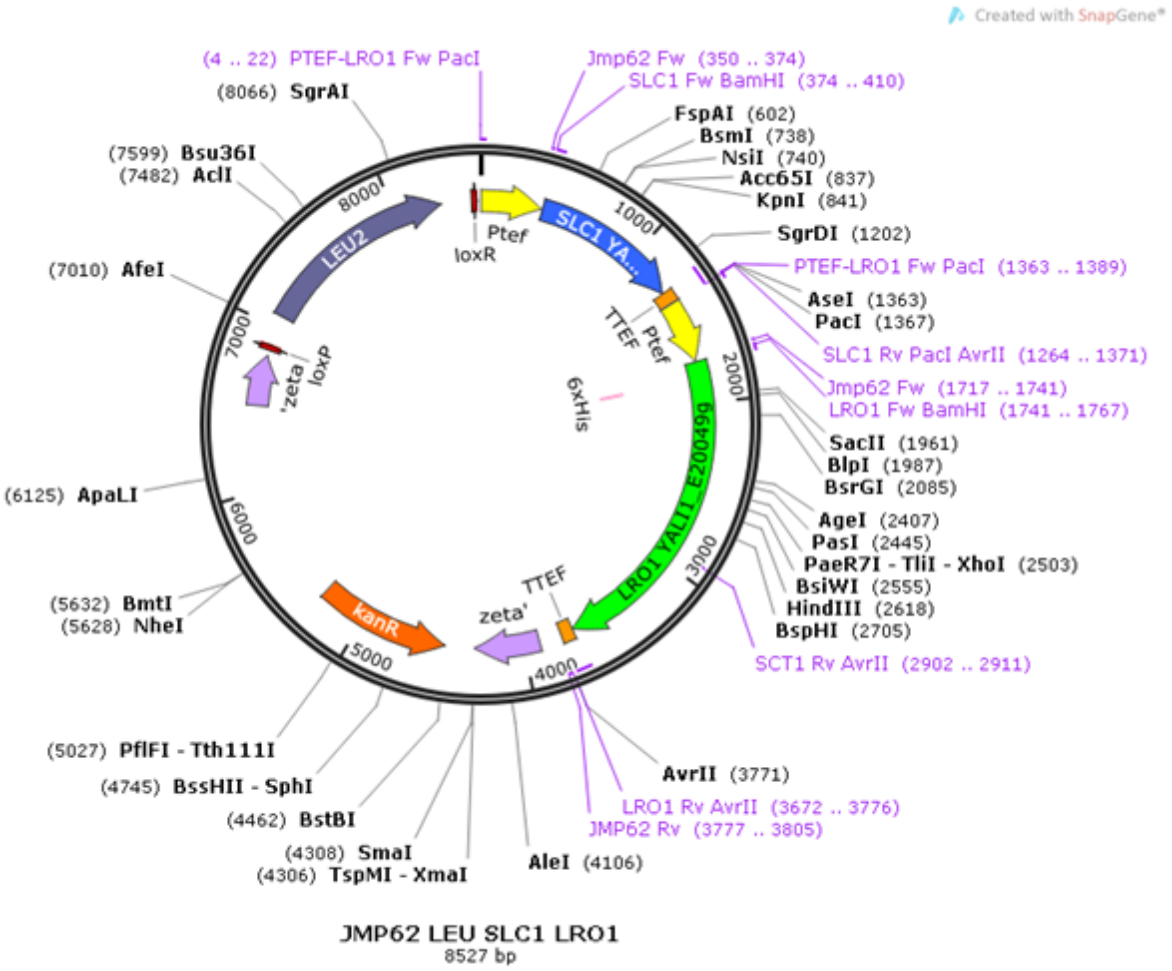
## Map of plasmid JMP62 LEU SCL1-SCT1



## Map of plasmid JMP62 LEU SCT1-LRO1

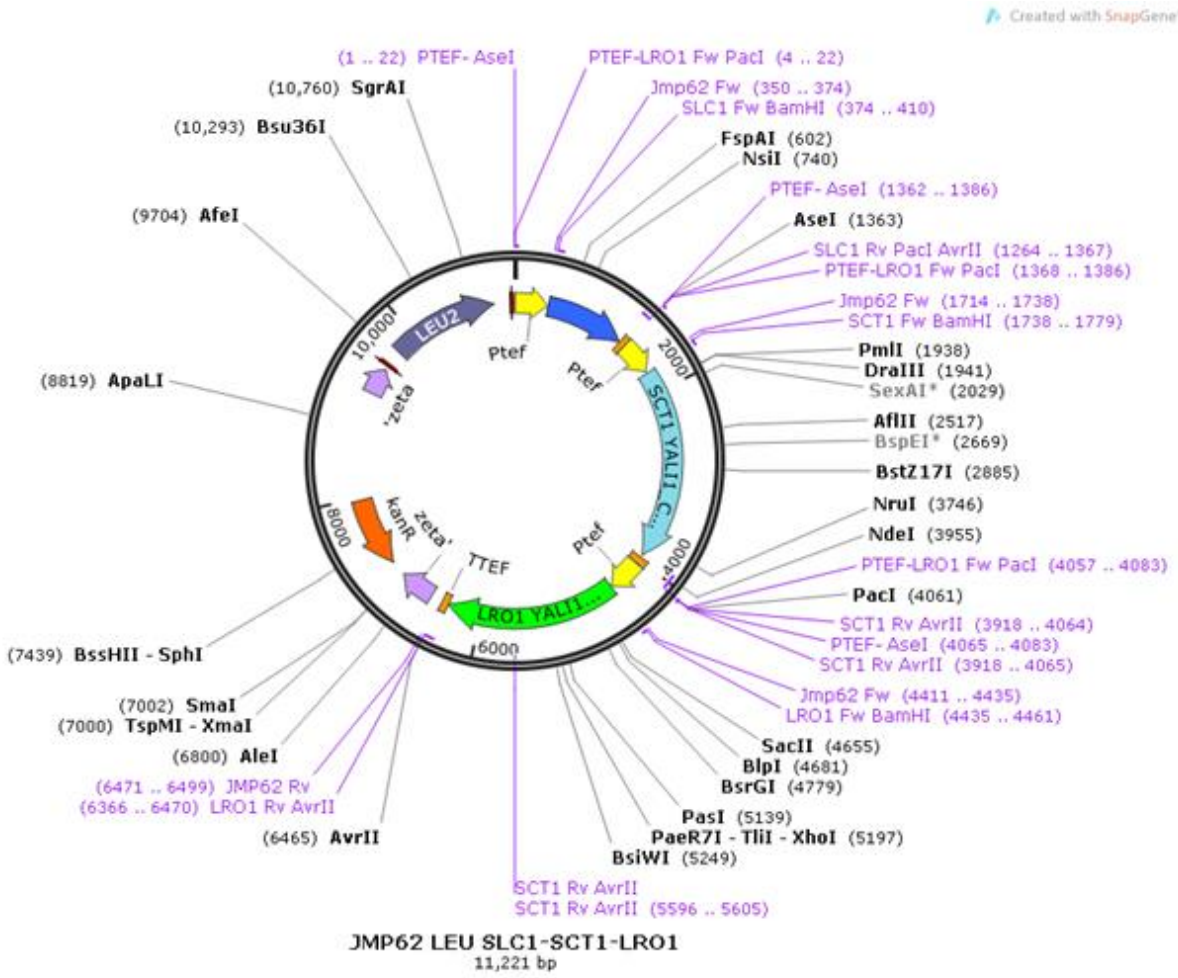


## Map of plasmid JMP62 LEU SCL1- LRO1

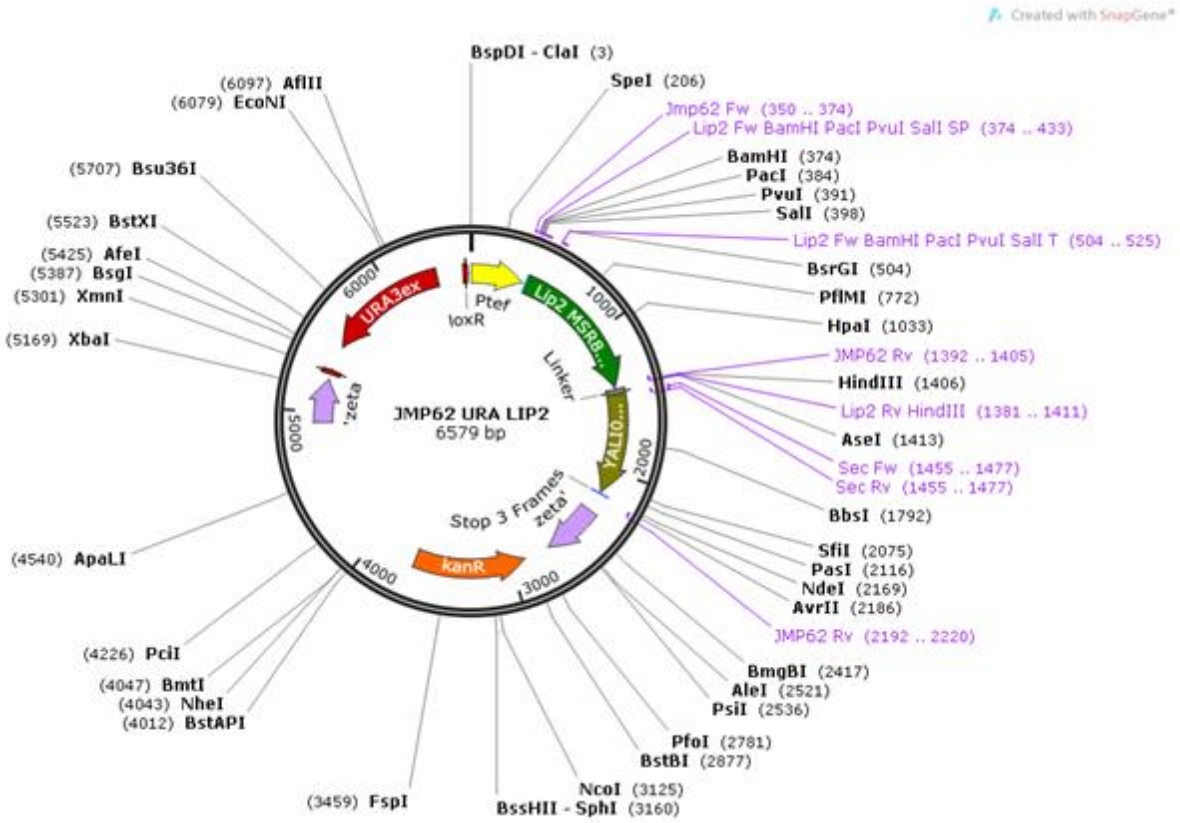



JMP62 LEU SLC1 LRO1
8527 bp

## Map of plasmid JMP62 LEU SCL1-SCT1-LRO1



## Map of plasmid JMP62 URA LIP2



## Map of plasmid JMP62 URA OPE