

# Improving the homologous recombination efficiency of *Yarrowia lipolytica* by grafting the heterogenous component from *Saccharomyces cerevisiae*

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## Abstract

The oleaginous non-conventional yeast *Yarrowia lipolytica* has enormous potential as a microbial platform for the synthesis of various bioproducts. However, while the model yeast *Saccharomyces cerevisiae* has very high homologous recombination (HR) efficiency, non-homologous recombination is dominant in *Y. lipolytica*, and foreign genes are randomly inserted into the genome. Consequently, the low HR efficiency greatly restricts the genetic engineering of this yeast. In this study, RAD52, the key component of the HR machinery in *S. cerevisiae*, was grafted into *Y. lipolytica* to improve HR efficiency. The gene *ade2*, whose deletion can result in a brown colony phenotype, was used as the reporter gene for evaluating the HR efficiency. The HR efficiency of *Y. lipolytica* strains before and after integrating the *ScRad52* gene was compared using insets with homology arms of different length. The results showed that the strategy could achieve gene targeting efficiencies of up to 95% with a homology arm length of 1000 bp, which was 6.5 times of the wildtype strain and 1.6 times of the traditionally used *ku70* disruption strategy. This study will facilitate the further genetic engineering of *Y. lipolytica* to make it a more efficient cell factory for the production of value-added compounds.

## 1 Introduction

The oleaginous non-conventional yeast *Yarrowia lipolytica*, a generally recognized as safe (GRAS) microorganism, is an attractive production host with great promise for industrial applications [1-4]. It has been used as a model organism to study the biosynthesis of peroxisomes, the accumulation of lipids, as well as the production and secretion of organic acids and heterologous proteins [5-7]. Due to its ability to grow using hydrophobic carbon sources, as well as generate and accumulate large amounts of lipids, it is widely used as a production host for valuable bioproducts, such as organic acids, omega-3 eicosapentaenoic acid, and other fatty acid derivatives [7-10]. In addition, it can secrete high levels of natural and heterologous proteins and has been used to produce various heterologous proteins, including proteases, lipases, and RNases [6, 11].

Targeted deletion or insertion of genes and functional nucleic acid sequences at the target sites of a microbial genome is not only an effective method to study the gene function and regulate gene expression, but also a basic tool for genome editing for strain improvement. With the deciphering of the whole-genome sequence of *Y. lipolytica* [12], a series of platforms for molecular biology and genetics were established in this yeast, including transformation methods for gene deletion and integration [13, 14], which made it possible to redesign whole metabolic pathways [15]. However, in *Y. lipolytica*, the vast majority of foreign genes are randomly inserted into the genome, and the probability of targeted insertion by homologous recombination

(HR) is extremely low [16-18]. When the 5'- and 3'-flanking regions of the target gene are 0.5-1.0 kb long, HR occurs only at a rate of 0-36% [19]. This is due to the fact that *Y. lipolytica* has a dominant non-homologous end joining (NHEJ) recombination pathway that it uses to repair the DNA double strand breaks (DSB) rather than the HR pathway [20]. These two repair mechanisms are independent and competitive [21]. Most eukaryotic microorganisms use NHEJ as the main DSB repair pathway, and knocking out or destroying NHEJ-related genes is an effective strategy to increase the efficiency of gene targeting. However, many studies also showed that disrupting the NHEJ pathway can make the cells prone to mutation, which is not conducive to industrial application [22, 23]. Moreover, the cell growth can be severely affected under non-optimal conditions, such as high temperature, ultraviolet irradiation and the presence of chemical DNA-damaging agents [24, 25]. In addition, in strains lacking components of the NHEJ pathway, the integration efficiency is highly dependent on the targeted gene locus.

Consequently, strengthening the HR repair is also an effective approach for improving the gene targeting efficiency. The HR repair pathway is mediated by a class of conserved enzymes called DNA recombinase enzymes, most of which were initially identified in *Saccharomyces cerevisiae* [26]. Notably, the RAD51/RAD52 complex is a major participant in the targeted integration of foreign DNA in eukaryotes and plays a key role in the HR pathway [27] (Fig. 1). *S. cerevisiae* has very high HR efficiency (up to 100%), and ScRAD52 has been identified as an important component that plays a vital role in mitotic and meiotic recombination in this organism [28-30]. The heterologous expression of *S. cerevisiae* HR related genes such as *ScRad51* and *ScRad52*, in mammalian cells (e.g., monkey embryonic cells) and plant cells (e.g., *Arabidopsis*), or its combination with the CRISPR/Cas9 system, can greatly increase the efficiency of HR and reduce the off-target effects [31-36].

Here, we focused on improving the HR efficiency of *Y. lipolytica* by grafting heterologous component that is crucial for the HR mechanisms of *S. cerevisiae*. Since *ScRad52* is a key repair gene mainly responsible for DSB repair, we hypothesized that heterologous expression of the *Sc RAD52* protein in *Y. lipolytica* would improve its HR efficiency. To validate this concept, the *ScRad52* gene was first heterologously expressed in *Y. lipolytica* and the HR efficiency was examined by knocking out the adenosine succinic acid synthase gene (*ade2*, YALI0B23188g) using cassettes with homology arms of different lengths. The corresponding endogenous gene of *Y. lipolytica* (*YlRad52*, YALI0F02431g) was further knocked out to explore its role in *Y. lipolytica* and whether the heterologous expression of ScRAD52 could replace the function of the native YlRAD52 protein. This work provides an effective strategy for precise genome editing, which may contribute to the molecular biology and genetics of *Y. lipolytica* and will greatly facilitate the rapid development of genome editing in other similar microorganisms.

## 2 Materials and methods

### 2.1 Strains and culture conditions

*Y. lipolytica* Po1f (ATCC MYA-2613), a leucine and uracil auxotrophic strain, was used as the base strain for all genome editing and expression experiments in this study. The *ku70*-disrupted *Y. lipolytica* strain Po1f- $\Delta ku70$  was constructed using the URA3-blaster method and a *ku70* deletion cassette as described by Jang et al. [16]. *Escherichia coli* DH5 $\alpha$  was used for plasmid construction and propagation. It was grown in Luria-Bertani medium supplemented with 100 mg/L ampicillin at 37°C. Yeast cultures were grown in YPD medium (20 g/L glucose, 10 g/L yeast extract, and 20 g/L tryptone) at 28 °C. The synthetic complete medium without uracil (SC-Ura) plates contained 20 g/L glucose, 6.7 g/L yeast nitrogen base (YNB) without amino acids (BBI, Sangon Biotech (Shanghai) Co. Ltd., China), 0.77 g/L CSM-Ura (Complete supplement mixture minus uracil, MP Biomedicals, USA), and 20 g/L agar. The synthetic complete medium without leucine (SC-Leu) plates contained 20 g/L glucose, 6.7 g/L YNB without amino acids (BBI, Sangon Biotech (Shanghai) Co. Ltd., China), 0.77 g/L CSM-Leu (Complete Supplement Mixture minus leucine, MP Biomedicals, USA), and 20 g/L agar. The URA3 maker was removed and selected against on SC+FOA plates, which contained 6.7 g/L YNB, 0.77 g/L CSM (Complete Supplement Mixture, MP Biomedicals, USA), 0.8 g/L 5-fluoroorotic acid, and 20 g/L agar.

## 2.2 General molecular biology techniques

The plasmids and primers used in this study are listed in Table 1 and Supplementary Table 1. Genomic DNA from the yeast strains was purified using an E.Z.N.A.<sup>®</sup> Yeast DNA Kit (Omega Bio-Tek Inc., USA). The transformation of *Y. lipolytica* was performed using the Frozen-EZ Yeast Transformation II Kit (Zymo Research, Orange, CA). Gene deletion was performed by HR based on direct repeats, using URA3 as a counter-selection marker on SC+FOA plates. The recombinant strains were spread on SC+FOA plates and cultured for 2-3 days. Strains expressing URA3 did not grow in the presence of 5-FOA.

## 2.3 Construction of expression cassettes expressing *ScRad52*

The *S. cerevisiae Rad52* sequence (GenBank accession No. CAA86623.1) was codon-optimized for expression in *Y. lipolytica* and ordered as fully synthetic DNA (GeneScript, Nanjing, China, Supplementary Table 2). The vector pUC-Leu-A08-ScRad52 expressing the gene of interest under the control of the endogenous Rad52 gene promoter P<sub>YlRad52</sub> and terminator T<sub>YlRad52</sub> as generated using the ClonExpress<sup>™</sup> MultiS one step cloning kit (Vazyme, Nanjing, China), according to the manufacturer's instructions. The promoter P<sub>YlRad52</sub> and terminator T<sub>YlRad52</sub> were respectively amplified by PCR using the primer pairs PYlRad52-F/R and TYlRad52-F/R with genomic DNA extracted from *Y. lipolytica*. The codon-optimized *ScRad52* gene was then amplified using the primers ScRad52-F/R. The PCR-amplified sequences were digested with *Pac* I and *Sna* BI, and then one-step cloned into the digested pUC-Leu-A08 backbone to generate the recombinant vector pUC-Leu-A08-ScRad52. We used the A08 gene site in the *Y. lipolytica* genome whose disruption is known to not affect cell growth [37]. All the newly constructed plasmids were confirmed by DNA sequencing.

## 2.4 Construction of disruption cassettes

Disruption cassettes targeting *YlRad52* (YALI0F02431g) and *ade2* (YALI0B23188g) were constructed according to the URA3-blaster protocol [16]. For the construction of the YlRad52 deletion cassette, 2 kb 5'- and 3'- homology arms of the *YlRad52* gene, named YlRad52-up and YlRad52-down, were respectively amplified from *Y. lipolytica* genomic DNA using the primer pairs YlRad52-up-F/R and YlRad52-down-F/R. The resulting PCR fragments were respectively digested with *Eco* RI and *Pac* I, and one-step cloned into the pUC-HUH vector containing the HisG-URA3-HisG (HUH) blaster cassette, to generate the vector pUC-HUH-*YlRad52*.

For the construction of the *ade2* deletion cassettes with 5'- and 3'-homology arms of 1000, 500, 250 and 100 bp, the homology fragments were respectively amplified from the yeast genomic DNA using the primer combinations 1000-up-F, 500-up-F, 250-up-F, 100-up-F/Ade2-up-R and Ade2-down-F/1000-down-R, 500-down-R, 250-down-R, 100-down-R. The obtained PCR fragments were respectively digested with *Eco* RI and *Pac* I, and one-step cloned into the pUC-HUH vector containing the HisG-URA3-HisG blaster cassette, to generate the vectors of pUC-HUH- $\Delta$ ade2-1000, pUC-HUH- $\Delta$ ade2-500, pUC-HUH- $\Delta$ ade2-250, and pUC-HUH- $\Delta$ ade2-100.

## 2.5 Determination of the homologous recombination efficiency

The *ade2* gene was selected as the reporter, because its deletion causes the accumulation of phosphoribosyl amino imidazole, resulting in the disrupted mutants becoming brown (Supplementary Fig. 1). The HR efficiency was estimated by counting the number of *ade2*<sup>-</sup> colonies arising from cells transformed with the *ade2* disruption cassettes with different homology arm lengths (100, 250, 500 and 1,000 bp) [49]. The HR efficiency at the *ade2* locus was determined via the ratio of brown colonies. Transformation with the disruption cassettes was repeated three times.

## 3 Results

### 3.1 Construction of recombination strains expressing ScRad52

The expression cassette encoding the *ScRad52* gene driven by the endogenous promoter P<sub>YlRad52</sub> and URA3-blaster expression cassette were integrated into the A08 locus in the genomes of the *Y. lipolytica* strains Po1f and Po1f- $\Delta$ ku70. The resulting strains *Y. lipolytica* Po1f-ScRad52 and Po1f- $\Delta$ ku70-ScRad52 were successfully

selected on SC-Ura plates, and further confirmed by colony PCR as well as genomic DNA isolation followed by PCR. Microscopic examination and colony morphology observation throughout the growth cycle indicated that the engineered *Y. lipolytica* was not morphologically different from the parental strain.

### 3.2 *ScRad52* expression enhanced the homologous recombination efficiency

To explore the effect of heterologous expression of the *ScRad52* gene on the HR efficiency of *Y. lipolytica*, we selected genes whose deletion results in significant phenotypic changes (e.g. color, fluorescence, etc.) as target sites. In yeast, the adenylosuccinate synthetase gene *ade2* is responsible for the conversion of phosphoribosyl aminoimidazole (AIR) into 5-amino 4-carboxyimidazole ribose (CAIR). The deletion of *ade2* causes the accumulation of AIR, which turns the corresponding yeast colonies brown (Fig. 2). This phenotype was used for easy visual selection in both *S. cerevisiae* and *Y. lipolytica* [38-40]. Therefore, the *ade2* was chosen as the target site for gene knockout in this study.

By using the URA3 blaster method with homology arms of different targeting the *ade2* gene as a single variable, knockout plasmids containing different homology arm lengths were constructed via a single-fragment one-step assembly method. After the recombinant plasmid was linearized and introduced into *Y. lipolytica*, the positive transformants were screened according to their visible phenotype and confirmed using colony PCR. The HR efficiency of *Y. lipolytica* strains, including Po1f-*ScRad52* and Po1f- $\Delta$ ku70-*ScRad52*, as well as the parental strains Po1f and Po1f- $\Delta$ ku70, was obtained by calculating the ratio of the colonies with *ade2* knockout to the total number of strains on the plate, that is, the ratio of brown mutants.

As shown in Table 2, when the *ade2* knockout was performed with homology arms of 1000 bp, HR efficiency of the engineered strains *Y. lipolytica* Po1f-*ScRad52* and Po1f- $\Delta$ ku70-*ScRad52* with heterologous HR components from *S. cerevisiae* reached 90% and 95%, respectively. By contrast, the HR efficiency of the control strains *Y. lipolytica* Po1f and Po1f- $\Delta$ ku70 was only 14.7% and 60%, respectively. The best strain with the highest HR efficiency for *ade2* deletion was *Y. lipolytica* Po1f- $\Delta$ ku70-*ScRad52*. Its HR efficiency reached up to 95%, which was 6.5 times of the wild-type strain *Y. lipolytica* Po1f (Table 3). These results indicated that *ScRad52*, the main component of the HR machinery in *S. cerevisiae*, was functional and improved the HR efficiency in *Y. lipolytica*, simplifying genome editing.

### 3.3 Effect of homology arm length on homologous recombination efficiency

Disruption cassettes with homology arms of 1000, 500, 250, 100 and 50 bp targeting the *ade2* were constructed. As shown in Table 2, when the disruption cassette with 1000 bp homology regions was used for *ade2* deletion, the percentage of transformants generated by HR in the strains Po1f-*ScRad52* and Po1f- $\Delta$ ku70-*ScRad52* reached 60% and 95%, respectively. However, when the length of homology arms was less than 500 bp, the HR efficiency dropped sharply. At 100 bp, the HR efficiency of *Y. lipolytica* Po1f-*ScRad52* and Po1f- $\Delta$ ku70-*ScRad52* decreased to 6.1% and 11.9% respectively, which was only 6.8% and 12.5% of that with homology arms of 1000 bp (Fig. 3). The extension of homology arm length on each side of the disruption cassette increased the HR efficiency, which was consistent with previous reports [40-42].

### 3.4 Effect of endogenous *Rad52* disruption on homologous recombination efficiency

In order to further investigate the effect of the endogenous *Rad52* (*YlRad52*) on the HR efficiency, and whether the heterologous *ScRad52* can completely replace *YlRad52* in *Y. lipolytica*, the *YlRad52* gene was knocked out using the URA3 blaster method in the aforementioned *Y. lipolytica* strains. The growth rate of the engineered strains with the *YlRad52* gene knockout was slightly lower than that of the *YlRad52* wild-type strains. In addition, for the two strains Po1f-*ScRad52*- $\Delta$ YlRad52 and Po1f- $\Delta$ ku70-*ScRad52*- $\Delta$ YlRad52, there was almost no difference of colony morphology compared to the parental strains. However, the strains Po1f- $\Delta$ YlRad52 and Po1f- $\Delta$ ku70- $\Delta$ YlRad52 formed smaller and sharper colonies on YPD plates compared with the other two strains Po1f-*ScRad52*-YlRad52 and Po1f- $\Delta$ ku70-*ScRad52*-YlRad52 which express the *ScRad52* gene. This may be explained by the fact that *Rad52* is important for nuclear integrity in *Y. lipolytica*. This result is similar to a report by Campos-Gongora et al. [26]. The expression of the *ScRad52* gene alleviates the damage to nuclear integrity caused by the deletion of the endogenous

*Y. lipolytica*Rad52 to some extent. Consequently, the colony morphology of Po1f-ScRad52- $\Delta$ YIRad52 and Po1f- $\Delta$ ku70-ScRad52- $\Delta$ YIRad52 was not significantly different from the original strains.

The HR efficiency of the strains Po1f-ScRad52- $\Delta$ YIRad52 and Po1f- $\Delta$ ku70-ScRad52- $\Delta$ YIRad52 was significantly lower than the HR efficiency of the strains with their own YIRad52 genes (Table 2 and Fig. 3). When the homology arm length was 1000 bp, the HR efficiency of strain Po1f-ScRad52- $\Delta$ YIRad52 reached 53.3%, which was only 40.8% that of Po1f-ScRad52. Moreover, the HR efficiency of Po1f- $\Delta$ ku70-ScRad52- $\Delta$ YIRad52 was 6.7%, which was even lower than that of the wild-type strain *Y. lipolytica* Po1f. Compared with Po1f- $\Delta$ ku70-ScRad52, the HR efficiency of Po1f- $\Delta$ ku70-ScRad52- $\Delta$ YIRad52 was reduced by 92.9%, and there was practically no recombination with homology arms of 250 and 100 bp. These results show that the *YIRad52* gene plays a crucial role in the native HR mechanism of *Y. lipolytica*, which is consistent with the previous report that the Rad52 gene is crucial for DNA repair in this yeast [26].

## 4 Discussion

With the development of artificial nucleases in recent years, the possibilities of performing accurate genome editing based on HR have been greatly expanded. Although the efficiency is still relatively low, double-strand breaks can be introduced at practically any target sites, but there are great differences in knockout efficiency for different organisms [43, 44]. RAD52 protein is considered to be the crucial recombinase of the HR pathway. Previous studies have reported that integrating the *S. cerevisiae* RAD52 can significantly enhance the efficiency of HR in mammalian cells [36]. In the present study, the gene encoding RAD52 from *S. cerevisiae* was codon-optimized and expressed in *Y. lipolytica*. The results showed that the heterologous expression of *ScRad52* significantly improved the HR efficiency of *Y. lipolytica*, and the endogenous *Rad52* gene also plays a crucial role in the *Y. lipolytica* HR mechanism. When the homology arm length was 1000 bp, the HR efficiency of the strains, Po1f, Po1f- $\Delta$ ku70, Po1f-ScRad52, and Po1f- $\Delta$ ku70-ScRad52 was 14.7%, 60%, 90%, and 95%, respectively. The highest HR efficiency was observed in the strain Po1f- $\Delta$ ku70-ScRad52, and it was 6.5 times of the wildtype strain Po1f as well as 1.6 times of the traditionally used *ku70* disruption strategy. However, when the endogenous RAD52 gene (YIRad52) of *Y. lipolytica* was knocked out, the HR efficiency was reduced. Although the mechanism of HR mediated by YIRad52 is currently unclear, the results show that it plays a very important role in nuclear integrity and DSB repair. Interestingly, the HR efficiency of the Po1f- $\Delta$ ku70-ScRad52- $\Delta$ YIRad52 strain was lower than that of Po1f-ScRad52- $\Delta$ YIRad52, which may be due to the deletion of the KU70 protein related to the NHEJ mechanism in this strain, further compromising nuclear integrity and DNA repair in this strain compared to Po1f-ScRad52- $\Delta$ YIRad52. In addition, in the engineered strain with the YIRad52 knockout, the increase of homology arm length improved the HR efficiency. Therefore, HR mediated by YIRAD52 plays a very important role in nuclear integrity and DNA repair.

In conclusion, the HR efficiency of *Y. lipolytica* was improved by integrating the key gene *ScRad52* governing HR in *S. cerevisiae*. The HR efficiency of the best engineered strain Po1f- $\Delta$ ku70-ScRad52 reached 95% with 1000 bp homology arms. Additionally, the results proved that the endogenous YIRAD52 plays an important role in HR. Generally, most industrially important non-model eukaryotic microorganisms have a complicated genetic background and are inherently inefficient in terms of HR, making it difficult to achieve large-scale, high-throughput gene editing in these cells. This study provides a reference for improving the HR efficiency of *Y. lipolytica* and other similar eukaryotic microorganisms, which can further accelerate the genome editing and metabolic engineering of important industrial hosts.

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## Conflict of interest

The authors declare no financial or commercial conflict of interest.

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**Table 1. Strains and plasmids used in this study**

Strains or plasmids	Genotypes or properties
<b>Strains</b>	
<i>E. coli</i> DH5 $\alpha$	supE44 [?]lacU169 ( $\phi$ 80 lacZ [?]M15) hsdR17 recA1 endA1 gyrA96 thi-1 relA
<i>Y. lipolytica</i> Po1f (ATCC MYA-2613)	MATa, leu2-270, ura3-302, xpr2-322, axp1-2
<i>Y. lipolytica</i> Po1f- $\Delta$ ku70	Po1f, $\Delta$ ku70
<i>Y. lipolytica</i> Po1f-ScRad52	Po1f, <i>ScRad52</i> ::A08
<i>Y. lipolytica</i> Po1f- $\Delta$ ku70-ScRad52	Po1f, $\Delta$ ku70, $\Sigma$ 5 <i>Pa</i> $\delta$ 52 ::A08
<i>Y. lipolytica</i> Po1f- $\Delta$ YlRad52	<i>Il</i> o1 $\phi$ , $\Delta$ $\Psi$ $\lambda$ <i>Pa</i> $\delta$ 52
<i>Y. lipolytica</i> Po1f- $\Delta$ ku70- $\Delta$ YlRad52	Po1f, $\Delta$ ku70, $\Delta$ $\Psi$ $\lambda$ <i>Pa</i> $\delta$ 52
<i>Y. lipolytica</i> Po1f-ScRad52 - $\Delta$ YlRad52	Po1f, $\Sigma$ 5 <i>Pa</i> $\delta$ 52 ::A08, $\Delta$ $\Psi$ $\lambda$ <i>Pa</i> $\delta$ 52
<i>Y. lipolytica</i> Po1f- $\Delta$ ku70-ScRad52-YlRad52	Po1f, $\Delta$ ku70, $\Sigma$ 5 <i>Pa</i> $\delta$ 52 ::A08, $\Delta$ $\Psi$ $\lambda$ <i>Pa</i> $\delta$ 52
<b>Plasmids</b>	
pUC57	<i>ColE1 Ori</i> , <i>LacZ</i> , <i>Amp</i>
pUC-Leu-A08	A08 upstream and downstream homology arms and Leu auxotrophic selection
pUC-Leu-A08-ScRad52	P <sub>YlRad52</sub> -ScRad52 -T <sub>YlRad52</sub>
pUC-HUH	HisG-URA3-HisG (HUH) in pUC57
pUC-HUH- $\Delta$ YlRad52	YlRad52-up-HUH-YlRad52-down
pUC-HUH- $\Delta$ ade2-100	ade2-100-up-HUH-ade2-100-down
pUC-HUH- $\Delta$ ade2-250	ade2-250-up-HUH-ade2-250-down
pUC-HUH- $\Delta$ ade2-500	ade2-500-up-HUH-ade2-500-down
pUC-HUH- $\Delta$ ade2-1000	ade2-1000-up-HUH-ade2-1000-down



**Table 2.** Homologous recombination efficiency of different *Y. lipolytica* strains using different homology arm lengths.

<i>Y. lipolytica</i> strains	HR efficiency with different homology arm length (%)	HR efficiency with different homology arm length (%)
	100 bp	250 bp
Po1f- <i>ku70</i> – <i>ScRad52</i> – <i>YlRad52</i>	0	0
Po1f	0	2
Po1f- <i>ScRad52</i> - <i>YlRad52</i>	1.79	11.9
Po1f- <i>ku70</i>	3.3	13.3
Po1f- <i>ScRad52</i>	6.1	22.6
Po1f- <i>ku70</i> – <i>ScRad52</i>	11.9	32.8

### Figure legends

**Figure 1.** Pathways of DNA double-strand break (DSB) repair by homologous recombination (HR) and non-homologous end joining (NHEJ) in *Y. lipolytica*. *Y. lipolytica* is more inclined to use the NHEJ pathway for DSB repair rather than the HR pathway. Destroying NHEJ-related proteins (KU70/KU80) can increase the efficiency of gene targeting. The RAD51/RAD52 complex plays a key role in the HR pathway.

**Figure 2.** Screening method based on deleting the adenylosuccinate synthetase gene (*ade2*) involved in adenine biosynthesis in *Y. lipolytica*. The deletion of the *ade2* gene results in a brown colony phenotype. PRPP: phosphoribosyl pyrophosphate; PRA: 5-phosphoribosylamine, GAR: glycine ribotide, FGAR: formyl glycine ribotide, FGAM: formyl glycine ribotide, AIR: phosphoribosyl aminoimidazole, CAIR: 5-amino 4-carboxyimidazole ribotide.

**Figure 3.** Homologous recombination efficiency of wild-type *Y. lipolytica* Po1f and different engineered strains. Disruption cassettes with different homology arm lengths targeting the adenylosuccinate synthetase encoding gene (*ade2*) were constructed. The length of the homology arms from left to right is 100, 250, 500, and 1000 bp. The *S. cerevisiae* derived *ScRad52* was integrated into the genome of the *Y. lipolytica* strains Po1f and Po1f-*ku70*. Additionally, *YlRad52* was knocked out to investigate the function of endogenous *Rad52* in *Y. lipolytica*. Homology arms of d



