Construction of an IGR IRES-mediated reporter gene expression system based on RNA polymerase I for large scale screening of high-nucleic acid in *Saccharomyces cerevisiae*

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• Introduction

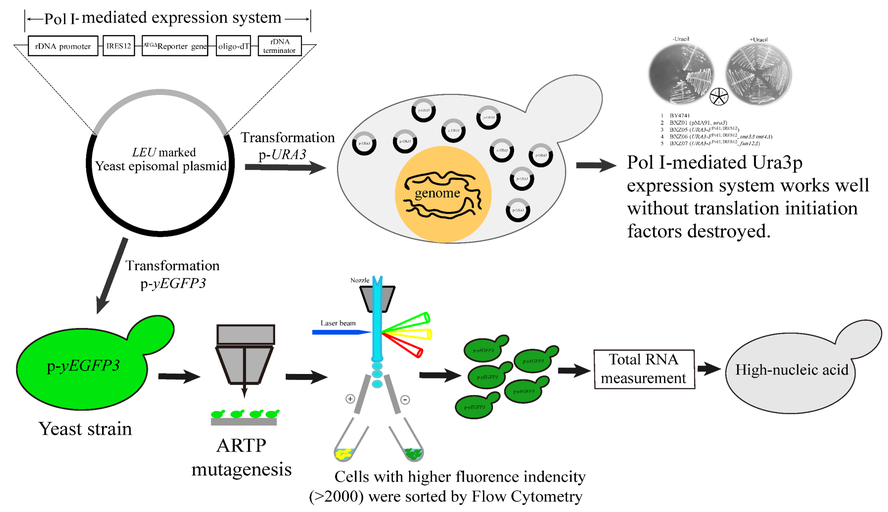
• Derivatives from Ribonucleic acid (RNA) are widely used in food and medicine homologous. rRNA, which is the most abundant RNAs, was synthesized by the tight regulation of RNA polymerase I (RNA Pol I) and other factors.

• *Saccharomyces cerevisiae* is recognized as the ideal source of RNA. At present, RNA is mainly extracted from discarded beer yeast. Affected by the raw material supply, RNA production is far from meeting market demand in China. Therefore, breeding of yeast with high RNA content is the basic measure to solve this problem. However, RNA extraction determination procedure limited the implement of above strategy. There is an urgent need to develop effective methods for large scale screening of high nucleic acid yeast.

• Since RNA Pol I does not contain an extra C-terminal domain (CTD), its transcript (rRNA) cannot be added with 5' cap and 3' poly (A) tail, and therefore cannot be translated into proteins. The intergenic region (IGR) IRES (internal ribosome entry site) from *cricket paralysis virus* does not require any translation initiation factors to initiate protein translation.

• In this study, we constructed an IGR IRES-mediated GFP expression system based on RNA Pol I in *S. cerevisiae* to reflect the intracellular change of RNA content by the fluorescence intensity change of GFP, and established a high throughput screening system based on fluorescent-activated cell sorting (FACS).

• Ideas and design



• Conclusions

• We designed and constructed of an IGR IRES-mediated reporter gene expression system based on RNA Pol I for large scale screening of high-nucleic acid in *S. cerevisiae.*

• In order to test this expression system working or not, we firstly designed a binary growth assay using the *URA3* gene (encoding for 5-phosphate decarboxylase) as the reporter gene. The results indicated that the reporter gene *URA3* was successfully expressed and the amount of Ura3p was sufficient to support the growth on an auxotrophic medium with no need to deletion any genes involved in protein translation initiation.

• We used another reporter gene (GFP) to replace the original *URA3* for high-throughout screening high rRNA synthesis cells. After a round of ARTP mutagenesis to disturb the rRNA synthesis, about 200,000 cells were analyzed using FACS, and 100 cells with fluorescence intensities higher than the threshold were large-scale sorted onto the selection medium. After single yeast separation, twelve pure mutants were finally obtained. Three mutants were randomly selected for further flow cytometry analysis and total RNA content measurement. The results showed that the mutants with higher fluorescence intensity than the threshold showed that RNA content improved by a maximum of 58% without changing its Pol I promoter sequence.

• Results

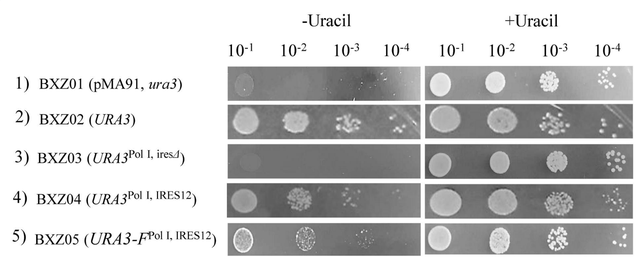


Fig.1. Cells containing Pol I-mediated *URA3* expression system grew well on plates minus uracil.
Line 1, cells with empty plasmid pMA91 as a negative control. Line 2, cells with Pol II-mediated expression cassette (*TEF1p-URA3-PGK1t*) as a positive control. Line 3, cells containing the Pol I-mediated *URA3* expression system with no IRES element showed no growth on the minus uracil plate. Line 4, cells containing the Pol I-mediated *URA3* expression system worked well. Line 5, FLAG labeling on the C-terminal end did not impact the Ura3p function.

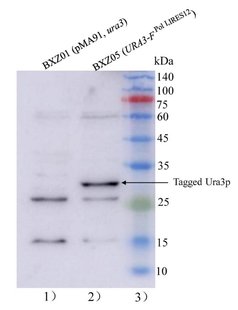


Fig.2. Pol I-mediated Ura3p expression was confirmed by western blot. The proteins produced by BXZ01 (pMA91, *ura3*), and BXZ05 (*URA3-F*Pol I, IRES12) were prepared and incubated with the anti-FLAG tag antibody to detect expression of Ura3p with a C-terminal FLAG tag.

----The reporter gene *URA3* was successfully expressed using the expression system based on RNA Pol I by the growth assay and western blot analysis.

----The amount of Ura3p expressed by the expression system based on RNA Pol I was sufficient to support the growth on an auxotrophic medium.
----There is no need to destroy the yeast cap-dependent translation initiation mechanism, the reporter gene *URA3* can also expressed well.

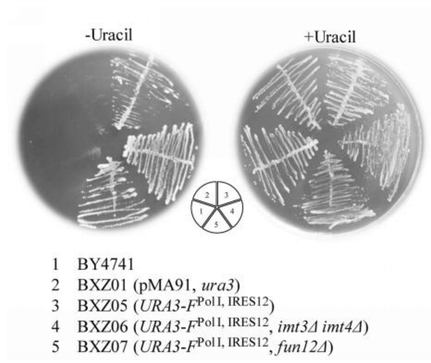
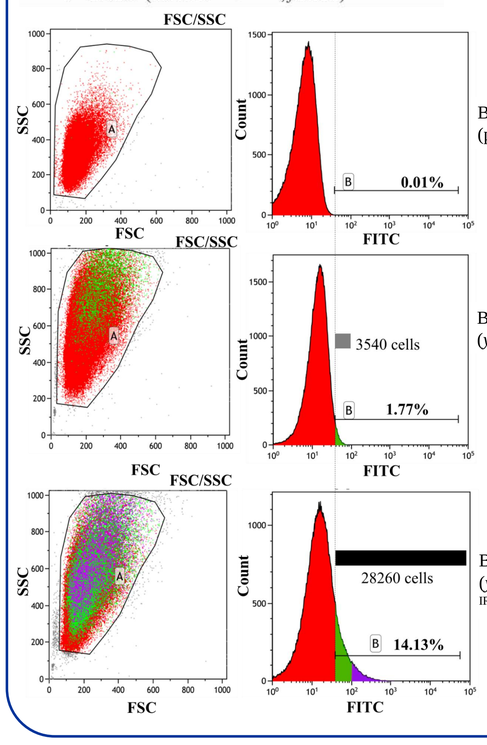


Fig.3. Destroying the cap-dependent translation initiation factors was unnecessary for Ura3p expressing strains. Regions 1 and 2, negative control. Region 3, the Pol I-mediated expression system worked well without destroying translation initiation factors related genes. Region 4, the Pol I-mediated expression system worked well with genes *IMT3* and *IMT4* deleted. Region 5, the Pol I-mediated expression system worked well with gene *FUN12* deleted.

----Large scale screening of the yeast cells with the fluorescence intensities higher than the threshold using FACS. The histogram of fluorescence intensity and counts for the ARTP mutants changed, which indicated that that ARTP can induce mutation of transcription factors related to rRNA synthesis.



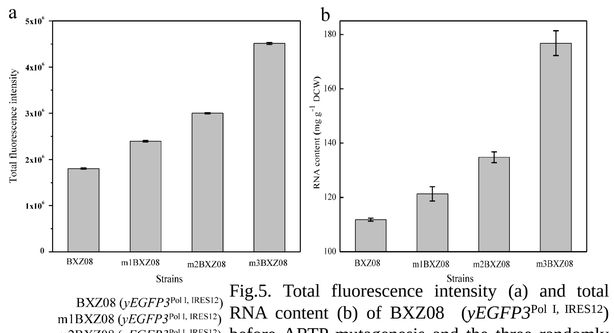


Fig.5. Total fluorescence intensity (a) and total RNA content (b) of BXZ08 (*yEGFP3*Pol I, IRES12) before ARTP mutagenesis and the three randomly selected mutants m1BXZ08, m2BXZ08, and m3BXZ08 after mutagenesis.

---- The mutants with higher fluorescence intensity than the threshold (>2000) showed that RNA content improved by a maximum of 58% without changing its Pol I promoter sequence.

Fig. 4. The Pol I-mediated GFP expression system enhanced strain fluorescence intensity and ARTP mutagenesis pushed it drift further. The fluorescence intensity distribution of strains were determined by flow cytometry (left panel). 200,000 cells of each strain were analyzed from the circled region in the FSC/SSC chart (right panel). FSC represents cell size. SSC represents cell refractive index. FITC represents the fluorescence intensity. Count represents number of cells at a certain fluorescence intensity.

• References

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