

Correction Notice: Single-step Precision Genome Editing in Yeast Using CRISPR-Cas9

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After official publication of our protocol in bio-protocol (<https://bio-protocol.org/e2765>), we noted some errors in the protocol and wished to correct the protocol. The edits to be performed are as the following:

1. The original Figure 1 does not appropriately indicate the generation of the Cas9 transcription unit. The process of how to create a Cas9 transcription unit with suitable promoters, terminators, and connectors has now been included in the new Figure 1. All the transcription units were made in the pYTK095 background, which has also been edited.
2. Table 2 now shows an additional reaction for generating the transcription unit for the Cas9, as shown in Figure 1. The new Table 2 is attached below.

Table 2. Golden Gate reaction for making Cas9 and gRNA transcription unit/cassette plasmids with appropriate connectors

| Cas9 cassette plasmid | | gRNA cassette plasmid | |
|-----------------------------------|----------|----------------------------|----------|
| Reagent | Amount | Reagent | Amount |
| Cas9 (pYTK036) | 20 fmol | gRNA in pYTK050 | 20 fmol |
| ConLS (pYTK002) | 20 fmol | ConL1 (pYTK003) | 20 fmol |
| ConR1 (pYTK067) | 20 fmol | ConRE (pYTK072) | 20 fmol |
| AmpR-ColE1 (pYTK095) | 20 fmol | AmpR-ColE1 (pYTK095) | 20 fmol |
| NEB T4 ligase buffer (10X) | 1.0 µl | NEB T4 ligase buffer (10X) | 1.0 µl |
| NEB T7 ligase | 0.5-1 µl | NEB T7 ligase | 0.5-1 µl |
| NEB BsaI | 0.5-1 µl | NEB BsaI | 0.5-1 µl |
| Promoter and terminator cassettes | 20 fmol | | |
| ddH ₂ O | to 10 µl | ddH ₂ O | to 10 µl |

3. Table 3 has also been edited that includes the title and footnote edits. The edits are the following:
 - a. The correct cassette plasmids with connectors are indicated in the reagents section.
 - b. The volumes of ligase and enzymes have been edited from 0.5 µl to 0.5 - 1 µl.
 - c. The Title shows that this plasmid now serves as an expression vector in a yeast cell.
 - d. The footnote for the table now indicates the End-On-Ligation step required at the end of the Golden Gate reaction cycle to generate a CEN6-URA-GFP vector.

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Table 3. Golden Gate reaction for making Cas9 and gRNA yeast expression vector

| Cas9 cassette plasmid | |
|---|----------|
| Reagent | Amount |
| gRNA cassette with connectors | 20 fmol |
| Cas9 transcription unit with connectors | 20 fmol |
| CEN6-URA-GFP plasmid* (KanR) | 20 fmol |
| NEB T4 ligase buffer (10X) | 1.0 µl |
| NEB T7 ligase | 0.5-1 µl |
| NEB BsmBI | 0.5-1 µl |
| ddH ₂ O | to 10 µl |

*Cen6-Ura is constructed by assembling YTK plasmids (008, 047, 073, 074, 081, and 084) using BsaI enzyme and End-ON-Ligation step for the Golden Gate reaction.

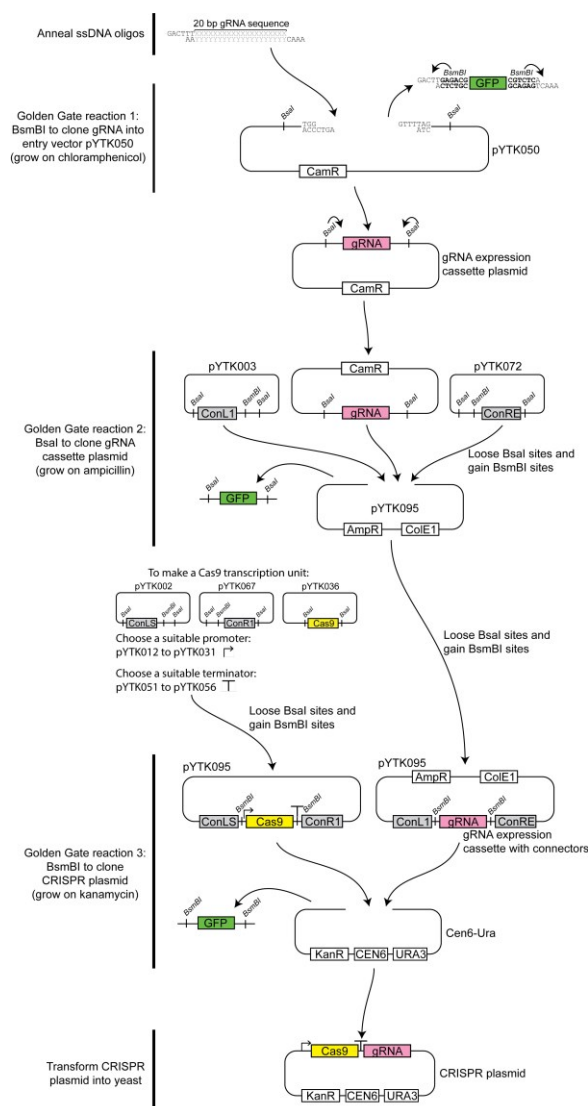


Figure 1. Overview of the CRISPR/Cas9-gRNA expression vector construction process. In the first step Xs and Ys represent the gRNA sequence selected, and BsmBI recognition site is indicated in bold.

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References

1. Akhmetov, A., Laurent, J. M., Gollihar, J., Gardner, E. C., Garge, R. K., Ellington, A. D., Kachroo, A. H. and Marcotte, E. M. (2018). [Single-step precision genome editing in yeast using CRISPR-Cas9](#). *Bio-protocol* 8(6): e2765.