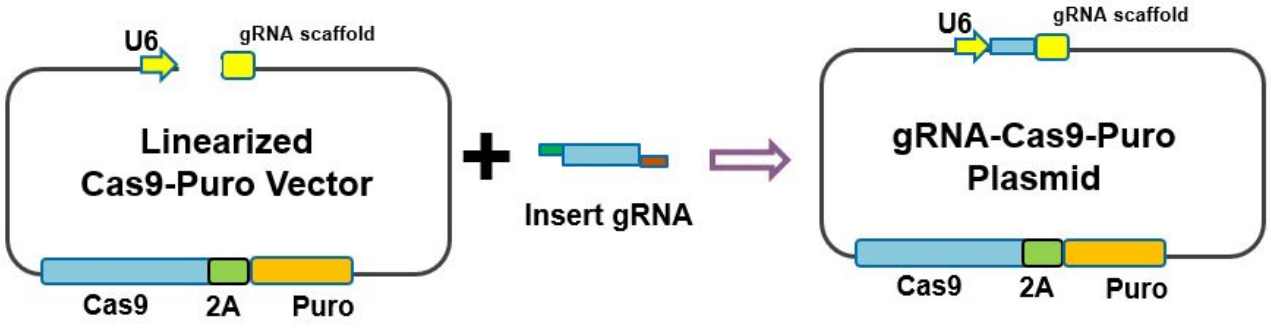
High-Efficiency gRNA-Cas9-Puro Plasmid (linear) Assembly Kit

Cat. No. L00691 Version 02202017

I Description

The High-Efficiency gRNA-Cas9-Puro plasmid (linear) Assembly Kit is used for easier and highly efficient cloning of gRNA fragments into Cas9 vectors. This vector has been constructed by integrating a suicide reporter gene into the gRNA insert site. Under the selection of this suicide report gene, almost 100% of clones are positive clones without screening. The High-Efficiency gRNA-Cas9-Puro plasmid is provided in both the circular and linear formats. The linear format allows omission of the digestion and purification process, which saves time. The Puromycin resistant gene is linked by 2A, a self-cleavage peptide, and is expressed separately from Cas9, keeping the maximum activity of Cas9.



II Kit Contents

Components	Quantity		Concentration
	10-reaction	25-reaction	
Linearized Cas9-Puro Vector	20 µL	50 µL	25 ng/µL
Control gRNA insert	20 µL	50 µL	0.005 µM
Control gRNA primer F	10 µL	25 µL	10 µM
Control Screening primer R	50 µL	125 µL	10 µM

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III Key Features

- **Simplicity** –Only a pair of primers is needed. The digestion and ligation can be done in a single reaction.
- **Efficiency** –Up to 100% positive clones.
- **Fidelity** –No mutations.
- **Time-saving** – The construction reaction can be completed within 30 minutes.

IV Quality Control Analysis

- Positive clone rate: up to 100%.
- None colonies could be grown in DH5α competent cells without the insert.

V Product Usage

Rapid construction of gRNA-Cas9 plasmid for the subsequent gene editing research.

VI Storage

Please store at -20°C.

VII Protocol

1. Design specific gRNA primers

Forward primer gRNA-F: CACC+N_{19-21} (gRNA target sequences)

Reverse primer gRNA-R：AAAC+ N_{19-21} (gRNA Reverse complementary sequence)

e.g：If the target sequence of gRNA is CATCATTGGAAGACGTTCTT, the forward primer is CACCCATCATTGGAAGACGTTCTT, and the reverse primer is AAACAAGAACGTCTTCCAATGATG.

2. Anneal the primers
   - Dissolve specific gRNA primers F and R to 10 μM.
   - Dilute to 0.01 μM.
   - Mix the primers F and R 1:1 by volume.
   - Denature at 95℃ for 5 minutes.
   - Place the mixture at room temperature for another 10 minutes, so that the primers cool down to room temperature gradually.

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3. Ligation reaction

	Experimental reaction	Positive control reaction
10×T4 DNA ligase buffer	1 μL	1 μL
Linearized Cas9-Puro Vector	1 μL	1 μL
annealed specific gRNA insert	1.5 μL	-
Control gRNA insert	-	1.5 μL
T4 DNA ligase(400U/μL)	0.25 μL	0.25 μL
H_2O	6.25 μL	6.25 μL
Total reaction volume	10 μL	10 μL

Reaction conditions :

Temperature	Time	Cycle
16℃	30 minutes	1×
4℃	Hold	-

4. Transformation
   - Add 5 μL of the above reaction mixture to 50 μL DH5α competent cells.
   - Place the cells on ice for 30 min.
   - Heat shock at 42℃ for 1 min followed by ice bath for 2 min.
   - Add 500 μL LB Broth and shake for 30 min at 37℃ under 180 rpm.
   - Add 100 μL to LB Agar plate with 100 μg/ml *Ampicillin* and incubate overnight at 37℃.

5. Colony PCR screening

	Experimental reaction screening	Positive control reaction screening
10×Taq buffer	2.5 μL	0.25 μL
10mM dNTP	0.5 μL	0.5 μL
Specific gRNA-F (10μM)	0.5 μL	-
Control gRNA primer F (10μM)	-	0.5 μL
Control Screening primer R (10μM)	0.5 μL	0.5 μL
Taq DNA polymerase (5U/μL)	0.25 μL	0.25 μL
Colony	a little	a little
H_2O	20.75 μL	20.75 μL
Total reaction volume	25 μL	25 μL

Note: Less than 10 clones is enough for screening in each experimental plate.

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Reaction conditions：

Temperature	Time	Cycle
94℃	5 minutes	1
94℃	10 seconds	25×
55℃	10 seconds	
72℃	10 seconds	
72℃	5 minutes	1
4℃	Hold	-

PCR products are detected using agarose gel electrophoresis. The positive control band runs at 270bp. The negative control should remain blank.

Note： This is a basic protocol. The reagent concentrations, conditions, and parameters may need to be optimized.

VIII FAQ

1. Which kinds of competent cells can be used for transformation?

Answer: Suitable competent cells include, but are not limited to DH5α, Stbl2, Stbl3, EPI300, EPI400, JM108, ER2925 and DH10B-N. Inappropriate competent cells include, but are not limited to Top10, XL1-Blue, and SURE2.

2. What is the size of the plasmid before and after transformation?

Answer: The size of linearized Cas9-Puro vector provided in the kit is 9155 bp, and the size of the target plasmid after transformation is about 9178-9180 bp depending on the inserted gRNA.

3. Based on this linearized vector, are further digestion and ligation still required?

Answer: Only one step of ligation reaction is required according to the procedure described on page 2. Further digestion is not required.

4. Are there any other versions of gRNA-Cas9 constructs?

Answer: GenScript carries a series of different versions of gRNA-Cas9 constructs, including “High-Efficiency gRNA-Cas9-GFP Plasmid (linear) Assembly Kit (Cat. No. L00690)”, “High-Efficiency gRNA-Cas9-GFP Plasmid Assembly Kit (Cat. No. L00692)”, and “High-Efficiency gRNA-Cas9-Puro Plasmid Assembly Kit (Cat. No. L00693)”.

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