Construction of pGGDestTol2LC-sgRNA vectors

Linlin Yin, Lisette A. Maddison, Mingyu Li, Nergis Kara, Matthew C. LaFave,Gaurav K. Varshney, Shawn M. Burgess, James G. Patton, and Wenbiao Chen

Abstract

This protocols is from:
Linlin Yin, et al. (2015) Multiplex Conditional Mutagenesis Using Transgenic Expression of Cas9 and sgRNAs. Genetics 200:431-441; doi:10.1534/genetics.115.176917

Please see the full manuscript for additional details.

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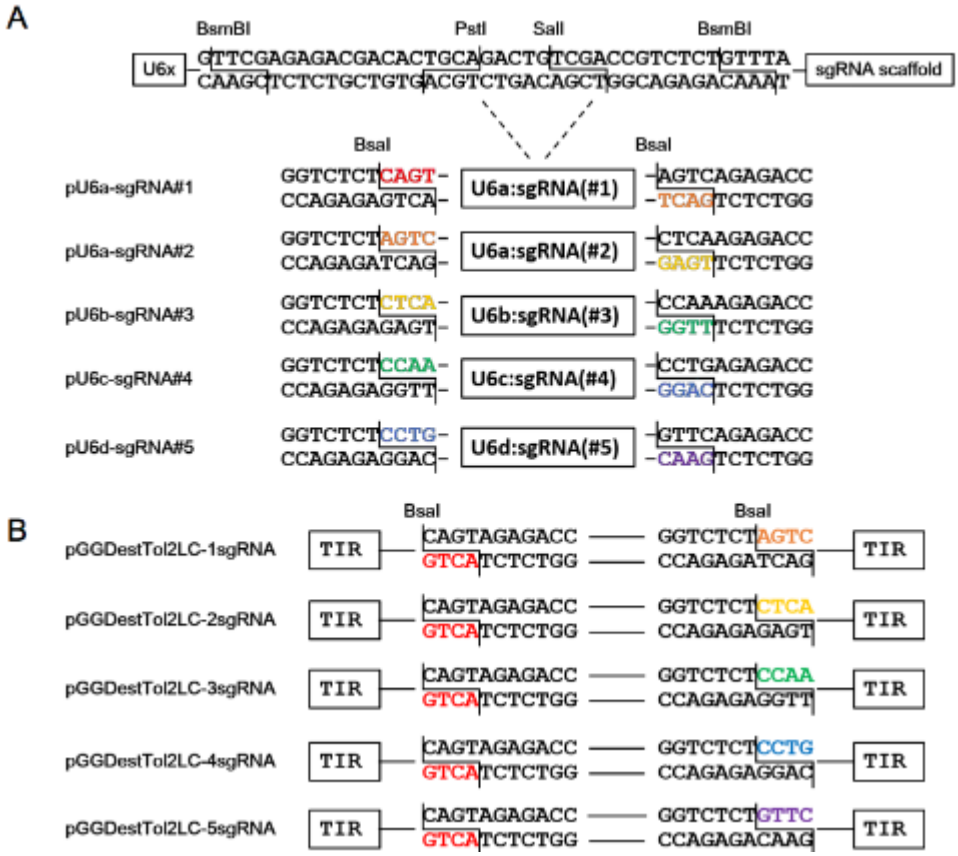
Guidelines



List of plasmids deposited at Addgene (Deposit 71794)

Plasmid ID Plasmid Name
64237 pME-Cas9

64239 pGGDestTol2LC-1sgRNA
64240 pGGDestTol2LC-2sgRNA
64241 pGGDestTol2LC-3sgRNA
64242 pGGDestTol2LC-4sgRNA
64243 pGGDestTol2LC-5sgRNA
64245 pU6a:sgRNA#1
64246 pU6a:sgRNA#2
64247 pU6b:sgRNA#3
64248 pU6c:sgRNA#4
64249 pU6d:sgRNA#5
64250 pU6a:sgRNA(tyr)

Reference:
JAO, L. E., S. R. WENTE and W. CHEN, 2013 Efficient multiplex biallelic zebrafish genome editing using a CRISPR nuclease system. Proc Natl Acad Sci U S A 110: 13904- 13909.

Protocol

Step 1.
Ligate the U6x:sgRNA cassettes into a pGGDestTol2LC vector by mixing the following components:

PROTOCOL

. pGGDestTol2LC-sgRNA ligation mixture

CONTACT: Tracey DePellegrin
Step 1.1.
2µl 10x CutSmart buffer

Step 1.2.
2 µl T4 DNA ligase buffer

Step 1.3.
100ng of each pU6x-sgRNA plasmid

Step 1.4.
50ng empty pGGDestTol2LC-sgRNA vector of choice (see plasmid list and diagrams in Guidelines),

Step 1.5.
1µl BsaI

Step 1.6.
1µl T4 DNA ligase

Step 1.7.
adjust volume to 20µl with H2O

Step 2.
Incubate mixture at 37 °C for 20 min

DURATION
00:20:00

Step 3.
Incubate at 16 °C for 15min

DURATION

00:15:00

Step 4.

Incubate at 80 °C for 15min

DURATION

00:15:00

Step 5.

The reaction is ready for transformation (use 5 µl of the ligation and plate 50% of the transformants). Transform and spread onto ampicillin (100 µg/ml) plates.