SLIC sub-cloning using T4 DNA polymerase treated inserts without RecA

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Method Article

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Abstract

Introduction

We describe a novel cloning method SLIC that allows the assembly of multiple DNA fragments in a single reaction using _in vitro_ homologous recombination and single-strand annealing.

Procedure

1. Digest 2 µg of vector with restriction enzymes. Gel purify the vector and isolate the DNA using QIAEX II gel extraction kit. Quantitate the vector. 2. Amplify inserts using Taq DNA polymerase. Set up a 100 µl PCR reaction with 250 µM of each dNTP, 0.5 µM of each primer, and 2.5 U of Taq DNA polymerase \(from Eppendorf). Cycle as follows: 94°C for 45 seconds; 30 cycles of 94°C for 45 seconds, 54°C for 45 seconds, and 72°C for 1 minute; 72°C for 10 minutes. Add 20 U of DpnI to 100 µl of PCR products after PCR, incubate at 37°C for 1 hour \(not necessary if going from a MAGIC vector to ColE1 origin). Purify the PCR products by QIAquick PCR purification column. Quantitate the inserts. We typically have 20 bp homology between the vector and the inserts. 3. Take 1 µg of the vector and 1 µg of the inserts and treat separately with 0.5 U of T4 DNA polymerase in T4 buffer \(NEB) plus BSA in a 20 µl reaction at room temperature for 30 minutes. Stop the reaction by adding 1/10 volume of 10 mM dCTP and leave on ice. 4. Set up a 10 µl annealing reaction using 1:1 insert to vector ratio with 150 ng of a 3.1 kb vector \(0.074 pmol), 1x ligation buffer \(NEB), appropriate amount of insert, and water. Incubate in 37°C for 30 minutes. Leave on ice or store in -20°C. 5. Add 5 µl of the annealed mixture into 150 µl of BW23474 chemical competent cells, incubate on ice for 30 minutes, heat shock at 42°C for 45 seconds, return to ice for 2 minutes, add 0.9 ml of SOC, and recover at 37°C for 1 hour. 6. Plate 100 µl onto Cl-Phe/Kan 50 µg/ml plate; incubate in 37°C for overnight. \(Cl-Phe is used for vectors that have PheS-G294 between restriction enzyme sites. We have found that most background comes from uncut vector in our preps and therefore we can select against it with Cl-Phe. However, Cl-Phe is not an essential step and usually only has a 2-fold effect on background.) For Cl-Phe/antibiotic plates: 0.5% yeast extract, 1% NaCl, 0.4% glycerol, 15 mM DL-p-chlorophenylalanine \(Sigma C6506), 2% agar. DL-p-chlorophenylalanine will not dissolve until after autoclaving. Glycerol and antibiotics are added after autoclaving. \(We use a higher Cl-Phe concentration for this vector than in normal MAGIC recipients because the PheS gene in pMAGIC is expressed at a much lower level than in normal MAGIC recipients and therefore requires a higher Cl-Phe concentration.) For SOC: 2% tryptone, 0.5% yeast extract, 0.05% NaCl, 2.5 mM KCl, 10 mM MgCl2, and 20 mM glucose.