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Modifications in the Transformation Step of Commercially Available Site-directed Mutagenesis Kit Increase Its Price-performance

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[Abstract] Site-directed mutagenesis helps unravel the relationship between the structure and function of a protein at the level of amino acid contributions. Many techniques have been established to carry out site-directed mutagenesis on a daily routine. QuikChange II Site-Directed Mutagenesis Kit™ is one of such methods available commercially. But this kit is expensive. Here we describe modifications in the transformation step by reducing the usage of competent cells of this protocol that will increase its price-effectiveness. Since the kit is used widely, these modifications might have global application.
Keywords: Site-directed mutagenesis, Transformation, DNA isolation, Competent cells, Chaperone proteins

[Background] Physiological function of a protein is meticulously controlled by its structure. Conformation changes in a protein from its native state have disastrous implications on human health (Carrell and Gooptu, 1998; Chiti and Dobson, 2006). For example, a single amino acid change in hemoglobin leads to sickle cell disease (Ingram, 2004) and a single amino acid change in α-synuclein is the reason for familial parkinsonism (Krüger *et al.*, 1998). Thus, study of amino acids in relation to protein structure and function is highly relevant and useful with respect to human health.

In 1982 Winter *et al.*, devised a method for selective replacement of single amino acid in a protein, tyrosyl tRNA synthetase. In their site-directed replacement, they mutated Cys35 of the molecule to Ser and observed that single amino acid change caused reduction in the tyrosyl tRNA synthetase enzyme activity (Winter *et al.*, 1982). Since then many different methods have been developed for achieving site-directed mutagenesis of proteins. QuikChange mutagenesis method is one such method (Papworth *et al.*, 1994; Braman *et al.*, 1996). Further, in using QuikChange II Site-Directed Mutagenesis Kit™ from Agilent Technologies, the need for specific restriction sites and the strand status is completely eliminated and the process can be completed in a short period of time. However, QuikChange II Site-Directed Mutagenesis Kit is expensive and cost to product ratio is very high. In this study, we describe modifications in the transformation step of the protocol, which will significantly reduce the use of materials in carrying out each site-directed mutation and will also reduce the time taken in the whole process. These modifications will maximize the yield of QuikChange II Site-Directed Mutagenesis Kit and will significantly improve its price-performance ratio.

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Material and Reagents

1. Pipette tips (Fisher Scientific, many different sizes and types)
2. Bacteriological Petri Dishes (100 x 15 mm) (Fisher Scientific, catalog number: 08-757-100D)
3. Falcon tube (Corning, catalog number: 352059)
4. NaCl (Fisher Scientific, catalog number: S671-3)
5. Tryptone (Sigma, catalog number: T7293-250G)
6. Yeast extract (Fisher Scientific, catalog number: BP14220-500)
7. Agar (Apex Chemicals and Reagents, catalog number: 20-248)
8. $MgSO_4$ (Fisher Scientific, catalog number: M-63 75626)
9. $MgCl_2$ (Fisher Scientific, catalog number: BP214-500)
10. Glucose (Fisher Scientific, catalog number: D16-1)
11. Ampicillin (Fisher Scientific, catalog number: BP1760-25)
12. Luria-Bertani Broth (Difco™ Becton and Dickinson, catalog number: 244610)
13. QuikChange II Site-directed mutagenesis kit with XL-super competent cells (Agilent Technologies, catalog number: 200523-5)
14. PureLink™ Quick Plasmid Miniprep kit (Thermo Fisher Scientific, catalog number: K210010)
15. Tris hydroxymethyl aminomethane hydrochloride (Fisher Scientific, catalog number: BP152-500)
16. LB-media (see Recipes)
17. SOC buffer (see Recipes)
18. Agar plates (see Recipes)

Equipment

1. Pipettes (Eppendorf North America)
2. Water bath (Fisher Scientific, catalog number: 15-462-15)
3. Incubator 37 °C (Fisher Scientific, catalog number: 11-690-650D)
4. Shaker incubator (New Brunswick Scientific, model: classic C24, catalog number: M12470004)
5. Centrifuge swing bucket (Eppendorf, catalog number: AG 22331)
6. Tabletop fixed angle (Beckman Coulter Inc., catalog number: 367160)
7. Biospec-nano spectrophotometer (Shimadzu, catalog number: 206-26300-32)
8. Weighing scales (Mettler Toledo, models: AE240 and PE200)
9. -80 °C freezer (Thermo Fisher Scientific, model: ULT2586-4-A48)
10. -20 °C freezer (GE 20.8 cu. Ft. Top Freezer)

Procedure

A. Transformation of plasmid of choice into the XL1 blue cells

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1. Prepare LB media, LB-Agarose plates and SOC as described in the recipes section below. Procure QuikChange II Site-Directed Mutagenesis Kit and PureLink™ Quick Plasmid Miniprep kit.
2. Aliquot XL1 blue super competent cells from QuikChange II Site-Directed Mutagenesis Kit™ or cell stock in 10 or 5 µl sizes to prevent repeated freeze/thaw. Store at -80 °C until next use.
3. Start your circulating water bath and set its temperature at 42 °C. It is a good practice to use an additional external thermometer for monitoring temperature. Make sure its temperature is steady at 42 °C before going to Step A8.
4. Place the LB-agar plate(s) containing desired antibiotics and SOC at 37 °C.
5. When ready for transformation thaw 10 or 5 µl cells (or any volume from 1-20 µl) on ice. Also, thaw your desired plasmid to be transformed on ice (for best results plasmid concentration should be ~100 ng/µl). This step takes 15-20 min.
6. Transfer competent cells into a Falcon tube and mix 1 µl plasmid with it.
7. Incubate on ice for 10 min without disturbance.
8. After incubation, immerse the falcon tube in the 42 °C circulating water bath for 30 s. Check Step A3 for extra precautions before starting this step. If using a stationary water bath, keep moving the falcon tube in water to ensure uniform exposure to temperature.
9. Incubate the heat shocked sample on ice for 2 min.

B. Plasmid amplification in XL1 blue cells
1. Add 300 µl SOC to transformed sample (Step A9 above) and incubate in shaker incubator for 60-90 min; with shaking at 250 rpm, 37 °C.
2. Take 30 µl from this SOC mixture and spread uniformly on the LB-agar-antibiotic plate(s) preincubated at 37 °C.
3. Incubate the plate(s) with your transformed material overnight at 37 °C. For best results don't exceed 16 h of incubation.
4. A successful transformation will produce a floor of transformed bacterial (XL1 cell) colonies.
5. Pick an isolated colony and inoculate 4 ml LB culture media containing desired antibiotics. This is your starter culture. Incubate it for 6-8 h or until the media is slightly turbid.
6. Inoculate a fresh 10 ml LB-antibiotic media with 100 µl starter culture and incubate overnight (12-16 h).
7. The next morning centrifuge the cells at 3,381 *x g* for 20 min. Discard the supernatant and store the cell pellet at -20 °C until further use. To purify plasmid from these cells or to check the transformed plasmid is exactly as you desire, proceed to Procedure C.

C. Plasmid/DNA purification
1. When ready thaw the cell pellet stored at -20 °C. Lyse the cell pellet and isolate the plasmid/DNA using your choicest method or a kit. For example, we used PureLink™ Quick Plasmid Miniprep kit to purify the transformed plasmid.

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2. Determine the concentration of the plasmid. We used 2 µl plasmid on Shimadzu Biospec-nano spectrophotometer. The machine uses algorithm and automatically calculates the final concentration of DNA.
3. We recommend getting the plasmid sequenced to know the exact sequence of the plasmid before proceeding to the next step of your project.
4. Gel electrophoresis can be used to purify the plasmids further if needed.

Data analysis

The protocol described above reduces the amount of cells required to perform a transformation using the QuikChange Site-directed mutagenesis kit. The results (Figure 1) show similar cell colonies produced irrespective of the amount of XL1 blue cells used in transformation. The difference in the amount of purified plasmid DNA produced under both conditions was < 20% (Table 1). Therefore the modifications proposed in this protocol are going to increase the price-performance of QuikChange II Site-directed mutagenesis kit.

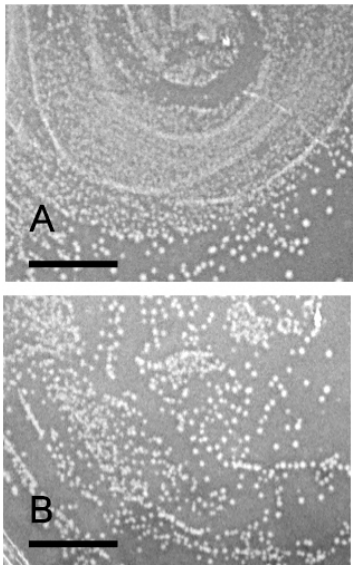

Figure 1. Bacterial colonies produced from transformation of 10 µl (A) or 5 µl (B) XL1 blue cells with the same plasmid at the same concentration. Scale bars = 10 mm.

Table 1. The concentration for purified plasmid produced by transforming 10 or 5 µl XL1 blue cells

XL1 blue cells used in the experiment	$(A_{260} - A_{280}) \times 50$
10 µl	58 ng/µl
5 µl	49 ng/µl

Recipes

1. LB-media
   Dissolve 25 g of powdered broth (from Difco™) per liter of double distilled water, autoclave it, add ampicillin when it cooled (~60 °C)
2. SOC buffer
   a. Autoclave NaCl (0.05%), Tryptone (2%), Yeast extract (0.5%) in double distilled water
   b. Wait for it to cool and add the following filter sterilized compounds: 1 ml of 1 M $MgSO_4$, 1 ml of 1 M $MgCl_2$ and 2 ml 20% (w/v) Glucose
   c. Store at 4 °C
3. Agar plates
   a. Autoclave NaCl (1%), Tryptone (1%), Yeast extract (0.5%), agar (2%) in double distilled water
   b. Upon cooling, add ampicillin and pour (~20 ml) into each plate. Let solidify (you can get away with ~10 ml/plate)
   c. Store at 4 °C until use

   Note: Transformation of plasmid, Plasmid amplification, Plasmid/DNA purification, Refer to *Procedure section above.*

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Contribution: AA developed the idea, interpreted data and wrote the article. DFJ, HK and AA collected data. CPE, MAF and JLS provided reagents.
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