QuikChange HT Protein Engineering System

Protocol

Version B.0, June 2015

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Notices

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In this Guide...

This document describes how to use the QuikChange HT Protein Engineering System to construct mutant plasmid libraries.

1 Before You Begin

Make sure you read and understand the information in this chapter and have the necessary equipment and reagents listed before you start an experiment.

2 Procedures

This chapter contains protocols for Agilent's QuikChange HT Protein Engineering System.

3 Reference

This chapter contains reference information related to the protocol.

What's New in Version B.0

- Updated product labeling statement

Content

1 Before You Begin

Make sure you read and understand the information in this chapter and have the necessary equipment and reagents listed before you start an experiment.

Required Reagents

Table 1 Required Reagents for the QuikChange HT Protein Engineering System protocol

Description	Vendor and part number
QuikChange HT Protein Engineering System Kit*	Agilent
150 nt, 10 sites, Non-academic Users	p/n G5900A
150 nt, 20 sites, Non-academic Users	p/n G5900B
200 nt, 10 sites, Non-academic Users	p/n G5901A
200 nt, 20 sites, Non-academic Users	p/n G5901B
150 nt, 10 sites, Academic Users	p/n G5902A
150 nt, 20 sites, Academic Users	p/n G5902B
200 nt, 10 sites, Academic Users	p/n G5903A
200 nt, 20 sites, Academic Users	p/n G5903B
PCR-grade water	Ambion p/n AM9930, or equivalent
Ethanol, molecular biology grade	Sigma-Aldrich p/n E7023, or equivalent
5 mM Tris-HCl, pH 8.0, 0.1 mM EDTA	General laboratory supplier
NZY$^+$ broth	Prepare as directed on page 32
LB–ampicillin agar plates	Prepare as directed on page 32
isopropyl-1-thio-β-D-galactopyranoside (IPTG)	Agilent p/n 300127
5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal)	Agilent p/n 300201

* The custom oligonucleotide libraries and custom primers provided in each kit are designed using the Agilent eArray design system. You must complete the design process in eArray before ordering the kit.

Required Equipment

Table 2 Required Equipment for the QuikChange HT Protein Engineering System protocol

Description	Vendor and part number
Thermal Cycler	Agilent SureCycler 8800, p/n G8800A or equivalent
8-well PCR strip tubes	Agilent, p/n 410092
Tube cap strips (8 domed caps per strip)	Agilent, p/n 410096
P20 and P200 pipettes	Pipetman or equivalent
Ice bucket	General laboratory supplier
Vortex mixer	General laboratory supplier
Microcentrifuge	Eppendorf Centrifuge model 5804 or equivalent

Procedural Notes

- The plasmid to be mutagenized must be isolated from a dam$^+$ *E.coli* strain. The majority of commonly-used *E. coli* strains are dam$^+$. Plasmid DNA isolated from dam$^-$ strains, such as the JM110 and SCS110 strains, is not a suitable target for mutagenesis using this system.
- Efficiency of the mutagenesis reaction and of mutant plasmid recovery by transformation will vary for each custom library. The protocol in this manual includes a pilot transformation experiment to determine the number of transformation reactions required to reach the target library size. Additional competent cells may be required to recover the targeted number of mutagenized plasmids.
- The provided SoloPack Gold Supercompetent cells are resistant to tetracycline and chloramphenicol. If the plasmid to be mutagenized contains only the tet^r and chl^r resistance markers, an alternative tetracycline-sensitive strain of competent cells must be used.
- The custom Mutagenesis Library is used for both control and protein engineering mutagenesis reactions. The oligonucleotide required to mutate the positive control pWS4.5 plasmid in the positive control reaction is present in each custom oligonucleotide library mixture.
- The Positive Control Primer Mix contains primers with the following sequences:

  5´-CCACTAGTTCTAGAGCGGC-3´

  5´-ACAGCTATGACCATGATTACG-3´

2 Procedures

This chapter contains protocols for Agilent's QuikChange HT Protein Engineering System.

Outline of the Protocol and Positive Control

Overview of the Workflow

The QuikChange HT Protein Engineering System allows you to construct a diverse collection of engineered mutant clones using Agilent's QuikChange technology, using the workflow summarized in Figure 1.

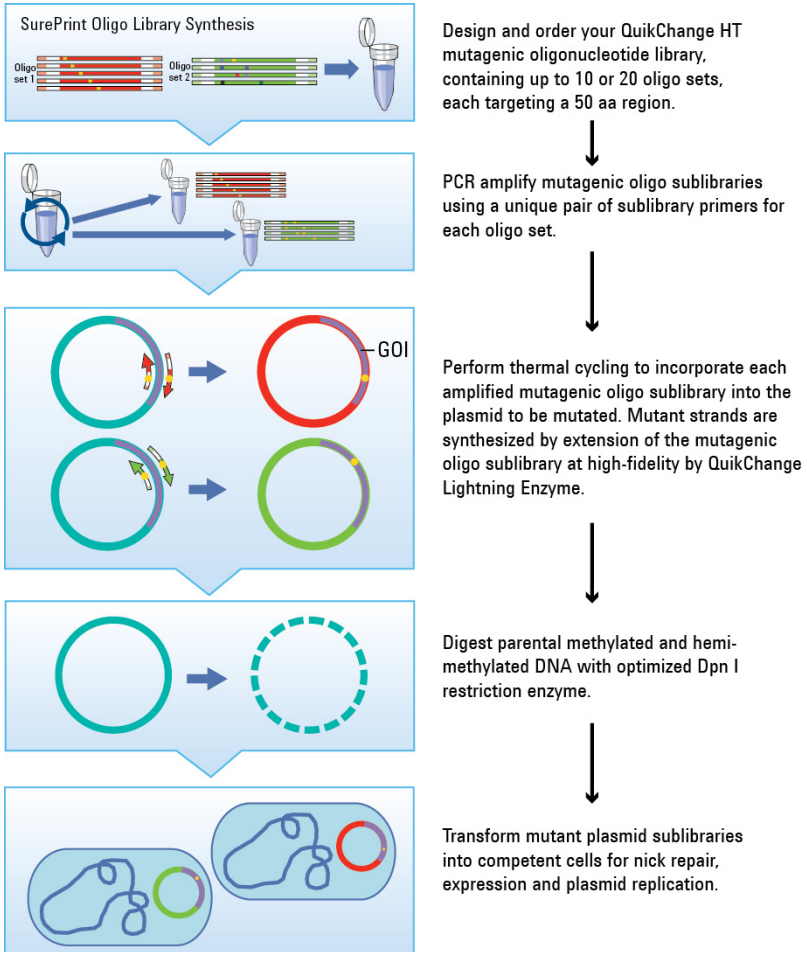

Figure 1 Overall QuikChange HT workflow.

Outline of the Protocol

The QuikChange HT Protein Engineering System protocol uses a library of custom mutagenic oligonucleotides and custom primers to produce an engineered mutant plasmid library. The mutagenic oligonucleotide library and custom primers must be designed using Agilent's eArray website before ordering a custom QuikChange HT Protein Engineering System kit.

The protocol steps for mutant plasmid library construction are summarized below. Detailed instructions are provided starting on page 17.

- In step 1 (see page 17) the provided custom mutagenic oligonucleotide library is PCR amplified using the appropriate pair of provided custom primers.
- In step 2 (see page 19) the amplified mutagenesis library is purified using kit-provided components.
- In Step 3 (see page 21) the mutagenic oligonucleotide library is incorporated into the plasmid to be mutagenized by a linear amplification reaction using a thermocycler. Components of the thermal cycling reaction include the supercoiled, double-stranded DNA template, the amplified oligonucleotide library containing the desired mutations, and the kit-provided enzyme blend featuring a *Pfu* Fusion DNA polymerase, which extends the mutagenic primers with high fidelity. After thermal cycling, the parental DNA template is removed by treating the reaction products with the restriction endonuclease *Dpn* I (target sequence: 5´-Gm6ATC-3´). *Dpn* I acts specifically on methylated and hemimethylated DNA, and does not cleave the newly synthesized mutagenized DNA library. DNA isolated from almost all *E. coli* strains is dam methylated and therefore susceptible to digestion.
- In step 4 (see page 23) the mutagenized plasmid library is transformed into the provided SoloPack Gold supercompetent cells. The instructions provided are for the initial pilot transformation to determine the appropriate clone recovery strategy for the custom library.

Use of the Positive Control

The kit includes a *LacZ*-based control template and control primers to verify performance of the QuikChange HT Protein Engineering System custom library, reagents, and protocol.

The supplied 4.5 kb pWS4.5 control plasmid contains the *LacZ* coding sequence with a stop codon mutation that prevents expression of full-length *LacZ*. The *LacZ* gene product is responsible for the production of blue colonies when appropriate *E. coli* transformants are grown on media containing X-gal in the presence of the inducer IPTG. The provided SoloPack Gold supercompetent cells transformed with the pWS4.5 control plasmid appear white on LB–ampicillin agar plates containing IPTG and X-gal, because the stop codon in the control plasmid prevents production of active beta-galactosidase.

In addition to the custom-designed oligonucleotides, each QuikChange HT Mutagenesis library also contains mutagenic oligonucleotides that direct reversion of the mutant stop codon in the pWS4.5 control plasmid. After completing the QuikChange HT Protein Engineering System protocol using the provided Positive Control Primer Mix for amplification from the custom library, a mutagenized control plasmid library is constructed. Following transformation of the control library into SoloPack Gold supercompetent cells, colonies are scored for the blue color production phenotype, where a blue colony indicates the production of a mutagenized control plasmid expressing the full length *LacZ* gene product. Each custom mutagenesis library should be used to construct a positive control plasmid library in parallel with the custom mutant plasmid library to verify efficient amplification and transformation of the custom library.

Step 1. PCR Amplify the Custom Mutagenesis Library

1 For each Forward Custom Primer and Reverse Custom Primer to be used in the experiment, dilute 1 µL of the supplied primer solution with 19 µL of PCR-grade water, for a final concentration of 5 µM.

2 Prepare 50-µL custom library and control PCR amplification reactions by combining the reagents in Table 3. Mix thoroughly by vortexing.

Table 3 Amplification of Mutagenesis Library

Component	Custom Library Amplification	Positive Control Amplification
2× PfuUltra II HS Master Mix AD	25 µL	25 µL
QuikChange HT Mutagenesis Library solution	1 µL	1 µL
Forward Custom Primer dilution (5 µM, prepared in step 1)	5 µL	—
Reverse Custom Primer dilution (5 µM, prepared in step 1)	5 µL	—
10× Positive Control Primer Mix (5 µM each primer)	—	5 µL
PCR-grade H_2O	14 µL	19 µL

3 Cycle the amplification reactions according to Table 4 below.

Table 4 Thermal cycler program for Mutagenic Oligo Library amplification

Segment	Cycles	Temperature	Time
1	1	95°C	30 seconds
2	30	95°C	20 seconds
		primer Tm –5°C*	10 seconds
		72°C	30 seconds
3	1	72°C	2 minutes
4	1	4°C	Hold

* For custom library amplification, obtain custom primer Tm values from the supplied Custom Primer tube labels. For the positive control reaction, use an annealing temperature of 55°C.

4 Analyze 5-µL samples of the custom library amplicons and of the positive control amplicon by electrophoresis on a 2% (w/v) agarose gel. Load 5 µL of the provided 1.1 kb Gel Standard marker DNA solution in an adjacent lane.

5 After staining the gel with a suitable DNA-staining agent, the intensity of each amplicon band should comparable to the intensity of the band in the 5-µL 1.1 kb Gel Standard lane.

If the library amplicon band (from 5 µL sample) is substantially less intense than the 1.1 kb Gel Standard band (from 5 µL of the standard solution), troubleshoot the amplification reaction before proceeding to purification and plasmid library construction steps.

Step 2. Purify the Amplified Mutagenesis Library

1 Before starting the purification procedure with a new set of reagents, prepare 1× buffer solutions according the instructions in Table 5.

After adding the ethanol, be sure to mark the ethanol-added checkbox on the label for reference by later users.

Table 5 Preparation of 1× Buffer Solutions

1× Buffer to be Prepared	Preparation Instructions
1× DNA Binding Buffer	Add 2.5 mL of 80% ethanol to the provided DNA Binding Buffer bottle (shipped containing 2.5 mL of 2× buffer)
1× PCR Wash Buffer	Add 20 mL of 100% ethanol to the vial of 5× PCR Wash Buffer (shipped containing 5.0 mL of 5× buffer)

2 Purify the amplicons from each library amplification reaction using the provided microspin cups and receptacle tubes according to the following protocol.

a To the remaining 45 µL of amplification reaction, add 100 µL of 1× DNA Binding Buffer and mix well by vortexing.

b For each library amplification reaction, seat a microspin cup (provided) in a receptacle tube (provided).

c Transfer each amplicon plus binding buffer mixture (approximately 145 µL) into a seated microspin cup. Avoid touching the fiber matrix of the microspin cup with the pipet tip.

d Cap the spin cup with the provided receptacle tube cap, then spin in a microcentrifuge for 30 seconds at maximum speed (13,000 to 14,000 rpm).

e Retain the microspin cup, with bound DNA, and discard the flow-through solution in the receptacle tube (retaining the receptacle tube).

f Re-seat the spin cup in the receptacle tube, and add 500 µL of 1× Wash Buffer.

g Cap the spin cup, then spin in a microcentrifuge for 30 seconds at maximum speed.

h Retain the microspin cup, and discard the flow-through solution in the receptacle tube (retaining the receptacle tube).

i Re-seat and cap the spin cup, then spin in a microcentrifuge for an additional 2 minutes at maximum speed.

j Transfer the microspin cup to a fresh 1.5-mL microcentrifuge tube, then add 40 µL of TE elution buffer (5 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) to the spin cup.

k Incubate the spin cup at room temperature for 1 minute.

l Cap the spin cup with the tube cap, then spin in a microcentrifuge for 1 minute at maximum speed.

m Retain the 40-µL purified amplicon solution in the microcentrifuge tube, and discard the microspin cup.

3 Keep the amplified library solutions on ice until use on page 21.

Stopping Point If you do not continue immediately to the next step of the protocol, store the samples at –20°C until use.

Step 3. Construct the Mutant Plasmid Library using Thermal Cycling

Prepare the custom mutant plasmid library using the linear amplification mutant strand synthesis protocol detailed below. In this step, the plasmid to be mutagenized serves as template and the mutagenesis sublibrary amplicons from page 20 serve as primer in the mutant strand synthesis reaction.

For the positive control reaction, use the provided Positive Control Plasmid pWS4.5 as template and the control mutagenesis library amplicons as primer.

After amplification, the products are digested with *Dpn* I restriction enzyme to remove the parental (non-mutated) supercoiled dsDNA.

1 Prepare the 25-µL mutant plasmid library synthesis reactions by combining the reagents listed in Table 6.

Table 6 Mutant plasmid library synthesis

Component	Custom Library Preparation	Positive Control
10× QuikChange Lightning Buffer	2.5 µL	2.5 µL
dNTP Mix	1 µL	1 µL
QuikSolution	0.75 µL	0.75 µL
Mutagenesis target plasmid (25 ng/ µL)	1 µL	—
Positive Control Plasmid pWS4.5 (25 ng/ µL)	—	1 µL
Purified custom mutagenesis library amplicon (from step m on page 20)	15 µL	—
Purified control mutagenesis library amplicon (from step m on page 20)	—	5 µL
QuikChange Lightning Enzyme	1 µL	1 µL
PCR-grade H_2O	3.75 µL	13.75 µL

2 Cycle the reactions using the cycling parameters in Table 7 below.

Table 7 Thermal cycler program for mutant plasmid library synthesis

Segment	Cycles	Temperature	Time
1	1	95°C	2 minutes
2	18	95°C	20 seconds
		60°C	10 seconds
		68°C	30 seconds/kb target plasmid length*
3	1	68°C	5 minutes

* For the pWS4.5 positive control reaction, use a 2.5-minute extension time.

3 Add 1 µL of the provided *Dpn* I Enzyme directly to each amplification reaction.

4 Gently and thoroughly mix each reaction mixture by pipetting the solution up and down several times. Briefly centrifuge the reaction tube and then immediately incubate at 37°C for 5 minutes.

Dpn I-digestion is required to remove the non-mutated dsDNA supplied as template in the amplification reaction.

NOTE

The protocol detailed on the following pages is designed to determine the number of transformants obtained from 1.5 µL of the mutagenesis library. Store the remaining library solution at –20°C for additional rounds of transformation following the pilot transformation experiment.

Step 4. Design the Library Recovery Strategy using a Pilot Transformation Reaction

Efficiency of the mutagenesis reaction and of mutant plasmid recovery by transformation may vary for each custom library. The purpose of the pilot transformation is to determine the number of transformation reactions required to reach the target library size and to determine the optimal plating strategy for the transformation reactions.

If desired, the transformation efficiency of the competent cells may be verified by transforming a tube of cells with 0.01 ng of pUC18 DNA. (Dilute the provided pUC18 DNA prior to use.) Using the conditions specified below, the expected efficiency is $\geq 1 \times 10^9$ cfu/µg pUC18 DNA.

1 Preheat NZY$^+$ broth to 42°C for use as the outgrowth medium in step 8.

NOTE

The optimized transformation protocol uses NZY$^+$ broth for cell outgrowth (see page 32 for a recipe). SOC medium may also be used for cell outgrowth, but transformation efficiency will be reduced.

2 For each custom sublibrary and positive control sample to be transformed, thaw one tube of SoloPack Gold supercompetent cells on ice.

3 When the cells have thawed, swirl the tube gently to mix the cells.

4 Transfer 1.5 µL of the *Dpn* I-treated DNA from each mutant sublibrary construction reaction and control reaction to a separate tube of cells.

5 Swirl the transformation reaction gently to mix and then incubate the tube on ice for 30 minutes.

6 After removing any ice from the outside of the tube, heat-pulse the tube in a **42°C** water bath for 30 seconds. The temperature and duration of the heat pulse are critical for maximum efficiency.

7 Incubate the tube on ice for 2 minutes.

8 Add 250 µL of preheated (42°C) NZY$^+$ broth and incubate the tube at 37°C for 1 hour with shaking at 225-250 rpm.

9 Plate the appropriate volume of each transformation reaction, as indicated in the table below, on agar plates containing the appropriate antibiotic for the plasmid vector. Prior to plating the cells, pipet 100 µL of NZY$^+$ broth onto the surface of the agar. Pipet the transformation mixture into the pool of liquid medium and then spread the mixture with a sterile spreader.

Table 8 Transformation reaction plating volumes and media

Reaction Type	Volume to Plate	Plating Medium
pWS4.5 positive control mutagenesis	2 µL and 10 µL	LB-ampicillin agar containing 20 mM IPTG and 80 µg/mL X-gal
Custom mutant plasmid library	10 µL and 50 µL on each of two plates	LB agar containing the appropriate antibiotic (and mutagenesis indicators, where applicable)

10 Incubate the plates at 37°C overnight. For the pWS4.5 positive control plates and any other plates used for blue-white color screening, incubate the plates at 37°C for at least 16 hours to allow full color development. Blue color may also be enhanced by subsequent incubation of the plates at 4°C for 2 hours.

11 Evaluate the transformation results and plan the optimal large-scale library transformation plating strategy.

- For the pWS4.5 positive control mutagenesis library, ≥80% of the total colony forming units (cfu) should be blue-colored, indicating a mutagenesis efficiency ≥80%.
- For the custom mutagenesis library, colony number and mutagenesis efficiency will vary, depending on the plasmid size and sequence, library design and sublibrary primer design.

  Determine the number of transformation reactions required to reach the target library size (target number of mutagenized plasmid clones) and to determine the optimal plating strategy for each transformation reaction using the steps below:

  a Count and record the number of cfu on each of the custom mutagenesis library transformation plates.

b Determine the total number of cfu expected from transformation of each 1.5-µL sample of the *Dpn* I-digested mutagenesis reaction. A sample calculation is shown below.

Table 9 **Sample calculation of total cfu expected per transformation**

Volume of transformation reaction plated	Average cfu obtained	Average cfu obtained per µl of transformation reaction	Calculated cfu per 300-µl transformation reaction	Average calculated cfu per 300-µl transformation reaction containing 1.5 µl of mutagenesis library
10 µL	50	5	1500	1350
50 µL	200	4	1200	

c Determine the number of library transformation reactions required to generate the target library size. Using the example plating data in Table 9, if your target library size is 1 x 10^4 clones, you would need to complete eight transformation reactions.

d Determine the optimal plating volume for your custom mutagenesis library. Typically, the optimal plating volume will yield approximately 200 well-isolated cfu per 100-mm plate. Using the example plating data in Table 9, plating the full-scale transformation reactions at 50 µL per plate is expected to produce a suitable cfu density for clone recovery.

Once the appropriate plating strategy is determined for your custom library, transform additional 1.5-µL aliquots of each *Dpn* I-treated mutagenized plasmid sublibrary into additional tubes of SoloPack Gold supercompetent cells, using the transformation protocol starting on page 23. Additional tubes of SoloPack Gold supercompetent cells may be purchased separately (Agilent p/n 230350).

Troubleshooting

Observation	Suggestions
No product or low yield of mutagenesis library amplicons (from step 5 on page 18)	Amplification yield may be improved for some custom library and custom primer pair combinations by substitution of the 2× PfuUltra II HS Master Mix AD with QuikChange Lightning components. Prepare the amplification reaction using the following reagents: 32 µL PCR-grade water 5 µL 10× QuikChange Lightning Buffer 1 µL QuikChange HT Mutagenesis Library solution 5 µL Forward Custom Primer dilution (5 µM) 5 µL Reverse Custom Primer dilution (5 µM) 1 µL dNTP Mix 1 µL QuikChange Lightning Enzyme Use the cycling conditions described inTable 4 on page 17 and continue with the protocol according to the instructions provided.
	Titrate the concentration of the Forward and Reverse Custom Primers between 0.5 µM and 2.5 µM in the final reaction.
	For library designs with high GC content or secondary structures, it may be beneficial to include a cosolvent in the PCR reaction. Titrate addition of QuikSolution in the reaction at 1% to 10% (v/v) final concentration or glycerol at 5% to 20% (v/v) final concentration.
	Increase the cycle number to greater than 30.
Multiple bands produced in the mutagenesis library amplification reaction (from step 5 on page 18)	Ensure that the primer annealing temperature is optimal. Increase the annealing temperature in 5°C increments or try an annealing temperature gradient from 55°C to 70°C.

Observation	Suggestions
Low colony number or low mutagenesis efficiency after transformation of the LacZ control mutant plasmid library synthesis reaction (results from step 11 on page 24)	Verify the transformation efficiency of the SoloPack Gold Supercompetent Cells by transforming a tube of cells with 0.01 ng of the provided pUC18 DNA. (Dilute the provided pUC18 DNA prior to use.) Using the provided transformation protocol, the expected efficiency is $\geq 1 \times 10^9$ cfu/μg pUC18 DNA. Ensure that supercompetent cells are stored at the bottom of a –80°C freezer immediately upon receipt.
	Verify that the agar plates were prepared correctly. See Preparation of Media and Reagents on page 33 and follow the recommendations for IPTG and X-Gal concentrations carefully. For best visualization of the blue (β-gal$^+$) phenotype, the control plates must be incubated for at least 16 hours at 37°C and stored at 4°C overnight.
	Increase amount of control mutagenesis library amplicon added in Table 6 on page 21 from 5 μL to 15 μL.
	Allow sufficient time for the *Dpn* I enzyme to completely digest the parental template; increase the digestion time to 30 minutes.
	Avoid multiple freeze-thaw cycles for the dNTP mix. If necessary, prepare single-use aliquots of the provided dNTP mix, and store the aliquots at –20°C.
Low colony number or low mutagenesis efficiency after transformation of the custom mutant plasmid library synthesis reaction (results from step 11 on page 24 to page 25)	Analyze the plasmid DNA used in the reaction by gel electrophoresis to verify the quantity and quality of the template. Nicked or linearized plasmid DNA will not generate complete circular products; verify that the template DNA is at least 80% supercoiled.
	Repeat the strand synthesis reaction using 50 to 100 ng plasmid DNA.
	Titrate addition of QuikSolution in the strand synthesis reaction at 0.5 μL increments from 0 to 2.5 μL.
	Increase the amount of the mutagenesis library amplicon added to the strand synthesis reaction by eluting the amplicon in 20 μL of elution buffer (step j on page 20). Alternatively, concentrate the 40-μL eluate before use using a method of choice.
	Increase extension time in the strand synthesis reaction from 30 seconds/kb to 45 second/kb.
	Increase the amount of *Dpn* I-treated DNA used in the transformation reaction to 4 μL. If needed, you can purify and precipitate DNA from the entire strand synthesis reaction. After resuspending the DNA in a small volume, transform competent cells using the entire volume of DNA.
	Allow sufficient time for the *Dpn* I enzyme to completely digest the parental template. Increase the digestion time to 30 minutes or repeat the *Dpn* I digestion if too much DNA was present.
	Avoid multiple freeze-thaw cycles for the dNTP mix. If necessary, prepare single-use aliquots of the provided dNTP mix, and store the aliquots at –20°C.

3 Reference

This chapter contains reference information related to the protocol.

Kit Contents

QuikChange HT Protein Engineering System Kits contain the following component kits:

Table 10 QuikChange HT Protein Engineering System Kit Contents

Component Kits	Storage Condition	10 Sites	20 Sites
QuikChange HT Mutagenesis Library and Primers*	–20°C	5190-7454	5190-7454
QuikChange HT Mutagenesis Reagents	–20°C†	5190-7452	5190-7455
QuikChange HT DNA Cleanup Kit	Room Temperature	5190-7456	5190-7456
SoloPack Gold Supercompetent Cells	–80°C	230350	230350 × 2

* Kits may also be provided with the QuikChange HT Mutagenesis Library and the Sublibrary Primers in separate boxes (p/n 5190-7451 and 5190-7454, respectively).

† Store at –20°C upon receipt. After thawing the 2× PfuUltra II HS Master Mix AD, store the master mix at 4°C; do not subject to multiple freeze-thaw cycles. Store the remainder of the QuikChange HT Mutagenesis Reagents kits at –20°C throughout use of the product.

The contents of the multi-component kits listed in Table 10 are described in Table 11 through Table 14 below.

Table 11 QuikChange HT Mutagenesis Library and Primers Content

Reagent	Format
QuikChange HT Mutagenesis Library	single tube with clear cap
QuikChange HT Mutagenesis Sublibrary Primers	variable number of primer-containing tubes (one pair of primer tubes per sublibrary in custom design)

.

Table 12 QuikChange HT Mutagenesis Reagents Content

Reagent	Format
2× PfuUltra II HS Master Mix AD	tube with clear cap
10× QuikChange Lightning Buffer	tube with clear cap
QuikChange Lightning Enzyme	tube with red cap
40 mM dNTP	tube with yellow cap
QuikChange Solution	tube with green cap
Positive Control Plasmid pWS4.5 (25 ng/µL)	tube with green cap
10× Positive Control Primer Mix	tube with green cap
1.1 kb Gel Standard	tube with blue cap
Dpn I Enzyme	tube with black cap

Table 13 QuikChange HT DNA Cleanup Kit Content

Kit Component	Format
DNA Binding Solution	bottle (contains 2× solution; see page 19 for dilution instructions)
PCR Wash Buffer	bottle (contains 5× solution; see page 19 for dilution instructions)
Microspin cups and receptacle tubes	bag containing 24 cups and tubes

Table 14 SoloPack Gold Supercompetent Cells Kit Content

Reagent	Format
SoloPack Gold Supercompetent Cells	15 single-use transformation tubes (yellow caps)*
pUC18 control plasmid (0.1 ng/µl in TE buffer)	tube with blue cap*

* Two sets of 15 transformation tubes and two tubes of pUC18 are provided with 20 Site QuikChange HT Protein Engineering System Kits.

Preparation of Media and Reagents

NZY+ Broth (per Liter)

10 g NZ amine (casein hydrolysate)
5 g yeast extract
5 g NaCl
Add deionized H_2O to a final volume of 1 liter
Adjust pH to 7.5 using NaOH
Autoclave
Add the following filter-sterilized supplements prior to use:
- 12.5 mL of 1 M $MgCl_2$
- 12.5 mL of 1 M $MgSO_4$
- 20 mL of 20% (w/v) glucose, or 10 mL of 2 M glucose

LB Agar (per Liter)

10 g tryptone
5 g yeast extract
10 g NaCl
20 g agar
Add deionized H_2O to a final volume of 1 liter
Adjust pH to 7.0 with 5 N NaOH
Autoclave
Pour into petri dishes (approximately 25 mL per 100-mm plate)

LB-Ampicillin Agar (per Liter)

Prepare 1 liter of LB agar
Autoclave
Cool to 55°C
Add 10 ml of 10 mg/mL filter-sterilized ampicillin
Pour into petri dishes (approximately 25 mL per 100-mm plate)

LB-Ampicillin Agar with IPTG and X-gal for blue-white screening (per Liter)

Prepare 1 liter of LB agar
Autoclave
Cool to 55°C, then add the following components:
10 ml of 10 mg/mL filter-sterilized ampicillin
X-gal to a final concentration of 80μg/mL
IPTG to a final concentration of 20 mM
Pour into petri dishes (approximately 25 mL per 100-mm plate)

NOTE Alternatively, IPTG and X-gal may be spread on pre-poured agar plates using the guidelines provided below.

10 mM IPTG (per 10 mL) for spreading on pre-poured agar plates

24 mg isopropyl-1-thio-β-d-galactopyranoside (IPTG)
10 mL sterile dH2O
Store at –20°C
Spread 100 μL per 100-mm LB-agar plate for blue-white color screening
(do not mix the IPTG and X-gal solutions prior to spreading)

2% X-gal (per 10 mL) for spreading on pre-poured agar plates

0.2 g 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal)
10 mL dimethylformamide (DMF)
Store at –20°C
Spread 100 μL per 100-mm LB-agar plate for blue-white color screening
(do not mix the X-gal and IPTG solutions prior to spreading)

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In This Book

This guide contains information to run the QuikChange HT Protein Engineering System protocol.

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