SureGuide Custom CRISPR Guide Library

Guidelines for Amplification and Cloning Assembly

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About the Agilent SureGuide Custom CRISPR Guide Library (UnAmplified)

The SureGuide Custom CRISPR Guide Library (UnAmplified), Agilent Part Number G7555B, is composed of linear DNA fragments that encode user-defined guide RNAs with custom flanking sequences. In order to create a plasmid library, first PCR-amplify the CRISPR guide library and then clone the amplicons into a suitable linearized plasmid that contains overlapping flanking sequences for homologous recombination.

Table 1 Materials provided with the SureGuide Custom CRISPR Guide Library (UnAmplified), p/n G7555B

Materials provided	Quantity	Storage condition
Custom CRISPR Guide Library (UnAmplified)	10 pmol, lyophilized	–20°C
Herculase II Fusion DNA Polymerase	40 µL	–20°C
5× Herculase II Reaction Buffer	1.5 mL	–20°C
100 mM dNTP Mix	40 µL	–20°C
DMSO	1 mL	–20°C

Amplification Guidelines

Use the guidelines listed below when optimizing a PCR protocol for amplification of the DNA fragments in the unamplified Custom CRISPR Guide Library. Adherence to these guidelines helps ensure amplification of the full-length DNA fragments.

- In the PCR reactions, keep the concentration of the full-length library fragments high (200 pM) while minimizing the number of amplification cycles (≤15 cycles). These conditions help ensure adequate representation of all the guide sequences in the library. Full-length fragments generally represent ~25% of the unamplified CRISPR guide library.
- Keep the volumes of the PCR reactions at 100 µl. If you need to increase yield, increase the number of replicate reactions and not the number of cycles.
- The PCR primers should be standard PCR quality primers 25–30 nucleotides in length.
- Use the Herculase II Fusion DNA Polymerase and 5× Herculase II Reaction Buffer for high-fidelity, high-yield amplification.
- The tables below offer suggested starting points for PCR optimization. A well optimized reaction will yield a robust band of the correct size on an Agilent BioAnalyzer DNA 1000 chip (or comparable electrophoresis-based analysis method).

Table 2 Suggested Reagent Concentrations

Reagent	Recommended concentration in PCR reactions
Custom CRISPR Guide Library (UnAmplified)	200 pM of the full-length portion of the library
dNTP Mix	1 mM (250 µM each nucleotide)
Forward Primer (not provided)	250 nM
Reverse Primer (not provided)	250 nM

Table 3 Suggested Cycling Conditions

Cycle	Temperature (°C)	Duration
1	95	2 minutes
15	95	20 seconds
	55	20 seconds
	72	30 seconds
1	72	3 minutes

Once amplified, the library needs to be purified before it is suitable for cloning into the plasmid. Use SPRI magnetic bead purification or another method of similar quality. A final concentration ≥ 5 ng/µL is required for optimal cloning efficiency.

Cloning Assembly Guidelines

Use the guidelines listed below when cloning the amplified Custom CRISPR Guide Library into a linearized plasmid using the reagents in the Agilent SureVector CRISPR Cloning Kit (p/n G7556A).

NOTE: Every Custom CRISPR Guide Library is different. You may need to optimize your cloning procedure to obtain a high-quality plasmid library.

- Both the PCR-amplified Custom CRISPR Guide Library and the plasmid need to be in double-stranded, linear form.
- The 3' end of the linear plasmid and the 5' end of the PCR-amplified Custom CRISPR Guide Library need to have 40–60 nucleotides of homologous overlapping sequence.
- The 5' end of the linear plasmid and the 3' end of the PCR-amplified Custom CRISPR Guide Library need to have 40–60 nucleotides of homologous overlapping sequence.
- The linearized plasmid needs to be purified and free of all parental (circular) plasmid. If you linearized the plasmid using PCR, then use the restriction enzyme Dpn I to digest any parental plasmid.
- Perform a test cloning reaction and transformation using the procedure outlined in the Agilent SureVector CRISPR Cloning Protocol (Agilent publication G7556-90000; available at www.agilent.com/genomics). Calculate the library size of the test reaction and then extrapolate that number to determine the projected library size if the reaction were scaled up to 12–15 transformations. If the projected library size is adequate for the library complexity (see guidelines in publication G7556-90000), then proceed with making a full-sized library. If the projected library size is too small, then continue to optimize the cloning reaction and/or transformations.
- In the cloning reactions, Agilent has found the following factors to be most influential in maximizing the number of individual clones in the plasmid library: 1) the quantity of Custom CRISPR Guide Library; 2) the quantity of linearized plasmid; 3) the volume of SureSolution.

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