SLALOM 1.0 ™

CRISPR-Cas9 sgRNA Library Synthesis Kit

User Manual

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Support

Thank you for purchasing a SLALOM 1.0 ™ sgRNA Library synthesis kit.
If you have any question or need support, please contact us at:

support@pioneerbiolabs.com

Kit Components

Each kit contains enough reagents to synthesize 10 individual sgRNA libraries, including libraries synthesized from control DNA. Reagents included in the kit and additional materials supplied by the user are listed below.

Supplied Reagents

- C1 Buffer
- C1 Enzyme
- L1 Buffer
- L1 Adapter
- L1/L2 Enzyme
- Capture Beads
- 10x Wash Buffer
- C2 Buffer
- C2 Enzyme
- L2 Buffer
- L2 Adapter
- Stop Buffer
- E1 Buffer
- E1 Enzyme
- PCR Primers
- Control DNA

Reagents or Materials Supplied by the User

- > 2 µg DNA (per library)
- Magnetic Rack
- Tubes for Magnetic Rack
- DNA purification columns

Magnetic Rack can use either 1.5 ml microcentrifuge tubes or 0.2 ml PCR tubes.

Overview

SLALOM 1.0™ sgRNA Library Synthesis Kits, S. pyogenes

SLALOM 1.0™ sgRNA Library Synthesis Kits, S. pyogenes provide a simple and quick method for enzymatically synthesizing high quality sgRNA libraries through a sequence of fast reactions, using the supplied reagents and source of DNA supplied by the user.

The CRISPR Cas9 complex from S. pyogenes can be programmed to bind to a specific DNA sequence by altering the first 20 nucleotides of a single guide RNA (sgRNA)[1]. Libraries of sgRNA molecules have enabled new techniques including live cell chromatin imaging and functional genomic screening, but the synthesis of custom libraries can often be a bottleneck in these experiments. SLALOM 1.0 ™ sgRNA Library Synthesis Kits permit the synthesis of a custom library in a few hours and without specialized bioinformatic techniques.

SLALOM 1.0™ sgRNA Library Synthesis Kits, S. pyogenes are based on SLALOM (sgRNA Library Assembly by Ligation Onto Magnetic beads)[2], which works on the principle of eznymatically extracting valid spacer sequences from a source of DNA. These kits were designed to be quick and highly efficent and include optional control DNA and a troubleshooting guide to ensure fast synthesis of custom libraries.

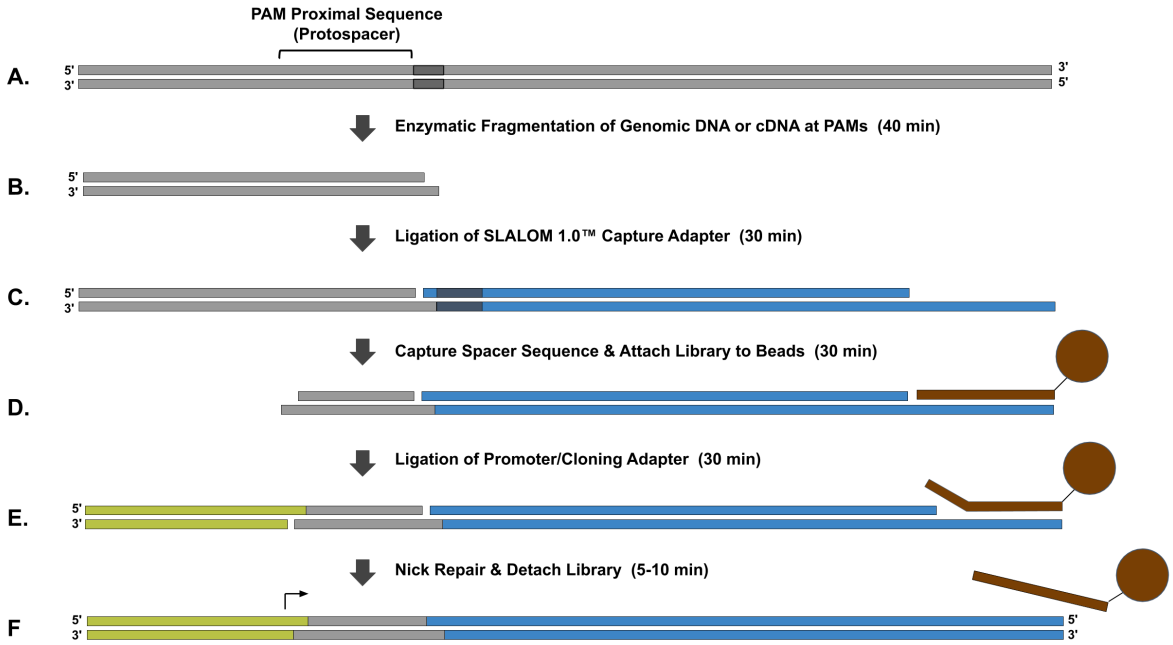

Figure 1: Workflow of SLALOM 1.0 ™ sgRNA Library Synthesis Kits, S. pyogenes.

(A). A source of DNA such as Genomic DNA or cDNA is isolated. (B). The DNA source is enzymatically digested at protospacer adjacent motifs (PAMs) to create DNA fragments with valid spacer sequences on each end of the fragments. (C). The SLALOM 1.0™ Capture Adapter is ligated to the ends of the fragments. (D). Using a restriction enzyme that cleaves approximately 20 bp away from its reqconition sequence, the spacer sequences are captured and the remaining fragments are washed away using bead purification. (E). A promoter or cloning adapter is ligated to the spacer sequence. (F). The final library is detached from the beads and nicks in the library are repaired. The library can be used as a template for in vitro transcription or cloned into plasmids depending on the downstream application.

Working with Magnetic Beads

Magnetic beads provide a fast and convienient way of handling oligonucleotides and reduces the amount of material lost during purification. Below are a few tips for working with magnetic beads.

- Do not freeze magnetic beads.
- Do not let the beads dry out.
- When resuspending the beads, avoid air bubbles by pipetting slowly.
- Ensure that the tube containing the beads securely fits into the magnetic rack.

Use the following procedure to exchange the buffer.

Exchanging Buffer

1. Apply a magnet to the side of the tube by placing it into a magnetic rack (see “Additional Materials Required” p. 5).
2. Wait for approximately 30-60 seconds for the solution to clear.
3. Remove the supernatant from the tube and discard it.
4. Add the new buffer to the beads.
5. Remove the tube from the rack and resuspend the beads by gently pipetting up and down to mix thoroughly.

DNA Input Recommendations

The second step of the kit (ligation of DNA adapter to DNA fragments) is sensitive to the amount of input DNA added to the reaction. If there is insufficient input DNA in the reaction, DNA byproducts can form. A ratio of at least 1:2 DNA adapter (10 pmol) to DNA fragments (20 pmol) will inhibit byproducts from forming. The amount of input DNA needed is dependant on the number of CCGG sites in the input DNA. For most DNA samples, the recomended starting point is 2-3 µg of DNA.

If there are unexpected bands on an agarose gel after PCR amplification, this is likely due to insufficient input DNA. To resolve this issue, the method can be rerun with a higher concentration of input DNA.

The control DNA (Lambda DNA) is at a concentration of 500 ng/µl. When using the control DNA as the input, it is reccomend that 4 µl of control DNA is used for a total of 2 µg.

Input DNA Purification

Standard spin column purification methods have been shown to be an effictive way of preparing DNA for use with this kit. However, excess salt from these columns sometimes decreases ligation efficiency. if there are problems in the ligation efficiency, is can lead to the same DNA byproducts described above. If excess salt in the input DNA sample is suspected, drop dialysis is recommended to purify the excess salt from the buffer.

Stopping Points

The reaction can be frozen after the completion of step 3, step 5, and step 19. Once the library is attached to the magnetic beads the solution should not be frozen.

SLALOM 1.0 ™ Kit Protocol

We recommend finishing the protocol using 4 µl of the control DNA provided before using DNA from a sample, especially if the sample is difficult to obtain.

Fragment DNA

1. Assemble the following reaction components in a clean 0.2 ml PCR tube at room temperature in the order listed.

Nuclease-free Water	Total reaction volume of 50 µl
C1 Buffer	5 µl
DNA	> 2 µg (see details on p. 7)
C1 Enzyme	1 µl

2. Thoroughly mix the solution by pipetting gently. If necessary, spin the tube to collect the liquid into the bottom of the tube.
3. Using a thermocycler with a heated lid, incubate at 37 °C for 40 minutes then 80°C for 20 minutes.

Ligate Scaffold Adapter

4. When the incubation from step 3 is complete, remove the tube from the thermocycler and add the following components.

L1 Buffer	7 µl
L1 Adapter	2 µl
L1/L2 Enzyme	1 µl

5. Thoroughly mix the reaction by pipetting gently. Using a thermocycler with a heated lid, incubate at 22°C for 1 hour, then 65 °C for 10 minutes.
6. During the incubation, prepare 1 ml of wash buffer by mixing 100 µl 10x Wash Buffer and 900 µl Nuclease free water and prepare the capture beads.

   Preparing Capture Beads - Resuspend the beads by pipetting gently. Aliquot 50 µl of the beads into a clean tube that is compatable with a magnetic rack (see “Additional Materials Required” p. 3) and exchange the buffer twice (2x) with 50 µl wash buffer (see “Exchanging Buffer” p. 6).

Attach Library to Magnetic Beads

7. When the incubation from step 5 is complete, place the tube containing the beads from step 6 into a magnetic rack to pellet the beads. Discard the supernatant.
8. Tranfer the solution from step 5 into the tube containing the pelleted beads and resuspend the beads by pipetting up and down to mix thoroughly.
9. Incubate the tube at room temperature for 30 minutes (keep the beads in suspension by pipetting gently when needed).

Isolate Spacer Sequences

10. When the incubation from step 9 is complete, assemble the following rection compnents in a clean 0.2 ml PCR tube on ice in the order listed.

C2 Buffer	48 µl
C2 Enzyme	2 µl

11. Place the tube containing the beads into a magnetic rack to pellet the beads. Exchange the buffer twice (2x) with 50 µl wash buffer.
12. Resuspend the beads in the solution prepared in step 10 and incubate the tube at room temperature for 1 hour (keep the beads in suspension by pipetting gently when needed).

Ligate Promoter Adapter

13. When the incubation from step 12 is complete, assemble the following rection compnents in a clean 0.2 ml PCR tube on ice in the order listed.

L2 Buffer	46 µl
L2 Adapter	3 µl
L1/L2 Enzyme	1 µl

14. Place the tube containing the beads into a magnetic rack to pellet the beads. Exchange the buffer twice (2x) with 50 µl wash buffer.

(continued on next page)

15. Resuspend the beads in the solution prepared in step 13 and incubate the tube at room temperature for 1 hour (keep the beads in suspension by pipetting gently when needed).

Detach sgRNA Library from Magnetic Beads

16. When the incubation from step 15 is complete, assemble the following rection compnents in a clean 0.2 ml PCR tube on ice in the order listed.

E Buffer	49 µl
E Enzyme	1 µl

17. Place the tube containing the beads into a magnetic rack to pellet the beads. Exchange the buffer twice (2x) with 50 µl wash buffer.
18. Resuspend the beads in the solution prepared in step 16 and incubate the tube at room temperature for 15 minutes (keep the beads in suspension by pipetting gently when needed).
19. When the incubation from step 16 is complete, place the tube containing the beads into a magnetic rack to pellet the beads. Collect the supernatant and discard the beads.

Column Purify

20. Use a DNA purification column (see Additional Materials Required) to purify the sgRNA library and elute in 12 µl of nuclease-free water or elution buffer.
21. Measure the concentration of DNA using a spectophotometer. The expected yield is about 25-50 ng/µl.
22. The sgRNA library can be stored at -20°C or amplified using PCR (see next section).

sgRNA Library Amplification

Below is an example of a 2 step PCR protocol for amplifying the sgRNA library using NEBNext® Ultra™ II Q5® 2x Master Mix. Other high fidelity polymerases can be used, but the annealing temperature should be adjusted accordingly.

1. Assemble the following rection components in a clean 0.2 ml PCR tube on ice in the order listed.

Nuclease-free Water	Total reaction volume (50 µl)
Ultra™ II Q5® Master Mix	25 µl
SLALOM 1.0™ PCR Primers	5 µl
Column Purified Library	(20 ng)

2. Thoroughly mix the solution by pipetting gently. If necessary, spin the tube to collect the liquid into the bottom of the tube.
3. Using a thermocycler with a heated lid, run the following program.

STEP	TEMPERATURE	TIME
Initial denaturation	98°C	30 seconds
10 cycles	98°C	10 seconds
	70°C	20 seconds
Final Extension	70°C	2 minutes
Hold	8°C	

4. Use a DNA purification column to purify the sgRNA library.
5. Measure the concentration of DNA using a spectophotometer. Run 50-100 ng on an agarose gel along with a 100 bp ladder.
6. The sgRNA library can be stored at -20°C or transcribed or cloned (see the following sections.

Sequencing

Sequence Information

Library oligonucleotides

A FASTA file containing the sgRNA library sequence as well as the PCR amplification primers for each version of the kit are available online. It should be noted that the scaffold sequence is slightly different from standard sgRNA scaffold sequences.

Downstream Applications

In vitro Transcription

Experiments such as live cell chromatin labeling require invitro transcription of the sgRNA library. Below is an expample protocol of in vitro transcription using the HiScribe T7 Quick High Yield RNA syntheis Kit from New England Biolabs (NEB).

HiScribe T7 Quick High Yield RNA Synthesis Kit (NEB)

1. Assemble the following rection components in a clean 0.2 ml PCR tube at room temperature in the order listed.

Nuclease-free Water	Total reaction volume of 20 µl
NTP Buffer Mix	10 µl
sgRNA Template	250-300 ng
T7 RNA Polymerae Mix	2 µl

2. Incubate at 37°C for 1-2 hours.
3. Add 28 µl Nuclease-free Water and 2 µl DNAse.
4. Incubate at 37°C for 15 minutes.
5. Purify using an RNA purification column.

Cloning into lentiCRISPRv2

The restriction enzymes Esp3I & NheI, T4 DNA ligase, lentiCRISPRv2 (addgene #52961) supplied in Stbl3 cells to prevent recombination, and High-efficiency electrocompetent cells such as Endura™ Electrocompetent cells are reccomended for cloning.

Libraries generated using the lentiCRISPRv2 version of the kit should be digested with the resriction enzyme Esp3I. The lentiCRISPRv2 plasmid should be double digested with Esp3I & NheI. Digesting the plasmid with these enzymes will produce 4 bp sticky ends compatible with the library and position the sgRNA for transcription under the U6 promoter. Gel purification of the digested library and of the digested plasmid can increase ligation efficiency. A molar ratio of 3:1 digested library to digested plasmid are reccomended as a starting point for optimizing the ligation efficiency.

Cloning into Mobile CRISPRi pJMP2846

The restriction enzyme BsaI, T4 DNA ligase, pJMP2846 (addgene #160676) supplied in BW25141 cells for replication of R6K plasmids, and High-efficiency electrocompetent cells such as TransforMax™ EC100™ Electrocompetent cells are reccomended for cloning.

Libraries generated using the Mobile CRISPRi version of the kit should be digested with the resriction enzyme BsaI. The pJMP2846 plasmid should be digested with BsaI. Digesting the plasmid with this enzymes will produce 4 bp sticky ends compatible with the library and position the sgRNA for transcription under the Lac promoter. Gel purification of the digested library and of the digested plasmid can increase ligation efficiency. A molar ratio of 3:1 digested library to digested plasmid are reccomended as a starting point for optimizing the ligation efficiency.

Troubleshooting

We recommend finishing the protocol using the provided control DNA before using a DNA sample, especially if the sample is difficult to obtain.

If there are unexpected bands on an agarose gel after PCR amplification, this is likely due to insufficient input DNA. To resolve this issue, the method can be rerun with a higher concentration of input DNA.

Unexpected bands can also be the result of excess salt in the input DNA which decreases ligation efficiency. If excess salt in the input DNA sample is suspected, drop dialysis against nuclease free water is recommended to purify the excess salt out the buffer.

If the unexpected bands persist after adding addition DNA and drop dialysis, the correct size band can be cut out of an agarose gel and reamplified.

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