Cloning high-quality CRISPR libraries with oPools Oligo Pools

Contributed by Ben Herken, BA, Department of Urology, Gilbert Lab, University of California, San Francisco

The method presented here is provided by customers who have used oPools Oligo Pools. This method can serve as a starting point for using oPools oligos in similar experiments but may not be fully optimized for your genes or application. IDT does not guarantee these methods, and application specialists at IDT can only provide general guidance with limited troubleshooting and support.

Overview

CRISPR screening libraries can be a fast way to perform high-throughput experiments to associate target DNA regions with functions. This protocol describes how to clone oPools Oligo Pools that contain guide RNA sequences into a lentiviral expression vector to create CRISPR guide RNA screening libraries.

In brief, oPools oligos are converted to double-stranded DNA using a polymerase and a primer that anneals to the 3′ end of the oligo. Then, restriction digestion and fragment purification allow ligation of the CRISPR guide RNA sequences into the expression vector.

Figure 1 provides a representative oPools oligo sequence. In this example the remainder of the guide RNA constant region is in the expression vector (i.e., pCRISPRia-v2, plasmid #84832, Addgene). In this vector, use of the BlpI site is known to introduce a single base pair mismatch compared to the *S. pyogenes* CRISPR consensus sequence but has not resulted in detectable loss of editing activity.

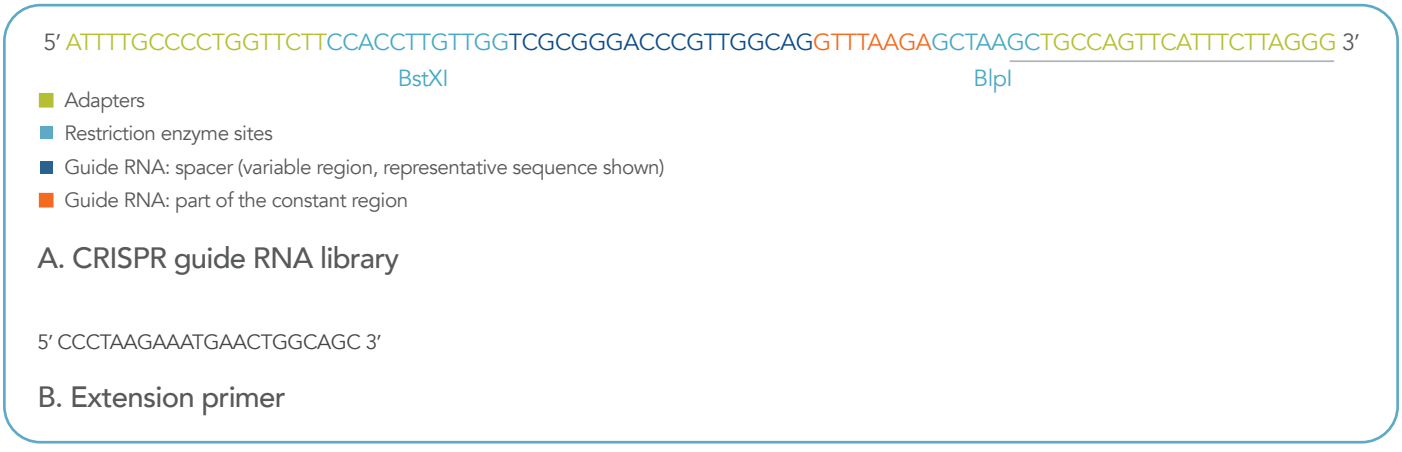

Figure 1. Representative sequences for a CRISPR library and primer. (**A**) Example sequence of an oligo in a CRISPR guide RNA library made as oPools Oligo Pools. (**B**) The extension primer binds to the 3′ end (underlined sequence in part A) of the CRISPR guide RNA library and is used to create a double-stranded library for cloning into an expression vector.

Consumables and equipment

Consumables from IDT

Item	Catalog #
oPools Oligo Pools	www.idtdna.com/oPools
Primers	www.idtdna.com/site/order/oligoentry

Consumables from other suppliers

Item	Supplier	Catalog #
NEBuffer™ 2.1 (10X)	New England Biolabs	B7202S
Klenow Polymerase	New England Biolabs	M0210S
Deoxynucleotide (dNTP) Solution Mix	New England Biolabs	N0447S
Restriction enzymes (based on your vector sequence)	New England Biolabs	Varies
100% EtOH	Various suppliers	Varies
NaOAc	Various suppliers	Varies
GlycolBlue™ Coprecipitant	Thermo Fisher Scientific	AM9515
SYBR® Gold Stain, or equivalent	Thermo Fisher Scientific	S11494
DNA Clean and Concentrator™– 5 kit	Zymo Research	D4013
Corning® Costar® Spin-X® centrifuge tube filters	Millipore-Sigma	CLS8160-24EA

Equipment

Item	Supplier	Catalog #
Thermal cycler	Various suppliers	Varies

Generate double-stranded oPools library

1. Mix 1 µg of library (i.e., oPools oligos) with an equimolar amount of primer.
2. Add 5 µL of NEBuffer 2.1 (10X), then dilute with ultra-pure water to a final volume of 48 µL.
3. Anneal the primer to the library sequences in a thermal cycler using the following conditions:

	Temperature	Time
Denature	94°C	5 minutes
Anneal	Ramp 0.1°C/sec to primer T_m*	—
	Primer T_m	5 minutes
	Ramp 0.1°C/sec to 37°C	—
	4°C	Hold

* For assistance with determining primer T_m, use the OligoAnalyzer Tool at www.idtdna.com/OligoAnalyzer.

4. Add 1 µL of Klenow Polymerase and 1 µL of 10 mM dNTPs.
5. Complete the primer extension in a thermal cycler using the following conditions:

	Temperature	Time
Extend	37°C	1 hour
Inactivate enzyme and anneal DNA	75°C	20 minutes
	Ramp 0.1°C/sec to 42°C	—
	4°C	Hold

6. Process the resulting dsDNA library through a DNA Clean and Concentrator-5 kit according to manufacturer's instructions. Elute in 10 µL of 70°C DNA Elution Buffer (from the DNA Clean and Concentrator kit).

Gel purification of the double-stranded oPools library

1. Set up an overnight restriction digest using all your double-stranded library in a 50 µL reaction according to the manufacturer's instructions.
2. Run all 50 µL of digested library on a 20% acrylamide gel for 2.5 hours.
3. Visualize DNA with SYBR Gold reagent (Figure 2) according to manufacturer's instructions and excise the desired band from the gel.

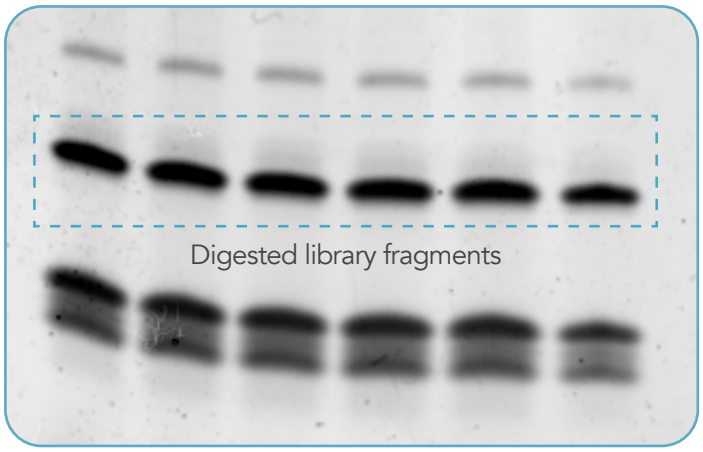

Figure 2. Representative band pattern of a digested library. Results may vary to the extent to which the digestion reaction was carried to completion and the design of your oPools oligos.

4. Fragment your gel pieces:
   a. With an 18-gauge sterile needle, carefully poke a hole in the bottom of a microfuge tube.
   b. Place your gel piece inside this tube.
   c. Place the punctured tube on top of another microfuge tube.
   d. Centrifuge at max speed for 5 minutes.
5. Add 400 µL ultra-pure water to the gel fragments and heat at 70°C for 45 minutes to elute the DNA.
6. Place the heated gel slurry into a Spin-X column and centrifuge at 20,000 x*g* for 3 minutes.
7. Recover 300 µL of eluted DNA and add to it: 3 µL of GlycolBlue reagent followed by 37.5 µL of 3M NaOAc and 900 µL of 100% EtOH.

   Note: After adding each reagent, mix thoroughly, especially after adding the EtOH.
8. Incubate overnight at –20°C.
9. Centrifuge DNA mixture at 20,000 x*g* and 4°C for 30 minutes.
10. Discard supernatant, being sure not to disturb the blue pellet while keeping on ice at all times.
11. Wash pellet with 1 mL of 80% EtOH. Centrifuge again at 20,000 x*g* and 4°C for 5 minutes, then repeat.
12. Remove all liquid from the tube and let air dry until completely dry.
13. Resuspend in 25 µL of ultra-pure water and allow the pellet to completely dissolve.

Cloning of the oPools library into expression vector

Your oPools library is now ready to ligate into a target vector for transformation. We recommend transforming your ligated oPools library using a large volume of bacteria with extremely high competency. This is important to maintain elements that might be present at a low concentration in the pool and to minimize variation in abundance among the elements in your pool.

We then use next generation sequencing (NGS) to test our oPools libraries to confirm the presence of all elements and determine variation.

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Technical support:

applicationsupport@idtdna.com