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Barcode plasmid library cloning

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ABSTRACT

Protocol for cloning Time Machine barcode plasmid library.

MATERIALS

NAME	CATALOG #	VENDOR
Gibson Assembly Master Mix - 10 rxns	E2611S	New England Biolabs
Electroporation Cuvette 1mm	1652089	BioRad Sciences
Monarch® PCR & DNA Cleanup Kit (5 µg)	T1030	New England Biolabs
LB plates with 100 µg/ml ampicillin		
LB Broth with 100ug/mL ampicillin	244620	Gibco, ThermoFisher
Lucigen Endura electrocompetent cells	60242	Lucigen
ssDNA barcode template	View	IDT
Linearized vector backbone	View	
EndoFree Plasmid maxiprep kit	12362	Qiagen

Gibson assemnbly

1 ⚠

Perform Gibson assembly usiing 100ng linearized vector and 5:1 molar excess of ssDNA template per 20 microliter reaction.

1.1 Pre-heat thermal cycler to 50ºC with lid set to 65ºC.

50 °C Pre-heat thermal cycler

1.2 Combine 100ng linearized vector with ~21.7 fmol ssDNA template (1.08 microliters of 100nM oligo) to a final volume of 10 microliters in nuclease free water. In parallel, perform a negative control reaction with plasmid and nuclease free water without the ssDNA insert. Keep on ice.

100 ng Linearized vector

1.08 µl ssDNA oligo (160nt)

10 µl **Final volume in NF water**

1.3 Thaw Gibson assembly master mix on ice then add 10 microliters to plasmid plus insert. Pipette mix then spin down if needed.

10 µl **Gibson Assembly master mix**

1.4 Incubate on thermal cycler at 50ºC for 1 hour.

50 °C 01:00:00 **incubation**

2 Purify Gibson reaction using PCR clean-up column according to manufacturer's protocol eluting in 20 microliters of

2.1 Add 40 microliters of DNA binding buffer to 20 microliters of Gibson assembly.

40 µl **DNA binding buffer**

20 µl **Gibson assembly**

2.2 Re-load eluant and re-spin.

2.3 Add 200 microliters DNA wash buffer. Make sure ethanol has been added to wash buffer before use.

200 µl **Wash buffer**

2.4 Centrifuge at 12,000 x g for 1 minute.

12000 x g

00:01:00

2.5 Repeat wash and centrifugation (steps 2.4 and 2.5)

2.6 Transfer column to a new eppendorf or collection tube then centrifuge at 12,000 x g for 2 minutes to dry column

12000 x g 00:02:00

Sometime residual liquid can collect on a lip inside the column. It may be helpful to rotate the column 180º after a 1 minute spin, then repeat another 1 minute spin to get rid of this liquid.

2.7 Add 10 microliter of nuclease free water pre-heated to 50ºC to column and incubate for ≥ 2 minutes

10 µl **nuclease free water** 50 °C **Pre-heat water** 00:02:00 **Incubate**

2.8 Centrifuge at 12,000 x g for 1 minute to elute

12000 x g **00:01:00**

2.9 Optionally nanodrop the recovered DNA. Expected concentration is 10-15ng/uL.

Electroporation

3 Elecroporation of competent cells

3.1 Pre-warm Lucigen recovery media to 37ºC

37 °C Pre-warm recovery media

3.2 If using Biorad Gene Pulser, pre-chill cuvettes to 4ºC.

4 °C Pre-chill electroporation cuvettes

3.3 Thaw Lucigen Endura electrocompetent cells on ice. Each DUO vials contains 50uL of cells, enough for 2 electroporations. Use one reaction's worth of cells to electroporate the negative control Gibson assembly.

3.4 Pipet vial of electrocompetent cells into 2 25 microliter aliquots.

3.5 Add 2 microliters of cleaned Gibson assembly to 25 microliters of electrocompetent cells.

2 µl Gibson assembly per **25 µl electrocompetent cells**

3.6 Transfer electrocompetent cells to pre-chilled cuvette. Keep on ice.

3.7 Using the GenePulserXCell, electroporate with the following manual pulse setting: PL - 25msec, C - 10uF, PC- 600Ohms, V-1800

Pulse length - 25msec, Capacitance - 10 microFaraday, Resistance - 600 ohms, Voltage - 1800 Volts.

Expected time constant around 5-6 msec.

3.8 Immediately add 975 microliters of pre-warmed recovery media to cuvette then transfer to clean eppendorf or vial.

3.9 Incubate on shaker at 250 rpm at 37ºC for 1 hour.

37 °C **01:00:00** **On shaker at 250rpm**

3.10 Plate dilutions of electroporated cells for plating and calculating electroporation efficiency.

Prepare first dilution by adding **10 µl electroporated cells** to **990 µl LB Broth**

Prepare second dilution by adding **100 µl First dilution** to **900 µl LB broth**

Plate **100 µl first dilution** on pre-warmed LB/ampicillin agar plate for a 1:1000 dilution plate.

Plate **100 µl second dilution** on pre-warmed LB/Ampicllin agar plate for a 1:10,000 dilution plate.

After incubating the plates overnight, expect to see >1,000 colonies on the 1:1000 dilution plate and >200 colonies on the 1:10,000 plate.

3.11 Add the remainder of the electroporated cells to 150mL LB broth containing 100ug/mL ampicillin. Incubate 12-14 hours on shaker at 32ºC.

150 ml LB broth

32 °C

12:00:00 - **14:00:00** **shaker**

3.12 Pellet the overnight culture by centrifugation in 50mL falcon tubes then store pellets at -20ºC or proceed immediately with maxiprep.

6000 x g **00:15:00** **4 °C** Pellet e. coli cultures.

-20 °C **Store e. coli pellets**

If centrifuge capable of 6,000 x g is unavailable, can pellet at ≥3,000 x g.

4 PCR screen colonies to confirm barcode insertion.

5 Maxiprep barcode plasmid library.