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Cost-efficient Yeast genome Flongle library

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Protocol status: Working
We use this protocol and it's working

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Abstract

A cost-efficient protocol for constructing a Flongle library.

Materials

PEG/NaCl precipitation buffer (see Rocky Mountain adventure protocol)

	A	B
	PEG 8000	9% (w/v)
	NaCl	1 M
	Tris-HCl (pH 8.0)	10 mM

PEG wash buffer

Dilute PEG/NaCl precipitation buffer with the same amount of water.

1 Assemble the following components to end repair and add A to the genomic sample: 10m

	A	B
	Ultra II buffer	1.75
	End repair buffer	1.75
	Ultra II enzyme	1
	End repair enzyme	1
	DNA	x (500 ng)
	MQ	44.5 - x

Incubate at 20 °C for 00:05:00, then 65 °C for 00:05:00.

2 Add 50 µL of Ampure beads (or equivalent beads) and mix well.
Wash with 75 % EtOH twice and elute with 11 µL of [M] 10 millimolar (mM) Tris-HCl (pH 8.0).
Keep the tube on the magnetic rack and do not disturb the beads while washing with 75% EtOH.
Use 1 µL of the solution to check the concentration with Qubit. The concentration needs to be at least [M] 30 ng/uL.

3 Assemble the following components for ligation: 1h 21m

	A	B
	DNA	10 uL
	TLB	4 uL
	Quick ligase	1.5 uL
	AMX	0.8 uL

Incubate at Room temperature for 00:30:00.

Add 2.3 µL of [M] 5 Molarity (M) NaCl and incubate at Room temperature for 00:30:00 and then cfg at max speed for 00:20:00 .
Remove sup and add PEG wash buffer, cfg at max speed for 00:01:00 .
Remove sup and add 11 µL of 10mM Tris-HCl (pH 8.0) and incubate at 4 °C for Overnight .
Check the concentration with Qubit.

4 Assemble the following priming solution:

	A	B
	FLB	2.5 uL
	FB	97.5 uL

and the library mix solution:

	A	B
	DNA	x uL (100 ng)
	SB II	10 uL
	LB II	4 uL
	MQ	6 - x uL

. Load them and start sequencing.