



DEC 16, 2022

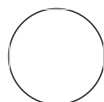
WORKS FOR ME

1

Cost-efficient Yeast genome Flongle library

COMMENTS 0

DOI

dx.doi.org/10.17504/protocols.io.e6nvwjb2wlmk/v1Yutaro Hori¹¹The University of Tokyo

Yutaro Hori

ABSTRACT

A cost-efficient protocol for constructing a Flongle library.

DOI

dx.doi.org/10.17504/protocols.io.e6nvwjb2wlmk/v1

PROTOCOL CITATION

Yutaro Hori 2022. Cost-efficient Yeast genome Flongle library . **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.e6nvwjb2wlmk/v1>



LICENSE

————— This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

CREATED

Dec 16, 2022

LAST MODIFIED

Dec 16, 2022

PROTOCOL INTEGER ID

74086

MATERIALS TEXT

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.

10m

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

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 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 30 ng/uL .

1h 21m

- 3 Assemble the following components for ligation:

A	B
DNA	10 uL
TLB	4 uL

A	B
Quick ligase	1.5 uL
AMX	0.8 uL

Incubate at Room temperature for 00:30:00 .

Add 2.3 μ L 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 μ L 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.