# DNA extraction protocol for yeast PacBio sequencing

**Douda Bensasson**

## Abstract

A very detailed four-day protocol for DNA extraction for yeast PacBio sequencing modified from Promega's protocol for yeast p13-14.

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## Guidelines

# Promega's Wizard® Genomic DNA Purification Kit

Wizard® Genomic DNA Purification Kit (Catalog number: A1125) Other reagents:

- 0.5M EDTA (Life Technologies, Catalogue number: AM9260G). Dilute 1:10 for 50mM EDTA.
- lyticase (Sigma, Catalogue number: L2524-50KU). For a 25 units/ul stock, suspend in 2ml UltraPure H2O and dispense into 2x 1ml aliquots, store at -20C

Note: This is a very detailed protocol because it is actually just our standard lab protocol x 8 with a couple of extra steps to increase purity for PacBio.

Modified from Promega's protocol for yeast p13-14:

Some of the changes to our standard protocol might have been unnecessary e.g. We spun samples at 16,162g and for longer than suggested: for 10 minutes after the addition of Nuclei Lysis Solution (Promega) and Protein Precipitation Solution (Promega); for 10 minutes after DNA precipitation with isopropanol; and for 5 minutes after washing the DNA pellet with 70% ethanol. I am not sure the extra time on spins is really necessary if you know your centrifuge is good: we had just had problems with a lab centrifuge, which lowered DNA quality and yield.

## Before start

Future improvements: This is a mini-prep kit for DNA extractions from 1.5ml of yeast culture. Consider using a midi-prep kit for higher yield without needing 8 reactions per strain.

## Materials

Wizard® Genomic DNA Purification Kit A1125 by Promega
O.5M EDTA AM9260G by Thermo Fisher Scientific
Lyticase L2524-50KU by Millipore Sigma

## Protocol

Day One
***Step 1.***

For each strain, inoculate 15 ml YPD in 50 ml Falcon tubes from single colonies on YPD plates using a sterile inoculation loop.

AMOUNT
15 ml : YPD for innoculation

Day One
***Step 2.***

Incubate at overnight at 30°C (for at least 20 hours, e.g. 24 hours).

TEMPERATURE
30 °C : Incubation
DURATION
20:00:00 : Incubation

Day Two
***Step 3.***

Take 1.4 ml of each overnight culture and add to a 1.5 ml microcentrifuge tube.

*Do this a total of 8 times for each strain (so there are 8 microcentrifuge tubes for each strain).

AMOUNT
1.4 ml : culture

Day Two
***Step 4.***

Centrifuge at high speed (14,000 rpm on Eppendorf centrifuge or 16,162g on other) for 2 minutes to pellet the cells.

DURATION

00:02:00 : Centrifugation

Day Two

***Step 5.***

Remove and discard the supernatant into a yeast waste jar containing Virkon solution (make this up with Virkon powder and water to a deep pink color).

Day Two

***Step 6.***

Pipette to resuspend the pellet in 296 µl 50mM EDTA pH 8.

AMOUNT

296 µl : 50mM EDTA pH 8

Day Two

***Step 7.***

Add 4 µl Lyticase (25U/ul, diluted stock from Sigma) and incubate overnight at 37°C.

AMOUNT

4 µl : Lyticase

TEMPERATURE

37 °C : Incubation Temperature

NOTES

We used only 100 units of lyticase (Sigma, L2524) in an overnight incubation at 37 °C to save money (past experiments showed no change in yield).

Day Three

***Step 8.***

Centrifuge the sample at 16,162g for 2 mins.

DURATION

00:02:00 : Centrifugation

Day Three

***Step 9.***

Remove and discard the supernatant.

Day Three

***Step 10.***

Add 300 µl Promega Nuclei Lysis Solution.

AMOUNT

300 µl : Promega Nuclei Lysis Solution

Day Three

***Step 11.***

Add 100 µl Promega Protein Precipitation Solution. Stir with the pipette tip and pipette up and down to resuspend the pellet.

AMOUNT
100 µl : Promega Protein Precipitation Solution

NOTES

The combination of Protein Precipitation Solution and Nuclei Lysis Solution makes the mixture cloudy (not the pellet or protein).

Day Three
***Step 12.***

Let the sample sit on ice for 5-30 minutes.

DURATION
00:05:00 : on ice

Day Three
***Step 13.***

Centrifuge at high speed for 10 minutes.

DURATION
00:10:00 : Centrifugation

Day Three
***Step 14.***

Carefully pipette the supernatant to a clean 1.5 ml microcentrifuge tube containing 300 µl Isopropanol.

AMOUNT
300 µl : Isopropanol

NOTES

Be careful to avoid the protein pellet and leave some residual liquid to avoid contaminating the DNA solution.

Day Three
***Step 15.***

Invert the samples 50 times, and a wispy mass might be visible.

Day Three
***Step 16.***

Centrifuge at high speed for 10 minutes.

DURATION
00:10:00 : Centrifugaiton

Day Three

***Step 17.***

- Carefully pipette off and discard the supernatant without dislodging the pellet.
- Drain the tube on clean absorbent paper. At this stage, you should see a pellet for all samples except negative controls (if you see a pellet in the negatives, then this is probably not DNA and will be purified away in subsequent steps).

NOTES

If the pellet is dislodged during the removal of the supernatant, respin the sample. You may need to leave some residual liquid in the tube to avoid dislodging the pellet (the pellet after the ethanol wash below will be whiter and less easy to dislodge).

Day Three

***Step 18.***

Add 300 µl of room temperature 70% ethanol and gently invert the tube 20 times to wash the DNA pellet.

AMOUNT

300 µl : 70% ethanol

Day Three

***Step 19.***

Centrifuge at 16,162g for 5 mins. Carefully pipette off all the ethanol.

DURATION

00:05:00 : Centrifugation

NOTES

Note: the pellet at this stage contains impurities, so the size (or visibility) of the pellet is not an indicator of final yield.

Day Three

***Step 20.***

Drain the tube on clean absorbent paper, and allow the pellet to air dry for 15 minutes.

DURATION

00:15:00 : Drying time

Day Three

***Step 21.***

Add 25 µl Promega DNA rehydration solution, and gently resuspend the pellet by pipetting.

AMOUNT

25 µl : Promega DNA rehydration solution

Day Three

***Step 22.***

Add 1.5 µl of Promega RNase solution and pipette to mix the RNase into each sample.

AMOUNT
1.5 µl : Promega RNase solution

NOTES

Also, a 1:2 dilution of the RNase solution from Qiagen (i.e. 5.25 units, Qiagen, Cat. no. 19101) can be used in place of the Promega RNase solution.

Day Three
***Step 23.***

Incubate at 37°C for 1-2 hours.

TEMPERATURE
37 °C : Incubation
DURATION
00:01:00 : hours

Day Three
***Step 24.***

Vortex for 1 second, and spin quickly to collect liquid.

DURATION
00:00:01 : Vortex

Day Three
***Step 25.***

Leave at room temperature overnight, then store DNA extracts in the fridge at 2-8°C until ready for ethanol precipitation (could be several days later).

TEMPERATURE
2 °C : Storage

Day Three
***Step 26.***

Check yield of every sample on gel. If all samples give a good yield for each strain, then it is ok to pool them all. Discard any samples where 1 µl is not visible on a gel.

Day Four
***Step 27.***

Pool samples for each strain and remove RNA from purified DNA.

NOTES

(modified from Qiagen's protocol, Apendix D)

*I am pretty sure any ethanol precipitation protocol would be fine here. I only used the protocol and 'Protein Precipitation Solution' from Qiagen because it was to hand.

Day Four

***Step 28.***

Cool DNA extracts 15 minutes on ice.

DURATION

00:15:00 : Cooling

Day Four

***Step 29.***

Spin at high speed for 10 minutes.

DURATION

00:10:00 :

Day Four

***Step 30.***

Pool the supernatants of the four extracts for each strain into a fresh tube, bringing the final volume for each strain to approximately 180 µl.

Day Four

***Step 31.***

Add 20 µl (or other amounts) of Promega (or Qiagen's) DNA Hydration solution, bringing the final volume of DNA extract up to 200 µl for each strain.

AMOUNT

20 µl : Promega DNA Hydration solution

Day Four

***Step 32.***

Add 6 µl RNase A from Promega (or 3 µl RNase A Solution from Qiagen), mix by pipetting, quick 1 second vortex and spin.

AMOUNT

6 µl : RNase A

DURATION

00:00:01 : vortex

Day Four

***Step 33.***

Incubate at 37°C for 1 hour, then transfer to ice.

TEMPERATURE
37 °C : incubation
DURATION
01:00:00 : incubation

Day Four

***Step 34.***

Add 100 µl Promega (or Qiagen) Protein Precipitation Solution (0.5 volume), 400 µl 96-100% Ethanol from freezer (2 volumes).

Day Four

***Step 35.***

Mix by inverting 50 times (visible mass formed in all except negatives). Leave on ice for 15 mins.

DURATION
00:15:00 : on ice

Day Four

***Step 36.***

Centrifuge at high speed for 10 minutes.

DURATION
00:10:00 : Centrifugation

Day Four

***Step 37.***

Carefully, pipette off supernatant.

Day Four

***Step 38.***

Add 600 µl room temperature 70% ethanol (3 volumes).

AMOUNT
600 µl : 70% ethanol

Day Four

***Step 39.***

Centrifuge at high speed for 3 minutes.

DURATION
00:03:00 : Centrifugation

Day Four

***Step 40.***

Carefully, pipette off and discard supernatant and air dry the pellet for 15 minutes.

DURATION
00:15:00 : air dry

Day Four

***Step 41.***

Resuspend pellet in 110 µl Qiagen DNA Hydration Solution (1xTE) by gentle pipetting.

AMOUNT
110 µl : Qiagen DNA Hydration Solution

Day Four

***Step 42.***

Incubation at 37 ºC for 1-1.5 hours to fully dissolve the DNA.

TEMPERATURE
37 °C : Incubation
DURATION
01:00:00 : Incubation

Day Four

***Step 43.***

Quick spin, pipette to mix, 15 minutes at room temperature.

DURATION
00:15:00 :

Day Four

***Step 44.***

Centrifuge at high speed for 10 minutes in order to pellet any impurities that might have precipitated together with the DNA.

DURATION
00:10:00 : Centrifugation

NOTES

The DNA should now be fully dissolved and will no longer form a pellet.

Day Four

***Step 45.***

Transfer the supernatants to new tubes (even though no pellets were visible at this stage).

NOTES

The quality and quantity of DNA was assessed by running alongside a high molecular weight ladder (GeneRuler High Range DNA Ladder, SM1351) on a 0.5% agarose gel stained with 1x Gel Red in 1x TAE buffer, by NanoDrop, and using a Qubit fluorometer.

## Warnings

See SDS (Safety Data Sheet) for warnings and safety hazards.