

Bioingentech Genomic DNA Purification

Isolation of Genomic DNA from Yeast Culture or Plant Tissue

Prepare Yeast Lysate

1. Pellet cells from 0.5ml of culture by centrifugation at 13,000-16,000 x g* for 2 minutes.
2. Suspend the cell pellet in 150µl of 50mM EDTA.
3. Add 4µl 20mg/ml lyticase and mix gently.
5. Centrifuge as in Step 1. Discard the supernatant.
6. Add 120µl of Nuclei Lysis Solution. Proceed to **Protein Precipitation and DNA Rehydration** Step 1 (below).

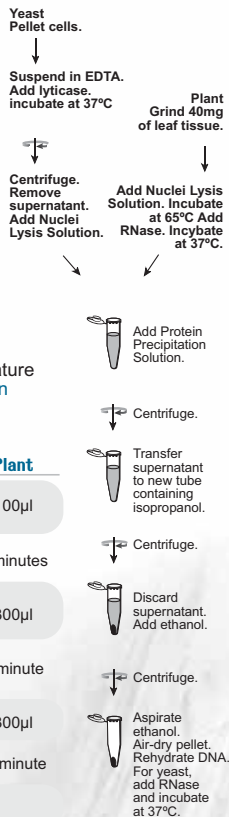
Prepare Plant Lysate

1. Grind approximately 10mg of leaf tissue in liquid nitrogen.
2. Add 120µl of Nuclei Lysis Solution. Incubate at 65°C for 15 minutes.
3. Add 2µl of RNase Solution. Incubate at 37°C for 15 minutes. Cool sample to room temperature for 5 minutes. Proceed to **Protein Precipitation and DNA Rehydration** Step 1 (below).

Protein Precipitation and DNA Rehydration

	Yeast	Plant
1. Add Protein Precipitation Solution. Vortex. For yeast only: Incubate 5 minutes on ice.	50µl	100µl
2. Centrifuge at 13,000-16,000 x g*.	3 minutes	3 minutes
3. Transfer supernatant to clean tube containing room temperature isopropanol.	150µl	300µl
4. Mix by inversion and centrifuge at 13,000-16,000 x g*.	2 minutes	1 minute
5. Decant supernatant and add room temperature 70% ethanol.	150µl	300µl
6. Centrifuge at 13,000-16,000 x g*.	2 minutes	1 minute
7. Aspirate the ethanol and air-dry the pellet.		
8. Add DNA Rehydration Solution.	20µl	40µl
9. For yeast only: Add RNase. Incubate at 37°C for 15 minutes.	1µl	
10. Rehydrate at 65°C for 5 minutes or overnight at 4°C.		

*Maximum speed on a microcentrifuge
See additional protocol information in Technical Manual, available upon request from Bioingentech on line www.bioingentech.com



Bioingentech Genomic DNA Purification

Isolation of Genomic DNA from Whole Blood

Simple size	Lysis solution Cell	Proteinase K Solution	RNase Solution	Protein Precipitation solution	Isopropanol	DNA Rehydration solution
300µl	900µl	120µl	2µl	1µl	42µl	20µl
1ml	3ml	500µl	6µl	3µl	175µl	50µl
3ml	9ml	1.5ml	18µl	1.5µl	525µl	80µl
10ml	30ml	5ml	60µl	5µl	1.75ml	250µl

As little as 20µl can be processed using this system.

Red Blood Cell Lysis

1. Using Volumes from the table above, combine the appropriate volumes of Cell Lysis Solution and blood. Mix by inversion.
2. Incubate for 5 minutes at room temperature.
3. Centrifuge:
 - <300µl sample 13,000 -16,000 x g*; 2 minutes
 - 1-10ml sample 2,000 x g; 2 minutes
4. Discard supernatant. Vortex pellet.
5. The pellet must be completely white. Otherwise repeat steps 1 to 4.

Nuclei Lysis

6. Using volumes from the table above, add Nuclei Lysis Solution an mix by inversion.
7. Add 2µl proteinase K solution, incubate 30 minutes at 55-65°C.
8. Incubate at 90°C for 3 minutes.
9. Add RNase Solution, incubate 20 minutes at 35-40°C.

Protein Precipitation

10. Add Protein Precipitation Solution; vortex for 20 seconds.
11. Centrifuge as in step 3.

DNA Precipitation and rehydration

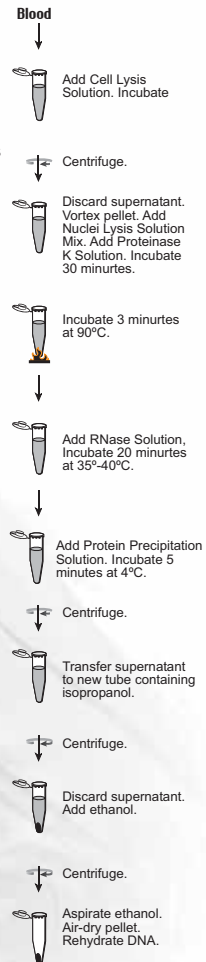
12. Transfer supernatant to a new tube containing isopropanol (using volumes from table above). Mix.
13. Centrifuge as in step 3.
14. Discard supernatant. Add 70% ethanol (same volume as isopropanol).
15. Centrifuge as in step 3.
16. Aspirate the ethanol and air-dry the pellet (10-15 minutes).
17. Rehydrate the DNA in the appropriate volume of DNA Rehydration Solution for 5 minutes at 65°C or overnight at 4°C.

*Maximum speed on a microcentrifuge
See additional protocol information in technical manual Bighth0075, available upon request from Bioingentech or online at www.bioingentech.com

NOTE: The reagents in the kit, are designed for 50 DNA purifications, from samples of 300µl of whole blood.

ORDERING/TECHNICAL INFORMATION:

www.bioingentech.com · phone (056-41) 2790435



Isolation of Genomic DNA from Animal Tissue and Tissue Culture Cells

Prepare Tissues

Tissue Culture Cells : Centrifuge 200µl (10⁶ Cells) at 13,3000-16,000 x g* for 2 minutes. Wash the cell pellet with PBS, vortex and then add 120µl of Nuclei Lysis Solution and mix by pipetting.

Animal Tissue : Add 5-10mg of fresh or thawed tissue to 120µl of chilled Nuclei Lysis Solution and homogenize for 10 seconds. Alternatively, use 5-10mg of ground tissue. Incubate at 65°C for 15-30 minutes.

Mouse Tail : Add 120µl of chilled Nuclei Lysis Solution to 0.2 -0.5 cm of fresh or thawed mouse tail. Add 5µl of 20mg/ml Proteinase K and incubate 30 minutes at 55-65°C with gentle shaking.

Lysis and Protein Precipitation

1. Add 1.5µl of RNase Solution to the cell or animal tissue nuclei lysate and mix. Incubate for 15-30 minutes at 37°C. Cool to room temperature.
2. Add 100µl of Protein Precipitation Solution. Vortex and chill on ice for 5 minutes.
3. Centrifuge at 13,000-16,000 x g* for 3 minutes.

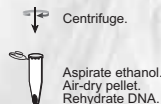
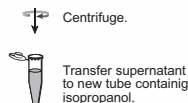
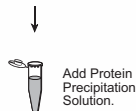
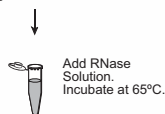
DNA Precipitation and Rehydration

4. Transfer supernatant to a fresh tube containing 300µl of room temperature isopropanol.
5. Mix gently by inversion.
6. Centrifuge at 13,000-16,000 x g* for 2 minutes.
7. Remove supernatant and add 300µl of room temperature 70% ethanol. Mix
8. Centrifuge as in Step 6.
9. Aspirate the ethanol and air-dry the pellet for 15 minutes.
10. Rehydrate the DNA in 40µl of DNA Rehydration Solution for 5 minutes at 65°C or overnight at 4°C.

*Maximum speed on a microcentrifuge

See additional protocol information in technical manual B1gth0075, available upon request from Bioingentech or online at www.bioingentech.com

Cells or tissue
with Nuclei
Lysis Solution.



Isolation of Genomic DNA from Gram Positive and Gram Negative Bacteria

Pellet Cells

Centrifuge 0.5ml of overnight culture for 2 minutes at 13,000-16,000 x g*. Discard the supernatant.

A. For Gram Positive Bacteria

1. Suspend cells in 150µl 50mM EDTA.
2. Add Lytic enzyme(s) (30µl) [lysozyme and/or lyostaphin].
3. Incubate at 37°C for 30-60 minutes.
4. Centrifuge for 2 minutes at 13,000-16,000 x g* and remove supernatant.
5. Go to Step 1, Lyse Cells (below)

B. For Gram Negative Bacteria

Go to Step 1, Lyse Cells (below)

Lyse Cell

1. Add 120µl Nuclei Lysis Solution. Pipet gently to mix.
2. Incubate for 5 minutes at 80°C, then cool to room temperature.
3. Add 1.5µl of RNase Solution. Mix, incubate at 37°C for 20-40 minutes, then cool to room temperature.

Protein Precipitation

4. Add 100µl of Protein Precipitation Solution. Vortex.
5. Incubate on ice for 5 minutes.
6. Centrifuge at 13,000-16,000 x g* for 3 minutes.

DNA Precipitation and Rehydration

7. Transfer the supernatant to a clean tube containing 300µl of room temperature isopropanol. Mix.
8. Centrifuge as in Steps 6, and discard the supernatant.
9. Add 300µl of room temperature 70% ethanol. Mix.
10. Centrifuge for 2 minutes at 13,000-16,000 x g* .
11. Aspirate the ethanol and air-dry the pellet for 10-15 minutes.
12. Rehydration the DNA pellet in 40µl of rehydration Solution for 5 minutes at 65°C or overnight at 4°C.

*Maximum speed on a microcentrifuge
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