PBS Buffer (Phosphate Buffered Saline) (1x pH 7.4)

Date: 2024-06-18
Category: Protocols
Created by: Tim Velden

Component	Amount	Concentration
Sodium Chloride (mw: 58.44 g/mol)	8 g	0,137 M
Potassium Chloride (mw: 74.55 g/mol)	0,2 g	0,0027 M
Sodium Phosphate Dibasic (mw: 141.96 g/mol)	1,44 g	0,01 M
Potassium Phosphate Monobasic (mw: 136.09 g/mol)	0,245 g	0,0018 M

1. Prepare 800 mL of distilled water in a suitable container.
2. Add 8 g of Sodium chloride to the solution.
3. Add 0.2 g of Potassium Chloride to the solution.
4. Add 1.44 g of Sodium Phosphate Dibasic to the solution.
5. Add 0.245 g of Potassium Phosphate Monobasic to the solution.
6. Adjust solution to desired pH (typically pH ≈ 7.4).
7. Add distilled water until the volume is 1 L.

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Genomic DNA Extraction from bacteria for obtaining PCR template

Date: 2024-06-10
Tags: K. xylinus | Genomic DNA | gDNA extraction
Category: Protocols
Created by: Kayra Sofie Rüffer

Genomic DNA Extraction from bacteria for obtaining PCR template

1. Pre-heat a thermoblock to 95°C
2. Transfer 1 ml of liquid bacterial culture into a 1.5 ml Eppi
3. Centrifuge for 3 mins at 4,000 g and decant the supernatant
4. Wash the pellet with PBS buffer and vortex briefly
5. Centrifuge for 3 mins at 4,000 g and decant the supernatant
6. Repeat the previous two steps of washing and centrifugation
7. Re-suspend the pellet in MilliQ water
8. Boil at 95°C for 10 mins
9. Centrifuge for 3 mins at 11,000 g
10. Re-suspend the pellet in the supernatant

Note: This lab protocol is currently designed for gDNA extraction in Vibrio, but we will need to modify/adapt it for use in K. xylinus.

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Monarch_Genomic DNA Purification Kit Protocol

Date: 2024-06-05
Tags: K. xylinus | Genomic DNA
Category: Protocols
Status: Work in progress
Created by: Meggie Weber

Before You Begin:

Store RNase A and Proteinase K at -20°C.
Add ethanol (≥ 95%) to the gDNA Wash Buffer concentrate as indicated on the bottle label.
Set a thermal mixer (e.g. ThermoMixer®) or, if not available, a heating block to 56°C for sample lysis.
Set a heating block to 60°C. Preheat the appropriate volume of elution buffer to 60°C (35-100 µl per sample).
Do not load a single column with the lysed sample more than once; over-exposure of the matrix to the lysed sample can cause the membrane to expand and dislodge.
Genomic DNA Purification Consists of Two Stages:

PART 1: Sample Lysis (Not needed for our experiment)

PART 2: Genomic DNA Binding and Elution

PART 2: GENOMIC DNA BINDING AND ELUTION

1. Add 400 µl gDNA Binding Buffer to the sample and mix thoroughly by pulse-vortexing for 5-10 seconds. Thorough mixing is essential for optimal results.

2. Transfer the lysate/binding buffer mix (~600 µl) to a gDNA Purification Column pre-inserted into a collection tube, without touching the upper column area. Proceed immediately to step 3. Do not reload the same column with more sample; over-exposure of the matrix to the lysed sample can cause the membrane to expand and dislodge. Avoid touching the upper column area with lysate/binding mix and avoid transferring foam that may have formed during lysis. Any material that touches the upper area of the column, including any foam, may lead to salt contamination in the eluate.

3. Close the cap and centrifuge: first for 3 minutes at 1,000 x g to bind gDNA (no need to empty the collection tubes or remove from centrifuge) and then for 1 minute at maximum speed (> 12,000 x g) to clear the membrane. Discard the flow-through and the collection tube.

4. Transfer column to a new collection tube and add 500 µl gDNA Wash Buffer. Close the cap and invert a few times, so that the wash buffer reaches the cap. Centrifuge immediately for 1 minute at maximum speed (12,000 x g), and discard the flow through. The collection tube can be tapped on a paper towel to remove any residual buffer before reusing it in the next step.

5. Replace the column into the collection tube. Add 500 µl gDNA Wash Buffer and close the cap. Centrifuge immediately for 1 minute at maximum speed (>12,000 x g), then discard the collection tube and flow through.

6. Place the gDNA Purification Column in a DNase-free 1.5 ml microfuge tube (not included). Add 35-100 µl preheated (60°C) gDNA Elution Buffer, close the cap and incubate at room temperature for 1 minute. Elution in 100 µl is recommended, but smaller volumes can be used and will result in more concentrated DNA but a reduced yield (20–25% reduction when using 35 µl). Eluting with preheated elution buffer will increase yields by ~20–40% and eliminates the need for a second elution. For applications in which a high DNA concentration is required, using a small elution volume and then eluting again with the eluate may increase yield (~10%). The elution buffer (10 mM Tris-Cl, pH 9.0, 0.1 mM EDTA) offers strong protection against enzymatic degradation and is optimal for long term storage of DNA. However, other low-salt buffers or nuclease-free water can be used if preferred.

7. Centrifuge for 1 minute at maximum speed (> 12,000 x *g*) to elute the gDNA.

Source:
https://www.neb.com/en/protocols/2019/04/30/quick-protocol-for-extraction-and-purification-of-genomic-dna-using-the-monarch-genomic-dna-purification-kit-neb-t3010

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Xyloglucan Extraction from Tamarind Seeds

Date: 2024-06-04
Tags: xyloglucan
Category: Protocols
Status: Verified
Created by: Zio Kim

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1. Tamarind fruits were deseeded and dried overnight at 50°C.

2. Dried seeds were ground in a knife mill (Retsch GM 200).

- 4,000 rpm for 10 sec with 2 sec interval x 4
- 6,000 rpm for 6 sec x 3
- 8,000 rpm for 8 sec x 2
- 10,000 rpm for 6 sec x 5

3. Defat with hexane (seed powder to hexane in 1:3 ratio)

- stir for 60-90 minutes at 400 rpm
- wait 30 min for powder to settle and decant
- repeat the process three times

4. Dry the powder overnight inside a fume hood

5. The seed powder was suspended in water (5 g/100 mL) and stirred at RT overnight.

6. The suspension was transferred to a falcon tube and centrifuged at 2,000 g for 10 min in a table-top centrifuge and the supernatant, containing solubilized xyloglucan (XGs), was decanted into a graduated cylinder. The pellet was discarded.

7. Three volumes of absolute ethanol were added to the supernatant and stirred at 4°C overnight.

8. The solution was transferred to culture flask and centrifuged at 10,000 g for 15 min in a Beckman Avanti centrifuge (Beckman, Ontario, CA USA).

9. The supernatant was discarded, and the pellet was dissolved in water and centrifuged at 10,000 g for 15 min.

10. The supernatant, containing the tamarind XGs, was frozen and freeze dried.

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Genomic DNA Purification from Gram-negative Bacteria (NEB #T3010)

Date: 2024-06-04
Tags: K. xylinus | Genomic DNA | DNA purification
Category: Protocols
Created by: Said Laibacher

Genomic DNA Purification from Gram-negative Bacteria (NEB #T3010)

Up to 2 x 10^9 Gram-negative bacteria can be processed using either a quick protocol which employs Lysozyme for bacterial cell wall lysis, or a longer protocol that does not require enzymatic lysis with Lysozyme. Both protocols are available below.

Before You Begin:

- Store RNase A and Proteinase K at -20°C.
- Add ethanol (≥ 95%) to the Monarch gDNA Wash Buffer concentrate as indicated on the bottle label.
- Cold PBS or 10 mM Tris-HCl pH 8.0 is required (not supplied).
- Set a thermal mixer (e.g. ThermoMixer or similar device), or a heating block to 56°C for sample lysis.
- For Lysozyme-based Lysis:
    - Set a thermal mixer or heating block to 37°C.
    - Prepare or thaw a stock solution of Lysozyme (not supplied) (25 mg/ml in water or 10 mM Tris-Cl, pH 8.0)
- To prepare for elution, set a heating block to 60°C. Preheat the appropriate volume of elution buffer to 60°C (35–100 µl per sample). Confirm the temperature, as temperatures are often lower than indicated on the device.
- Do not load a single column with the lysed sample more than once; over-exposure of the matrix to the lysed sample can cause the membrane to expand and dislodge.

Rapid Protocol (requires Lysozyme)

1. Harvest a maximum of up to 2 x 10^9 Gram-negative bacteria by centrifugation for 1 minute at > 12,000 x g. Discard supernatant.

2. Add 90 µl of cold PBS or 10 mM Tris-Cl pH 8.0 and resuspend bacterial pellet by vortexing.

3. Add 10 µl Lysozyme solution (25 mg/ml) and vortex briefly, then add 100 µl Tissue Lysis Buffer and vortex thoroughly.

4. Incubate at 37°C for 5 minutes or until clear. Most lysates will become fully clear, but for some bacteria a slight haze may remain.

5. Add 10 µl Proteinase K, vortex briefly, and incubate at 56°C for a minimum of 30 minutes in a thermal mixer with agitation at full speed (~1400 rpm).

6. Add 3 µl of RNase A to the lysate, vortex briefly, and incubate for a minimum of 5 minutes at 56°C with agitation at full speed (~1400 rpm).

7. Proceed to Genomic DNA Binding and Elution.

Simplified Protocol (no Lysozyme required)

1. Harvest a maximum of up to 2 x 10^9 Gram-negative bacteria by centrifugation for 1 minute at > 12,000 x g. Remove supernatant.

2. Add 100 µl of PBS or 10 mM Tris-Cl pH 8.0 and resuspend bacterial pellet by vortexing.

3. Add 10 µl Proteinase K and vortex briefly, then add 100 µl Tissue Lysis Buffer and vortex thoroughly.

4. Incubate at 56°C for 1–4 hours in a thermal mixer with agitation at full speed until the lysate is mostly clear and ceases to change in appearance (lysis is usually complete within 2 hours).

5. Add 3 µl of RNase A to the lysate, vortex briefly, and incubate for a minimum of 5 minutes at 56°C with agitation at full speed (~1400 rpm).

6. Proceed to Genomic DNA Binding and Elution.

GENOMIC DNA BINDING AND ELUTION

1. **Add 400 µl gDNA Binding Buffer to the sample and mix thoroughly by pulse-vortexing for 5-10 seconds.** Thorough mixing is essential for optimal results.

1. **Transfer the lysate/binding buffer mix (~600 µl) to a gDNA Purification Column pre-inserted into a collection tube, without touching the upper column area. Proceed immediately to step 3.** Do not reload the same column with more sample; over-exposure of the matrix to the lysed sample can cause the membrane to expand and dislodge. Avoid touching the upper column area with lysate/binding mix and avoid transferring foam that may have formed during lysis. Any material that touches the upper area of the column, including any foam, may lead to salt contamination in the eluate.

1. **Close the cap and centrifuge: first for 3 minutes at 1,000 x *g* to bind gDNA (no need to empty the collection tubes or remove from centrifuge) and then for 1 minute at maximum speed (> 12,000 x *g*) to clear the membrane. Discard the flow-through and the collection tube.** For optimal results, ensure that the spin column is placed in the centrifuge in the same orientation at each spin step (for example, always with the hinge pointing to the outside of the centrifuge); ensuring the liquid follows the same path through the membrane for binding and elution

can slightly improve yield and consistency.

1. **Transfer column to a new collection tube and add 500 µl gDNA Wash Buffer. Close the cap and invert a few times, so that the wash buffer reaches the cap. Centrifuge immediately for 1 minute at maximum speed (12,000 x *g*), and discard the flow through.** The collection tube can be tapped on a paper towel to remove any residual buffer before reusing it in the next step. Inverting the spin column containing wash buffer prevents salt contamination in the eluate.

1. **Reinsert the column into the collection tube. Add 500 µl gDNA Wash Buffer and close the cap. Centrifuge immediately for 1 minute at maximum speed (>12,000 x *g*), then discard the collection tube and flow through.**

1. **Place the gDNA Purification Column in a DNase-free 1.5 ml microfuge tube (not included). Add 35-100 µl preheated (60°C) gDNA Elution Buffer, close the cap and incubate at room temperature for 1 minute.** Elution in 100 µl is recommended, but smaller volumes can be used and will result in more concentrated DNA but a reduced yield (20–25% reduction when using 35 µl). Eluting with preheated elution buffer will increase yields by ~20–40% and eliminates the need for a second elution. For applications in which a high DNA concentration is required, using a small elution volume and then eluting again with the eluate may increase yield (~10%). The elution buffer (10 mM Tris-Cl, pH 9.0, 0.1 mM EDTA) offers strong protection against enzymatic degradation and is optimal for long term storage of DNA. However, other low-salt buffers or nuclease-free water can be used if preferred. For more details on optimizing elution, please refer to “Considerations for Elution & Storage” in the product manual.

1. **Centrifuge for 1 minute at maximum speed (> 12,000 x *g*) to elute the gDNA.**

Reference:

https://www.neb.com/en/protocols/2018/10/25/genomic-dna-purification-from-gram-negative-bacteria-t3010

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K. xylinus Cultivation and Bacterial Cellulose Pellicle Harvest

Date: 2024-05-30
Tags: Protocol | K. xylinus | property testing
Category: Protocols
Status: Verified
Created by: Zio Kim

Pre-culture:

- Prepare a YPD agar plate.
- In a sterile laminar flow, add 100 mL aliquot from the primary (or passaged) inoculum to the plate.
- Streak the liquid culture evenly using a cell spreader.
- Incubate on room temperature up to 3 weeks to be used as a cell culture to be used for preparing primary inoculum.

Primary Inoculum:

- Prepare a 500 mL beaker.
- In a sterile laminar flow, add 150 mL aliquot from the YPD media.
- Streak out colonies of *K. xylinus* from the pre-culture (approx. 1 colony per 1 mL).
- Incubate without agitation at 30°C for 72 hours.

For Chemical (HPLC) Test:

- Prepare three sterile 200 mL beakers with 65 mm diameter.
- In a sterile laminar flow, add 18 mL aliquot from the composite HS + hemicellulose growth media (or YPD + hemicellulose media).
- add 2 mL inoculum of *K. xylinus*.
- Incubate without agitation at 30°C for 168 hours (one week).
- After incubation, autoclave the pellicles submerged in deionised water.
- After autoclave, the composite pellicle is removed with forceps and washed at room temperature by gentle agitation (50 rpm), in a sterile 3 L glass beaker containing excess ice-cold sterile deionised water.
    - This process is carried out until the pellicle changes from off-white to white colour. This is typically achieved by carrying out at least six washes (three times 30 min, followed by three times 10 min washes).
- Before testing: dry the pellicle under the fume hood, covered in foil overnight.

For Tensile Strength Test: