Lectin C gene analysis

Forked from Lectin C gene analysis

Tran Vinh Phuong[1], Nguyen Ngoc Phuoc[1], Nguyen Quang Quang Linh[1]

[1]Hue University

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Nguyen Quang Quang Linh
Hue University of Agriculture and Forestry

ABSTRACT

Mammalian Tissue Total RNA Purification Protocol by GeneJET RNA Purification Kit (Thermo Scientific, USA)

Before starting:

• Supplement the required amount of Lysis Buffer with β-mercaptoethanol or DTT. Add 20 µL of 14.3 M β-mercaptoethanol or 2 M DTT to each 1 mL volume of Lysis Buffer required.

• Prepare the required amount of Proteinase K solution: dilute 10 µL of Proteinase K to 590 µL of TE buffer (10 mM Tris HCl, pH 8.0, 1 mM EDTA).

Step Procedure

Step 1. Weigh the tissue (use up to 30 mg of fresh or frozen tissue)
Disruption using a mortar and pestle: Place up to 30 mg of tissue into liquid nitrogen and grind thoroughly with a mortar and pestle. Transfer the tissue powder immediately into a 1.5 mL microcentrifuge tube containing 300 µL of Lysis Buffer supplemented with β-mercaptoethanol. Vortex for 10 s to mix thoroughly.

Step 2. Add 600 µl of diluted Proteinase K (10 µL of the included Proteinase K diluted in 590 µL of TE buffer). Vortex to mix thoroughly and incubate at 25°C for 10 min.

Step 3. Centrifuge at 12000 rpm/ 10 min/ 4ºC. Transfer the supernatant into a new RNase-free microcentrifuge tube

Step 4. Add 450 µL of ethanol (96-100%) and mix by pipetting.

Step 5. Transfer up to 700 µL of lysate to the GeneJET RNA Purification Column inserted in a collection tube. Centrifuge the column at 12000 rpm/ 1 min/ 4ºC. Discard the flowthrough and place the purification column back into the collection tube.
Repeat this step until all of the lysate has been transferred into the column and centrifuged. Discard the collection tube containing the flow-through solution. Place the GeneJET RNA Purification Column into a new 2 mL collection tube.

Step 6. Add 700 µL of Wash Buffer 1 (supplemented with ethanol) to the GeneJET RNA Purification Column and centrifuge at 12000 rpm/ 1 min/ 4ºC. Discard the flow-through and place the purification column back into the collection tube.

Step 7. Add 600 µL of Wash Buffer 2 (supplemented with ethanol) to the GeneJET

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RNA Purification Column and centrifuge at 12000 rpm/ 1 min/ 4^{o}C. Discard the flowthrough and place the purification column back into the collection tube.
Step 8. Add 250 µL of Wash Buffer 2 to the GeneJET RNA Purification Column and centrifuge at 12000 rpm/ 2 min/ 4^{o}C
Discard the collection tube containing the flow-through solution and transfer the GeneJET RNA Purification Column to a sterile 1.5 mL RNase-free microcentrifuge tube.
Step 9. Add 40 µL of Water, nuclease-free to the center of the GeneJET RNA Purification Column membrane. Centrifuge at 12000 rpm/ 1 min/ 4^{o}C to elute RNA.
Step 10. Discard the purification column. Use the purified RNA for downstream applications or store RNA at -40°C until use.

First Strand cDNA Synthesis by RevertAid First Strand cDNA Synthesis kit (Thermo Fisher Scientific, USA)

After thawing, mix and briefly centrifuge the components of the kit. Store on ice.
*Step 1.*Add the following reagents into a sterile, nuclease-free tube on ice in the indicated order:
- Template RNA: 1µg total RNA
- Primer: 15 pmol specific primer
- Water, nuclease-free to 12 µL
*Step 2.*Add the following components in the indicated order:
- 5X Reaction Buffer: 4 µL
- RiboLock RNase Inhibitor (20 U/µL): 1 µL
- 10 mM dNTP Mix: 2 µL
- RevertAid M-MuLV RT (200 U/µL): 1 µL
Total volume: 20 µL
*Step 3.*Mix gently and centrifuge briefly.
Step 4. Incubate for 60 min at 42°C.
*Step 5.*Terminate the reaction by heating at 70°C for 5 min.
The reverse transcription reaction product can be directly used in PCR applications or stored at -20°C for less than one week. For longer storage, -70°C is recommended.

PCR products purification by GeneJET Gel Extraction kit (Thermo Scientific, USA)

Step 1. Excise gel slice containing the DNA fragment using a clean scalpel or razor blade. Cut as close to the DNA as possible to minimize the gel volume. Place the gel slice into a pre-weighed 1.5 mL tube and weigh. Record the weight of the gel slice.
Step 2. Add 1:1 volume of Binding Buffer to the gel slice (volume: weight).
Step 3. Incubate the gel mixture at 60°C for 10 min or until the gel slice is completely dissolved. Mix the tube by inversion every few minutes to facilitate the melting process. Ensure that the gel is completely dissolved. Vortex the gel mixture

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briefly before loading on the column.
Check the color of the solution. A yellow color indicates an optimal pH for DNA binding. If the color of the solution is orange or violet, add 10 µL of 3 M sodium acetate, pH 5.2 solution and mix. The color of the mix will become yellow.
*Step 4.*For ≤500 bp.
Add 1 gel volume of 100% isopropanol to the solubilized gel solution. Mix thoroughly.
Step 5. Transfer up to 800 µL of the solubilized gel solution (from step 3 or 4) to the GeneJET purification column. Centrifuge for 1 min. Discard the flow-through and place the column back into the same collection tube.
Step 6. Add 100 µL of Binding Buffer to the GeneJET purification column. Centrifuge for 1 min. Discard the flow-through and place the column back into the same collection tube.
Step 7. Add 700 µL of Wash Buffer (diluted with ethanol) to the GeneJET purification column. Centrifuge for 1 min. Discard the flow-through and place the column back into the same collection tube.
Step 8. Centrifuge the empty GeneJET purification column for an additional 1 min to completely remove residual wash buffer.
Step 9. Transfer the GeneJET purification column into a clean 1.5 mL microcentrifuge tube. Add 50 µL of Elution Buffer to the center of the purification column membrane. Centrifuge for 1 min.
Step 10. Discard the GeneJET purification column and store the purified DNA at -20 °C.

Cloning with pGEM T-easy vector(Promega, USA)

1. Briefly centrifuge the pGEM®-T Easy Vector and Control Insert DNA tubes to collect contents at the bottom of the tubes.
2. Set up ligation reactions as described below.
2X Rapid Ligation Buffer, T4 DNA Ligase: 5µl
pGEM®-T or pGEM®-T Easy Vector (50ng): 1µl
PCR product: 3µl
T4 DNA Ligase (3 Weiss units/µl): 1µl
3. Mix the reactions by pipetting. Incubate the reactions 1 hour at room temperature. After that incubate the reactions overnight at 4°C.

Isolation of plasmids using the GeneJET Plasmid Miniprep Kit (Thermo Fisher Scientific, USA).

- *Harvest bacteria:* Harvest the bacterial culture by centrifugation at 8000 rpm (6800 x g) in a microcentrifuge for 2 minutes at room temperature. Decant the supernatant and remove all remaining medium.
- *Resuspend Cells, Lyse and Neutralize:* Add to the pelleted cells:

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250 µL of Resuspension Solution and vortex.
250 µL of Lysis Solution and invert the tube 4-6 times.
350 µL of Neutralization Solution and invert the tube 4-6 times.
Centrifuge 5 minutes.
- *Bind DNA:*Transfer the supernatant to the Thermo Scientific GeneJET Spin Column. Centrifuge 1 minute.
- *Wash the column:*Add 500 µL of Wash Solution and centrifuge for 1min. Discard the flow-through. Centrifuge empty column for 1 minute. Repeat for two times.
- *Elute purified DNA:* Transfer the column into a new tube. Add 50 µL of Elution Buffer to the column and incubate 2 minutes. Centrifuge 2 minutes. Collect the flow-through.

Phylogenetic tree building using MEGA 11 software

- Preparing the sequencing file with fasta format.
- Click **Align**, select Edit/Built Alignment, select Creat a new alignment/DNA.
- From the Alignment Explorer window, select **Edit/**Select sequence from file
- Click **Alignment/**Align with ClustalW
- From the main MEGA launch bar, select **Phylogeny** | Construct/Test Neighbor-Joining Tree menu option.
- In the Analysis Preferences window select the p-distance option from the Model/Method drop-down.
- Click **Compute**to accept the defaults for the rest of the options and begin the computation. A progress indicator will appear briefly before the tree displays in the Tree Explorer window.

Amino acid sequence translation with Expasy

- Go to https://web.expasy.org/translate/
- Copy, paste the nucleotide sequence to the Box
- Click **Translate**
- See the result

Prediction of protein domain using SMART

- Go to http://smart.embl-heidelberg.de/
- Copy, paste the translated protein sequence to the Box
- Click **Sequence SMART**
- See the result

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Prediction of protein structure using Phyre2

- Go to http://www.sbg.bio.ic.ac.uk/~phyre2/html/page.cgi?id=index
- Fill the email address
- Copy, paste the translated protein sequence to the Box
- Click **Phyre search**
- See the results

Prediction of isoelectric point using IPC2

- Go to http://www.ipc2-isoelectric-point.org/
- Copy, paste the translated protein sequence to the Box
- Click **Calculate**
- See the result

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FORK NOTE

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