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RNAi synthesis for soaking

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We use this protocol and it's working

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Abstract

RNAi synthesis for elegans soaking

Materials

Primers (synthesized by company), **cDNA for PCR**, **ddH_2O, 2×Phanta Max Master Mix** (Vazyme), **T7 RNAi Transcription Kit** (Vazyme), **NTP Mix**, **10× Transcription Buffer, T7 Enzyme Mix**, **DNase I**, **RNase-free H_2O, RNA Clean Beads** (for magnetic bead purification), **80% ethanol** (prepared with RNase-free H_2O), **isopropanol** (if transcript <100 bp), **PCR tubes**, **magnetic stand**, **pipettes**.

Safety warnings

Be careful not to stir the beads when sucking the supernatant.
Do not suck the beads.
Dry until there is no gleaming water on the surface of the magnetic beads, excessive drying will affect the elution of RNA.
In order to avoid the influence of magnetic beads on subsequent experiments, when transferring the product, please reserve 1 - 2 µl solution to prevent absorption of the magnetic beads.

Design primers

1 Copy CDS sequences of targeted genes.
* If there are multiple transcripts, pick the shortest one that could apply to all the transcripts.

2 Design primers.
* 58–60 °C, ~20 bp
- If the CDS is shorter than 2000 bp, design primers to cover ~80% of the CDS; if the CDS is longer than 2000 bp, design primers to cover ~2000 bp.

3 Add T7 sequence in the 5' prime of both forward and reverse primers.

4 Primers synthesized by company.

DNA template synthesis

5 Set up experiment with necessary material: primers, template (cDNA for PCR, ddH_2O, enzyme (2×Phanta Max Master Mix, Vazyme).

6 Prepare reaction system using following sheet.
2×Phanta Max Master Mix 12.5 µL
Primer F 1 µL
Primer R 1 µL
Template 0.5 µL
ddH_2O up to 25 µL

7 PCR program (list as follow) for replicating target DNA.
95°C 3 min
(95°C 15 sec
60°C 15 sec
72°C 1 min) 35 cycles
72°C 5 min
4°C Forever

8 Electrophoresis of 10 µL product to check correctness.

RNA transcription and dsRNA purification

9 Prepare reaction system using following sheet.
NTP Mix 8 µL
10× Transcription Buffer 2 µL
T7 Enzyme Mix 2 µL
DNA template 2 µL
RNase-free H_2O 6 µL

10 PCR program for transcription: 37°C, 4h or overnight.

11 Annealing to form dsRNA: 72°C, 10 min; let naturally cool to room temperature, ~10 min.

12 Digest DNA template.
Transcription Product 20 µL
DNase I 1 µL
RNase-free H_2O 19 µL
37°C, 30 min.

13 dsRNA purification following instruction.

14 The RNA product can be purified by the methods of magnetic beads, column, phenol/chloroform extraction or gel recovery. This kit is recommended to purify RNA by magnetic beads.
Magnetic beads purification can quickly and efficiently remove proteins, free nucleic acids and salts. The 80% ethanol used for purification needs to be prepared with RNase-free H_2O (self-prepared).

15 Take the RNA Clean Beads out of 4°C and equilibrate for 30 min at room temperature. Please invert or vortex before use.

16 Add 80 µl magnetic beads solution to the transcript and pipette 10 times or more to mix the solution thoroughly.
▲ If fragment length of the transcript is less than 100 bp, add 200 µl isopropanol and mix thoroughly.

17 Incubate for 8 min at room temperature to allow RNA to bind to the beads.

18 Place the PCR tube on the magnetic stand for about 5 min, and carefully remove the supernatant after the solution is clarified. Please be careful not to stir the beads when sucking the supernatant.

19 Keep the PCR tube on the magnetic stand and add 200 µl 80% ethanol prepared freshly. Be careful not to stir the beads, incubate for 30 sec at room temperature, and carefully remove the supernatant. Repeat this step once.

20 Open the lip and dry the magnetic beads with air for 5 - 10 min.
▲ Dry until there is no gleaming water on the surface of the magnetic beads, excessive drying will affect the elution of RNA.

21 Remove the PCR tube from the magnetic stand, add 40 µl RNase-free H_2O, use pipette to rinse the magnetic beads on the tube wall, mix thoroughly, and incubate for 3 min at room temperature.

22 Place the PCR tube on the magnetic stand, and carefully remove the supernatant to a new RNase-free EP tube after the solution is clarified. Do not suck the beads.
▲ In order to avoid the influence of magnetic beads on subsequent experiments, when transferring the product, please reserve 1 - 2 µl solution to prevent absorption of the magnetic beads.

23 Measure the absorbance of the product at A_{260}, determine its concentration, and store the purified product at -20°C.

24 Detect the transcripts by electrophoresis: add 1 µL of product to 9 µL ddH_2O and run gel to check correctness.