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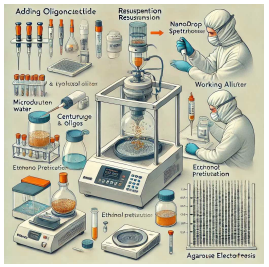
Resuspension, Purification, and Preparation of Working Aliquots of Oligos



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Manuscript citation:

- Romo-García, M. F., Bastian, Y., Zapata-Zuñiga, M., Macías-Segura, N., & Castillo-Ortiz, J. D. (2019). *Identification of putative miRNA biomarkers in early rheumatoid arthritis by genome-wide microarray profiling: a pilot study*. Gene, 720, 144081. https://doi.org/10.1016/j.gene.2019.144081
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- López-Ramos, J. E., Macías-Segura, N., Cuevas-Córdoba, B., Araujo-Garcia, Z. (2018). *Improvement in the diagnosis of tuberculosis combining Mycobacterium tuberculosis immunodominant peptides and serum host biomarkers*. Archives of Medical Research, 49(3), 147-153.e1. https://doi.org/10.1016/j.arcmed.2018.09.001
- Serrano, C. J., Cuevas-Córdoba, B., Macías-Segura, N., González-Curiel, R. A. (2018). *Transcriptional profiles discriminate patients with pulmonary tuberculosis from non-tuberculous individuals depending on the presence of non-insulin diabetes mellitus*. Clinical Immunology, 162, 107-117. https://doi.org/10.1016/j.clim.2015.12.002
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Protocol status: Working
We use this protocol and it's working

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Keywords: Oligonucleotide resuspension, Oligo purification, Quantitative PCR (qPCR), Molecular biology protocol, DEPC water, Ethanol precipitation, Nanodrop quantification, Agarose gel electrophoresis

Abstract

This protocol details the steps for resuspending, purifying, and preparing working aliquots of oligonucleotides (oligos) for use in molecular biology experiments. The procedure ensures the oligos are properly dissolved, purified, quantified, and aliquoted for consistent and reliable experimental results. This protocol was adapted for work conducted in the following publications: https://doi.org/10.1016/j.gene.2019.144081; https://doi.org/10.1371/journal.pone.0194205; https://doi.org/10.1016/j.arcmed.2018.09.001; https://doi.org/10.1016/j.clim.2015.12.002; https://doi.org/10.1111/iji.12355

Keywords:

- Oligonucleotide resuspension
- Oligo purification
- Quantitative PCR (qPCR)
- Molecular biology protocol
- DEPC water

Method Overview:

1. Resuspend lyophilized oligos in sterile DEPC H2O.
2. Purify the oligos using sodium acetate and ethanol precipitation.
3. Quantify the oligos with a Nanodrop and verify integrity by agarose gel electrophoresis.
4. Aliquot the purified oligos and store at -20°C.

Guidelines

- Always work under a sterile hood to prevent contamination.
- Ensure all equipment and reagents are properly sterilized before starting the protocol.
- Wear appropriate personal protective equipment (PPE), including gloves and lab coat.
- Use filtered pipette tips to avoid contamination.
- Follow proper waste disposal protocols for hazardous materials, including tips and tubes.
- Document all steps and any deviations from the protocol in your lab notebook.

Materials

Materials:

- 70% ethanol
- Oligos
- Sterile DEPC H2O
- 3M Sodium Acetate
- Absolute Ethanol
- Agarose
- 1X TBE
- Loading buffer
- Molecular weight marker
- Ethidium bromide solution (0.5 µg/µL)
- Micropipettes and filtered tips (20 µL, 200 µL, 1000 µL)
- Centrifuge
- Hood
- Vortex
- Nanodrop
- Electrophoresis chamber
- Gel documenter
- Gloves
- 2 mL screw-cap tubes
- Sterile 1.5 mL and 0.5 mL Eppendorf tubes
- Waste container for tips and tubes
- Marker or labels
- Freezer (-20°C)

Safety warnings

- Handle ethidium bromide with care as it is a potent mutagen; always wear gloves and work in a designated area.
- Ensure proper ventilation when working with volatile chemicals like ethanol.
- Dispose of all biological and chemical waste according to your institution's safety guidelines.
- Avoid direct contact with DEPC water as it is toxic; use appropriate handling techniques.
- Follow centrifuge safety protocols to prevent accidents due to imbalanced tubes.
- Make sure to seal all tubes and capillaries tightly to avoid spillage or contamination.

Before start

Before starting the protocol, make sure to:

1. Verify that all required materials and reagents are available and properly labeled.
2. Prepare a clean and organized workspace under the sterile hood.
3. Calibrate and check all equipment, including micropipettes and the Nanodrop spectrophotometer.
4. Read through the entire protocol to understand each step and its purpose.
5. Ensure that all reagents, especially DEPC water and ethanol, are at the correct temperatures (DEPC water should be at room temperature and ethanol should be cold).

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1 RESUSPENSION

1. Record all data or attach the technical sheet of the oligo in the logbook.
2. Ensure the lyophilized tube is well-sealed and centrifuge for 1 minute at 5000 rpm.
3. In a sterile, airflow-free hood, open the tube and add 200 µL of sterile DEPC H2O. Seal and vortex at medium speed for 30 seconds.
4. Spin briefly in the centrifuge, resuspend gently six times with a 200 µL micropipette. Transfer 100 µL to a sterile 1.5 mL Eppendorf tube. Store the original tube at -20°C until use.

2 PURIFICATION

1. Add 20 µL of 3M Sodium Acetate to the Eppendorf tube containing 100 µL. Mix thoroughly, then add 700 µL of cold absolute ethanol. Mix by inversion eight times.
2. Incubate at -70°C for 1 hour.
3. Centrifuge at 4°C for 20 minutes at 14,000 rpm.
4. Discard the supernatant without touching the pellet. Wash with 100 µL of cold 70% ethanol (adding along the tube wall). Mix by inversion three times.
5. Centrifuge at 4°C for 10 minutes at 14,000 rpm. Decant the supernatant and dry in a concentrator for around 5 minutes at medium temperature.
6. Resuspend in 30-50 µL of sterile DEPC H2O, depending on the pellet size. Mix thoroughly by flicking the tube.

3 QUANTIFICATION

1. Dilute 1:50 and measure with a Nanodrop (menu > nucleic acids > ssDNA-33).
2. Calculate to prepare a 20 µM working solution (www.idtdna.com).
3. Verify integrity and concentration by densitometry of the oligo on a 2% agarose gel in 1X TBE (run electrophoresis for 10 minutes at 100 volts, then 60 minutes at 80 volts, and 10 minutes at 100 volts).

4 ALIQUOTING

1. Label 0.5 mL Eppendorf tubes with: oligo name, concentration, date, preparer’s name, and volume.
2. Aliquot the working solutions and store at -20°C until use.

Protocol references

- Romo-García, M. F., Bastian, Y., Zapata-Zuñiga, M., Macías-Segura, N., & Castillo-Ortiz, J. D. (2019). *Identification of putative miRNA biomarkers in early rheumatoid arthritis by genome-wide microarray profiling: a pilot study*. Gene, 720, 144081. https://doi.org/10.1016/j.gene.2019.144081
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