prepIT®•L2P

Laboratory protocol for manual purification of DNA from 0.5 mL of sample

Ethanol precipitation protocol and prepIT®•L2P reagent for the purification of genomic DNA from the Oragene® and ORAcollect® families of collection kits.

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The following step-by-step protocol describes how to purify DNA from a 500 µL aliquot of sample.

Reagents included

- prepIT•L2P (catalog #: PT-L2P)

Equipment and reagents

- Microcentrifuge capable of running at 15,000 × *g*
- 1.5 mL microtubes (e.g., Axygen #MCT-150-C)
- Air or water incubator at 50°C
- Ethanol (95% to 100%) at room temperature
- Ethanol (70%) at room temperature
- DNA storage buffer: TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) or similar solution

Procedure

Purification steps	Notes
1. Mix the sample in the DNA Genotek kit by inversion and gentle shaking for a few seconds.	• This is to ensure that viscous samples are properly mixed.
2. Incubate the sample at 50°C in a water incubator for a minimum of 1 hour or in an air incubator for a minimum of 2 hours. **Note:** The use of an air incubator may be preferable since the sample tubes may float in a water bath. If a water bath must be used, ensure the sample-containing portion of the tube remains immersed in water.	• This heat-treatment step is essential to ensure that DNA is adequately released and that nucleases are permanently inactivated. • This incubation step may be performed at any time after sample is collected and before it is purified. • The entire sample must be incubated in the original collection tube before aliquoting to ensure sample homogeneity. • The sample may be incubated at 50°C overnight if it is more convenient. • A longer time is required in an air incubator because temperature equilibration is slower than in a water incubator.
3. Transfer 500 µL of the mixed sample to a 1.5 mL microcentrifuge tube.	• The remainder of the sample can be stored at room temperature or frozen (-15°C to -20°C).
4. For 500 µL of sample, add 20 µL (1/25th volume) of PT-L2P to the microcentrifuge tube and mix by vortexing for a few seconds.	• The sample will become turbid as impurities and inhibitors are precipitated.

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PD-PR-006 Issue 11/2015-01
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Purification steps	Notes
5. Incubate on ice for 10 minutes.	• Room temperature incubation can be substituted but will be slightly less effective in removing impurities.
6. Centrifuge at room temperature for 5 minutes at 15,000 × *g*.	• A longer period of centrifugation (up to 15 minutes) may be beneficial in reducing the turbidity (high A_{320}) of the final DNA solution.
7. Carefully transfer the clear supernatant with a pipette tip into a fresh microcentrifuge tube. **Discard the pellet containing impurities.**	• The pellet contains turbid impurities. If accidentally disturbed, the tube should be re-centrifuged.
8. To 500 µL of supernatant, add 600 µL of room temperature 95% to 100% ethanol. Mix gently by inversion 10 times.	• During mixing with ethanol, the DNA will be precipitated. This may appear as a clot of DNA fibers or as a fine precipitate, depending upon the amount of DNA in the sample. • Even if no clot is seen, DNA will be recovered by carefully following the next steps.
9. Allow the sample to stand at room temperature for 10 minutes to allow the DNA to fully precipitate.	• Incubation at -20°C is not recommended because impurities may co-precipitate with the DNA.
10. Place the tube in the microcentrifuge in a known orientation. Centrifuge at room temperature for 2 minutes at 15,000 × *g*.	• For example, place each tube in the microcentrifuge with the hinge portion of the cap pointing away from the centre of the rotor. After centrifugation, the position of the pellet can be located (even if too tiny to be easily visible), it will be at the tip of the tube below the hinge.
11. Carefully remove the supernatant with a pipette tip and discard it. Take care to avoid disturbing the DNA pellet.	• This pellet contains DNA. Loss of the pellet will result in loss of the DNA. • Rotating the tube such that the pellet is on the upper wall will allow you to safely move a pipette tip along the lower wall and remove all of the supernatant. • The supernatant may contain impurities and should be removed as completely as possible. • Excessive drying of the pellet can make the DNA more difficult to dissolve.
12. Ethanol wash: Carefully add 250 µL of 70% ethanol. Let stand at room temperature for 1 minute. **Completely remove the ethanol without disturbing the pellet.**	• **It is important to remove all ethanol from the sample. Carryover of ethanol may impact the performance of the assay.** • Take care not to disturb the DNA pellet. • The DNA pellet may be small. • Should the pellet detach, centrifuge the sample for 5 minutes at 15,000 x *g*. • After removing the 70% ethanol the tube can be pulse-spun to allow removal of residual ethanol.

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Purification steps	Notes
13. Add 100 µL of TE solution (see Page 1) to dissolve the DNA pellet. Vortex for at least 5 seconds.	• If a higher concentration of DNA is desired, 50 µL of TE should be used. • **Note:** large amounts of high molecular weight DNA can be slow to hydrate (dissolve) completely. • Incomplete hydration of the DNA is a cause of inaccuracy in estimating DNA concentration and of failure of downstream applications such as PCR.
14. To ensure complete rehydration of the DNA (pellet and smear) incubate at room temperature overnight followed by vortexing or at 50°C for 1 hour with occasional vortexing.	• Incomplete rehydration of the DNA is a cause of inaccuracy in estimating DNA concentration and potential failure of downstream applications such as PCR.
15. Options for storage of the fully rehydrated DNA: a) **Recommended** in TE, in aliquots at -20°C for long-term storage, or b) In TE at 4°C for up to 2 months.	• Freezing of purified DNA in TE will cause DNA to precipitate. When thawing a sample of frozen purified DNA, pay careful attention to rehydration, as discussed in step 14.

Quantification of DNA

By fluorescence method

Assays that use fluorescent dyes are more specific than absorbance at 260 nm for quantifying the amount of double-stranded DNA (dsDNA) in a DNA sample. We recommend using fluorescent dyes such as PicoGreen® or SYBR® Green I to quantify dsDNA since there is less interference by contaminating RNA. An inexpensive protocol using SYBR Green I is described in PD-PR-075, *DNA quantification using SYBR Green I Dye and a micro-plate reader*[1]. Alternatively, commercially available kits such as Invitrogen's Quant-iT™ PicoGreen dsDNA Assay Kit (Cat. No. Q-33130) can be used. For either protocol, we recommend that the purified DNA be diluted 1:50 with TE solution and that 5 µL be used in the quantification assay.

By absorbance method

If you choose to quantify DNA by absorbance, we recommend that you first treat the purified sample with RNase to digest contaminating RNA and then remove the RNA fragments by ethanol precipitation of the DNA. A detailed protocol is described in PD-PR-040, *RNA removal by double-RNase digestion*[2]. Please note that DNA from an oral sample typically contains appreciably more RNA than found in blood samples. Ensure that alcohol-precipitated DNA is fully dissolved before reading the absorbance.

Conversion factor: An absorbance of 1.0 at 260 nm corresponds to a concentration of 50 ng/µL (50 µg/mL) for pure dsDNA.

Ensure that absorbance values are within the linear range of the spectrophotometer. Re-dilute and re-measure samples that fall outside of the linear range. See your instrument documentation for more information.

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Method:

1. Dilute a 10 µL aliquot of purified RNase-treated DNA with 90 µL of TE (1/10 dilution). Mix by gently pipetting up and down. Wait for bubbles to clear.
2. Use TE in the reference (blank) cell.
3. Measure absorbance at 320 nm, 280 nm and 260 nm.
4. Calculate corrected A_{280} and A_{260} values by subtracting the absorbance at 320 nm (A_{320}) from the A_{280} and A_{260} values.
5. DNA concentration in ng/µL = corrected $A_{260} \times 10$ (dilution factor) $\times 50$ (conversion factor).
6. A_{260}/A_{280} ratio: Divide corrected A_{260} by corrected A_{280}.

Example

1. Assume the measured A_{320}= 0.025, A_{280}= 0.175 and A_{260}= 0.295
2. The DNA concentration of the undiluted sample will be:
   $(A_{260} - A_{320}) \times 10$ [dilution factor] $\times 50$ [conversion factor]
   $= (0.295 - 0.025) \times 10 \times 50$
   $= 0.270 \times 10 \times 50$
   = 135 ng/µL or 135 µg/mL
3. The corrected A_{260}/A_{280} ratio will be:
   $(A_{260} - A_{320}) \div (A_{280} - A_{320})$
   $= (0.296 - 0.025) \div (0.175 - 0.025)$
   $= 0.270 \div 0.150$
   $= 1.80$

References

[1] DNA quantification using the Fluorescence/DNase (F/D) assay. Replaced by DNA quantification using SYBR Green I dye and a micro-plate reader. DNA Genotek. PD-PR-075.

[2] RNA removal by double-RNase digestion. DNA Genotek. PD-PR-040.

Technical support is available Monday to Friday (9h00 to 17h00 EST):

- Toll-free (North America): 1.866.813.6354, option 6
- All other countries: 613.723.5757, option 6
- Email: support@dnagenotek.com

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Quick reference guide:

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2. Incubate the sample at 50°C in a water incubator for a minimum of 1 hour or in an air incubator for a minimum of 2 hours.
3. Transfer 500 µL of the sample to a microcentrifuge tube.
4. Add 20 µL of PT-L2P and mix by vortexing for a few seconds.
5. Incubate on ice for 10 minutes.
6. Centrifuge at room temperature (RT) for 5 minutes at 15,000 x *g*.
7. Carefully transfer the majority of the clear supernatant with a pipette to a fresh microcentrifuge tube. **Discard the pellet.**
8. Add 600 µL of RT 95% to 100% ethanol to the clear supernatant. Mix gently by inversion 10 times.
9. Let the sample stand at RT for 10 minutes to allow the DNA to fully precipitate.
10. Place the tube into the centrifuge with a known orientation. Centrifuge at RT for 2 minutes at 15,000 × *g*.
11. Carefully pipette off the supernatant and discard it. Take care to avoid disturbing the DNA pellet.
12. Add 250 µL of 70% ethanol and let stand at RT for 1 minute. **Completely remove the ethanol, without disturbing the pellet.**
13. Add 100 µL of TE solution and vortex the sample for at least 5 seconds.
14. Incubate overnight at RT or at 50°C for 1 hour vortexing occasionally.
15. Storage: In aliquots at -20°C for long-term storage (recommended) or at 4°C for up to 2 months.

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