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IDEEL- Protocol for DNA Extraction- Chelex-Tween

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We use this protocol and it's working

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Disclaimer

It is very important to completely seal the plate at the end of extraction, ensuring that each well is sealed, and that the edges are pressed down. If the seal is not properly placed, DNA evaporation will occur more rapidly during storage.

Abstract

This SOP describes the procedures necessary extract genomic DNA (gDNA) from punched blood spots using Chelex resin in 96 well plates (punched using IDEEL- Protocol for DBS Punching on protocols.io). The blood spots will be on filter paper deposited in a 96-well deep-well plate which will undergo a two-day procedure to extract gDNA using Chelex 100 (Bio-Rad, Richmond, CA), a chelating ion-exchange resin. This procedure requires approximately 15 minutes on day one and three hours on day two, with an overnight incubation in between.



Materials

Reagents

- 10X Phosphate-Buffered Saline (PBS), pH 7.4 $\pm$ 0.1, liquid without calcium and magnesium (Corning – 46-013-CM)
- TWEEN® 20 (Sigma – P2287-100ML)
- Chelex 100 Resin (Bio-Rad – 143-2832)
- Molecular grade water

Consumables and Supplies

- P20 filtered tips; Thermo Scientific 2749-RI (Refill)/ 2749-HR (Box)*
- p200 filtered tips; Thermo Scientific 2769-RI (Refill)/ 2769-HR (Box)*
- p1000 filtered tips; Thermo Scientific 2779-RI (Refill)/ 2779-HR (Box)*
- p200 Rainin wide-orifice tips; Rainin –30389241*
- Sterile reservoirs 50ml ; Genesee – 28-127
- non-skirted 96 well plate; VWR – 82006-636
- Lobind DNA plates; eppendorf # 0030129504 (Semi-skirted)/0030129512 (Skirted)
- Lobind Protein Deepwell plate, VWR – 86-1636 (Eppendorf # 951032905)
- P1000 multichannel pipette
- P200 multichannel pipette
- P20 multichannel pipette
- Microplate Shaker (Fisherbrand – 88861023)
- Centrifuge for Plates
- Glass Media Bottles (500ml)
- Conical Tubes (50ml)
- eXTreme FoilSeal (Genesee Scientific – 12-156)
- Clear plate seals (Genesee Scientific – 12-167) does not have to be qPCR optical clear seal
- Plate sealer
- Scale

Preparation

1 Put on gloves. Spray work area with 70% ethanol.

2 Preparing buffers and Chelex

2.1 For ~200 samples or 2 × 96-well plate with samples (punched DBS) make the following dilutions:

2.2 1X PBS (need 2 ml/sample on Day1 26 on Day2)

Mix 40 ml of 10X PBS + 360 ml of molecular grade water.

2.3 0.5% TWEEN® 20 in 1X PBS (need 1 ml/sample on Day1)

1 ml of 100% TWEEN® 20 + 199 ml of 1X PBS

Recommended approach: Add 9 ml of 1X PBS and 1 ml of 100% TWEEN® 20 into the 50 ml conical tube and vortex it, then pour the solution into the 500 ml glass media bottle. Add 190 ml of 1X PBS into the bottle and shake it well.

Make this solution well ahead of planned starting extraction time so that bubbles can resolve.

2.4 10% Chelex (need 150µL/sample on Day2)

Use 2 × 50ml Conical tubes (each Conical tube for ~100 samples or 1 × 96-well plate)
Place 1.5 g of Chelex in 50 ml Conical tube then fill with molecular grade water up to 15ml. (Repeat for x2)

Chelex settles out of solution very easily. Ensure that mixture is vortexed sufficiently before diluting. (Mixture should be a homogenous cloudy white liquid).

3 **Preparing Plates:** (for Day2)

3.1 **DNA plate**

o Use the label printer to print two labels ("SamplePlateName_DNA") and label the final DNA plates (Lobind DNA plates).

3.2 **Protein plate (If eluted plasma needs to be saved)**



- o Use the label printer to print two labels ("SamplePlateName_Protein") and label the Protein plates
(Lobind Protein Deepwell plates).

Section 1- Day 1

- 4 If pulling punched plates from storage, check to make sure that no DBS are stuck to the clear seal of the plate. If some are, centrifuge the plate to spin the DBS to the bottom of the wells. If still stuck, then gently peel back the clear seal, use tweezers to move DBS to the appropriate well(s), cleaning the tweezers with 70% ethanol and wiping with a Chemwipe afterwards.
- 4.1 Fill a sterile reservoir with 0.5% TWEEN® 20 in 1X PBS, and using the P1000 multichannel, add 1ml of 0.5% TWEEN® 20 in 1X PBS to the wells with punched DBS.
- 4.2 Make sure DBS are submerged or floating in 0.5% TWEEN® 20 in 1X PBS, before sealing the plate with a foil seal.
- 5 Seal the plate(s) with foil seals. Secure the plate to a shaker (up to 4) and shake the plate overnight at room temperature, 300rpm.

Section 2- Day 2

- 6 Turn on the water bath at this time to allow time for 95°C (203°F) to be reached.
- 7 Remove the Deep well DBS plates from the shaker. Centrifuge DBS plates briefly at 300 rpm for 1 min. Make sure to balance the centrifuge if extracting a single plate.
- 8 Match the prepared Protein storage plate label with the plate being extracted. If not, go to step 8.
- 8.1 Using a P1000 multichannel pipet, aspirate 300µL of the supernatant (0.5% TWEEN® 20 in 1X PBS) from the DBS plate and place in the corresponding wells in the final Protein Plate (keep the well location the same, so the same plate map can be used).
- 8.2 Make sure to change tips between each sample.
- 8.3 Seal the final protein plate with a foil seal.

8.4 Freeze plate at -80C.

8.5 Aspirate all remaining 0.5% TWEEN® 20 in 1X PBS from the DBS wells and discard.

When pipetting up, move the tips back and forth gently along the bottom of the wells to dislodge the DBS and aspirate all the liquid underneath.

9 **If not collecting eluant for protein:** Aspirate all the supernatant (0.5% TWEEN® 20 in 1X PBS) from the DBS wells and discard. When pipetting up, move the tips back and forth gently along the bottom of the wells to dislodge the DBS and aspirate all the liquid underneath.

10 Fill a sterile reservoir of 1X PBS (no Tween® 20 in this PBS), and using the P1000 multichannel, add 1ml of this buffer to each well with DBS. Vortex briefly, and incubate at 4°C for 15-30min.

11 Centrifuge the plate at 800 rpm for 1 min and aspirate as much fluid as possible. Check underneath the plate to visualize any remaining liquid. Afterwards use the tip to press the filter paper down into the lower third of the well without packing it excessively.

12 Prepare a full row of p200 Rainin wide-orifice tips and a sterile reservoir. **Wide tips are necessary because Chelex beads have trouble fitting through normal pipette tips.**

NOTE: Chelex quickly settles out of suspension, make sure you are not just transferring water.

12.1 Vortex the 10% Chelex solution. Ensure that the mixture is a homogenous white cloudy liquid and then pour it into the reservoir.

12.2 Using a single row of p200 Rainin wide-orifice tips, transfer 150µL of 10% Chelex solution into every well.

12.3 Make sure to hover above the well and depress the liquid directly into each row. This allows for quick pipetting of the Chelex, as well as avoids cross contamination of the wells.

12.4 If Chelex begins to settle at the bottom of the reservoir, move the pipette tips gently back and forth along the bottom as well as pipetting up and down when loading to re-suspend the Chelex. The liquid aspirated into the tips should have a homogenous cloudy white appearance.

13 Ensure that the Chelex solution and the DBS are in the bottom of each well. Seal plate with a foil seal. Centrifuge briefly at 300rpm to collect all liquid at the bottom and no droplets remain on the walls.



- 14 Extract the genomic DNA by incubating the plate for 10 min in a 95°C water bath, vigorously vortex the plate every 2 minutes throughout the incubation.
- 15 After incubation, centrifuge the plate at 1500 rpm for 5 minutes.

Use a proper balance for the centrifuge, if extracting an odd number of plates.
- 16 Using a multichannel pipet, transfer as much solution as possible from the wells of the spun plate to a labeled transfer plate, not worrying if some Chelex is carried over as well at this point.
- 16.1 Use a non-skirted 96 well plate as transfer plate, this allows for better visualization.
- 16.2 Gently move the tip along the bottom of the well to dislodge the DBS and ensure all liquid is aspirated. Check underneath the deep well plate to visualize any remaining liquid, as well as the transfer plate for any wells lacking appropriate volume.
- 17 Ensure all DNA elution is carried over. Seal the transfer plate.
- 18 Spin the transfer plates for 10 minutes at 1500 rpm.
- 19 Transfer the final, white-to-yellowish supernatant to the labeled DNA Lobind Plate, a minimum of 100 µL per well. Avoid disturbing the Chelex beads pelleted at the bottom by drawing slowly and from the middle/top with a P20 multichannel repeatedly. The goal is to aspirate supernatant without Chelex beads.
- 20 Visualize the aspirated DNA before depressing into the final DNA plate. If pelleted beads are disturbed and Chelex beads are present in the pipette, dispense back into the transfer plate and let beads settle for a few minutes.
- 21 Change tips when moving to a new row.
- 21.1 Ensure that no tip comes into contact with more than one sample, and that each DNA sample is only added to the appropriate well corresponding to the plate-map.
- 22 Carefully seal the final DNA plate, and ensure plate is properly labelled before storing at -20°C.

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