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IDEEL- Protocol for DBS punching

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We use this protocol and it's working

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Abstract

This SOP describes the procedures necessary to efficiently and reliably punch and organize dried blood spots. The blood spots will be on filter paper and will be deposited in a 96-well deep-well plate in which they will undergo a two-day procedure to extract gDNA using Chelex 100 (Bio-Rad, Richmond, CA), a chelating ion-exchange resin.

Materials

1.1 Reagents
- NA

1.2 Consumables and Supplies
- Deep-well 96 well plates (Genesee Scientific – 22-491)
- Clear seals (Genesee Scientific – 12-167) not the qPCR optical clear seals
- Hole puncher
- Filter Paper
- Tweezers
- Paper or computer to keep record of sample wells
- Label maker or Sharpie marker
- Plate sealer (Genesee Scientific – 12-202)

Before start

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

Methods

1 Label one empty 96-well deep-well plate using a label maker or marker.

Ensure that the name of the plate is legible and corresponds to a paper or computer record. As you punch DBS into each well, you must update the computer record to indicate which well contains which sample, (this is the "plate-map"). All plates following extraction including qPCR, DNA, and Protein storage, must be carefully labelled with the same unique plate name so that samples/data can be tracked using the plate-map.

2 Selecting one bagged DBS at a time:

2.1 Place the end of the hole puncher within the target well so that punches will fall directly into the desired well.

If new to punching DBS, you can cut a small square hole (about the size of one well) in a large sheet of filter paper. Place this sheet on top of the plate you are punching into. Ensure the hole is aligned with the proper well before punching, leaving all other wells in the plate covered. This ensures that no punched spot will land in another well.

2.2 Without removing the puncher, line the blood spot up in order to punch one 6mm circular punch of fully saturated filter paper from the card directly into the well. Make sure that each punch falls into the well and doesn't stick to the puncher.

2.3 If the study only requires one 6mm punch move on to step 3. Number of punches should be determined beforehand for each study, and kept consistent throughout that study.

2.4 If the study requires three punches in total, move the DBS to punch two additional fully saturated blood spots into the same well.

If three full spots cannot be punched due to smaller size of the blood smear on the card, then approximate a second/third using the edges of the DBS. If three full spots cannot be punched at all, then make note of it on the plate-map.

2.5 Record the sample ID from the DBS bag and the corresponding sample well of your deep-well plate into the plate-map record.

It is very important to ensure that sample wells are listed correctly on the plate-map record.

2.6 Carefully place the punched DBS back into its original sample bag and re-seal. Make sure each bag contains desiccant. If the desiccant is no longer active, replace it with a new pack of desiccant.

2.7 **Punch and discard five punches from a blank piece of filter paper to clean the hole puncher.** If the tweezers were used to move the punched blood spots, spray them with ethanol, wipe with a fresh Chem-wipe (do not re-use for multiple samples), and allow to dry before reusing.

3 Repeat Step 2 for each well of the deep well plate. Stopping at H06 after 90 total samples.

(This leaves six wells empty, H07-H12. These will make it easier to include controls during future/downstream PCR assays).

4 Once the plate has 90 sample wells filled:

4.1 If proceeding immediately to DNA extraction, take the open plate containing DBS punches directly into the Chelex-Tween gDNA Extraction steps.

5 If not proceeding immediately to DNA extraction (**IDEEL- Protocol for DNA Extraction-Chelex-Tween** on protocols.io) then carefully seal the plate with a clear seal, press hard onto the cover to seal it to the plate, and ensure that all edges are covered as well. Store at 4C overnight, or at -20C long term.

6 Ensure the plate-map and Plate name are correct. Make sure to save the plate-map computer file, and to keep a backup of the file in a different location for future reference.

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