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Method for Chelex Extraction of DNA

1 PURPOSE AND SCOPE

This method describes the use of 20% (w/v) Chelex 100 in the extraction of DNA from human cellular material. The cellular material referred to includes blood, semen, saliva, hair, nucleated cells and mixtures of these materials.

Only one sample type is extracted per batch. The method is designed so the appropriate page can be attached to the sample worksheet and filed as part of the record for that batch of samples.

Reference samples and casework samples **must** be extracted separately.

2 DEFINITIONS

Samples	Samples awaiting DNA extraction
DNA extracts	Samples that had DNA extraction process performed

3 PRINCIPLE

Chelex 100 is a chelating resin that has high affinity for polyvalent metal ions. The Chelex 100 resin is composed of styrene divinylbenzene copolymers containing paired iminodiacetate ions, which act as chelating groups. It has been postulated that the presence of Chelex 100 during boiling prevents the degradation of DNA by chelating metal ions that may catalyse the breakdown of DNA subjected to high temperatures in low ionic strength solutions. The basic Chelex 100 procedure consists of boiling the sample in a 20% (w/v) Chelex 100 solution, then adding a portion of the supernatant directly to the PCR mix. This procedure results in denatured sample DNA.

4 REAGENTS AND EQUIPMENT

4.1 Reagents

- Proteinase K (Pro K) 10mg/mL
- Dithiothreitol (DTT) 1M, 10mL (For semen and differential lysis extractions only)
- 20% (w/v) Chelex 100
- 10% Bleach 7x solution
- 70% Ethanol

Table 1. Reagent storage locations.

Reagent	Device	Storage Location
Pro K	Freezer	6122
DTT	Freezer	6122
Decon® 90	Bench shelves	6112,6120,6121 & 6122
20% (w/v) Chelex 100	Desiccator	6112
10% bleach	Bench shelves	6120,6121 & 6122
70% ethanol	Bench shelves	6120,6121 & 6122

4.2 Equipment

Table 2. Equipment used and location.

Equipment	Asset No.	Location
Water bath	30435253	Room 6120
Water bath	10074257	Room 6121
STORstar	10233252	Room 6117
Vortex	30435255	Room 6120
Vortex	30087062	Room 6121
Eppendorf 5415C	10074282	Room 6120
Eppendorf 5424	10233209	Room 6120
Eppendorf 5415C	10074265	Room 6121
Eppendorf 5424	102332108	Room 6121
Sigma 1-15	10076023	Room 6121
Sigma 1-15	10076022	Room 6122
Magnetic stirrer	N/A	Room 6112

5 SAFETY

As per the procedures in the QIS document "*Operational Practices in the DNA Dedicated Laboratories*" (QIS 17120), PPE is to be worn by all staff when performing this procedure.

The benches, hoods and pipettes should be cleaned with the 10%bleach/7x solution and 70% ethanol prior and after use.

Where possible, extraction processes should be conducted within a Class II Biological safety cabinet.

Sample racks used for the extraction process should be soaked in the prepared Decon® 90 solution for at least 15 minutes after each use. They are then to be rinsed in tap water and left to dry on a drying rack.

DTT is a reducing agent that destroys disulphide bonds. It is an irritant. Confine use to a fume cupboard or Biohazard safety cabinet. Wear appropriate PPE.

Warning: TRIS base, Ethlyenediaminetetraacetate (EDTA) and SDS are irritants. Handle carefully and wear appropriate PPE.

6 SAMPLING AND SAMPLE PREPARATION

Samples waiting to be extracted are stored in freezers as described in Table 3.

Table 3. Sample storage locations.

Sample type	Storage Device	Storage Location
Samples	Freezer	6117-2

QC samples

All extraction batches require two controls to be registered. These controls, UR numbers and positions are listed below in Table 4.

Table 4. Extraction Quality Controls

QC	UR Number	Extraction types
Neg Control	FBOT33	All
QC-C dot (cells)	FBOT34	Cell (Chelex 100)
QC-B dot (blood)	FBOT35	Blood (Chelex 100)
Diff control	FBOT36	Diff Lysis (Chelex 100)
Semen control	FBOT37	Semen (Chelex 100)
QC-Hair	FBOT38	Hair (Chelex 100)

1. Log into the **AUSLAB Main Menu**.
2. Select **1. Request Registration**.
3. Select **2. Full Reception Entry**.
4. Scan in barcode of control.
5. Enter the UR number as per Table 4 and press **[Enter]**.
6. Enter the appropriate Specimen type (e.g. Blood for blood extraction).
7. Request a **9PLEX** test, when prompted to enter the processing comment, enter **EXTP** (Positive extraction control) or **EXTN** (Negative extraction control).
8. Enter **LAB** in the Billing code field.
9. Press **[F4]** ***Save*** to save the Billing details.
10. Press **[F4]** ***Save*** to save the registration details.

Differential lysis controls

As method above except:

The positive and negative controls should have two laboratory numbers registered per control.

1. One will have the specimen type Sperm lysate with the client reference XXXXXXXX-S where XXXXXXXX is the barcode of the control.
2. The other will have the specimen type Epithelial lysate with the client reference XXXXXXXX-E where XXXXXXXX is the barcode of the sperm lysate control.

N.B Quality controls should not have a DNA priority allocated at time of registration to ensure they are included in the top positions of a batch

Create the Extraction Batch

1. Log into the **AUSLAB Main Menu**.
2. Select **5. Workflow management**.
3. Select **1. DNA workflow table**.
4. Highlight the appropriate Extraction batch type and press **[F5]** ***Batch Allocation***.
5. Press **[F6]** ***Create batch***.
6. Press **[F8]** ***Print menu***.
7. Press **[F6]** ***Print Batch label***.
8. Press **[F7]** ***Print Sample labels***. (print two sets of labels for all extractions expect Differential lysis which is four sets of labels)
9. Press **[F8]** ***Print Worksheet***.
10. Press **[SF5]** ***Main menu***.
11. Press **[SF11]** ***Print***.
12. Press **[SF6]** ***Accept batch***.
13. Press **[Pause/Break]** to exit to the **Main Menu**.
14. Obtain worksheets (FBLASER3) and labels (FBLABEL13-17) from the Analytical Section printing bench (Room 6117).

Locating Samples

Determine the storage locations of the required samples using the Batch Creation table/Batch details table print out. The columns ***Rack*** and ***Pos*** respectively identify the rack and the grid location where the sample has been stored. Remove the samples from the storage rack and place in an orange rack (12x8).

When all samples have been located:

1. Log into the **AUSLAB Main Menu**.
2. Select **2. Sample Processing**.
3. Select **7. Search Sample storage**.
4. Scan in the sample barcode that is affixed to the sample tube.
5. Press **[F6]** ***Remove Sample***.
6. AUSLAB prompts "***Are you sure you want to remove XXXX-XXXX? (Y/N)***", Enter **Y** and press **[Enter]**.
7. AUSLAB prompts "***Please enter remove comment***", No comment is required. Press **[Enter]**.
8. Press **[Scroll lock]** to clear.
9. Repeat steps **5 - 8** until all of the samples have been removed from their rack.

Sequence Check the tubes

1. Thaw samples and vortex briefly.
2. Sequence check the tubes as per the QIS document "*Sequence Checking with the STORStar Instrument*".
3. Add the sequence check details into AUSLAB.
4. Log into **AUSLAB Main Menu**.
5. Select **5. Workflow Management**.
6. Select **2. DNA Batch Details**.
7. Scan in the appropriate extraction batch ID barcode.
8. Press **[F5]** ***Sequence Check***.
9. Scan in the appropriate extraction batch ID barcode.
1. Press **[Pause/Break]** to exit to **Main Menu**.

7 PROCEDURE

See Appendices 14.1 -14.6 for individual procedures.

8 SAMPLE STORAGE

1. Log into **AUSLAB Main Menu**.
2. Select **2. Sample Processing**.
3. Select **6. Sample Storage**.
4. Scan in Rack barcode.
5. Press **[SF5]** ***Fill Rack***.
6. Scan in sample barcode and place in rack in scanned position.
7. Repeat for all samples.
8. Press **[Esc]**.
9. Press **[Pause/Break]** to return to the **Main Menu**.
10. Select **3. Patient Enquiry**.
11. Scan in Rack barcode.
12. Tab down to the next blank ***DNA Batch No*** field and press **[F2]** ***Edit***.
13. Scan in the Batch ID of the samples stored.

14. Press **[Pause/Break]** to return to the **Main Menu**.

9 QUALITY ASSURANCE/ACCEPTANCE CRITERIA

- A reagent blank is included with each batch of extractions. This reagent blank is processed as a normal sample through to completion. If any results are obtained from this sample, either at the quantitation step or the Genescan analysis step, then the possible source of the contamination is investigated. The samples extracted with this control are thoroughly checked and repeated if possible.
- Positive and negative controls are included in each extraction batch as per table 4.

10 REFERENCES

- BIO-RAD "Chelex® 100 and Chelex 20 Chelating Ion Exchange Resin Instruction Manual." LIT200 Rev B. Current in 2007.
- Singer-Sam J., Tanguay R., "Use of Chelex to improve the PCR signal from a small number of cells", Amplifications, A Forum for PCR Users, Sept 1989, Issue 3..
- Rapid DNA Extraction Protocols, Cetus Corporation
- Walsh S, Metzger D.A.,& Higuchi R., "Chelex 100 as a Medium for Simple Extraction of DNA for PCR_Based Typing from Forensic Material" Cetus Corporation and Illinois State Police, BioTechniques 1991 Vol 10. No 4.

11 STORAGE OF DOCUMENTS

- All worksheets are stored in the Analytical area (Room 6117).
- All old original 5mL sample tubes are stored in the Walk-in Cold Room in case further extraction is required. This is only for old samples since 5mL tubes are no longer stored.

12 ASSOCIATED DOCUMENTS

QIS 17120 Operational Practices in the DNA Dedicated Laboratories
QIS 17199 Preparation of Reagents for use in Analytical Methods
QIS 24469 Batch functionality in AUSLAB
QIS 24256 Sequence Checking with the StorStar Instrument
QIS 24255 Analytical Sample Storage

13 AMENDMENT HISTORY

Revision	Date	Author/s	Amendment
3	20 Jul 2001		
4	21 Feb 2001	V Ientile	
5	18 Mar 2001	V Ientile	
6	27 Apr 2005	M Gardam	Added areas to record lot numbers, transfer tube sequence checks and additional operators. Outlined PPE requirements for extraction process & decontamination of racks. Added note to cells protocol to outline when spin baskets are required.
7	14 Aug 2006	M Gardam	Added QC dot as pos control. Removed reference to

			staining slides with H&E.
8	14 Oct 2006	M Gardam	Slot for writing chelex lot number.
9	07 Feb 2007	T Nurthen	Removed worksheet details. Updated and added details of new AUSLAB workflow.

14 APPENDICES

14.1 Chelex 100 Extraction of DNA from Reference Bloods/Dried Bloodstains
14.2 Chelex 100 Extraction of DNA from Hair Samples
14.3 Chelex 100 Extraction of DNA from Semen Stains (not containing epithelial cells).
14.4 Chelex 100 Extraction of DNA from Cells
14.5 Chelex 100 Extraction of DNA from Reference Buccal Swabs
14.6 Differential Lysis of Semen Stains using Chelex 100
14.7 Differential Lysis of Semen Stains using Chelex 100 - (Appendix for wall of Extraction Room)

14.1 Chelex 100 extraction of DNA from Reference Blood/Dried blood stains

Steps 1-13 to be performed in Rooms 6120, 6121 or 6122

1 Obtain samples, worksheets and labels.

2 Label the 1.5mL sample tubes and the sterile 1.0mL Nunc™ Bank-It™ tubes (Transfer tubes).

3 Sequence check the samples and transfer tubes. Add the Sequence check details in AUSLAB and on the extraction worksheet.

4 Pipette 1mL of autoclaved nanopure water into each tube, vortex gently.

5 Incubate at room temperature for 30 minutes.

6 Vortex, then spin in centrifuge for 3 minutes at 15800g.

7 Carefully remove all but 50µL of supernatant. Leave substrate in tube with pellet.

8 Add 150µL of 20% (w/v) Chelex 100 to each tube and vortex.

Note: When pipetting Chelex 100, the resin beads must be distributed evenly in the solution. Use magnetic stirrer in beaker of Chelex 100 and wide bore pipette tips.

9 Check and record temperature of water bath on worksheet. Incubate in 56°C water bath for 30 minutes.

10 Vortex, then incubate in boiling water bath for 8 minutes.

11 Vortex, then spin in centrifuge for 3 minutes at 15800g.

12 Transfer supernatant to the labelled 1.0mL Nunc™ Bank-It™ tube leaving Chelex 100 beads and substrate behind.

13 Store the samples to Freezer 6117-3. In Analytical, file worksheets in Extraction batches folder.

14 Complete batch in AUSLAB.

14.2 Chelex 100 extraction of DNA from hair samples

Steps 1 – 10 to be performed in Rooms 6120, 6121 or 6122

1 Obtain samples, worksheets and labels.

2 Label the 1.5mL sample tubes and the sterile 1.0mL Nunc™ Bank-It™ tubes (Transfer tubes).

3 Sequence check the sample and transfer tubes. Add the Sequence check details in AUSLAB.

4 Add 200µL of 20% (w/v) Chelex 100 (20g in 100mL of autoclaved nanopure water) and 4µL of Proteinase K (10mg/mL) to each tube.

Note: When pipetting Chelex 100, the resin beads must be distributed evenly in the solution. Use magnetic stirrer in beaker of Chelex 100 and wide bore pipette tips.

5 Check and record temperature of water bath on worksheet. Incubate in 56°C water bath overnight (or for at least 6-8 hours).

6 Vortex for 5-10 seconds.

7 Incubate in boiling water bath for 8 minutes.

Note: Hair must be completely immersed in solution at all times.

8 Vortex, then spin samples in centrifuge at 15800g for 3 minutes.

9 Transfer supernatant to the labelled 1.0mL Nunc™ Bank-It™ tube leaving Chelex 100 beads and substrate behind.

10 Store the samples to Freezer 6117-3. In Analytical, file worksheets.

11 Complete batch in AUSLAB.

14.3 Chelex 100 extraction of DNA from semen stains (not containing epithelial cells)

Steps 1 – 19 to be performed in Rooms 6120, 6121 or 6122

1 Obtain samples, worksheets and labels.

2 Label the 1.5mL sample tubes and the sterile 1.0mL Nunc™ Bank-It™ tubes (Transfer tubes).

3 Sequence check the sample and transfer tubes.

4 Add the Sequence check details in AUSLAB.

5 Pipette 1mL of autoclaved nanopure water into each tube, vortex gently.

6 Incubate at room temperature for 30 minutes.

7 Twirl the swab/fabric substrate with a sterile toothpick for 2 minutes then transfer it to a spin basket.

8 Spin substrates (in spin basket) for 30 seconds at 15800g.

9 Discard Spin basket with substrate. Vortex supernatant and pool with the original extract.

10 Spin samples in centrifuge for 3 mins at 15800g.

11 Carefully remove all but 50µL of supernatant and discard.

12 Add 150µL of 20% (w/v) Chelex 100 (20g of Chelex 100 in 100mL autoclaved nanopure water) to each tube.

Note: When pipetting Chelex 100, the resin beads must be distributed evenly in the solution. Use magnetic stirrer in beaker of Chelex 100 and wide bore pipette tips.

13 Add 4µL of Proteinase K (10mg/mL) and 7µL of 1M DTT. Mix gently by vortexing.

14 Check and record temperature of water bath on worksheet. Incubate in 56ºC water bath for 45 minutes.

15 Vortex, then incubate in boiling water bath for 8 minutes.

16 Vortex, then spin in centrifuge for 3 minutes at 15800g.

17 Transfer supernatant to the labelled 1.0mL Nunc™ Bank-It™ tube leaving Chelex 100 beads and substrate behind.

18 Store the samples to Freezer 6117-3. In Analytical, file worksheets.

19 Complete batch in AUSLAB.

14.4 Chelex 100 extraction of DNA from cells

Steps1 – 16 to be performed in Rooms 6120, 6121 or 6122

1 Obtain samples, worksheets and labels.

2 Label the 1.5mL sample tubes and the sterile 1.0mL Nunc™ Bank-It™ tubes (Transfer tubes).

3 Sequence check the sample and transfer tubes.

4 Add the Sequence check details in AUSLAB.

5 Pipette 1mL of autoclaved nanopure water into each tube, vortex gently.

6 Incubate at room temperature for 30 minutes.

7 Twirl the swab/fabric with a sterile toothpick for 2 minutes then transfer substrate to a spin basket.

Note: a. For cigarette butts, envelopes & stamps twirling time may be reduced to 1 minute to prevent disintegration of the substrate and further interference of the substrate during later steps.
b. Toothbrush bristles, cigarette butts, envelopes, stamps, tapelifts, fingernails/scraping implement, chewing gum etc should not be spun in the spin basket, discard substrate & go to Step 8.

8 Spin tubes with spin basket for 30 seconds at 15800g. Discard spin basket with substrate.

9 Vortex supernatant and pool with the original extract - then pipette back into original extract tube.

10 Spin samples in centrifuge for 3 mins at 15800g.

11 Carefully remove all but 50µL of supernatant and discard.

12 Add 150µL of 20% (w/v) Chelex 100 (20g of Chelex 100 in 100mL autoclaved nanopure water) to each tube.

Note: When pipetting Chelex 100, the resin beads must be distributed evenly in the solution. Use magnetic stirrer in beaker of Chelex 100 and wide bore pipette tips.

13 Add 4µL of Proteinase K (10mg/mL). Mix gently by vortexing.

14 Check and record temperature of water bath on worksheet. Incubate in 56^{o}C water bath for 30 minutes.

15 Vortex, then incubate in boiling water bath for 8 minutes.

16 Vortex, then spin in centrifuge for 3 minutes at 15800g.

17 Transfer supernatant to new labelled 1.0mL Nunc™ Bank-It™ tube leaving Chelex 100 beads behind.

18 Samples are stored at –20^{0}C into Freezer 6117-3.

19 Complete Batch in AUSLAB.

14.5 Chelex 100 extraction of DNA from reference buccal swabs

Steps1 – 16 to be performed in Rooms 6120, 6121 or 6122

1 Obtain samples, worksheets and labels.

2 Label the 1.5mL sample tubes and the sterile 1.0mL Nunc™ Bank-It™ tubes (Transfer tubes).

3 Sequence check the sample and transfer tubes.

4 Add the Sequence check details in AUSLAB.

5 Pipette 1mL of autoclaved nanopure water into each tube, vortex gently.

6 Incubate at room temperature for 30 minutes.

7 Place tubes on multitube vortex for 5 minutes at 1200 rpm.

Note: Vortex FTA punch samples then go to Step 9.

8 Transfer swab into spin baskets.

9 Spin tubes with spin basket for 30 seconds at 15800g. Discard spin basket with swab.

10 Vortex supernatant then pipette back into original extract tube.

11 Spin samples in centrifuge for 3 minutes at 15800g.

12 Carefully remove all but 50µL of supernatant and discard.

13 Add 150µL of 20% (w/v) Chelex 100 (20g of Chelex 100 in 100mL autoclaved nanopure water) to each tube.

Note: When pipetting Chelex 100, the resin beads must be distributed evenly in the solution. Use magnetic stirrer in beaker of Chelex 100 and wide bore pipette tips.

14 Add 4µL of Proteinase K (10mg/mL). Mix gently by vortexing.

15 Check and record temperature of water bath on worksheet. Incubate in 56^{o}C water bath for 30 minutes.

16 Vortex, then incubate in boiling water bath for 8 minutes.

17 Vortex, then spin in centrifuge for 3 minutes at 15800g.

18 Transfer supernatant to new labelled 1.0mL Nunc™ Bank-It™ tube leaving Chelex 100 beads behind.

19 Samples are stored at –20^{0}C into Freezer 6117-3.

20 Complete batch in AUSLAB.

14.6 Differential Lysis of semen stains using Chelex 100

Steps 1 – 30 to be performed in Rooms 6120, 6121 or 6122

1 Obtain samples, worksheets and labels.

2 Label sterile 1.0mL Nunc™ Bank-It™ tubes.

3 Label the 1.5mL sample tubes and the sterile 1.0mL Nunc™ Bank-It™ tubes (Transfer tubes).

4 Add the Sequence check details in AUSLAB.

5 Pipette 1mL of autoclaved nanopure water into each tube, vortex gently.

6 Incubate at room temperature for 30 minutes.

7 Twirl the swab/fabric substrate with a sterile toothpick for 2 minutes then transfer it to a spin basket.

8 Spin substrate (in spin basket) for 30 seconds at 15800g.

9 Pool supernatant with original extract. Place spin basket containing substrate into the original 5mL sample tube.

10 Spin samples in centrifuge for 3 mins at 15800g.

11 Carefully remove all but 50 µL of supernatant and discard.

12 Resuspend pellet and spot 3µL onto a microscope slide. (Label slide with lab#, date & Step 12. Sterilise before use, air-dry and heat fix).

13 Add 150µL of autoclaved nanopure water and 4µL of Proteinase K (10mg/mL). Vortex (gently).

14 Check and record temperature of water bath on worksheet. Incubate in a 56°C water bath for 1.5 hours (max time).

15 Centrifuge for 5 minutes at 15800g.

16 Remove 150µL of supernatant (epithelial lysate) to a new tube.

17 Add 50µL of 20% (w/v) Chelex 100 (20g in 100mL autoclaved nanopure water) to epithelial lysate and proceed to Step 26.

Note: When pipetting Chelex 100, the resin beads must be distributed evenly in the solution. Use magnetic stirrer in beaker of Chelex 100 and wide bore pipette tips.

18 Resuspend pellet in 500µL Digest buffer (10mM TRIS-HCl, 10mM EDTA, 50mM NaCl, 2% SDS, pH 7.5).

19 Vortex and centrifuge at 15800g for 5 minutes. Remove all but 50µL of supernatant and discard.

20 Repeat steps 17 and 18 two times, (i.e. total of 3 washes).

21 Resuspend pellet in 1mL of autoclaved nanopure water.

22 Vortex and centrifuge for 5 minutes at 15800g. Remove all but 50µL of supernatant and discard.

23 Repeat steps 20 and 21 two times (i.e. total of 3 washes).

24 Resuspend pellet in the 50µL of supernatant and spot 3µL onto a microscope slide (Label slide with lab #, date & Step 24. Sterilise before use, air-dry and heat fix).

25 Add 150µL of 20% (w/v) Chelex 100, 4µL of Proteinase K (10mg/mL) and 7µL of DTT.

Note: When pipetting Chelex 100, the resin beads must be distributed evenly in the solution. Use magnetic stirrer in beaker of Chelex 100.

26 Check and record temperature of water bath on worksheet. Incubate for 45 minutes in a 56ºC water bath.

27 Vortex tubes quickly, and then incubate for 8 minutes in a boiling water bath.

28 Vortex for 5 seconds and centrifuge for 3 minutes at 15800g.

29 Transfer supernatant to the labelled 1.0mL Nunc™ Bank-It™ tube leaving Chelex 100 beads and substrate behind.

30 Store the samples to Freezer 6117-3. In Analytical, file worksheets.

31 Complete batch in AUSLAB.

14.7 Differential Lysis of semen stains using Chelex 100

(Appendix for wall of Extraction Room)