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Chelex 100 as a Medium for Simple Extraction of DNA for PCR-Based Typing from Forensic Material

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Procedures utilizing Chelex 100 chelating resin have been developed for extracting DNA from forensic-type samples for use with the PCR. The procedures are simple, rapid, involve no organic solvents and do not require multiple tube transfers for most types of samples. The extraction of DNA from semen and very small blood stains using Chelex 100 is as efficient or more efficient than using proteinase K and phenol-chloroform extraction. DNA extracted from bloodstains seems less prone to contain PCR inhibitors when prepared by this method. The Chelex method has been used with amplification and typing at the HLA DQ α locus to obtain the DQ α genotypes of many different types of samples, including whole blood, bloodstains, seminal stains, buccal swabs, hair and post-coital samples. The results of a concordance study are presented in which the DQ α genotypes of 84 samples prepared using Chelex or using conventional phenol-chloroform extraction are compared. The genotypes obtained using the two different extraction methods were identical for all samples tested.

Methods of extracting DNA from a variety of biological materials have historically included separation and purification steps utilizing phenol chloroform extractions and/or ethanol precipitation (3,8). Inorganic extraction procedures have included the use of high salt concentration (4), excess proteinase K digestion (5) and the use of glass powder (13). Although these methods are successful in recovering high molecular weight DNA from large samples, they require several steps and may include transfers of DNA extracts to additional containers or washing/desalting procedures using various commercial filters or columns. These additional steps allow increased opportunities for cross-transfer of samples or introduction of contaminants.

The polymerase chain reaction (PCR) (12) is a method of amplifying small quantities of relatively short target sequences of DNA using sequence specific oligonucleotide primers and thermostable Taq DNA polymerase. PCR does not require native high molecular weight DNA in order to amplify target sequences. Only the target sequence itself needs to be intact. Thus, PCR can amplify partially degraded and/or denatured DNA. PCR has great potential benefit for the analysis of forensic casework samples, in which the amount and molecular

weight of DNA may be less than optimal for analysis by other methods.

Recently, Singer-Sam et al. reported the use of Chelex 100 as a means of increasing the signal from the PCR amplification of small amounts of DNA released from small numbers of tissue culture cells that have been boiled (11). Chelex 100 is a chelating resin that has a high affinity for polyvalent metal ions. The Chelex resin is composed of styrene divinylbenzene copolymers containing paired iminodiacetate ions, which act as chelating groups. Singer-Sam et al. postulated that the presence of Chelex during boiling prevents the degradation of DNA by chelating metal ions that may act as catalysts in the breakdown of DNA at high temperatures in low ionic strength solutions (11).

The procedures adapted for forensic samples are based on those used by Singer-Sam et al. and involve boiling suspensions of cells in a 5% suspension of Chelex, followed by amplification of a fraction of the supernatant by PCR. The alkalinity of Chelex suspensions (pH 10-11) and the exposure to 100°C temperatures result in disruption of the cell membranes and denaturation of the DNA. Because these Chelex procedures result in denatured sample DNA, the DNA is not suitable for RFLP analysis.

In this paper, we demonstrate that the Chelex extraction method efficiently makes available to PCR amplification DNA from sperm and from blood dried onto cloth. Also, we show that for all 84 different forensic-type samples examined, there are no differences between the HLA DQ α genotypes obtained by PCR amplification of DNA prepared by either conventional phenol-chloroform extraction or the Chelex-based procedure.

Materials and methods

Assessment of Chelex DNA extraction from semen

Fresh semen, which had been stored frozen, was thawed and its sperm content measured using a hemocytometer. Five microliters of this semen were added in duplicate either to 200 μ l of 5% Chelex 100 (Bio-Rad, Richmond, CA) or to 200 μ l of sterile distilled water. Both Chelex- and water-treated samples were carried through the remainder of the extraction protocol described below for semen. To estimate DNA released, 4',6'-diamidino-2-phenylindole (DAPI) fluorescence measurements were taken on the resultant supernatant (2,7). DNA in amounts estimated by DAPI fluorescence and input sperm count were added to HLA-DQ α PCR

Table 1. Environmental Exposure Samples

Sample #	Description	Exposure	DQ α Types
1	Bloodstain, cotton cloth	10% SDS	1.1, 4
2	"	0.1 M KPO ₄ , pH 6.8	1.1, 4
3	"	household bleach	1.1, 4
4	"	unleaded gasoline	1.1, 4
5	"	10W40 motor oil	1.1, 4
6	"	1 N NaOH	1.1, 4
7	"	1 M acetic acid	1.1, 4
8	"	none	1.1, 4
9	"	2 wk greenhouse	1.1, 4

For treatments 1–7 above, 50 μ l of each substance were applied to cotton cloth, dried for 1.5 h, then 50 μ l of whole blood of known HLA DQ α type were spotted on top. A bloodstain control (sample #8) was also prepared by spotting 50 μ l of whole blood on cotton cloth. Sample #9 was prepared by spotting 50 μ l of whole blood of known HLA DQ α type on cotton cloth and then exposing the stain to light in a greenhouse for two weeks.

Table 2. β Test Site Samples

Sample #	Description	DQ α Types
B7	Bloodstain	1.1, 1.1
B8	"	1.1, 4
B9	"	1.1, 2
B11	"	1.2, 1.2
B13	"	1.3, 4
B16	"	1.1, 3
B17	"	1.2, 3
B18	"	1.1, 4
B20	"	3, 4
B21	"	1.3, 4
B23	"	3, 3
B24	"	4, 4
B27	"	1.1, 1.2
S26	Semen stain/collected 1980	3, 4
M1	Buccal swab	1.1, 3
M2	"	1.1, 1.1
M3	"	1.1, 3
M4	"	1.2, 4
M6	"	1.3, 4
M29	"	1.3, 4
H4	Plucked hair w/sheath	3, 3
H6	"	3, 4
H7	"	1.2, 1.3
H8	"	1.1, 3
H9	"	1.2, 1.3
V1 M	Post-coital	1.2, 4
V1 F	"	1.1, 3
V7 M	"	1.1, 3
V7 F	"	mix 1.1, 2, 3
V8 M	"	1.1, 3
V8 F	"	mix 1.1, 3
V9 M	"	3, 4
V9 F	"	1.3, 4

as described below, and the yield of PCR product was compared by gel electrophoresis (1,8) to that obtained from comparable amounts of highly-purified control DNA extracted with phenol-chloroform.

Assessment of Chelex DNA extraction from bloodstains

Fresh blood from a fingerprick was spotted in duplicate onto cotton cloth in 5-, 2-, and 1- μ l volumes. The same volumes of blood were also spotted onto Saran Wrap (Dow Brands, Indianapolis, IN). The stains were kept in the dark for 1 week. The stains on cotton cloth were either treated as described below for DNA extraction using Chelex or by organic extraction (1,8). The dried blood on Saran Wrap was resuspended in 50 μ l of sterile distilled water, and 10- or 3- μ l aliquots of these resuspensions were extracted either by the Chelex procedure or by the use of organic solvent. Ten percent of the total extract of a stain by either method was added to an HLA-DQ α PCR, as described.

Forensic-Type Samples used in Concordance Study

Environmental exposure samples. Nine bloodstains were obtained from C.T. Comey (Forensic Science Research and Training Center, F.B.I. Academy, Quantico, VA) that had been exposed to a variety of substances to which evidential bloodstains from crime scenes might have been exposed. These stains, all from the same individual (DQ α genotype 1.1,4) and the substances to which each was exposed are listed in Table 1.

Forensic-type samples given to "β-test" sites. Thirty-three samples that had also been given to five different crime labs which had participated in a "β-test" of the AmptiType HLA DQ α kit were also tested using the Chelex extraction method (14). The samples and their DQ α genotypes that had been obtained after organic extraction of their DNA are listed in Table 2.

Samples used in a proficiency test. Reminders or duplicates of 28 samples that had been given out as part of a blind proficiency test sponsored by the California Association of Crime Lab Directors (CACL D) were extracted using the Chelex-based procedures below and their DQ α genotypes determined. The samples and their DQ α genotypes, as obtained after organic DNA extraction, are listed in Table 3.

Additional bloodstains. Additional bloodstains spotted on cotton cloth were obtained from J. Maholovich (Forensic Science Associates, Richmond, CA). The DQ α genotypes of these samples, deter-

mined after organic extraction of their DNA, are listed in Table 4.

Typing from single, stained threads

In order to test the limits of usefulness of the Chelex extraction method, two bloodstains and one semen stain previously typed were again tested. New 3 x 3-mm samples were cut from the stains. These pieces of stained fabric were then dissected into individual 3-mm yarns representing various portions of the original stains. Single, 3-mm stained yarns representing between 5.5% and 9% of the original 3 x 3-mm stains were extracted with Chelex and amplified and typed.

Chelex-based extraction procedures

Note on handling Chelex solutions: When pipetting Chelex solutions, the resin beads must be distributed evenly in solution; this can be achieved by gentle mixing with a stir bar in a beaker. Make 20% and 5% (wt/vol) stock solutions in sterile, distilled water to a final volume of 100-300 ml. Add Chelex to individual samples as follows: For each set of samples, pour ca. 15 ml of Chelex stock solution into a 50-ml beaker containing a stir bar. Pipet the volume needed for each sample directly from the beaker while the stir bar is mixing. The pipette tip used must have a relatively large bore- 1000- μ l pipettor tips are adequate.

HLA DQ α amplification and typing

The HLA DQ α sequence in samples were amplified using the AmpliType HLA-DQ α kit (Cetus; 1) following the manufacturers directions. The cycling reaction was done in a programmable heat block (DNA Thermal Cycler, Perkin-Elmer Cetus, Norwalk, CT) set to heat at 94°C for 60 s (denature), incubate at 60°C for 30 s (anneal), and incubate at 72°C for 30 s (extend) by the "step-cycle" program. After 32 cycles, the samples were incubated an additional 10 min at 72°C. Determination of the DQ α alleles present in samples was done using the immobilized, allele-specific oligonucleotide probe system (10) provided in the kit.

Gel electrophoresis

Gelelectrophoresis of amplification products was performed using 3 NuSieve/1%GTG agarose gels (FMC, BioProducts, Rockland, ME). Both the gel buffer and running buffer were 89 mM Tris-borate, 2.5 mM EDTA pH 8.3 (1).

Results

Chelex DNA extraction from semen

Aliquots of DNA prepared from semen by Chelex extraction as described above were amplified for HLA each aliquot

Table 3. CACLD Proficiency Samples

Sample #	Description	DQ α Types
101	Bloodstain, cotton twill	1.1, 2
102	Bloodstain, twill	1.2, 3
103	Bloodstain, twill	1.2, 1.2
106	Bloodstain, sweaty T-shirt	3, 3
107	Bloodstain, red sweatshirt	1.2, 3
108	Bloodstain, twill	2, 4
109	Bloodstain, twill	1.2, 4
112	Bloodstain, terry cloth	4, 4
113	Bloodstain, sweaty T-shirt	1.2, 3
114	Bloodstain, twill	3, 3
116	Bloodstain, twill	3, 4
118	Bloodstain, twill	1.2, 3
119	Bloodstain, twill	1.3, 4
120	Bloodstain, dirt-rubbed twill	2, 4
124	Bloodstain, twill	2, 4
125	Bloodstain, blue denim	1.3, 2
126	Bloodstain, twill	1.2, 3
129	Bloodstain, twill	3, 3
130	Bloodstain, dirt-rubbed twill	1.2, 3
132	Bloodstain, twill	1.2, 3
105	Semen stain, black metallic fabric	2, 4
110	Semen stain, 1:1 mix rayon print	Mix 1.1, 1.3, 3, 4
115	Semen stain, tan cotton	1.3, 2
123	Semen stain, blue polyester	1.3, 3
127	Semen stain, twill	1.2, 1.2
128	Semen stain, cotton	2, 4
131	Semen stain, twill	1.1, 2
133	Semen stain, black cotton	1.1, 4

Table 4. Additional Bloodstains

Sample #	Description	DQ α Types
445-1	Bloodstain, cotton cloth	2, 4
278-5	Bloodstain, cotton cloth	1.1, 2
90-041-1	Bloodstain, cotton cloth	1.2, 4
436-17	Bloodstain, cotton cloth	1.1, 4
402-1	Bloodstain, cotton cloth	4, 4
349-11-2	Bloodstain, cotton cloth	1.1, 3
349-13	Bloodstain, cotton cloth	4, 4
395-1	Bloodstain, cotton cloth	3, 4
442-1	Bloodstain, cotton cloth	1.1, 3
442-17	Bloodstain, cotton cloth	1.1, 1.1
90-041-02	Bloodstain, cotton cloth	1.2, 1.2
421-14	Bloodstain, cotton cloth	2, 4
421-1	Bloodstain, cotton cloth	1.2, 3
RH	Bloodstain, cotton cloth	1.1, 3

was estimated by DAPI fluorometry and the input number of sperm cells. For DNA prepared using either Chelex or using water without Chelex, the fluorescence measurement of DNA was about 45%-80% of that predicted by the number of sperm. The yield of PCR product DNA was revealed by gel electrophoresis as

shown in Figure 1. The input amount of DNA estimated by fluorometry is given, as are the amounts of input highly-purified control DNA. The yield of PCR product from Chelex-prepared DNA is comparable to that from purified DNA, while DNA prepared without Chelex results in much less amplified DNA.

Figure 1. Amplification from DNA prepared from semen. DNA was prepared from semen as described in Materials and Methods. Chelex 100 was either present or omitted from the boiling step. The amount of DNA released was estimated by DAPI fluorescence. Increasing amounts of this DNA were subjected to PCR amplification for the DQα locus (32 cycles). The amplification products were resolved by electrophoresis on a NuSieve agarose gel as shown. 5% of each sample was loaded on the gel. See Reference 15 for a description of "primer-dimer" artifacts.

Figure 2. Amplification from DNA prepared from bloodstains: extraction with proteinase K/phenol:chloroform vs. boiling with Chelex. Duplicate bloodstains on either cotton cloth (A) or Saran Wrap (B) were prepared using either extraction with organic solvents or boiling with Chelex as described in Materials and Methods. The volume of blood in the stain is indicated. 10% of each extract was used in an amplification. The PCR products are shown resolved by electrophoresis on a NuSieve agarose gel. 5% of each sample was loaded on the gel. Very small volume stains were simulated on Saran Wrap by resuspension of the dried blood and using a fraction of the resuspension in the extraction procedures.

Chelex DNA extraction from bloodstains

Small bloodstains had been prepared as described above. Stains were prepared either on cloth or on plastic wrap. The stains on plastic were done to allow complete resuspension and aliquoting of fractions of the resuspension in order to quantify very small volumes of blood that had dried. Extracts of various size stains were prepared either by using Chelex or phenol-chloroform extractions. Ten percent of each extract was subjected to PCR amplification for the DQα locus. The results of the amplification as assayed by gel electrophoresis are shown in Figure 2. In general, there is little difference in PCR product yield from DNA prepared by either method. When there is a difference, the yield from Chelex-prepared DNA is greater.

Forensic-type samples: concordance study

The different forensic-type samples listed in Tables 1-4 (a total of 84 samples) have all been previously typed at the DQα locus after their DNA had been extracted using traditional phenol-chloroform methodology (1, 8). After repeating the extraction of another portion or replicate of each of these samples, but using the Chelex-based procedures described above, the typings were repeated. As summarized in Table 5, there were no discrepancies for any samples in the DQα genotype obtained using either extraction method. Representative probe strip typings of 16 of these samples are shown in Figure 3.

Among these samples, one blood stain (#107, Table 3) and one semen stain (#133, Table 3) failed to amplify initially using the Chelex procedure. The semen stain was successfully amplified and typed after repeating the extraction from a fresh cutting from the fabric substrate. The bloodstain substrate was a red fabric which colored the extract a red color. This red dye apparently inhibited the amplification.

An additional 3 x 3-mm piece of this bloodstain was allowed to soak for 30 min at room temperature, and then it was subjected to vortexing to dislodge cellular material from the fabric. The fabric was then removed and the solution centrifuged to pellet the cellular debris. The supernatant was removed and discarded. The cellular debris was washed three times with water washes, followed by centrifugation. All but 50 μ l of the last wash was discarded, and 150 μ l of 5% Chelex was added. The remainder of the protocol was as described above. At this time the sample was successfully amplified and typed.

Typing from single, stained threads

The single 3-mm stained yarns cut from 2 different bloodstains and one semen stain were successfully typed.

Discussion

A set of protocols have been described here which effectively and simply extract DNA from a variety of materials, including those encountered in forensic science laboratories. The extracted DNA can be amplified by PCR and hybridized to allele-specific oligonucleotide probes. Because of the relative simplicity of these procedures, it is anticipated that this will make the use of PCR in forensic analyses more easily accomplished. The reduction of the number of steps in sample preparation in which sample is transferred will also help reduce the chance of operator-introduced DNA contamination or of sample mix-up.

It is not certain what the role of Chelex is in DNA preparation for PCR. When we have subjected purified DNA to the boiling treatment in these procedures with Chelex, or else in distilled water alone, the DNA treated by boiling without Chelex becomes inactive in PCR. If the DNA is boiled in 0.01 M Tris-HCl, pH 8.0 with 0.1 M EDTA, it is also protected for PCR. This is in agreement with the protective role for Chelex that Singer-Sam et al. (11) suggest in which Chelex sequesters divalent heavy metals that would otherwise introduce DNA damage. A model for the extraction process is that the boiling treatment is useful for releasing DNA from low numbers of cells, and that Chelex protects the DNA from the effects of the boiling. Note that we have found that, unlike other cells, sperm do not release DNA from the boiling treatment alone; pretreatment with proteinase K and DTT treatment is required. Chelex can chelate a large amount of divalent ions that may be donated by the sample, and the Chelex beads can be easily removed so that they will not interfere with subsequent PCR amplifications that require Mg²⁺.

An additional motivation for our testing alternative sample preparation methods is that we have found that DNA prepared from bloodstains some times contains inhibitors of PCR (6; E. Blake, personal communication). We have found that extractions of bloodstains prepared by the Chelex-based procedure are less likely to have inhibitors of PCR than extracts prepared by proteinase treatment and phenol-chloroform extraction. For example, two of the CACLD bloodstain samples initially showed inhibition of PCR when extracted using conventional phenol-chloroform

Table 5. Results of Typing Concordance Study in Typing Chelex vs. Organic Extraction

Sample Type	Number	% Concordance
Bloodstains	56	100
Semen Stains	9	100
Post-coital	8	100
Hairs	5	100
Buccal swabs	6	100
Total # samples:	84	
# Different genotypes present: 18 of 21 possible (all except 1.2, 2; 1.3, 1.3; 2, 2)		
Different alleles present: 6 of 6 possible

Figure 3. Examples of HLA-DQα typings on DNAs prepared using the Chelex method from forensic-type bloodstains. Shown are probe strips that have been hybridized to PCR product made from the indicated samples. The PCR product is made with biotinylated primers. After the hybridization, biotinylated PCR product specifically captured by immobilized oligonucleotide probes is detected by a streptavidin horseradish peroxidase conjugate that catalyzes the production of the colored precipitate seen as the "dots" in this photo. Presence of a dot indicates presence of that DQα allele in the sample. The positive control probe will hybridize with any DQα allele. Reference 1 contains information on interpretation of probe strips.

methods, but no inhibition was observed when these samples were extracted using the Chelex methods. Previous work (6) has suggested that porphyrin compounds from blood are the cause of the inhibition.

Because no proteinase treatment is used that would release heme from globin, it is possible that less free porphyrin compounds are in the Chelex prepared extracts. Also, it is possible that such inhibitors are binding to the Chelex bead matrix itself.

In summary, we have presented procedures that should permit the rapid preparation of DNA from forensic-type samples. Since far fewer manipulations are required, these procedures should also reduce the chance of inadvertent contamination of samples by extraneous DNAs.

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