Chapter 5

DNA Extraction: Organic and Solid-Phase

Wafa Altayari

Abstract

DNA extraction remains a critical step in DNA profiling of biological material recovered from scenes of crime. In the forensic community several methods have gained popularity, including Chelex®, organic extraction, and solid-phase extraction. While some laboratories streamlined their processes and only use one method we have retained several methods and continue to use these for different sample types. In this chapter we present three methods that have been used for several years in our laboratory.

Key words DNA extraction, Phenol–chloroform, Solid-phase extraction, PrepFiler®, EZ1® DNA Investigator®

1 Introduction

After the detection/characterization and collection of biological material the next step is the extraction of DNA, sometimes along with the extraction of RNA (*see* Chapters 2 and 3). In the field of molecular biology there are a multitude of methods available for DNA extraction, many of which are variations on the same principles. Chelex® 100 was one of the first methods to be adopted by the forensic community in the PCR era [1]. It is simple, inexpensive, and yields relatively high amounts of DNA [2]. However, it does have a limited capacity to remove PCR inhibitors [3]. Organic extraction, in particular, phenol–chloroform-based extraction, had widespread popularity in molecular biology, and yields high-molecular weight DNA (as long as the starting material is not degraded); when processing forensic samples the organic methods have proven to be effective at removing many PCR inhibitors (*see* Chapters 6 and 7). The popularity of organic methods has reduced in recent years, largely due to health and safety concerns because phenol is highly toxic, and also because of the limited potential for automation. Solid-phase DNA extraction techniques have become increasingly popular in forensic laboratories; these methods benefit from the potential for automation and also being efficient at removing

William Goodwin (ed.), *Forensic DNA Typing Protocols*, Methods in Molecular Biology, vol. 1420,
DOI 10.1007/978-1-4939-3597-0_5,

many PCR inhibitors [3–8]. The choice of method to be employed for DNA extraction depends on several variables, including the likely amount of DNA in the samples, the substrate that the sample is on, the type of biological material, and the presence of potential PCR inhibitors [3, 9, 10].

2 Organic Extraction (Phenol–Chloroform)

Despite the toxicity of phenol and the extra burden that this places onto laboratories in terms of specialist equipment, safety precautions and disposal of spent reagents the phenol–chloroform method is still popular in many forensic laboratories especially when extracting DNA from poor quality samples. The sample types that we have used this method for are detailed in Table 1.

2.1 Materials and Equipment

1. DNA-free 1.5 ml tubes.
2. Phase-lock 2 ml tubes (*see* **Note 1**).
3. DNA-free scalpel/scissors.
4. DNA extraction buffer (2 % SDS, 0.01 M EDTA pH 8.0).
5. Proteinase K, 20 mg/ml (Sigma-Aldrich).
6. Dithiothreitol (DTT) (0.4 M) (Sigma-Aldrich).
7. Phenol–chloroform–isoamyl alcohol (25:24:1) (Sigma-Aldrich).
8. *n*-Butanol (Sigma-Aldrich).
9. Microcon® 100 columns (Merck Millipore).
10. Benchtop vortex.
11. Benchtop minifuge.
12. Incubator (37–56 °C).

3 Method

1. Prepare the sample according to Table 1.
2. In the sample tube add the required amount of extraction buffer, typically we use 400 µl, but the reaction volume can be scaled up if necessary.
3. Add Proteinase K enzyme (20 mg/ml) in a ratio of 10:1 (extraction buffer: Proteinase K), for example, when using 400 µl of extraction buffer then 40 µl Proteinase K should be added.
4. Add the reducing agent dithiothreitol (0.4 M DTT) if semen, hair, nails, or bone is present in the extraction—add the same volume as proteinase K.

Table 1
Sample preparation using the organic extraction protocol developed by Abu Dhabi Forensic Evidence Department

Sample type	Sample preparation
Whole blood	Thaw and equilibrate up the whole blood sample at room temperature (15–25 °C). Transfer 10 µl of the sample to a 1.5 ml tube.
Buccal swabs	Cut 1/4 - 1/2 of the swab and place it into a 1.5 ml tube.
Blood swabs	If the blood on the swab is diluted the entire swab will be cut and placed in 1.5 ml tube. If the blood swab is concentrated, then a small part from the swab will be cut and placed in 1.5 ml tube
Saliva on fabric	Cut out an area indicated for saliva (25 mm^2).
Blood on fabric	Cut out an area indicated for blood (25 mm^2).
Manufactured cigarettes	Place 0.5–1 cm paper from the end of the filter in 1.5 ml tube.
Roll-ups cigarettes (smoked)	Place 1 cm of the paper taken around the base of the cigarette in 1.5 ml tube. Avoid taking any of tobacco as this will inhibit the extraction and PCR.
Roll-ups cigarettes (unsmoked)	Take 1–2 cm of the seam. Separate it before putting it in 1.5 ml tube.
Stamps and envelops	Access the gum from the reverse of the baking paper by making 4 dots at the corner of the stamp that you can see through the reverse backing paper. Use a sterilize scalpel and very gently score between the dots without going through the other side. Carefully peel the backing paper off to reveal the gum layer; the surface texture should go from fibrous paper to shiny, smooth and slightly tinted. Carefully peel away the gum layer till leaving the stamp intact. Put this paper in 1.5 ml tube.
Chewing gum	Cut third to half of the gum and then place it in 1.5 ml tube. Smear the gum around the inside of the tube using a DNA-free metal rod.
Hair	Place 1 cm from the root end in the 1.5 ml tube.
Fingernail scrapings/ cuttings	In case of scrapings, do not take more than will half fill 1.5 ml tube. In case of cutting, chop the cuttings into small pieces and this place it in 1.5 ml tube. Don't take more than will half fill a 1.5 ml tube.
Bone\teeth\muscle	Add 50 mg of powdered bone\tooth into 1.5 ml tube. Add small pieces of muscle into 1.5 ml tube

5. Vortex the sample for 5 s and then centrifuge briefly.
6. Incubate the sample either at 37 °C overnight or at 56 °C for 2 h.
7. Before sample purification with phenol–chloroform filter any samples that contain absorbent materials using the Spinaroo process, e.g., swabs or fabrics (*see* **Note 2**).

8. Add phenol–chloroform–isoamyl alcohol (25:24:1) to the sample tubes with the equivalent amount of the extraction buffer with the Proteinase K and DTT. Vortex and centrifuge at 14,000 × *g* for 5 min. Repeat this step 2–3 times until no pellicle is visible in the interphase (*see* **Note 1**).
9. Add an amount of *n*-butanol equal to the amount of the phenol–chloroform–isoamyl alcohol (*see* **Note 3**).
10. Vortex and then centrifuge at 14,000 × *g* for 5 min.
11. Transfer the lower aqueous phase to a Microcon® 100 Centrifugal Filter to re-concentrate the DNA. Centrifuge at 4000 × *g* for 20–30 min, or until the liquid has been reduced to the minimum retained volume (*see* **Note 4**).
12. Wash the filtrated Microcon with 500 μl of DNA-free water and centrifuge at 4000 × *g* for 20–30 min. Repeat this **steps 2–3** times.
13. Elute the DNA in 25–50 μl of DNA-free water (TE (Tris–EDTA) buffer could also be used) by inverting the Microcon into a 1.5 ml tube. Centrifuge at 4000 × *g* for 3–5 min.

The scheme for DNA extraction from epithelial cells using organic extraction is shown in Fig. 1.

4 Solid-Phase Nucleic Acid Extraction

Solid-phase nucleic acid purification is now widely used as the cleanest and easiest technique to separate DNA from inhibitors. Many commercial kits are available that use solid-phase extraction. It has gained popularity based on the increased levels of automation that are possible, the ability of the methods to remove many types of PCR inhibitor and the relatively high yields of DNA that can be recovered from many types of evidence. The methods all rely on DNA being released from the cellular material, and then binding to a solid substrate; for example, magnetic silica-based beads with QIAamp chemistry and polymer embedded magnetic beads with PrepFiler® chemistry (Life Technologies). After binding non-DNA components can be washed away, before releasing the DNA from the solid support to yield high purity DNA. The technology is suitable for a wide variety of samples (Table 2).

4.1 Qiagen Investigator® Kit Using the EZ1® Bio-Robot Workstation

Qiagen corporation has developed an automate method for DNA extraction based on solid-phase extraction using silica coated beads [11].

4.2 Materials and Equipment

1. DNA-free 1.5 ml tubes.
2. G2 Lysis Buffer (provided with the kit).

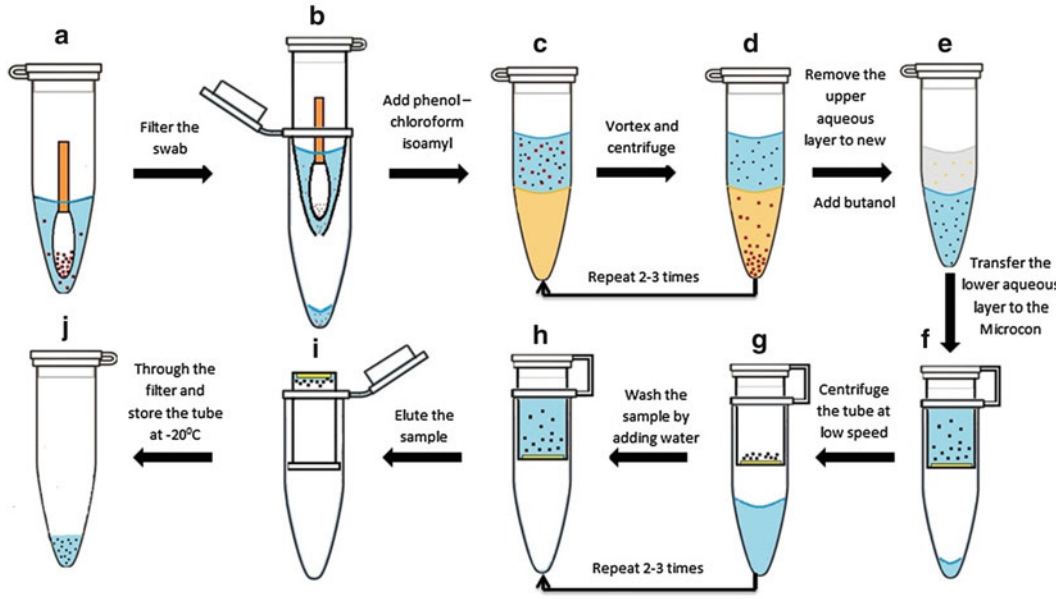

Fig. 1 Organic-based DNA extraction from epithelial cells (swab) using phenol–chloroform. (**a**) Cellular material is added to the lysis buffer and proteinase K and incubated at 56 °C for at least 2 h; (**b**) The digested swab is filtered by making a hole at the bottom of the tube and then placed it in a new 1.5 ml tube (spinaroo). The tube assembly is centrifuged at 4000 × *g*; (**c**–**d**) Phenol–chloroform–isoamyl alcohol is added to the filtrate solution, the solution is then vortexed and centrifuged. This step is repeated 2–3 times until there is no visible material at the interface (phase-lock gel tubes can be used when carrying out this step); (**e**) *n*-Butanol is added; this removes any residual of phenol–chloroform–isoamyl alcohol, which would inhibit downstream process such as PCR; (**f**) The aqueous phase is transferred to the Microcon 100 Centrifugal Filter assembly and centrifuged at low speed. (**g** and **h**) Sample is then washed using DNA-free water and centrifuged at 4000 × *g* for 20–30 min. This step is repeated 2–3 times depending on the sample condition and purity. Elution buffer is added to the Microcon and then the Microcon inverted in the elution tube. The inverted Microcon assembly is centrifuged for 3–5 min to collect the DNA at the bottom of the elution tube. (**i**) Elution tube is closed and stored at 4 °C short-term until the downstream analysis is finished. After that the samples are stored at –20 °C long-term

3. Proteinase K (provided with the kit).
4. Ultrapure (DNA-free) water.
5. EZ1 sample tubes, elution tubes, tips and holders, and cartridge.
6. Benchtop vortex.
7. Benchtop minifuge.
8. Heat block (56–95 °C).
9. EZ1 Bio-Robot workstation.

Table 2
Sample types suitable for processing as recommended by the manufacturers and validated for use under ISO17025 in Abu Dhabi Police Laboratories

	Manufacturers' recommendations		Internally validated	
Sample type	**PrepFiler®**	**DNA Investigator Kit**	**PrepFiler®**	**DNA Investigator Kit**
Whole blood	√	√	NT*	√
Dried blood	√	√	√	√
Buccal cells	√	√	NT	√
Surface and contact cells		√	√	X
Nails scraping		√	√	X
Chewing gum	√	√	√	√
Cigarette butts	√	√	√	√
Postage stamps and envelopes		√	√	NT
Hair	√	√	√	X
Bones or teeth	√	√	√	X
Soil		√	NT	NT
Stains on fabric	√	√	√	√
Human tissue		√	NT	NT
Epithelial cells mixed with sperm cells	√	√	√	X
Tape lifts	√		NT	NT

* NT stands for "Not Tested".

5 Method

1. Prepare the sample according to Table 3.
2. Pre-digest the samples by adding the recommended amount of lysis buffer and proteinase K provided with the kit (*see* Table 4). Dilute the lysis buffer with ultrapure water 1:1 when extracting DNA from absorbent material (*see* **Note 6**).
3. Vortex the samples thoroughly for 10 s and pulse spin them.
4. Incubate the sample under the recommended temperature and lysis time (Table 4).
5. Vortex the sample tube once or twice during the incubation or place it in the shaking incubator.
6. Incubate the sample at 95 °C for 5 min if you want to increase the yield of DNA.

Table 3
Shows sample preparation for samples to be used with EZ1® DNA Investigator® Kit. Adapted from manufacturer's manual—additional sample types are detailed in the manufacturer's manual)

Sample type	Sample preparation
Whole blood	Thaw and equilibrate up the whole blood sample at room temperature (15–25 °C). Transfer 10 µl of the blood sample to a 2 ml EZ1 sample tube, add 10 µl Proteinase K and 180 µl G2 Buffer (*see* **Note 5**).
Dried blood on FTA cards or Fabric	Punch 4 disks from the dried blood sample, each one is 3 mm in diameter and place it in 1.5 ml tube. If blood is on fabrics cut approximately 25 mm^2 section.
Buccal Swabs	Place 1/2 – 1/4 of the buccal swab into a 1.5 ml tube.
Chewing gum	Cut third to half of the gum and then place it in 1.5 ml tube. Smear the gum around the inside of the tube using a DNA-free metal rod.
Cigarette butts	Place 1 cm^2 paper from the end of the filter into 1.5 ml tube.
Stains on fabric	Place the fabric sample (25 mm^2) into 1.5 ml tube.

Table 4
Recommended protocols when using the EZ1 DNA Investigator Kit. Adapted from the manufacturer's manual—some variations from the manufacturer's recommendations are noted

Sample type	Recommended amount of lysis buffer (G2)	Recommended amount of Proteinase K (µl)	Recommended incubation time and temperature	Recommended EZ1 protocol
Whole blood (*see* **Note 5**)	180 µl Buffer G2	10	At 56 °C for 15 min	Trace protocol
Dried blood on FTA or fabric	190 µl Diluted buffer G2	10	At 56 °C for 15 min	Trace protocol (*see* **Note 7**)
Buccal swabs	190 µl Diluted buffer G2	10	At 56 °C for 15 min	Trace protocol (*see* **Note 7**)
Chewing gum	190 µl Buffer G2	10	At 56 °C for 15 min	Trace protocol
Cigarette butts	190 µl Diluted buffer G2	10	At 56 °C for 15 min	Trace protocol (*see* **Note 7**)
Stains on fabric	190 µl Diluted buffer G2	10	At 56 °C for 15 min	Trace protocol (*see* **Note 7**)

7. Flick or centrifuge the tube briefly to remove drops from inside the lid.
8. When extracting DNA from absorbent materials and using the trace protocol transfer the supernatant from the predigested tube (1.5 ml tube) to a 2 ml EZ1 tube using spinaroo method (*see* **Note 2**).

9. Load 2 ml EZ1 sample tubes, elution tubes, tips and holders, and cartridge into EZ1 Bio-Robot according to manufacturer's instructions.
10. Select the EZ1 Trace protocol.
11. Start the instrument according to manufacturer's instructions.
12. In the instrument:

    First, the sample will be added to the lysis buffer in the cartridge. This lysis buffer contains: guanidine thiocyanate (GuCN) and guanidine hydrochloride (GuCl) which work as chaotropic agents that lysis any remaining cells, denature the proteins, inhibit nuclease enzymes as well as provide the environmental conditions to promote the binding of the DNA to the paramagnetic silica beads. Secondly, the paramagnetic beads will be added to the lysis buffer. After that, the bound DNA will be washed to remove impurities twice and finally eluted under an alkaline condition using Tris–EDTA buffer. The extraction using the EZ1 instrument will take approximately 20 min. A schematic example of DNA extraction from epithelial cells using EZ1 instrument is shown below (Fig. 2).

6 PrepFiler® Express™ and Express BTA™ Forensic DNA Extraction Kits Using AutoMate Express™

PrepFiler® was initially introduced as a manual DNA extraction method; subsequently Applied Biosystems developed the AutoMate Express™, a small liquid handling robot similar to Qiagen EZ1 robot. This instrument use PrepFiler® chemistry which is similar to the EZ1 Qiagen kits except for the binding material. PrepFiler® uses encapsulated magnetic beads with polymer. This type of beads creates a large surface area with higher DNA binding capacity, which improved the extraction yield from some challenging samples. We use two kits: the PrepFiler® Express™ and PrepFiler® Express BTA™ Forensic DNA Extraction Kits.

6.1 Materials and Equipment

1. DNA-free 1.5 ml tubes.
2. PrepFiler lysis solution (provided with the kit).
3. Proteinase K (provided with the kit).
4. 1.0 M DTT (Sigma-Aldrich).
5. PrepFiler Lysep, PrepFiler sample tube, Bone/Tooth Lysate tube, extraction cartridge.
6. Benchtop vortex.
7. Benchtop minifuge.
8. Shaking Incubator (56–95 °C).
9. AutoMate Express™.

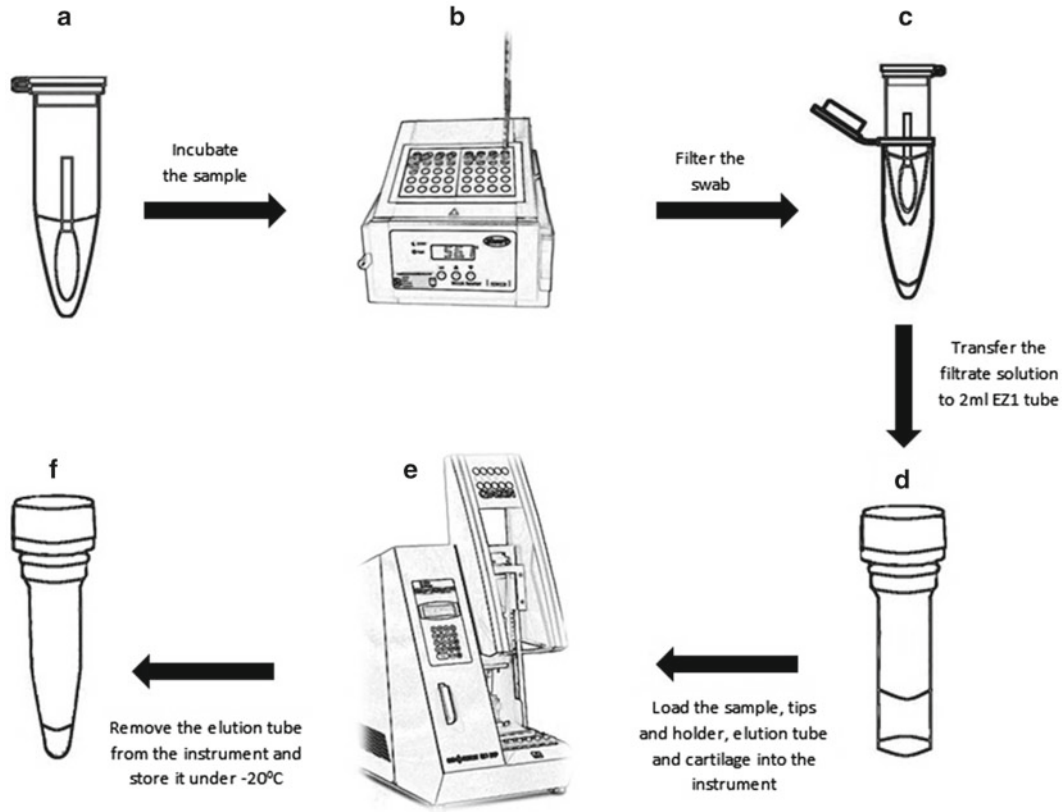

Fig. 2 DNA extraction from epithelial cells (swab) using solid-phase extraction method based on paramagnetic silica beads. (**a**) Cellular material is added to the lysis buffer and proteinase K which provided with the Qiagen kit. (**b**) The sample is then incubated at 56 °C for 15 min. (**c**) The digested swab is filtered by making a hole at the bottom of the tube and then placed it in a new 1.5 ml tube. Spin columns can also be used in this step. (**d**) Filtrate solution is transferred to 2 ml EZ1 tube. (**e**) The pre-digested sample, elution tube, tips and holders, and cartridge are loaded to the EZ1 instrument. The extraction process started using trace protocol installed in the instrument. The Tip Dance protocol can be used if the filtration step was not carried out. (**f**) After the run is complete the elution tube is closed and stored at 4 °C for short-term until the downstream analysis is finished (less than 1 week). After that the samples will be stored at –20 °C

7 Method

1. Prepare the column/tube assembly by inserting a spin column called PrepFiler LySep into a hingeless PrepFiler sample tube. Note that this assembly will work with all the samples except for the bone and tooth where the Bone and Tooth Lysate Tube will be used.
2. Prepare the sample according to Table 5 below.
3. Prepare the PrepFiler lysis solution. Each sample required:
   - Recommended amount of PrepFiler lysis buffer (provided with the kit) shown in Table 6.

Table 5
Samples preparation to be used with PrepFiler® Kits along with the AutoMate Express™ (information for additional sample types is provided in the manufacturer's manuals). Adapted from manufacturer's manual—differences to the manufacturer's recommendations are noted

Sample type	Sample preparation
Blood on FTA or fabric	Add up to 25 mm^2 cutting or punches to the column/tube assembly
Body fluids (semen, saliva) on fabric	Add up to 25 mm^2 cutting or punches to the column/ tube assembly
Body fluids on swab	Add up to 1 swab to the column/tube assembly
Hair	Add up to 5 mm from the root end to the column/tube assembly
Bone and teeth	Add 50 mg of powdered bone or tooth into PrepFiler Bone and Tooth Lysate Tube.
Chewing gum	Chewing gum is prepared by cutting third to half of the gum and then place it in 1.5 ml tube. By using a metal rod the gum is smeared around the inside of the tube. The recommended amount of PrepFiler lysis solution is added and the sample is incubated under the recommended temperature and lysis time (Table 6). The lysate is then transferred to the PrepFiler sample tube (*see* **Note 8**)
Cigarette butts	Remove the first 5 mm paper from the end of the filter. Cut it into small pieces then transfer the pieces to the column/tube assembly

- Recommended amount of 1.0 M DTT (not provided with the kit) shown in Table 6.
- Recommended amount of proteinase K (provided with the kit) shown in Table 6.

4. Pre-digest the samples by adding the recommended amount of PrepFiler lysis solution (Table 6).
5. Incubate the sample under the recommended temperature, lysis time, and shaking speed. Selection of those parameters depends on the sample type (Table 6).

   Note that when the temperature is above 56 °C, no proteinase K is added to the lysis solution. Most proteins denature when the temperature is above 50 °C, so there no need to add proteinase K when the incubation temperature is high (70 °C). A precautionary step has been taken by adding 1.0 M DTT to the solution to maximize the yield of DNA by breaking down any disulfide bonds that may be present in the substrate.
6. Centrifuge the column/tube assembly or the Bone and Tooth Lysate Tube for 2 min and 90 s respectively at 10,000 × *g*.

   In case of the column/tube assembly, if the volume after the centrifugation is less than 300 μl, centrifuge the column/tube assembly for additional 5 min to collect the rest of the

Table 6
Recommended parameters to be used with the AutoMate Express™ PrepFiler® Kits based on the manufacturer's recommendations—(adapted from manufacturer's manual—a variation from the recommendations is noted)

Sample type	PrepFiler lysis solution (µl)	PrepFiler lysis buffer (µl)	1.0 M DTT (µl)	Proteinase K (20 mg/ml)	Recommended incubation temperature and lysis time	Recommended shaking speed
Blood on FTA or fabric	500	500	5	–	At 70 °C for 40 min	750 rpm
Body fluids (semen, saliva) on fabric	500	500	5	–	At 70 °C for 40 min (*see* **Note 9**)	750 rpm
Body fluids on swab	500	500	5	–	At 70 °C for 40 min	750 rpm
Hair	500	500	5	–	At 70 °C for 40 min	750 rpm
Bone and teeth	230	220	3	7 µl	At 56 °C from 2 to 18 h	1100 rpm
Chewing gum	230	220	3	7 µl	At 56 °C for 40 min	750 rpm
Cigarette butts	230	220	3	7 µl	At 56 °C for 40 min	750 rpm

sample and then discard the column. If still less than 300 µl, add PrepFiler lysis buffer to bring the volume to 300 µl.
In case of Bone and Tooth Lysate Tube, transfer the clear lysate to new PrepFiler sample tube. If the lysate solution is less than 150 µl, bring the solution up to 150 µl by adding PrepFiler lysis buffer.

7. Load AutoMate Express sample tubes, elution tubes, tips and holders, and cartridge into the instrument according to manufacturer's instruction.
8. Start the instrument according to manufacturer's instruction.
9. In the instrument:
   First, the sample will be added to the lysis buffer in the cartridge. This lysis buffer contains chaotropic agents that lysis any remain cells, denature the proteins, inhibit nuclease enzymes as well as promote the binding of the DNA to the polymer embedded magnetic beads. Secondly, the magnetic beads will be added to the lysis buffer and the DNA will be bound to the bead and washed to remove impurities. Finally, the DNA is eluted under an alkaline condition using TE buffer. AutoMate Express takes approximately 30 min to finish the extraction. A schematic example of DNA extraction from epithelial cells using AutoMate Express instrument is shown in Fig. 3.

8 Notes

1. In this step, a Phase-Lock Gel tube (Eppendorf, North America) can be used to make the separation cleaner and easier. This technique utilizes gel inside the tube that migrates to between the organic and aqueous phases.
2. We use the spinaroo process to filter the pre-extracted samples from any absorbent material. Spinaroo is a filtration process done by making two holes in the top and bottom of the tube containing the pre-extracted sample, respectively. Then this tube is placed in another tube and centrifuged at 4000 × *g* for 3–5 min. In our laboratory we use a sterile metal needle to make the hole—this is heated in a Bunsen and then easily pierces the plastic tubes.
3. The n-butanol wash is not essential, but it helps to remove any trace phenol that can otherwise act as a PCR inhibitor.
4. DNA in the aqueous phase can be recovered using a standard ethanol precipitation protocol as an alternative; however, we find that that DNA extracts concentrated and washed using Microcon® 100 columns give higher yields and contain less PCR inhibitors.
5. The manufacturer recommends extracting 200 μl of liquid blood directly without predigestion step: we find this produces inhibitors which leads to dropout of some STR markers.
6. If the cellular material is on an absorbent substrate then the lysis buffer should be diluted 1:1 with DNA-free water.
7. Based on our validation study the recommended EZ1 protocol is Trace at all times. When processing absorbent materials using spinaroo followed by the Trace protocol gave higher DNA yield compared with the Tip Dance protocol.
8. Our protocol differs from the manufacturer's recommendation when handling chewing gum. The manufacturer's recommendation: (A) When using Automate Express is to place the gum in a clean petri dish, flatten the piece of gum into a pancake shape of approximately 5 mm thickness. Cover the petri dish and then place it in −80 °C freezer for at least 2 h. Cut and transfer around 50 mg to the column/tube assembly. (B) When using EZ1 instrument is to place 40 mg of chewing gum cut into small pieces to 2 ml EZ1 sample tube.
9. Based on our validation studies the recommended incubation temperature and lysis time for semen samples is 90 °C for 40 min.

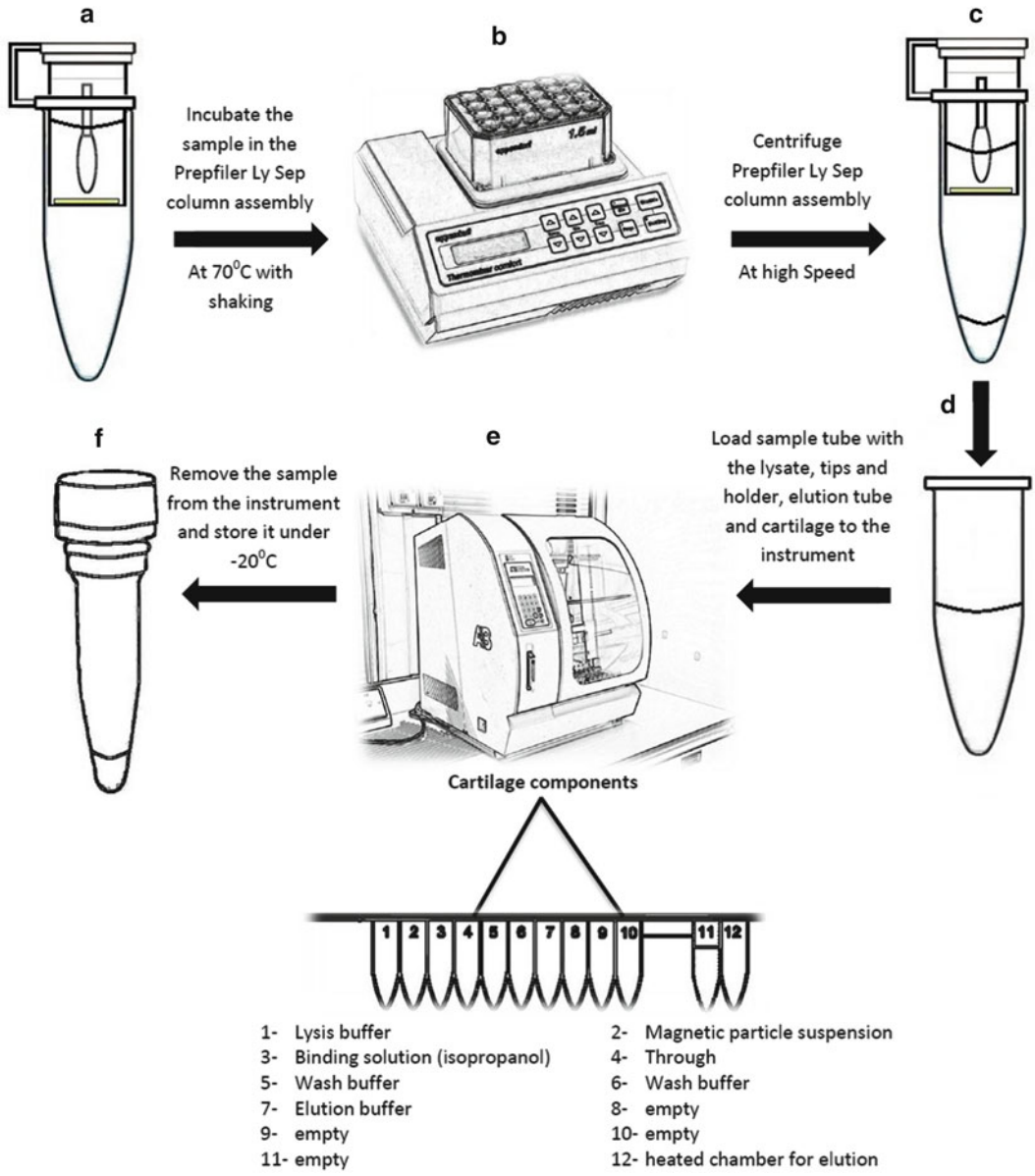

Fig. 3 DNA extraction from epithelial cells (swab) using solid-phase extraction method based on polymer embedded magnetic beads. (**a**) Cellular material is added to PrepFiler LySep column/tube assembly and lysis solution containing PrepFiler lysis buffer and DTT is added to the assembly. (**b**) The sample tube is incubated at 70 °C for 40 min. (**c**) Digested swab is centrifuged for 2 min at 10,000 × *g*. (**d**) Filtrate solution is checked to see if the volume in the sample tube is more than 300 μl if not, the volume will be adjusted to 300 μl with the PrepFiler lysis buffer. (**e**) The sample tube, elution tube, tips and holders, and cartridge are loaded to the AutoMate Express instrument. The extraction process started using the protocol installed in the instrument. (**f**) After the instrument has finished the running protocol each elution tube is closed and stored at fridge for short term until the downstream analysis is finished. After that the samples are stored at −20 °C

References

1. Walsh PS, Metzger DA, Higuchi R (1991) Chelex® 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 10:506–513
2. Phillips K, McCallum N, Welch L (2012) A comparison of methods for forensic DNA extraction: Chelex-100 ® and the QIAGEN DNA investigator kit (manual and automated). Forensic Sci Int Genet 6:282–285
3. Stangegaard M, Hjort BB, Hansen TN, Hoflund A, Mogensen HS, Hansen AJ et al (2013) Automated extraction of DNA from biological stains on fabric from crime cases. A comparison of a manual and three automated methods. Forensic Sci Int Genet 7:384–388
4. Amory S, Huel R, Bilic A, Loreille O, Parsons TJ (2012) Automatable full demineralization DNA extraction procedure from degraded skeletal remains. Forensic Sci Int Genet 6:398–406
5. Anslinger K, Bayer B, Rolf B, Keil W, Eisenmenger W (2005) Application of the BioRobot EZ1 in a forensic laboratory. Leg Med 7:164–168
6. Davis CP, King JL, Budowle B, Eisenberg AJ, Turnbough MA (2012) Extraction platform evaluations: a comparison of automate express™, EZ1® advanced XL, and maxwell® 16 bench-top DNA extraction systems. Leg Med 14:36–39
7. Keijzer H, Endenburg SC, Smits MG, Koopmann M (2010) Automated genomic DNA extraction from saliva using the QIAxtractor. Clin Chem Lab Med 48:641–643
8. Montpetit SA, Fitch IT, O'Donnell PT (2005) A simple automated instrument for DNA extraction in forensic casework. J Forensic Sci 50:555–563
9. Jakubowska J, Maciejewska A, Pawlowski R (2012) Comparison of three methods of DNA extraction from human bones with different degrees of degradation. Int J Legal Med 126:173–178
10. Verdon TJ, Mitchell RJ, van Oorschot RAH (2011) Evaluating the efficiency of DNA extraction methods from different substrates. Forensic Sci Int Genet 3:e93–e94
11. Butler JM, Shen Y, McCord BR (2003) The development of reduced size STR amplicons as tools for analysis of degraded DNA. J Forensic Sci 48:1054–1064