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Lab resource: Stem Cell Line

# Generation of human iPSCs from human prostate cancer-associated fibroblasts IBPi002-A

Zuzana Kahounová[a,f], Eva Slabáková[a], Lucia Binó[a], Ján Remšík[a,f,1], Radek Fedr[a,f], Jan Bouchal[c], Daniela Kurfürstová[c], Radek Vrtěl[d], Vladimír Študent[e], Lucie Jurečková[b], Volodymyr Porokh[b], Aleš Hampl[b,f], Karel Souček[a,f,*]

[a] Department of Cytokinetics, Institute of Biophysics of the Czech Academy of Sciences, Brno, Czech Republic
[b] Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, Czech Republic
[c] Department of Clinical and Molecular Pathology, Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic
[d] Department of Medical Genetics, University Hospital Olomouc, Faculty of Medicine and Dentistry, Palacky University Olomouc, Czech Republic
[e] Department of Urology, University Hospital Olomouc, Faculty of Medicine and Dentistry, Palacky University Olomouc, Czech Republic
[f] International Clinical Research Center, St. Anne's University Hospital Brno, Brno, Czech Republic

A B S T R A C T

A human induced pluripotent stem cell line was generated from cancer-associated fibroblasts of a 68-years old patient with diagnosed prostate adenocarcinoma (PCa). The fibroblast cell line was reprogrammed with Epi5™ Episomal iPSC Reprogramming Kit. Pluripotency of the derived transgene-free iPS cell line was confirmed both *in vitro* by detecting expression of factors of pluripotency on a single-cell level, and also *in vivo* using teratoma formation assay. This new iPS cell line may be used for differentiation into different prostate-specific cell types in differentiation studies.

Resource table

| | |
|---|---|
| Unique stem cell line identifier | IBPi002-A |
| Alternative name(s) of stem cell line | P71 hiPSCs |
| Institution | Department of Cytokinetics, Institute of Biophysics of the Czech Academy of Sciences, Brno, Czech Republic & International Clinical Research Center, St. Anne's University Hospital Brno, Brno, Czech Republic |
| Contact information of distributor | Karel Souček, ksoucek@ibp.cz |
| Type of cell line | iPSCs |
| Origin | Human |
| Additional origin info | Sex: male<br>Age 68<br>Ethnicity: Caucasian |
| Cell Source | Human prostate cancer-associated fibroblasts |
| Clonality | Clonal |
| Method of reprogramming | Epi5™ Episomal iPSC Reprogramming Kit (episomal vectors with the oriP/EBNA-1 backbone containing OCT4, SOX2, KLF4, LIN28 and L-MYC) |
| Genetic Modification | NO |
| Type of Modification | N/A |
| Associated disease | Prostate adenocarcinoma; pT2c; Gleason score 3 + 2 = 5 |
| Gene/locus | N/A |
| Method of modification | N/A |
| Name of transgene or resistance | N/A |
| Inducible/constitutive system | N/A |
| Date archived/stock date | April 2016–February 2018 |
| Cell line repository/bank | https://hpscreg.eu/cell-line/IBPi002-A |
| Ethical approval | Animal experiments were approved by the Academy of Sciences of the Czech Republic (approval nr. 13/2015); supervised by the local ethical committee of the Institute of Biophysics of the CAS; and performed by certified individuals. Human prostate tissue sample was obtained with approval of local ethical committee (Palacky University, Olomouc; approval nr. 127/14 and 163/08), and the donor gave written informed consent. |

## Resource utility

Generally, the models for studying normal prostate and disease development and progression are insufficient. Therefore, this iPS cell line was generated as a new model for use in both *in vivo* and *in vitro* studies.

* Corresponding author at: Department of Cytokinetics, Institute of Biophysics of the Czech Academy of Sciences, Brno, Czech Republic.
*E-mail address:* ksoucek@ibp.cz (K. Souček).
[1] Present address: Human Oncology & Pathogenesis Program, Memorial Sloan Kettering Cancer Center, New York, New York 10065, USA.

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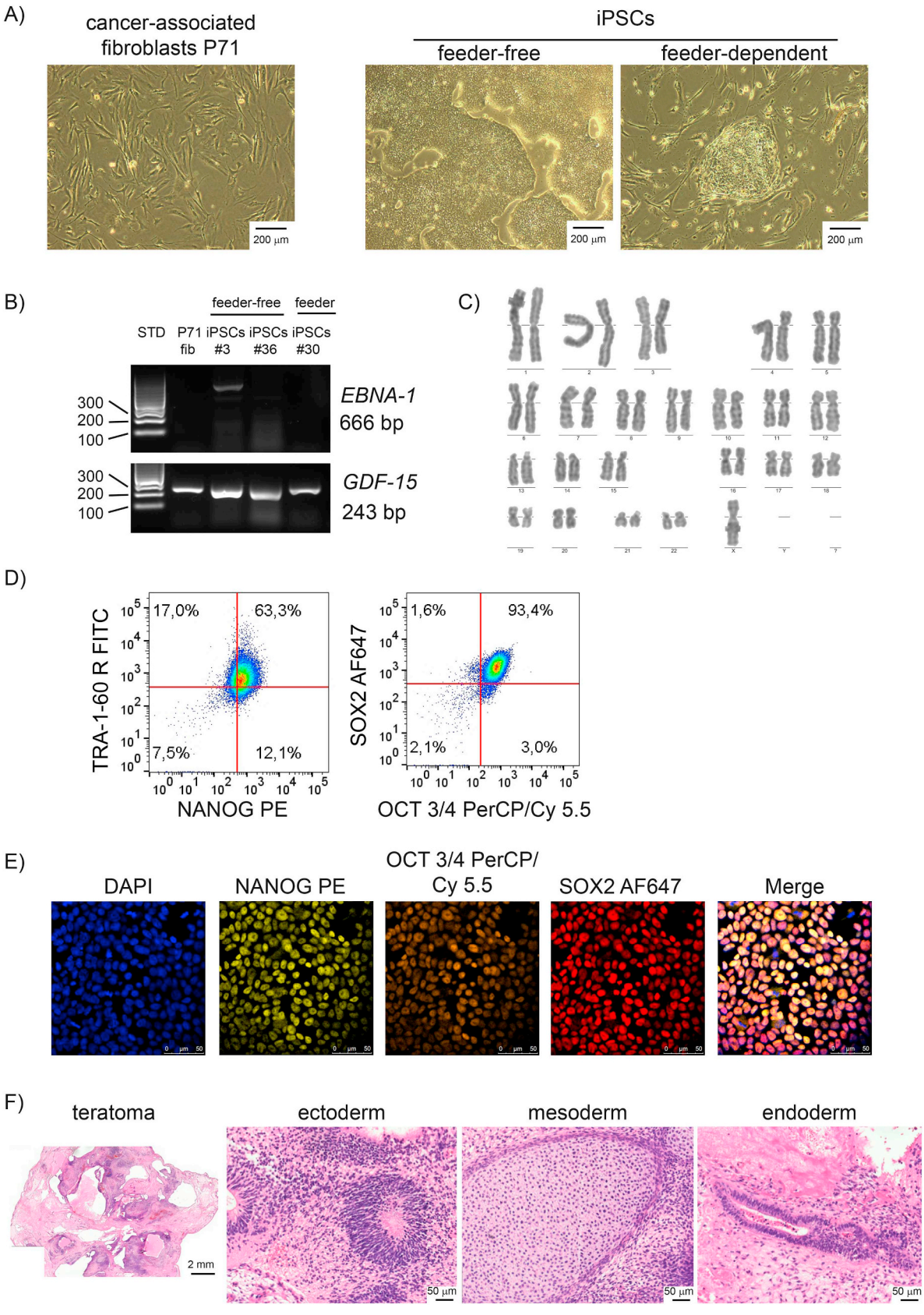


**Fig. 1.** Characterization of iPS cell line IBPi002-A. See text and Table 1 for details.

## Resource details

Human cancer tissue-derived fibroblasts were obtained from a patient diagnosed with PCa. Fibroblast origin of expanded cells was confirmed morphologically (Fig. 1A, left) and using flow cytometry - the cell line expressed the fibroblast marker detected by anti-fibroblast

**Table 1**
Characterization and validation.

| Classification | Test | Result | Data |
|---|---|---|---|
| Morphology | Photography | Confirmed fibroblast morphology of original P71 cells, and morphology of derived iPS cell lines | Fig. 1, panel A |
| Phenotype | Qualitative analysis - Immunocytochemistry | Expression of pluripotency markers OCT3/4, NANOG, SOX2 | Fig. 1, panel E |
| | Quantitative analysis - Flow cytometry | Expression level of pluripotency markers<br>OCT3/4: 96.4%<br>SOX2: 95%<br>TRA-1–60-R: 80.3%<br>NANOG: 75.4% | Fig. 1, panel D |
| Genotype | Karyotype (G-banding) and resolution | 45,X Resolution: 300 bands | Fig. 1, panel C |
| Identity | STR analysis | Match in 15 out of 16 tested alleles between parental cancer-associated fibroblasts P71 and derived iPSCs; discrepancy found in Amelogenin locus on Y chromosome – missing in iPSCs | Available with the authors |
| Mutational analysis | Sequencing | N/A | N/A |
| | Southern blot or WGS | N/A | N/A |
| Microbiology and virology | Mycoplasma | Mycoplasma testing by PCR, negative | Supplementary Fig. S1, panel A |
| Differentiation potential | Teratoma formation | Morphological proof of three germ layer formation - ectodermal (neuroepithelium), mesodermal (cartilage) and endodermal (gut-like) structures | Fig. 1, panel F |
| Donor screening | HIV 1 + 2 Hepatitis B, Hepatitis C | N/A | N/A |
| Genotype additional info | Blood group genotyping | N/A | N/A |
| | HLA tissue typing | N/A | N/A |

antibody and was negative for epithelial marker EpCAM (data not shown). Reprogramming was performed in feeder-free conditions using the commercially available system Epi5™ Episomal iPSC Reprogramming Kit, which uses episomal vectors containing 5 reprogramming factors (OCT4, SOX2, LIN28, KLF4, and L-MYC). The derived iPS cell line was successfully cultivated in both feeder-free (vitronectin coating, Essential 8 medium) and feeder-dependent (60 Gy irradiated mouse embryonic fibroblasts, iPS medium) conditions (Fig. 1A, right). Derived iPSCs were routinely tested for mycoplasma contamination. PCR analysis showed that P71 iPS cells were negative for mycoplasma-specific sequence (MYCO) (lane 7) when compared to positive control (PC, lane 11) and negative control (NC, lane 13) (Supplementary Fig. S1A). Lower part of the gel shows results for GDF-15, which was used as a reference gene (other lanes in the panel represent samples not relevant for this study).

Absence of reprogramming vector was confirmed using PCR detection of the EBNA vector (Fig. 1B). While iPSCs at early passage (#3) were positive for EBNA, the vector was no longer detected at passage #36 in feeder-free conditions and at passage #30 in feeder-dependent conditions. Reprogramming vector-free iPSCs were used in all following experiments. G-banding showed aberrant karyotype in all analyzed mitoses with absence of Y chromosome (45,X), which was confirmed in two independently tested passages (#36 and #80) (Fig. 1C). Further, to confirm the identity of parental P71 fibroblasts and the derived iPS cell line, STR analysis was performed. Identity was confirmed in 15 out of 16 tested alleles, the only discrepancy was found for Amelogenin (AM), which was not detected in iPSCs, therefore confirming the absence of Y chromosome in derived iPS cells (in accordance with karyotype analysis). Loss of Y chromosome is the most common mutation acquired during human life in the blood cells of men (Forsberg, 2017). For *in vitro* cultures, it was described that Y chromosome may be lost - this was seen in salivary gland epithelial cells transformed *in vitro* (Cowell, 1981) and in short term *in vitro* culture of normal kidney tissue (Elfving et al., 1990). Therefore, we assume that also in case of P71 iPS cells, the loss of Y chromosome may be associated with *in vitro* cultivation.

Pluripotency of the derived iPS cell line maintained in feeder-free conditions was confirmed in both *in vitro* and *in vivo* conditions. *In vitro*, expression of surface TRA-1-60-R, and intracellular OCT 3/4, SOX2, and NANOG was analyzed using multicolor flow cytometry (Fig. 1D). Only viable, single cells without debris were taken into analysis and isotype controls (and negative control in case of TRA-1-60-R biotin) were used to set the gating (Supplementary Fig. S1B). Expression of NANOG, OCT 3/4, and SOX2 was also detected in iPSCs colonies using immunofluorescence (Fig. 1E). Appropriate isotype controls for each marker after background subtraction are presented in Supplementary Fig. S1C. Both methods confirmed expression of selected pluripotency factors in newly derived iPSCs. *In vivo,* pluripotency was demonstrated in teratoma assay, where injection of iPSCs in testes of immunodeficient SHO mice gave rise to teratoma with identified structures from all three germ layers (ectoderm - neuroepithelium, mesoderm - cartilage, and endoderm - gut-like structure) (Fig. 1F). Altogether, we successfully prepared an induced pluripotent stem cell line derived from human prostate cancer-associated fibroblasts (Table 1).

## Material and methods

### *Cell culture and reprogramming*

Human prostate tissue sample was obtained with approval of local ethics committee (University Hospital Olomouc; approval nr. 127/14, 163/08), the donor gave written informed consent to generation of iPSCs from his cells. Tissue from prostatectomy was dissociated by collagenase (from Clostridium histolyticum, Sigma-Aldrich) for 3 h at 37 °C. Cell suspension was cultivated in a 1:1 mixture of Stromal Cell Growth Medium (SCGM, Lonza) and Prostate Epithelial Cell Growth Medium (PrEGM, Lonza). Reprogramming was performed using the Epi5™ Episomal iPSCs Reprogramming Kit (Thermo Fisher Scientific) using electroporation (Neon transfection system) based on manufacturer's recommendation. IPSCs were maintained in feeder-free [(0.5 μg/cm$^2$ vitronectin coat, Essential 8 media (Thermo Fisher Scientific)], or feeder-dependent conditions [60 Gy-irradiated MEFs, iPS media - KnockOut DMEM/F12, 20% KnockOut™ Serum Replacement, 1× GlutaMAX-1, 1× MEM NEAA Solution, 55 μM β-mercaptoethanol, 4 ng/ml bFGF (Thermo Fisher Scientific), penicillin/streptomycin (Biosera)]. Cells were cultivated on laboratory plastic (Corning Falcon) in humidified incubator (37 °C, 5% $CO_2$). iPS cells growing under feeder-free conditions were passaged every 3–5 days using 0.5 mM EDTA/PBS solution and split in the ratio 1:4 to 1:10. When thawed after cryopreservation, Y-27632 dihydrochloride (10 μM, Santa Cruz Biotechnology) was added to cultivation media for the first 24 h in both conditions.

*PCR analysis of vector clearance and mycoplasma contamination*

Lysis was performed in lysis buffer (10 mM Tris-HCl, pH 8.3; 50 mM KCl; 1% Triton X-100) containing proteinase K (100 µg/ml, Thermo Fisher Scientific; 50 °C, overnight). The next day, proteinase K was inactivated by incubation for 10 min at 95 °C. PCR was done with FastStart Taq DNA polymerase (Roche). Primers recommended by the manufacturer of the reprogramming kit were used for detection of EBNA and sequences are listed in Table 2. Following profile was used: initial denaturation 94 °C, 2 min; 36 cycles: denaturation (94 °C, 30 s), annealing (55 °C, 30 s), elongation (72 °C, 1 min); final elongation 72 °C, 7 min. For detection of mycoplasma, primer sequences described previously (Persing, 1993) were used with following PCR profile: initial denaturation 95 °C, 4 min; 30 cycles: denaturation (95 °C, 30 s), annealing (58 °C, 30s), elongation (72 °C, 1:30 min); final elongation 72 °C, 7 min. PCR was performed on PTC-200 Peltier Thermal Cycler (MJ Research). Products were resolved on 2% agarose gel (Sigma-Aldrich), visualized using GelRed Nucleic Acid Gel Stain (Biotium) and ChemiDoc ™ MP System (Biorad).

*Flow cytometry and immunocytochemistry*

For flow cytometry, IPS cells were harvested using 0.5 mM EDTA/PBS solution. Single cell suspension of iPSCs was stained with TRA-1-60-R (20 min, 4 °C) in 1% BSA/PBS/0.1% $NaN_3$ (Table 2), then with streptavidin-FITC (20 min, 4 °C), followed by viability dye (20 min, 4 °C). Fixation, permeabilization, and intracellular staining were performed using Human Pluripotent Stem Cell Transcription Factor Analysis Kit (BD Biosciences) according to manufacturer's recommendations. Simultaneously, isotype controls and negative control for TRA-1-60-R (incubation with streptavidin only) were prepared. Data were acquired using BD FACSVerse (BD Biosciences) and analyzed with FlowJo software (FlowJo, LLC, Data Analysis Software).

For immunofluorescence, iPSCs were grown on vitronectin-coated Labtek slides for 48 h, and fixed (4% PFA, 15 min, RT). Similar kit as for flow cytometry was used for intracellular staining. Permeabilization was done with 1× Perm/Wash buffer (15 min, RT) from Human Pluripotent Stem Cell Transcription Factor Analysis Kit (BD 560589). Next, samples were incubated with primary antibodies from the above mentioned kit (20 µl per sample, dilution 1:15, 30 min, RT). Simultaneously, nuclei were stained with DAPI. Samples were washed (1% BSA/PBS) and mounted in Mowiol + 0.6% DABCO. Images were acquired in sequential mode with a Leica SP5X confocal microscope (Leica Microsystems Vertrieb GmbH) and processed by background subtraction in Leica Application Suite X software (version 2.0.2.15022).

*Teratoma assay*

Teratoma assay was performed as described previously (Peterson et al., 2011). iPSCs were harvested using 0.5 mM EDTA/PBS and 1 × 10^6 cells in 30 µl PBS with bromophenol blue (0.005%) were injected into testes of Crl: SHO-*Prkdc*$^{scid}$*Hr*$^{hr}$ mice (Charles River) at the age of 8.5 weeks. After 49 days, teratomas were harvested and fixed in neutral buffered 4% formaldehyde solution (24 h, RT), after that processed for H&E staining and analyzed using TissueFAXS scanning system (TissueGnostics) using 20× objective and TissueFAXS Viewer v4.2 software.

*Karyotype analysis*

Cultured iPSCs (at passage #36 and passage #80) were treated in E8 medium with 2 µg/ml colcemid (Gibco) for 2–3 h, dissociated into single cells (0.1 mM EDTA, 37 °C, 3 min), treated with hypotonic solution (0.075 M KCl, 20 min), and fixed with methanol: acetic acid (3:1). Metaphases were spread on microscope slides. G-banding karyotypic analysis was performed. At least 15 metaphase spreads were examined for each sample.

**Table 2**
Reagents details.

| Antibodies used for immunocytochemistry and flow cytometry | | | |
|---|---|---|---|
| | Antibody | Dilution | Company Cat # and RRID |
| Pluripotency marker | TRA-1-60-R biotin | 1:200 | BioLegend Cat# 330603, RRID: AB_1186140 |
| Pluripotency marker | NANOG PE | 20 µl per sample | Human Pluripotent Stem Cell Transcription Factor Analysis Kit BD Biosciences, cat no:560589, RRID: AB_2722505 |
| Pluripotency marker | OCT 3/4 PerCP/Cy 5.5 | 20 µl per sample | Human Pluripotent Stem Cell Transcription Factor Analysis Kit BD Biosciences, cat no:560589, RRID: AB_2722505 |
| Pluripotency marker | SOX2 Alexa Fluor 647 | 20 µl per sample | Human Pluripotent Stem Cell Transcription Factor Analysis Kit BD Biosciences, cat no:560589, RRID: AB_2722505 |
| Secondary detection reagent | Streptavidin FITC | 1:2000 | eBioscience (Thermo Fisher Scientific), cat.no 11–4317-87 |
| Viability marker | LIVE/DEAD® Fixable Yellow Dead Cell Stain Kit | 1:500 | L34959, Thermo Fisher Scientific) |
| Isotype control | PE Mouse IgG1, kappa | 20 µl per sample | Human Pluripotent Stem Cell Transcription Factor Analysis Kit BD Biosciences, cat no:560589, RRID: AB_2722505 |
| Isotype control | PerCP/Cy5.5 Mouse IgG1, kappa | 20 µl per sample | Human Pluripotent Stem Cell Transcription Factor Analysis Kit BD Biosciences, cat no:560589, RRID: AB_2722505 |
| Isotype control | Alexa Fluor® 647 Mouse IgG2a, kappa | 20 µl per sample | Human Pluripotent Stem Cell Transcription Factor Analysis Kit BD Biosciences, cat no:560589, RRID: AB_2722505 |
| Primers | | | |
| | Target (product size) | | Forward/Reverse primer (5′-3′) |
| Reprogramming vectors | EBNA-1 (666 bp) | | pEP4-SF2-oriP 5′-ATC GTC AAA GCT GCA CAC AG-3′<br>pEP4-SR2-oriP 5′-CCC AGG AGT CCC AGT AGT CA-3′ |
| Reference gene | *GDF-15* (243 bp) | | ACA CAT CAA GGT TGC CCT TC<br>GGG CCT CAG TAT CCT CTT CC |
| Mycoplasma | MYCO (500 bp) | | GGC GAA TGG GTG AGT AAC ACG<br>CGG ATA ACG CTT GCG ACC TAT G |

*STR analysis*

DNA was isolated from cell lines using QIAamp DNA Blood Mini Kit (Qiagen). For STR analysis, 2 ng of DNA was amplified by PCR using AmpFlSTR Identifiler Plus PCR amplification kit (Thermo Fisher Scientific). The PCR products of amplified STR markers were separated through capillary electrophoresis on ABI PRISM 310 Genetic Analyzer (Applied Biosystems) in POP4 gel. Amplified 0.5 µl of sample in 10 µl of Hi-Di Formamide and 0.5 µl GeneScan-500 LIZ Size Standard was used for electrophoresis after prior 3 min denaturation. Data collection settings: 60 °C temperature, 15000 V injection voltage, 10 s injection duration, 25 min run duration. The data was analyzed by GeneScan v3.1 software. The Identifiler Allelic Ladder and control DNA sample 9947A was used to calibrate fluorescently labelled STR fragments during data analysis. Sample alleles were determined and are shown in Table available with the authors. The analysis provided alleles commonly used for authentication by ATCC or DSMZ cell line banks (*i.e.* D5S818, D13S317, D7S820, D16S539, VWA, TH01, Amelogenin, TPOX and CSF1PO), as well as additional ones (*i.e.* D8S1179, D21S11, D3S1358, D2S1338, D19S433, D18S51 and FGA).

## Author disclosure statement

There are no competing financial interests in this study.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2018.11.006.

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