



HAL
open science

Methods for simultaneous and quantitative isolation of mitochondrial DNA, nuclear DNA and RNA from mammalian cells

Jean Nakhle, Özkan Tülin, Lněničková Kateřina, Briolotti Philippe,
Marie-Luce Vignais

► To cite this version:

Jean Nakhle, Özkan Tülin, Lněničková Kateřina, Briolotti Philippe, Marie-Luce Vignais. Methods for simultaneous and quantitative isolation of mitochondrial DNA, nuclear DNA and RNA from mammalian cells. *Biotechniques*, 2020, Online ahead of print. 10.2144/btn-2020-0114 . inserm-02988728

HAL Id: inserm-02988728

<https://inserm.hal.science/inserm-02988728>

Submitted on 4 Nov 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Methods for simultaneous and quantitative isolation of mitochondrial DNA, nuclear DNA and RNA from mammalian cells

Jean Nakhle^{‡,1,2} , Tülin Özkan^{‡,1,3} , Kateřina Lněničková^{1,4} , Philippe Briolotti¹  & Marie-Luce Vignais^{*,1} 

¹Institute for Regenerative Medicine & Biotherapy (IRMB), INSERM, Univ Montpellier, F-34090 Montpellier, France; ²Institute of Molecular Genetics of Montpellier (IGMM), CNRS, Univ Montpellier, F-34090 Montpellier, France; ³Faculty of Medicine, Department of Medical Biology, University of Ankara, Ankara, Turkey; ⁴Department of Medical Chemistry & Biochemistry, Faculty of Medicine & Dentistry, Palacky University, Olomouc, Czech Republic; *Author for correspondence: marie-luce.vignais@inserm.fr;

[‡]Authors contributed equally

BioTechniques 69: 00–00 (December 2020) 10.2144/btn-2020-0114

First draft submitted: 20 July 2020; Accepted for publication: 25 September 2020; Published online: 26 October 2020

ABSTRACT

The aim of this study was to assess two protocols for their capacities to simultaneously isolate RNA, mtDNA and ncDNA from mammalian cells. We compared the Invitrogen TRIzol-based method and Qiagen DNeasy columns, using the HepG2 cell line and human primary glioblastoma stem cells. Both methods allowed the isolation of all three types of nucleic acids and provided similar yields in mtDNA. However, the yield in ncDNA was more than tenfold higher on columns, as observed for both cell types. Conversely, the TRIzol method proved more reproducible and was the method of choice for isolating RNA from glioblastoma cells, as demonstrated for the housekeeping genes *RPLP0* and *RPS9*.

METHOD SUMMARY

Here we compare two methods – Invitrogen TRIzol reagent and Qiagen DNeasy columns – for simultaneously extracting RNA, mtDNA and ncDNA from mammalian cells.

KEYWORDS:

mammalian cells • mitochondria • mitochondrial DNA (mtDNA) • nuclear DNA (ncDNA) • RNA

Mitochondria are endowed with essential cellular functions. Mitochondrial dysfunctions, which are caused by mutations in both ncDNA and mtDNA, are associated with a number of severe metabolic diseases [1–3]. Changes in mtDNA copy number have also been directly associated with aging and disease [4–9]. As demonstrated recently, mitochondria and their intrinsic mtDNA are also exchangeable between cells, resulting in the metabolic reprogramming of the recipient cells, in tissue repair and cancer progression [1,10–13]. This metabolic and functional reprogramming is also associated with modifications of the cellular gene expression pattern. In metabolic diseases originating from mtDNA mutations, the degree of heteroplasmy – the percentage of mutated mtDNA – is an essential criterion for the declaration of the pathology. For intercellular mitochondrial exchange, the concentration of exogenous mitochondria also determines the phenotype of the recipient cells. Therefore, in both cases, gene expression as measured by mRNA levels needs to be assessed as a function of the mtDNA concentrations in order to establish mechanistic links. This prompted us to identify robust methods to concurrently isolate the mtDNA and RNA in these eukaryotic cells. In addition, we were looking for a protocol that allowed the simultaneous isolation of RNA and DNA in order to save both the starting biological material and isolation time. A number of protocols have already been proposed to isolate RNA [14–16], mtDNA [15,17,18] or ncDNA [14,15,19] and most commercial kits are recommended for the exclusive isolation of either RNA or DNA. However, this is more costly and requires more biological material.

We assessed two experimental methods for nucleic acid isolation and tested their yields in mRNA, mtDNA and ncDNA. One of these methods was based on the Invitrogen TRIzol™ reagent, designed primarily for RNA isolation from the aqueous extraction phase [20]. The second method used the Qiagen DNeasy Blood & Tissue Kit, based on the known silica-adsorption properties of DNA [21] and designed for the rapid purification of total DNA (i.e., ncDNA and mtDNA). We tested whether the aqueous phase recovered in the TRIzol-based protocol also contained ncDNA and mtDNA and allowed their quantitative recovery. Conversely, we tested whether the DNeasy Blood & Tissue Kit also allowed the quantitative recovery of RNA. We performed these tests on the HepG2 adherent human hepatocyte cell line and on nonadherent human primary glioblastoma stem cells (GSCs) (clone Gli4) [22].

Materials & methods

Cell culture

The human hepatocyte cell line HepG2 was grown in Minimum Essential Medium Eagle, alpha modification (Lonza Bioscience BE12-169F), supplemented with 10% fetal bovine serum (Sigma-Aldrich F7524) and 2 mM L-glutamine (Gibco 25030024).

Human primary GSCs (clone Gli4 [22]) were grown as spheroids in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (Gibco 21331046), supplemented with 2 mM L-glutamine (Gibco 25030024), 0.3% D-glucose (Sigma-Aldrich G8769), 0.002% bovine insulin (Sigma-Aldrich I1882), N-2 supplement (Gibco 17502048), B-27 supplement (Gibco 12587010), 10 ng/ml human EGF (Miltenyi Biotec 130-097-750) and 10 ng/ml human FGF-2 (Miltenyi Biotec 130-104-924).

Human mesenchymal stem cells (MSCs) were isolated from the bone marrow of healthy donors at the authorized cell therapy unit (Biotherapy Team of General Clinic Research Center, French health minister agreement TCG/04/0/008/AA) at the Grenoble University Hospital. MSCs were cultured in Minimum Essential Medium Eagle, alpha modification (Lonza Bioscience), supplemented with 10% fetal bovine serum (Sigma-Aldrich), 2 mM L-glutamine (Gibco) and 1 ng/ml FGF-2 (Miltenyi Biotec). All cell types were cultured at 37°C in 5% CO₂.

Nucleic acid isolation by the TRIzol method & on column

Nucleic acids were isolated from 5×10^5 cells. The TRIzol reagent (Invitrogen 15596026) procedure was performed according to the manufacturer's instructions. Briefly, cells were homogenized in 700 μ l TRIzol reagent. The solution was separated into the two phases, aqueous and organic, by adding 200 μ l chloroform. The upper aqueous phase was gently recovered, without touching or aspirating the interphase, and transferred to a microtube containing 1.5 μ l glycogen (Invitrogen 10814010). The nucleic acids contained in this phase were precipitated with 500 μ l isopropyl alcohol, washed twice with 900 μ l ice-cold 70% ethanol, air-dried and resuspended in RNase-free water. The centrifugations for collecting and rinsing the nucleic acid pellets were all performed at 12,000 $\times g$, 4°C, for 15 min. Nucleic acid isolation with the Qiagen DNeasy Blood and Tissue kit (Qiagen 69506) was also performed according to the manufacturer's instructions. Briefly, cells were resuspended in 200 μ l phosphate-buffered saline to which 20 μ l proteinase K was then added. After the addition of 200 μ l buffer AL, the samples were vortexed. Ethanol (100%, 200 μ l) was then added and the samples vortexed again. The mixture was pipetted into a spin column which was then centrifuged at 6000 $\times g$ for 1 min on top of a collecting tube. The column was then rinsed twice, first with 500 μ l buffer AW1 (centrifugation at 6000 $\times g$ for 1 min) and second with 500 μ l buffer AW2 (centrifugation at 20,000 $\times g$ for 3 min). Nucleic acids were eluted with 200 μ l buffer AE (centrifugation at 6000 $\times g$ for 1 min). All centrifugations were performed at room temperature. Nucleic acid concentrations and purity were assessed with a NanoDrop spectrophotometer.

Transfer of MSC mitochondria to GSCs by MitoCeption

The transfer of MSC mitochondria to GSCs was performed as previously described [23,24]. Briefly, GSC spheroids were dissociated 24 h prior to the mitochondria transfer and seeded as single cells immediately before the mitochondria transfer. On the day of mitochondria transfer, MSCs (5×10^5) were trypsinized in the absence of EDTA (Gibco 15090046). MSCs were mechanically lysed using a syringe with 25- and 27-gauge needles. MSC mitochondria were isolated by two differential centrifugations of 10 min each, at 800 $\times g$ and 8000 $\times g$ respectively, using a buffer containing 200 mM mannitol (Sigma-Aldrich M1902), 70 mM saccharose (Sigma-Aldrich S0389), 1 mM EDTA (Sigma-Aldrich E9884), 10 mM HEPES (pH 7.4) (Sigma-Aldrich H3375) and 1 \times protease and phosphatase inhibitor cocktail (Roche, Meylan, France; 04693159001). MSC mitochondria were then added to the previously-seeded single-cell GSCs and centrifuged at 3000 rpm for 15 min. GSCs were incubated for 24 h at 37°C in 5% CO₂.

qPCR (mtDNA & ncDNA)

Real-time quantitative PCR to quantify mtDNA and ncDNA was performed on 30 ng nucleic acids, directly after the nucleic acid isolation step, by using the SYBR Green Master PLUS Mix (Roche 03515885001) and the LightCycler 480 instrument (Roche), with the following program: 10 min at 95°C, 50 cycles of 10 s at 95°C, 15 s at 67°C and 15 s at 72°C. ncDNA was quantified by using the following primers: β globin-F: 5'-ACACAAGTGTCTCACTAGC-3'; β globin-R: 5'-CCAACTTCATCCACGTTCA-3', targeting the nuclear β -hemoglobin gene. Total mtDNA was quantified by amplifying a DNA domain within the D-loop of mtDNA by using the following primers: Universal-F: 5'-TTAACTCCACCATTAGCACC-3'; Universal-R: 5'-GAGGATGGTGGTCAAGGGA-3' [25]. To specifically amplify mtDNA from MSCs (donor MSC119), the following set of primers was used: MSC-F: 5'-AAGCAAGTACAGCAATCAACCC-3'; MSC-R: 5'-TTAAGGGTGGGTAGGTTGTAGC-3'. The amplification efficacies with the different sets of primers were verified with serial DNA dilutions.

mRNA quantification

RNAs were reverse transcribed using the M-MLV RT kit (Invitrogen 28025013) and random hexamers (Invitrogen N8080127). Real-time quantitative PCR was performed using the SYBR Green Master Mix (Roche 04887352001), with 250 nM primer concentrations and the LightCycler 480 instrument (Roche), with the following program: 10 min at 95°C, 50 cycles of 10 s at 95°C, 15 s at 65°C and 15 s at 72°C. The following primer sets were used for *RPLP0* and *RPS9*: RPLP0-F 5'-TCGACAATGGCAGCATCTAC-3', RPLP0-R 5'-GCCTTGACCTTTTCAGCAAG-3', RPS9-F 5'-ATGAAGGACGGGATGTTAC-3', RPS9-R 5'-GATTACATCTGGGCCTGAA-3'.

Statistical analysis

Data were analyzed using GraphPad Prism 7 (GraphPad Software Inc., CA, USA). All data are presented as mean values with standard deviations. Unpaired Student *t*-tests with Welch's correction were performed to compare the concentrations of mtDNA, ncDNA and mRNA. Differences were considered statistically significant for $p < 0.05$ (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

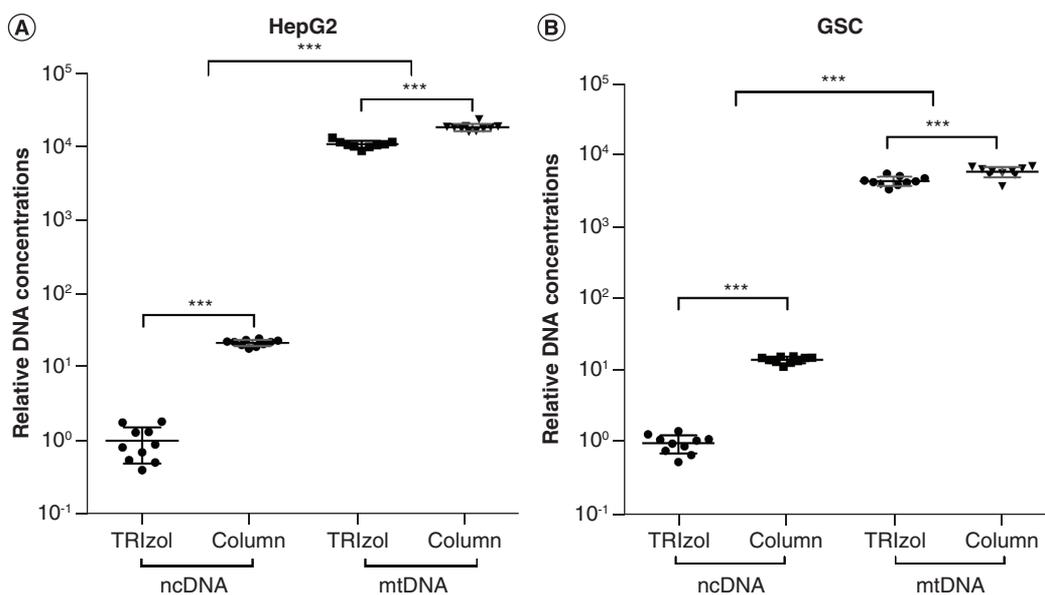


Figure 1. Comparison of the extraction yields by TRIZOL reagent and Qiagen DNeasy Blood and Tissue kit of nuclear and mitochondrial DNA. Samples of (A) hepatocellular carcinoma cells (HepG2) and (B) human primary glioblastoma stem cells (Gli4) were used to extract nucleic acids, by using either the Invitrogen TRIZOL-based method (n = 10) or the Qiagen DNeasy columns (n = 10). The relative concentrations of ncDNA and mtDNA were determined by qPCR using specific primers. Each dot represents an independent extraction. Represented are the means \pm standard deviation. Unpaired Student *t* tests with Welch's correction were performed. GSC: Glioblastoma stem cell.

Results & discussion

Isolation of mtDNA & ncDNA

We first showed that the TRIZOL-based method allowed the isolation of both nuclear and mitochondrial DNA from the HepG2 cell line (Figure 1A). Both ncDNA and mtDNA were isolated from the aqueous phase also containing RNA, which contributes to a simplified isolation procedure. Of note, we were unsuccessful in isolating DNA from the TRIZOL organic phase as we encountered both low isolation yields and reproducibility with the two human cell lines we tested. Isolation of mtDNA from the TRIZOL aqueous phase showed high reproducibility and the yield with the column method was only 1.7-fold higher than with the TRIZOL method. However, the column appeared superior for ncDNA isolation, providing a 21.3-fold higher yield than the TRIZOL method. Similar results were obtained when DNA was isolated from human primary GSCs (Gli4 clone [22]). While the mtDNA yields with the column were only slightly higher than with the TRIZOL method (1.3-fold), ncDNA isolation using the column was 14.5-times more efficient than with the TRIZOL method (Figure 1B). Overall, these data show that both methods are highly reliable for quantitatively isolating mtDNA, while the column proved more efficient than the TRIZOL-based method for ncDNA isolation.

Isolation of mRNA

We next checked whether the Qiagen DNeasy column procedure also allowed the isolation of mRNA. For this purpose, we prepared cDNA from the Qiagen DNeasy column samples, for both the HepG2 cells and the GSCs, and measured the expression of the two housekeeping genes *RPLP0* and *RPS9*. The same was done with the samples prepared by the TRIZOL method. Interestingly, the HepG2 samples prepared on the Qiagen DNeasy columns yielded mRNAs in amounts close to those obtained with the TRIZOL-based isolation method, albeit with yield differences depending on the analyzed genes (Figure 2A). For GSCs, the mRNA recovery appeared higher with the Qiagen DNeasy columns (Figure 2B). However, the isolation efficiency appeared less reproducible than with the TRIZOL procedure, with more widespread mRNA concentrations, as indicated by standard deviations that were on average twice as big for the column samples than for the TRIZOL-derived samples. These measures indicated that both the TRIZOL-based and column methods allow the concurrent isolation of mRNA, mtDNA and ncDNA. However, the TRIZOL-based method is to be preferred whenever experimental priority needs to be given to mRNA and mtDNA.

Application of the TRIZOL-based method to quantify exogenous mtDNA following mitochondrial intercellular transfer

One of our research goals is to determine changes of gene expression in GSCs following the acquisition of exogenous mitochondria from MSCs. On the basis of the above study, we opted for the TRIZOL-based method because it provides efficient and reproducible

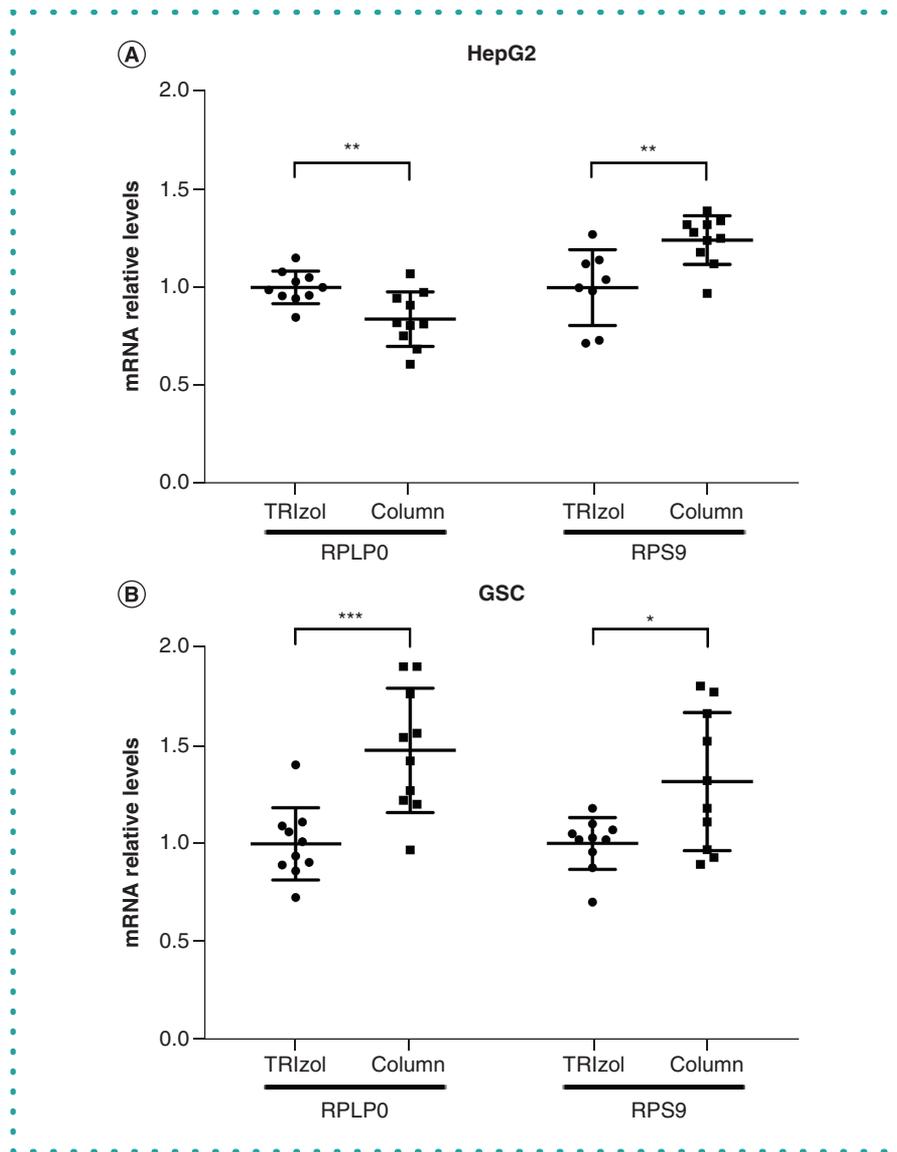


Figure 2. Comparison of the mRNA extraction yields by TRizol reagent and Qiagen DNeasy Blood and Tissue kit. Samples of (A) hepatocellular carcinoma cells (HepG2) and (B) human primary glioblastoma cells (Gli4) were used to extract nucleic acids, by using either the Invitrogen TRizol-based method ($n = 10$) or the Qiagen DNeasy columns ($n = 10$). cDNA was prepared and the relative concentrations of mRNA for the housekeeping genes *RPLP0* and *RPS9* were determined by qPCR. Each dot represents an independent extraction. Represented are the means \pm standard deviation. Unpaired Student *t* tests with Welch's correction were performed. GSC: Glioblastoma stem cell.

isolation of mRNA as well as mtDNA (required to quantify the amounts of acquired exogenous mitochondria). qPCR quantification of the exogenous mtDNA is based on the recognition of single nucleotide polymorphisms specific to the cell donor [23,24]. We first validated the qPCR conditions for specifically detecting MSC mtDNA (and not GSC mtDNA). For this, we isolated DNA from both the GSCs (Gli4) and the MSCs (MSC119) using the TRizol-based method. We then performed serial dilutions of the MSC DNA (30 to 6×10^{-2} ng) in a solution containing the GSC DNA (total DNA amount 30 ng) and PCR-amplified the MSC mtDNA. As shown by the linear regression, this provided the range of quantitative detection for MSC119 mtDNA in GSC-Gli4 by qPCR (Figure 3A). We then transferred mitochondria, isolated beforehand from MSCs (MSC119), to GSCs (clone Gli4) using the MitoCeption protocol [23,24]. Following the transfer, we used the TRizol-based method to isolate DNA from GSCs and determined the concentrations of MSC mtDNA by qPCR. This allowed us to demonstrate the dose-response acquisition of MSC mitochondria by the GSCs (Figure 3B). Additionally, the functional effects of MSC mitochondria acquisition were determined by quantifying mRNA concentrations and gene expression in these samples (data not shown). Overall, these data demonstrate that the TRizol-based method allows the simultaneous and quantitative isolation of both mtDNA and mRNA from mammalian cells, and from GSCs in particular, and can be exploited in the context of intercellular mitochondrial transfers.

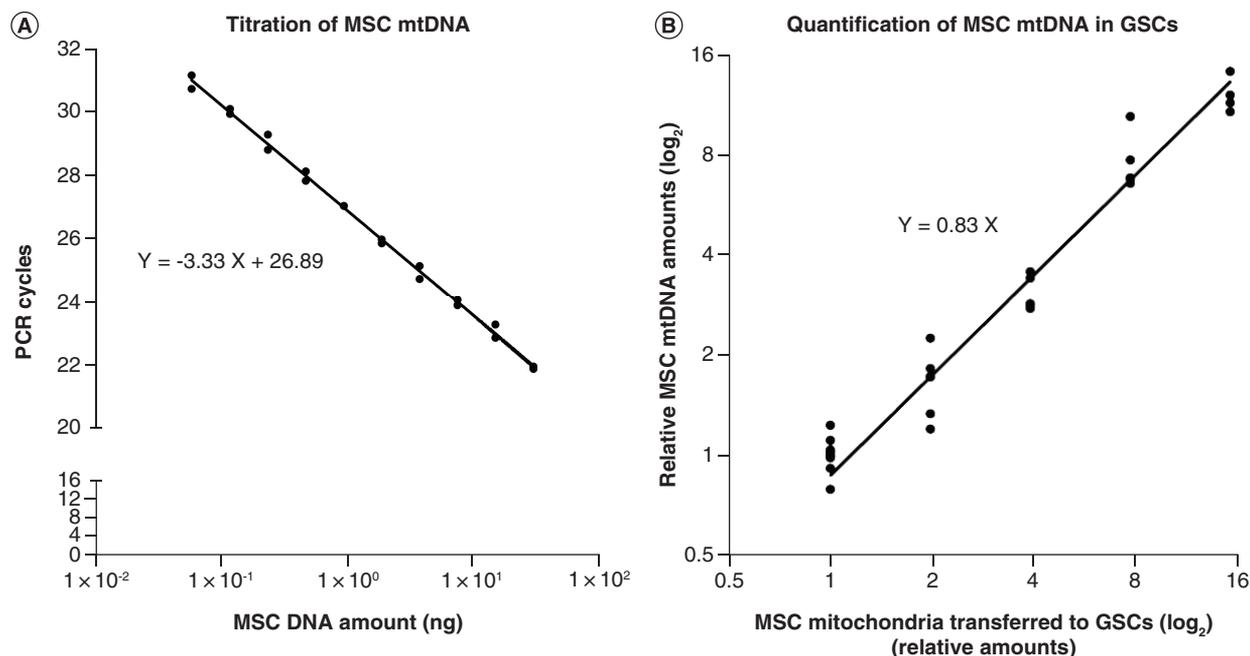


Figure 3. Quantification of mesenchymal stem cell mtDNA following the transfer of mitochondria to glioblastoma stem cells. (A) The range of detection of MSC (donor MSC119) mtDNA in GSCs (Gli4 clone) was determined by qPCR quantification of MSC mtDNA, following serial dilutions of MSC DNA with GSC DNA, both isolated by the TRIZOL method. Two independent experiments were performed. Data are presented on a semi-log scale and shown are the line and equation of interpolation best-fit. (B) MSC mitochondria were transferred in increasing amounts to GSCs by MitoCeption. The following day, total DNA was isolated by the TRIZOL-based method and the relative concentrations of MSC mtDNA were determined by qPCR and by using the titration curve shown in (A). Shown are the measured concentrations of MSC mtDNA as a function of transferred MSC mitochondria, expressed as relative values. Each dot corresponds to an individual TRIZOL extraction; data were obtained from four independent MitoCeption experiments. Data are presented on logarithmic scales (Log₂) and shown are the line and equation of best-fit. GSC: Glioblastoma stem cell; MSC: Mesenchymal stem cell.

As already observed [26–28], the respective yields in mitochondrial and nuclear DNAs are different depending on the DNA isolation method used. As the concentrations of mtDNA and ncDNA are often used to determine the number of mitochondria per cell, these method-related differences underline the caution required when drawing this type of conclusion.

Future perspective

The role played by mtDNA is beginning to emerge, in the fields of both mitochondrial intercellular exchanges and mitochondrial diseases (originating either from mutations in the mitochondrial DNA or from changes in mitochondrial DNA copy number). Studies in these fields need precise quantification of, first, the mtDNA concentrations of exogenous versus endogenous mitochondria and second, mutant versus wild-type mtDNA concentrations; therefore protocols enabling quantitative isolation are valuable. This further supports the need for robust and rapid mtDNA and mRNA isolation methods, as the characterization of the mtDNA sequence and copy number along with the gene expression pattern will be valuable for more accurate clinical evaluation. Studies on mtDNA characterization constitute an active and rapidly evolving field, as illustrated by novel technologies aiming at, for instance, single-cell mtDNA PCR detection [29,30]. In addition to qPCR, other technical approaches to determine mitochondrial DNA copy number have recently been proposed – for example, whole genome sequencing [31] – opening further possibilities for characterizing mitochondrial DNA sequences and copy number and, in the longer term, therapeutic manipulation of heteroplasmy [32–34].

Author contributions

The experiment was designed by J Nakhle, T Özkan and M Vignais and was undertaken by J Nakhle, T Özkan, K Lněničková and P Briolotti. The manuscript was prepared by J Nakhle, T Özkan and M Vignais. All authors had the opportunity to read and approve the manuscript.

Acknowledgments

We thank M Daujat-Chavanieu and S Gerbal-Chaloin for helpful discussions. The authors declare that there is no conflict of interest regarding the publication of this paper. No writing assistance was utilized in the production of this manuscript.

Financial & competing interests disclosure

This work was supported by grants from the Ligue Contre le Cancer-Comité du Gard and ARC. J Nakhle was supported by a PhD fellowship from the French Ministry of Research (MESRI), T Özkan by a post-doctoral research fellowship from the Scientific and Technological Research Council of Turkey (TUBITAK) and K Lněničková by the Mobility Support project at UP, CZ.02.2.69/0.0/0.0/16.027/0008482. P Briolotti and M Vignais are staff scientists from INSERM and CNRS, respectively. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

Human MSCs were isolated from bone marrow from healthy donors at the authorized cell therapy unit (Biotherapy Team of General Clinic Research Center, French health minister agreement TCG/04/0/008/AA) at the Grenoble University Hospital. The line of GSCs that was used was previously published.

Open access

This work is licensed under the Attribution-NonCommercial-NoDerivatives 4.0 Unported License. To view a copy of this license, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>

References

- Nakhle J, Rodriguez A-M, Vignais M-L. Multifaceted roles of mitochondrial components and metabolites in metabolic diseases and cancer. *Int. J. Mol. Sci.* 21(12), 4405 (2020).
- Gammage PA, Frezza C. Mitochondrial DNA: the overlooked oncogene? *BMC Biol.* 17(1), 53 (2019).
- Russell OM, Gorman GS, Lightowers RN, Turnbull DM. Mitochondrial diseases: hope for the future. *Cell* 181(1), 168–188 (2020).
- Castellani CA, Longchamps RJ, Sun J, Guallar E, Arking DE. Thinking outside the nucleus: mitochondrial DNA copy number in health and disease. *Mitochondrion* 53, 214–223 (2020).
- Dolcini J, Wu H, Nwanaji-Enwerem JC et al. Mitochondria and aging in older individuals: an analysis of DNA methylation age metrics, leukocyte telomere length, and mitochondrial DNA copy number in the VA normative aging study. *Aging (Albany NY)* 12(3), 2070–2083 (2020).
- Frederick M, Skinner HD, Kazi SA, Sikora AG, Sandulache VC. High expression of oxidative phosphorylation genes predicts improved survival in squamous cell carcinomas of the head and neck and lung. *Sci. Rep.* 10(1), 6380 (2020).
- Shukla P, Mukherjee S, Patil A. Identification of variants in mitochondrial D-loop and OriL region and analysis of mitochondrial DNA copy number in women with polycystic ovary syndrome. *DNA Cell Biol.* 39(8), 1458–1466 (2020).
- Sobenin IA, Zhelankin AV, Khasanova ZB et al. Heteroplasmic variants of mitochondrial DNA in atherosclerotic lesions of human aortic intima. *Biomolecules* 9(9), 455 (2019).
- Xu J, Chang W-S, Tsai C-W et al. Mitochondrial DNA copy number in peripheral blood leukocytes is associated with biochemical recurrence in prostate cancer patients in African Americans. *Carcinogenesis* 41(3), 267–273 (2020).
- Arazi J, Benowitz A, de Biasi V et al. Tunneling nanotubes and gap junctions—their role in long-range intercellular communication during development, health, and disease conditions. *Front. Mol. Neurosci.* 10, 333 (2017).
- Rodriguez A-M, Nakhle J, Griessinger E, Vignais M-L. Intercellular mitochondria trafficking highlighting the dual role of mesenchymal stem cells as both sensors and rescuers of tissue injury. *Cell Cycle* 17, 1–25 (2018).
- Hekmatshoar Y, Nakhle J, Galloni M, Vignais M-L. The role of metabolism and tunneling nanotube-mediated intercellular mitochondria exchange in cancer drug resistance. *Biochem. J.* 475(14), 2305–2328 (2018).
- Pinto G, Brou C, Zurzolo C. Tunneling nanotubes: the fuel of tumor progression? *Trends Cancer* (2020) (In Press).
- Merante F, Raha S, Reed JK, Proteau G. The simultaneous isolation of RNA and DNA from tissues and cultured cells. In: *Protocols for Gene Analysis*. Harwood AJ (Ed.). 113–120 <https://doi.org/10.1385/0-89603-258-2:113> Humana Press, NJ, USA (1994).
- Kong W, Wang Y, Wang Q, Han Y, Hu Y. Comparison of three methods for isolation of nucleic acids from membranate inner ear tissue of rats. *Chin. Med. J.* 119(12), 986–990 (2006).
- Alabi T, Patel SB, Bhatia S, Wolfson JA, Singh P. Isolation of DNA-free RNA from human bone marrow mononuclear cells: comparison of laboratory methods. *BioTechniques* 68(3), 159–162 (2019).
- Quispe-Tintaya W, White RR, Popov VN, Vijg J, Maslov AY. Fast mitochondrial DNA isolation from mammalian cells for next-generation sequencing. *BioTechniques* 55(3), 133–136 (2013).
- Devall M, Burrage J, Caswell R et al. A comparison of mitochondrial DNA isolation methods in frozen post-mortem human brain tissue – applications for studies of mitochondrial genetics in brain disorders. *BioTechniques* 59(4), 241–246 (2015).
- Clark DJ, Moore CM, Flanagan M et al. An efficient and novel technology for the extraction of parasite genomic DNA from whole blood or culture. *BioTechniques* 68(2), 79–84 (2019).
- Chomczynski P. A reagent for the single-step simultaneous isolation of RNA, DNA and proteins from cell and tissue samples. *BioTechniques* 15(3), 532–534 536–537 (1993).
- Vandeventer PE, Lin JS, Zwang TJ, Nadim A, Johal MS, Niemz A. Multiphasic DNA adsorption to silica surfaces under varying buffer, pH, and ionic strength conditions. *J. Phys. Chem. B.* 116(19), 5661–5670 (2012).
- Guichet P-O, Bieche I, Teiggell M et al. Cell death and neuronal differentiation of glioblastoma stem-like cells induced by neurogenic transcription factors. *Glia* 61(2), 225–239 (2013).
- Caicedo A, Fritz V, Brondello J-M et al. MitoCeption as a new tool to assess the effects of mesenchymal stem/stromal cell mitochondria on cancer cell metabolism and function. *Sci. Rep.* 5, 9073 (2015).
- Nzigou Mombou B, Gerbal-Chaloin S, Bokus A et al. MitoCeption: transferring isolated human MSC mitochondria to glioblastoma stem cells. *J. Vis. Exp.* 120, 55245 (2017). <http://www.jove.com/video/55245/mitoception-transferring-isolated-human-msc-mitochondria-to>
- Lyons EA, Scheible MK, Sturk-Andreaggi K, Irwin JA, Just RS. A high-throughput Sanger strategy for human mitochondrial genome sequencing. *BMC Genomics* 14(1), 1–16 (2013).
- Andreou AL, Martinez R, Marti R, Garcia-Arumi E. Quantification of mitochondrial DNA copy number: pre-analytical factors. *Mitochondrion* 9(4), 242–246 (2009).
- Guo W, Jiang L, Bhasin S, Khan SM, Swerdlow RH. DNA extraction procedures meaningfully influence qPCR-based mtDNA copy number determination. *Mitochondrion* 9(4), 261–265 (2009).
- Nacheva E, Mokretar K, Soenmez A et al. DNA isolation protocol effects on nuclear DNA analysis by microarrays, droplet digital PCR, and whole genome sequencing, and on mitochondrial DNA copy number estimation. *PLoS ONE* 12(7), e0180467 (2017).
- O'Hara R, Tedone E, Ludlow A et al. Quantitative mitochondrial DNA copy number determination using droplet digital PCR with single-cell resolution. *Genome Res.* 29(11), 1878–1888 (2019).

30. Maeda R, Kami D, Maeda H, Shikuma A, Gojo S. High throughput single cell analysis of mitochondrial heteroplasmy in mitochondrial diseases. *Sci. Rep.* 10(1), 10821 (2020).
31. Longchamps RJ, Castellani CA, Yang SY *et al.* Evaluation of mitochondrial DNA copy number estimation techniques. *PLoS ONE* 15(1), e0228166 (2020).
32. Jackson CB, Turnbull DM, Minczuk M, Gammage PA. Therapeutic manipulation of mtDNA heteroplasmy: a shifting perspective. *Trends Mol. Med.* 26(7), 698–709 (2020).
33. Jang Y-H, Lim K-I. Recent advances in mitochondria-targeted gene delivery. *Molecules* 23(9), 2316 (2018).
34. Mok BY, de Moraes MH, Zeng J *et al.* A bacterial cytidine deaminase toxin enables CRISPR-free mitochondrial base editing. *Nature* 583(7817), 631–637 (2020).

