Video Article

Detection of Intracellular Gene Expression in Live Cells of Murine, Human and Porcine Origin Using Fluorescence-labeled Nanoparticles

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Abstract

The reprogramming of somatic cells to induced pluripotent stem cells (iPS) has successfully been performed in different mammalian species including mouse, rat, human, pig and others. The verification of iPS clones mainly relies on the detection of the endogenous expression of different pluripotency genes. These genes mostly represent transcription factors which are located in the cell nucleus. Traditionally, the proof of their endogenous expression is supplied by immunohistochemical staining after fixation of the cells. This approach requires replicate cultures of each clone at this early stage to preserve validated clones for further experiments. The present protocol describes an approach with gene-specific nanoparticles which allows the evaluation of intracellular gene expression directly in live cells by fluorescence. The nanoparticles consist of a central gold particle coupled to a capture strand carrying a sequence complementary to the target mRNA as well as a quenched reporter strand. These nanoparticles are actively endocytosed and the target mRNA displaces the reporter strand which then start to fluoresce. Therefore, specific target gene expression can be detected directly under the microscope. In addition, the emitted fluorescence allows the identification, isolation and enrichment of cells expressing a specific gene by flow cytometry. This method can be applied directly to live cells in culture without any manipulation of the target cells.

Video Link

The video component of this article can be found at http://www.jove.com/video/53268/

Introduction

In 2006, a novel approach was described to reprogram somatic cells into a pluripotent state. After prescreening of an array of potential reprogramming factors murine fibroblasts could be successfully converted to so-called induced pluripotent stem cells (iPS) by retroviral transduction and overexpression of four transcription factors (*Oct4, Sox2, Klf4, c-Myc*) [1]. Subsequently this technique has effectively been reproduced using somatic cells of different mammalian species including humans [2], monkeys [3], rats [4], dogs [5], sheep [6] and pigs [7]. In addition, modified protocols omitting or replacing the potentially oncogenic transcription factor c-*MYC* have been published [8,9].

Regardless of the initial reprogramming approach the true pluripotency of growing colonies has to be confirmed, a laborious and time-consuming task. A major drawback of the majority of these analyses is that they cannot be performed on live cells. Established pluripotency factors such as *OCT4*, *SOX2*, *NANOG* or *REX1* represent transcription factors located in the nucleus and their detection requires fixation of the cells prior to immunohistochemistry or cell lysis for RT-PCR analysis [10-12]. Thereafter, these cells are lost for future experiments requiring replicate cultures of each colony.

Thus, a technology to analyze pluripotency on live cells is highly desirable. Several approaches can indeed be performed directly on live cells using antibodies directed against membrane-based antigens (*e.g.* TRA-1-60, SSEA4) [12], fluorescent reported dyes to detect alkaline phosphatase [13] or small molecules which specifically label embryonic stem (ES) and iPS cells [14]. However, the antibodies might nevertheless adversely affect the cells and each method is restricted to a single pluripotency marker. Finally, fluorescent molecular beacons, dual-antisense oligonucleotides with hairpin structures, which allow live staining of cells, have been described [15]. Although this approach may be applied to many genes, the technique requires nucleofection of a single cell suspension, which is not applicable to growing iPS colonies.

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A couple of years ago gene-specific nanoparticles have been described to analyze survivin expression in human SKBR3 breast cancer cells [16]. This manuscript describes a novel innovative approach to detecting pluripotency markers in live ES and iPS cells through the use of gold nanoparticle conjugates. These nanoparticles consist of a central gold particle with a coupled capture strand carrying the complementary RNA sequence and a Cy3-labeled reporter strand. The fluorescent signal of the reporter strand is quenched by the central gold particle. In addition, appropriate nanoparticles serve as negative and positive controls, respectively (**Figure 1**). For an application the nanoparticles are simply added to the culture medium (**Figure 2**) and enter the cells via endocytosis (**Figure 3**). If the target RNA is expressed it displaces the reporter strand which then emits a fluorescent signal. This method does not require manipulation of the target cell and the expression of the target gene can be monitored optically under a fluorescence microscope directly in live cells. Furthermore, the technology can be applied to any desired target gene across species in the case of iPS cells and thus may be employed in many different research areas. Finally, flow cytometric analyses allow the identification and enrichment of Cy3 positive cells.

Protocol

1. Preparation of Media, Reagents and Cell Culture Conditions

1. Human HEK 293 embryonic kidney cells
    1. Culture HEK 293 cells (CRL-1573, ATCC) in DMEM/Ham's F-12 medium supplemented with 5% FCS, L-glutamine (2 mM), and penicillin (100 U/ml)/ streptomycin (100 µg/ml).
    2. To passage the cells, wash cells once with PBS, add 0.05% Trypsin/EDTA and incubate for 3 min at 37 °C and 5% CO_2. Transfer cells into a 15 ml tube, centrifuge for 5 min at 1,200 rpm (300 x *g*) and transfer approximately $5x10^5$ cells into a fresh T25 culture flask.

2. Murine embryonic fibroblasts (MEFs)
    1. Add 1 ml of a 0.1% gelatin solution to individual 6-wells and incubate at room temperature (RT) for 1 hr. Aspirate liquid and store at RT until use.
    2. Culture MEFs in DMEM supplemented with 10% FCS, L-glutamine (2 mM), L-glucose (4.5 g/l), sodium pyruvate (1 mM), and penicillin (100 U/ml)/streptomycin (100 µg/ml) on gelatin-coated 6-well plates.
    3. Distribute MEFs into 6-well plates and let them grow until confluence. Add mitomycin (5 µg/ml) for 2.5 hr to arrest cells. Aspirate medium, wash cells twice with complete MEF medium and add 3 ml MEF medium to each 6-well. These cells serve as a feeder-layer for murine and porcine iPS cells.

3. Culture of murine ES and iPS cells
    1. To prepare mouse ES culture medium, supplement DMEM medium with 15% FCS, L-glutamine (2 mM), L-glucose (4.5 g/l), MEM non-essential amino acids (1X), β-mercaptoethanol (0.1 mM) and leukemia inhibitory factor (1.000 U/ml).
    2. Rinse 6-wells with growth-arrested MEFs once with serum-free DMEM, add 2 ml mouse ES medium and murine iPS cells (20% of the cells of a confluent dish). Every day wash cells once with serum-free DMEM and add 2 ml fresh mouse ES medium.
    3. To passage the cells, wash each well once with serum-free DMEM, add 0.25% Trypsin/EDTA and incubate for 3 min at 37 °C and 5% CO_2. Transfer cells into a 15 ml tube, centrifuge for 5 min at 1,200 rpm (300 x *g*), resuspend cells in 500 µl mouse ES medium and add 100 µl of the suspension to 2 ml mouse ES medium in a fresh 6-well with a MEF feeder-layer.

4. Culture of pig iPS cells
    1. To prepare pig iPS medium, supplement DMEM/Ham's F-12 medium with 20% knockout serum, L-glutamine (2 mM), MEM non-essential amino acids (1X), β-mercaptoethanol (0.1 mM), and penicillin (100 U/ml)/streptomycin (100 µg/ml). Filter through a 0.22 µM filter and store at 4 °C for 2 to 3 weeks. Upon first use, add bFGF (10 ng/ml final concentration).
    2. Rinse 6-wells with growth-arrested MEFs once with serum-free DMEM/Ham's F-12 and add 1.5 ml pig iPS medium and porcine iPS cells (10% of the cells of a confluent dish). Every day wash cells once with serum-free DMEM/Ham's F-12 and add 1.5 ml fresh pig iPS medium.
    3. To prepare dissociation buffer add 2.5% trypsin, collagenase IV (10 mg/ml), knockout serum (20%) and $CaCl_2$ (1 mM) to Ca^{2+}-free PBS. Wash cells twice with PBS and incubate with dissociation buffer for 5 min at 37 °C and 5% CO_2. Transfer cells into a 15 ml tube, centrifuge for 5 min at 1,200 rpm (300 x *g*).
    4. Resuspend porcine iPS cells in 500 µl fresh pig iPS medium and add 50 µl of the suspension to 1.5 ml pig iPS medium in a fresh 6-well with a MEF feeder-layer. Every day wash cells once with serum-free DMEM/Ham's F-12 and add 1.5 ml fresh pig iPS medium.

5. Feeder-free culture of human iPS cells
    1. Thaw the original vial containing the basement membrane preparation at 4 °C and dilute with ice-cold serum-free DMEM/Ham's F-12 to 8 mg/ml, aliquot and store at -20 °C until further use.
    2. Thaw an aliquot of the basement membrane preparation at 4 °C and dilute with ice-cold serum-free DMEM/Ham's F12 (90 µg/ml). Pipet 1 ml of this solution into 3 cm culture dishes and swirl gently to ensure that the whole surface is covered. Seal dishes with parafilm and store at 4 °C for up to one week. Prior to the use for cell culture incubate dishes for 30 min at 37 °C or 3 hr at RT to allow polymerization of the basement membrane preparation.
    3. Aspirate medium from dishes coated with the basement membrane preparation and wash twice with serum-free DMEM/Ham's F-12. Add 1.5 ml E8 medium to the culture dish and human iPS cells (10% of the cells of a confluent dish). Every day wash cells once with serum-free DMEM/Ham's F-12 and add 1.5 ml fresh E8 medium.
    4. To passage the cells, rinse dishes twice with PBS and add 1 ml PBS/0.5 mM EDTA. Incubate the dishes for 5 min at RT. Monitor the cells under a microscope. Note: When the colonies start to round up and appear to have holes they are ready for transfer.
    5. Aspirate the liquid, add 1.5 ml E8 medium and detach cells by gently pipetting up and down. Transfer cells to a 15 ml tube, centrifuge for 5 min at 1,200 rpm (300 x *g*), resuspend in 500 µl E8 medium and add 50 µl of the suspension to 1.5 ml E8 medium in a fresh culture dish coated with the basement membrane preparation.

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2. Design of Target-sequences Across Species Borders

1. Select reference human, murine and porcine cDNA sequences of the target genes (*GAPDH, NANOG, GDF3*) and perform a CLUSTAL multi-sequence alignment analysis.
   Note: This will indicate the sequences homologous between species.
   1. Use the following settings for multiple alignment: gap penalty (fixed) 10, gap penalty (varying) 5, transition weighting 0, gap separation penalty range 8, % identity for delay 40, maximum number of iterations to perform 3.

2. Submit desired target sequences to the manufacturer (see **Table of Materials**) for probe design and manufacturing.
   Note: The manufacturer will then confirm compatibility of desired sequence with the technology. If desired sequence is not compatible, the manufacturer will suggest alternative sequences.
   1. Ensure that homologous regions which can be used as potential capture strand sequences have a length of 27 bp.
      Note: It is advisable to order several nanoparticles with different capture strands for a selected target gene as only the real cellular experiment will give a definite proof of the functionality of an individual sequence. Therefore, two different sequences for *GAPDH* and *GDF3* were evaluated while three different nanoparticles were tested to evaluate *NANOG* expression. Their location and the exact sequences have been published elsewhere [17]. The manufacturer commercially manufactures the nanoparticles with desired sequences and provides them in lyophilized format.

3. Reconstitution of Nanoparticles and Addition to Cell Cultures

1. Store lyophilized nanoparticles upon receipt at 4 °C in the dark.
2. Reconstitute nanoparticles by addition of 50 µl double distilled water which gives a final concentration of 100 nM. Once reconstituted store nanoparticles at RT in the dark. Note: Under this condition they are stable for at least one year. Storage of reconstituted samples at 4 °C may adversely affect their stability.
3. Pre-dilute the nanoparticle stock solution with PBS to a concentration of 2 (HEK 293 cells) or 8 nM (iPS and ES cells) on the day of application. Note: This concentration is 20-fold higher than the final concentration in the culture dish. In ES cells and iPS cells, 400 pM of nanoparticles induced a fluorescent signal easily detectable under the microscope. For HEK 293 cells, a concentration of 100 pM is sufficient.
4. Pipet 12.5 µl of prediluted nanoparticles to a 24-well containing 237.5 µl complete cell culture medium. For wells of different sizes, adjust the volumes accordingly. Swirl the plate carefully to ensure equal distribution of the nanoparticles. Incubate the cells overnight (O/N) at 37 °C and 5% CO_2 in a humidified atmosphere.
5. The next day analyze cell cultures for gene-specific Cy3 fluorescence with an excitation wavelength of 546 nm (emission 575 – 640 nm) under a fluorescence microscope. Note: The period required to obtain a sufficiently strong fluorescence may depend on the cell type and the target gene. In these experiments, this was seen between 16 and 20 hr after the application of the probe.

4. Identification of Cy3 Positive Cell Populations by Flow Cytometry

1. Grow HEK 293 or murine ES cells using the conditions specified in section 1.1 or 1.3, respectively. One day prior to the FACS analysis add *GAPDH*-specific nanoparticles at different concentrations to HEK 293 cells (see **Figure 6A**) and at 400 pM to murine ES cells. Incubate the cells O/N at 37 °C and 5% CO_2 in a humidified atmosphere.
2. Analyze cell cultures for gene-specific Cy3-induced fluorescence under a fluorescence microscope.
3. Detach HEK 293 cells as specified under point 1.1.2. Centrifuge, transfer into a 1.5 ml tube and resuspend in 200 µl ice-cold PBS/0.5% BSA/2 mM EDTA (FACS buffer). Keep cells dark on ice until flow cytometric analysis.
4. Detach murine ES cells as specified under point 1.3.3. Centrifuge, transfer into a 1.5 ml tube and resuspend in 200 µl ice-cold PBS/0.5% BSA/2 mM EDTA. Keep cells dark on ice until flow cytometric analysis.
5. Press the cells through a 30 µM filter to prevent cell aggregation and add propidium iodide (2 µg/ml final concentration) 2 min prior to the FACS analysis to exclude dead cells.
6. To analyze Cy3-positive cells first set hierarchical gates on the FSC-A/SSC-A subset, the FSC-H/FSC-W-subset and the SSC-H/SSC-W subset. Use the forward and the side scatter to exclude cell debris and cells that are not scattered. Exclude dead cells by their propidium iodide staining displaying PE against PE-Texas Red. Perform the final sort gate on Cy3-PE positive cells (**Figure 4**).

Representative Results

In a first attempt to verify and establish the intracellular detection of specific gene expression in live cells using nanoparticles we decided to conduct pilot experiments using a constitutively expressed house-keeping gene (*GAPDH*) in human HEK 293 cells. The concentration of nanoparticles used in these experiments was chosen according to the recommendation of the manufacturer. The addition of a *GAPDH*-specific probe to the culture medium at a 100 pM final concentration induced a clear-cut Cy3-fluorescence in these cells which was visible under a fluorescence microscope after O/N incubation (**Figure 5A**). Two controls were included in these experiments to substantiate the result. One control comprises of a so-called “scramble” nanoparticle where the reporter strand does not have a matching sequence in mammalian or eukaryotic cells. The “scramble” nanoparticle determines the unspecific background fluorescence due to possible probe degradation or incomplete quenching of the fluorescence. The addition of the scramble probe produced an only marginal fluorescent signal, which is negligible (**Figure 5B**). A permanently fluorescing “uptake” control helps to determine the ability of cells to incorporate the nanoparticles by endocytosis which may vary between different cell types. These positive control particles produced a bright fluorescent signal in HEK 293 cells (**Figure 5C**). Thus, the feasibility of the technology to record a specific fluorescent signal of an intracellular gene with a negligible background signal was confirmed. It must be mentioned that the difference between the background signal and the specific fluorescence decreases over time. The background increases due to probe degradation or incomplete quenching while the specific fluorescence fades gradually (**Figure 5D**). The same *GAPDH*-specific nanoparticles have been tested in primary fibroblast cultures of murine, porcine and ovine origin [17]. These cells produce a much

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higher background signal than HEK 293 cells while the target-specific fluorescence is rather weak, possibly due to the relatively low proliferative capacity of these cultures.

These pilot experiments prompted us to use this technology for the detection of pluripotency gene expression in iPS derived from different species. For this purpose *NANOG* and *GDF3* were analyzed, both of which are known to be expressed in pluripotent cells [18]. At first iPS cells derived from tail-tip fibroblasts of an *Nkx2.5* cardiac enhancer transgenic mouse line [19] were investigated. For both genes a strong fluorescent signal was obtained (**Figure 6A**) and a negligible Cy3-fluorescence of the scramble control (data not shown). Similar results were obtained on human and porcine iPS cells (**Figure 6B** and **C**). Importantly, the fluorescence signal was restricted to the iPS colonies and did not label the fibroblasts used as a feeder layer for mouse and porcine iPS (see arrows in **Figure 6A** and **C**). The analysis of *NANOG* gene expression also revealed that not every sequence predicted *in silico* to be "good" finally is functional. One of three designs of a *NANOG*-specific nanoparticles induced almost no fluorescent signal comparable to that of the scramble control despite a 100% sequence match in murine iPS cells (**Figure 6D).** A similar result was obtained on iPS cells of human and porcine origin with this probe (data not shown). Thus, nanoparticles can be used to detect pluripotency gene expression across species borders while the functionality of every single designed probe needs to be evaluated beforehand.

The promising results of the *in situ* labeling of live cells in cell culture dishes prompted us to analyze gene expression quantitatively by flow cytometry. To this end *GAPDH*-specific nanoparticles were added at graded concentrations to HEK 293 monolayers in order to compare the labeling efficiency within the target cell population. As assessed by fluorescent microscopy an enhanced Cy3-induced fluorescence was seen with the lowest concentration compared to cells which did not receive the nanoparticles. Increasing concentrations of the nanoparticles induced a clearly concentration dependent fluorescence of HEK 293 cells *in situ* (**Figure 7A**). Subjecting these cells to a flow cytometric analysis confirmed the concentration dependent labeling efficiency. The frequency of Cy3 positive cells increased significantly (more than sevenfold) when the highest concentration of nanoparticles was applied (**Figure 7B**). In further experiments ES cells from the *Nkx2.5* cardiac enhancer transgenic mouse line [19] were used to analyze the expression of pluripotency genes by flow cytometry. Pilot experiments with 400 pM of the positive uptake control yielded clearly Cy3 positive live ES colonies under the microscope and flow cytometry detected almost 40% of Cy3 positive cells (**Figure 7C**). In contrast, comparable to untreated cells approximately 0.1% of cells treated with the scramble stained positive (data not shown). Similarly the addition of nanoparticles specific for *Gapdh*, *Nanog* and *Gdf3* induced a distinct fluorescence signal in individual colonies in the cell culture dish. The frequency of Cy3 positive cells for *Gapdh* as determined by flow cytometry was comparable to that seen for both pluripotency genes (**Figure 7C**). This result is expectable as *Nanog* and *Gdf3* are also thought to be expressed constitutively in pluripotent ES cells. Thus, these data clearly indicate that cells expressing a particular gene can be identified by flow cytometric analyses, qualitatively and by their relative amount.

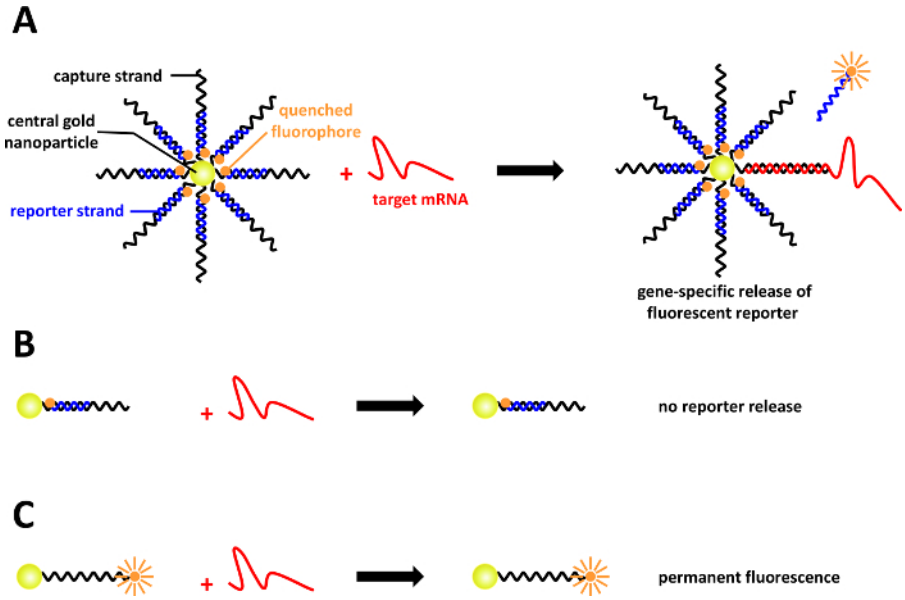


Figure 1: Schematic Representation of the Structure of the Nanoparticles. (A) Structure of a gene-specific nanoparticle. **(B)** Scramble nanoparticle (negative control). **(C)** Uptake nanoparticle (positive control). Adapted from Lahm *et al.* [17], reprinted with permission from Wiley.

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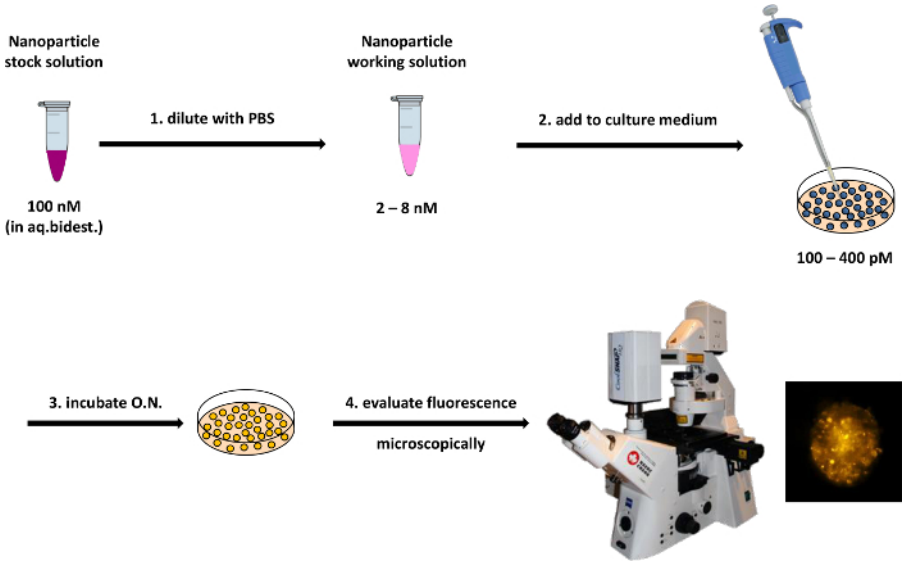


Figure 2: Application of Nanoparticles to Cell Cultures. A properly diluted solution of nanoparticles is pipetted simply into the culture medium. After O/N incubation gene-specific fluorescent cells are visible under a fluorescent microscope.

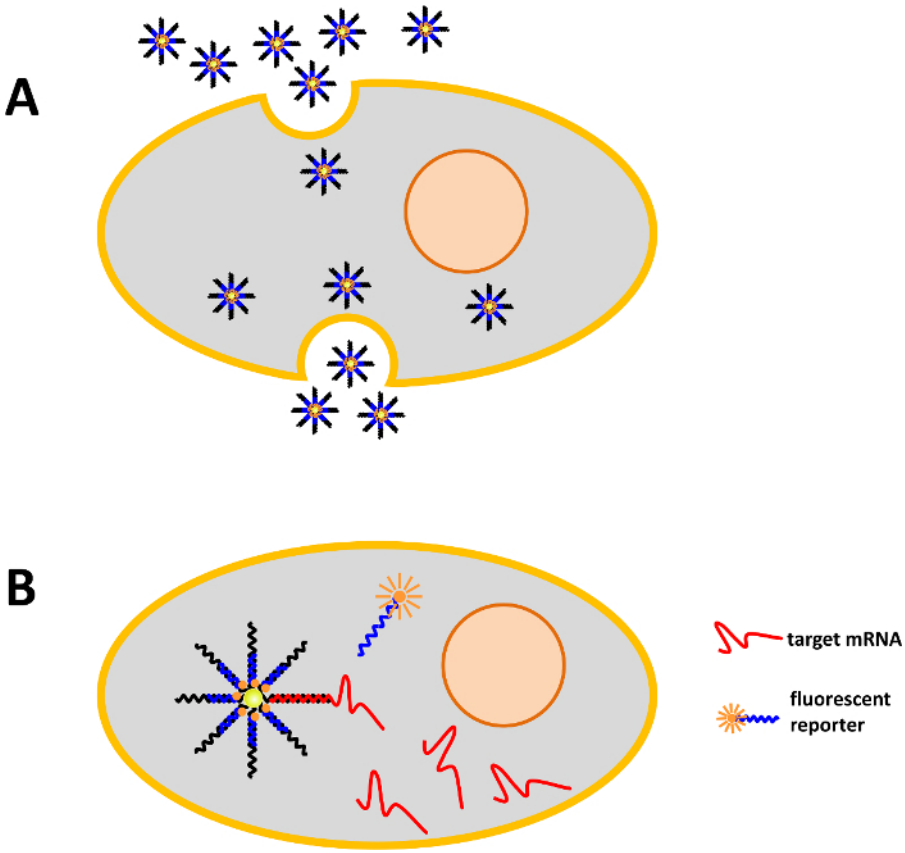


Figure 3: Active Uptake of Nanoparticles by Cells via Endocytosis. (A) Cells actively engulf the nanoparticles by endocytosis. **(B)** Target mRNA binds to the capture strand and displaces the fluorescing reporter.

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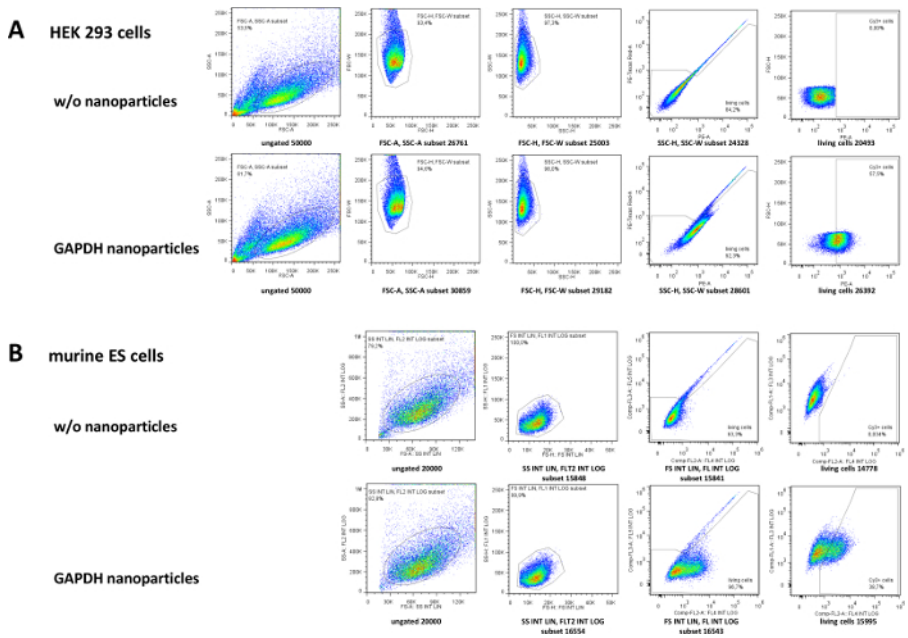


Figure 4: Gating Strategy for Flow Cytometry. (A) Strategy for HEK 293 cells. **(B)** Strategy for murine ES cells. Data were acquired using device 1 for HEK 293 cells or device 2 for murine ES cells and were subsequently analyzed by a software tool (see **Table of Materials**).

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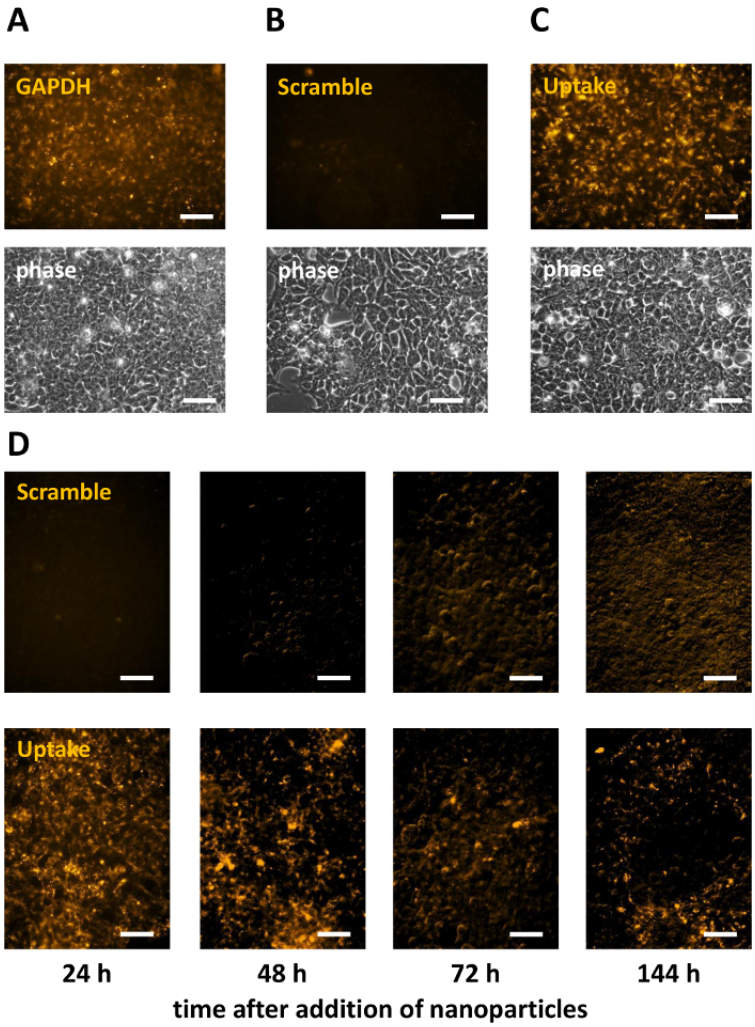


Figure 5: Analysis of *GAPDH*-expression in HEK 293 Cells. A *GAPDH*-specific nanoparticle was added to the cells at 100 pM (final concentration) and fluorescence was recorded after O/N incubation. **(A)***GAPDH*-specific nanoparticle. **(B)** Scramble (negative control). **(C)** Uptake (positive control). **(D)** Kinetics of scramble and uptake control. Fluorescence was determined at the indicated time-points after addition of *GAPDH*-specific nanoparticles. Scale bars represent 50 µM.

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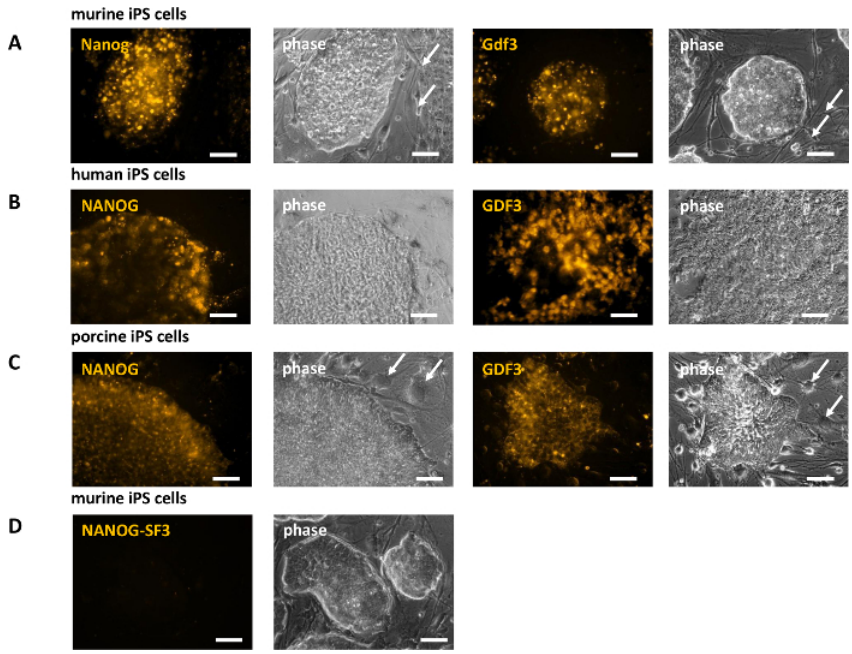


Figure 6: Analysis of Pluripotency Gene Expression in iPS Cells of Different Species. Nanoparticles specific for *NANOG* or *GDF3* were added to the cells at 400 pM (final concentration) and fluorescence was recorded after O/N incubation. **(A)** Murine iPS cells. **(B)** Human iPS cells. **(C)** Porcine iPS cells. Arrows in **(A)** and **(C)** designate unlabeled fibroblast feeder-layer cells. **(D)** Fluorescence in murine iPS cells after addition of the NANOG-design SF3 nanoparticle. Scale bars represent 50 µM.

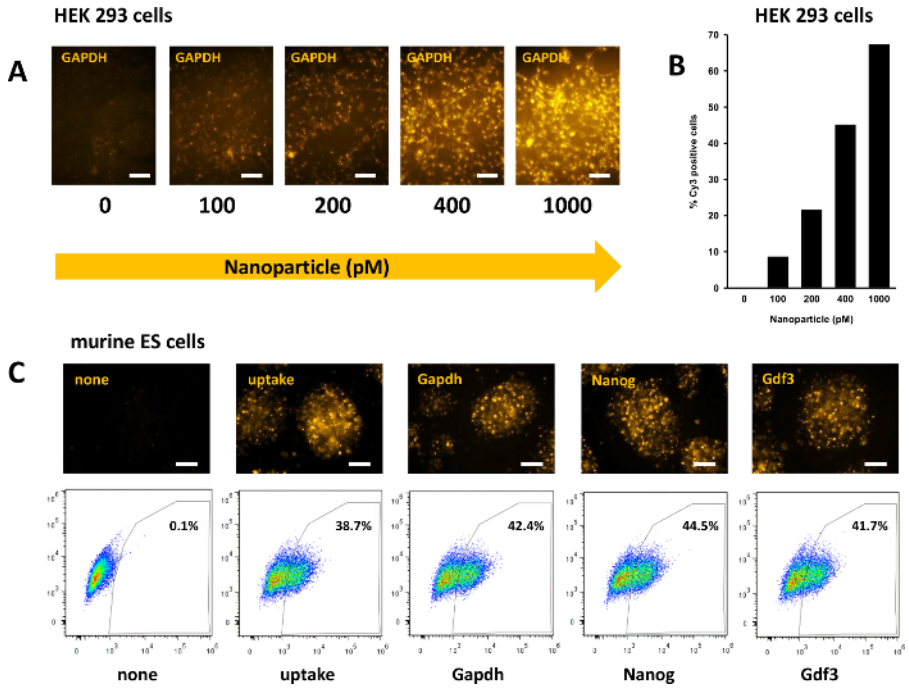


Figure 7: Nanoparticle Labeled Live Cells can be Identified by Flow Cytometry. (A) Microscopic view of Cy3 positive HEK 293 cells after addition of different concentrations of nanoparticles. **(B)** Frequency of Cy3 positive cells determined by flow cytometry. **(C)** Live staining of murine ES cell colonies and determination of the frequency of Cy3 positive cells. Nanoparticles were added at a final concentration of 400 pM. Scale bars represent 50 µM.

Discussion

The present work describes the use of fluorescence labeled nanoparticles which allow the reliable evaluation of intracellular gene expression directly in live cells from different mammalian species with diverse histogenetic origin under a fluorescence microscope. This approach has a couple of advantages compared to other published methods. First of all the researcher is not limited with respect to either the target gene or cell type. All antibody based detection methods are restricted to a single epitope. Although fluorescent molecular beacons can also be designed for a broad spectrum of genes, this method requires the dissociation of cells and subsequent nucleofection [15]. In contrast, the application of

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gene-specific nanoparticles does not require any manipulation of the target cell which engulfs the nanoparticles actively by endocytosis. During subsequent passaging the gold particles gradually leave the cells and the culture can be used in downstream experiments.

Nevertheless, the technology also still has some limitations. Some markers are obviously not detectable due to their carbohydrate nature such as SSEA-1 or TRA-1-81 [20]. At present, a large array of nanoparticles is commercially available. These particles have been pretested for their functionality in cellular experiments. The objective of the present work was to design customized probes which can be used across species borders. When following such an approach or when designing a probe for a novel target gene one has to be aware that the functionality of an *in silico* predicted probe needs to be thoroughly evaluated *in vitro*. The NANOG-SF3 probe which showed a 100% homology to the sequence homology did not work at all while NANOG-SF1 with one mismatched base in the murine and porcine sequence produced a nice fluorescence signal. Another question refers to the efficient uptake of the nanoparticles. The particles enter the cells by endocytosis, a process which is influenced by the size of the nanoparticles [21], the cell type and the differentiation status [22] and the cellular capability to perform phago- and macropinocytosis [23]. The optimum concentration to obtain a satisfying fluorescence signal has to be tested for each individual application or cell line. To that end, the first experiment should always be the application of the uptake control to evaluate the compatibility of the probes within the cell types and culture conditions before moving on to target-specific detection of gene expression.

A critical point to obtain reliable results when applying this protocol to a novel cell line or cell population is the inclusion of proper controls. The ever fluorescing uptake control will provide a hint which concentrations of nanoparticles will induce a sufficient fluorescent signal in the target cell population. The fluorescence must be evaluated the next day as the signal will fade with time [17]. At the same time the scramble control without a counterpart in the eukaryotic genome applied at an identical concentration must induce a negligible background signal. As a third control it is advisable to use a nanoparticle specific for a housekeeping gene (*e.g. GAPDH*, *β-Actin*). These nanoparticles serve as a positive control that the approach can be used to detect specific gene expression in the selected target cell population. If these controls turn out satisfactory one can expect to obtain reliable specific fluorescent signals using nanoparticles specific for other target genes. The concentration of the applied nanoparticles may also be critical as they have been shown to down-regulate the target sequence [24]. However, this effect requires much higher concentrations (5 nM) than the concentrations used in the experiments presented here (400 pM). At this concentration the nanoflares do not affect target gene mRNA or protein levels and concentrations as high as 2 nM do not impair the proliferation of HEK293 and murine ES cells [17]. In addition, a whole genome expression analysis did not reveal any significant changes in gene expression [25]. Therefore, at concentrations up to 1 nM the nanoflares should not exhibit any important adverse effects.

A very promising application of this technology is the possibility to label any given cell population which is marked by a specific gene. Recently, gene-specific nanoparticles have successfully been used to selectively stain live ventricular myocytes [26], subpopulations of melanoma cells [27], monocytes [28] and cerebellar cell cultures [29]. Such fluorescence-marked cell populations can be sorted and purified by flow cytometry as live cells. In the field of oncology it is imaginable to isolate live metastatic tumor cells in animal models based on their expression of *CEA*, most valuable for the search of potential metastasis promoting genes or target structures. We have recently shown that truly reprogrammed murine iPS colonies can be identified *in situ* with *Nanog*-specific nanoparticles based on the detected fluorescence intensity [17]. Therefore, the identification of truly reprogrammed iPS colonies could be performed on high throughput platforms, which use robotic fluorescence detection and picking devices to identify the most promising colonies. This technology appears to be suitable in many research areas to select specific cellular subpopulations and it seems possible to apply the nanoparticles in high throughput platforms. In conclusion, this protocol represents a novel approach using fluorescence labeled nanoparticles which allows the detection of intracellular gene expression directly in live cells. This technology may be a valuable tool to purify, expand and further characterize rare cellular subpopulations in many research areas.

Disclosures

H.L. has received the nanoparticles from EMD Millipore Corporation free of charge. All other authors have nothing to disclose.

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