March 2011

# qBiomarker iPSC Expression Lentivirus and Plasmid Handbook

qBiomarker iPSC Expression Lentivirus

qBiomarker iPSC Expression Plasmid

For iPSC creation using transduction-ready lentiviruses or transfection-ready plasmids

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## Product Use Limitations

qBiomarker iPSC Expression Lentiviruses and qBiomarker iPSC Expression Plasmids are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

# CONTENTS

# I. Background and Introduction

The discovery that mouse fibroblasts can be reprogrammed and generate induced pluripotent stem cells (iPSCs) with qualities remarkably similar to embryonic stem cells has created a valuable new source of pluripotent cells for drug discovery, cell therapy, and basic research.

SABiosciences (a QIAGEN company) has developed expression vectors for six iPSC related proteins (Nanog, Oct4, Sox2, c-Myc, Klf4 and Lin28) to create reagents for producing induced pluripotent stem cells. Ectopic expression of these factors has been shown to create pluripotent cells which resemble embryonic stem cells. The expression vectors for the six iPSC-related proteins are available as either ready-to-transfect plasmids or ready-to-transduce lentiviral particles.

# II. Materials Provided:

1. Reprogramming factors expression plasmid:

| Component/Description | Amount | Concentration | Volume |
|---|---|---|---|
| Ready-to-transfect expression vector | 10 μg | 500 ng/μl | 20 μl |

2. Lentiviral particles expressing reprogramming factors:

| Component/Description | Concentration | Volume |
|---|---|---|
| Ready-to-transduce Lentiviral particles | ≥ $0.8\times10^{7}$ TU/ml | 250 μl |

**Storage Conditions:**
Ready-to-transfect expression vectors should be stored at -20°C and ready-to-transduce lentiviral expression vectors should be stored at -80°C upon receipt.

**Description**
Available iPSC Induction vectors express human or mouse Nanog, Oct4, Sox2, c-Myc, Klf4 or Lin28 under the control of cytomegalovirus (CMV) promoter. For the map of parent vectors, see Appendix.

* The DYKDDDDK epitope tag is also referred to as the FLAG® epitope tag.

# III. Additional Materials Required:

- **Mammalian cell line cultured in the appropriate growth medium**
    - **Human fibroblast medium**
    - **hESC medium**
    - **MEF**
- **Cell culture medium and standard cell culture supplies**
- **Tissue culture plates**
- **Attractene Transfection Reagent** (QIAGEN, Catalog No. 301004) for ready-to-transfect expression vectors
- **SureENTRY Transduction Reagent** (QIAGEN; Catalog No. 336921) for ready-to-transduce lentiviral expression vectors
- **Opti-MEM™ I Reduced-Serum Medium (Invitrogen)**

# IV. Protocol:

**A. Before the Experiment:**

***Optimization of transfection conditions (For Plasmids):*** Optimizing transfection conditions for each cell type is important for the success of an experiment. Variables to consider when optimizing the transfection conditions include cell density, cell viability, amount of DNA, ratio of DNA to transfection reagent, transfection complex formation time, and transfection incubation time (see the detailed protocols for recommendations). The constitutive expressing CMV-mGFP vector (QIAGEN; Cat # 336881) can be used to determine the optimal transfection conditions.

***Optimization of transduction conditions (For Lentiviral Particles):*** Optimization of transduction conditions for each cell type is important for successful lentivirus particle use. Variables to consider, when optimizing the transduction conditions include Multiplicity of Infection (MOI), concentration of SureENTRY Transduction Reagent used, time of assay development and the cell density. The Cignal Lenti Reporter Control, Positive Control (GFP) (QIAGEN; Cat # 336891) can be used for determining the optimal transduction conditions.

***Multiplicity of Infection (MOI) (For Lentiviral Particles):*** The transduction efficiency of iPSC Induction expression vectors varies significantly for different cell types. Users should determine the Multiplicity of Infection (MOI), which is the number of transducing lentiviral

particles per cell, required for desired transduction efficiency of a new cell type. The MOI is typically adjusted by increasing or decreasing the amount of virus added per well to a series of wells containing the same number of cells. We recommend testing the Cignal Lenti Reporter Control, Positive Control (Cat # 336891) at MOIs of 5, 10, and 50 (each MOI in triplicate), in order to establish the optimal MOI for each cell type to be studied.

To calculate:

$$\text{Multiplicity of Infection (MOI)} = \frac{\text{Number of transducing units (TU) deposited in a well}}{\text{Number of target cells present in that well}}$$

Total transducing units needed per well (TU) = (Total number of cells per well) x (Desired MOI)

$$\text{Total mL of lentiviral particles to add to each well} = \frac{\text{Total TU needed per well}}{\text{TU/mL reported on Certificate of Analysis}}$$

SABiosciences has found that some commonly used cell lines (like HT1080, HEK293 and HepG2 etc.) can be effectively transduced using an MOI between 10 and 25, however, some cell types (like primary cells) are more resistant to transduction and efficient transduction of these cell types may require a higher MOI (~ 50).

**B. Transfection protocol for reprogramming plasmids:**

The following protocol is designed to transfect the adherent cell line using Attractene Transfection Reagent (Cat. # 301004) in a 96-well plate format. Transfection reagent from other vendors can be used with the iPSC Induction plasmids. *If you are using a transfection reagent other than Attractene Transfection Reagent, follow the manufacturer's protocol.* Moreover, the use of plates or wells of different size require the user to adjust the components in proportion to the surface area of the new plate or well. **The protocol below is just a general guideline; the optimal conditions/amounts should be adjusted according to the cell type and the study requirements.**

**Read the protocol completely before starting the experiment.**

1. One day before transfection, seed ($1\times10^5$ cells) in human fibroblast medium to each well of a 6-well plate.

2. On the day of transfection, add 5 μg of expression vector into separate 250 µl aliquots of Opti-MEM I Reduced-Serum Medium. Mix gently and incubate mixture for 5 minutes at room temperature (15–25ºC).

3. For each well, add 4.5 µl of Attractene Transfection Reagent into 250 µl of Opti-MEM separately. Mix gently and incubate mixture for 5 minutes at room temperature.
**IMPORTANT:** The amount of transfection reagent and DNA required for optimal performance may vary, depending on the cell line.

4. Add 250 µl of Attractene mix to 250 µl expression vector mix. Mix gently and incubate for 20 minutes at room temperature.

5. Add 500 µl expression vector-Attractene complexes in medium to the appropriate well containing cells and 1 ml of normal growth medium. This gives a final volume of 1.5 ml. Mix gently by rocking the plate back and forth.

6. Incubate cells at 37°C in a 5% $CO_2$ incubator for 16-24 hours.

7. One day after transduction, remove the medium containing lentiviral particles from wells. Wash the cells with 3 ml of 1 X PBS three times and add 3 ml of human fibroblast medium.

8. Four days after transduction, plate $5.5 \times 10^5$ MEFs in MEF medium on a 10-cm dish coated with 0.1% gelatin. Incubate until the next day.

9. Five days after transduction, incubate the infected fibroblasts with 1 ml 0.05% trypsin/EDTA for 3 min at 37°C. Stop the trypsinization with 11 ml human fibroblast medium and place all cells into one 10-cm dish plated with MEFs (prepared at step 8).

10. Seven days after infection remove human fibroblast medium and add 10 ml hESC medium (containing KOSR and 10 ng/ml of βFGF).

11. Replenish the cells with 10 ml hESC medium every day and observe the cells for any sign of colony formation.

Typically, sometime around three weeks after infection, various types of colonies will appear.

12. One day before the colonies are big enough to pick, plate $1.9 \times 10^4$ MEFs per well of a 24-well plate that have been coated with 0.1% gelatin.

13. Mark the colonies that show morphology similar to hES cells. Pick the marked colonies under a dissection microscope using a 20-µl pipette. Put each colony into one well of the 24-well plate prepared at step 12.

14. Follow standard human ES cells culture procedure to expand and maintain the colonies.

**C. Transduction protocol for <u>lentivirus-expressing reprogramming factors:</u>**

The following protocol is designed to transduce fibroblast using lentiviral particles expressing reprogramming factors in a 6-well plate format. If you are using plates or wells of different size, adjust the components in proportion to the surface area. **This is just a general guideline; the optimal transduction conditions and cell culture medium for the parental cells should be optimized according to the cell type and the study requirements**.

**Read the protocol completely before starting the experiment.**

1. One day before transduction, seed ($1 \times 10^5$ cells) in human fibroblast medium to each well of a 6-well plate.

2. On the day of transduction, replace the old medium from with 2 ml of fresh human fibroblast medium.

3. Add 125 μl of each lentiviral particles (at $0.8\times10^7$ TU/ml) expressing the reprogramming factors together. Adjust to a final volume of 2 ml using human fibroblast medium.

The amount of lentiviral particles should be adjusted accordingly if the concentration is not $0.8\times10^7$ TU/ml.

4. (Optional) Add SureENTRY Transduction Reagent to a final concentration of 8 μg/ml in each well. Gently swirl the plate to mix. Incubate at 37°C in a humidified incubator in an atmosphere of 5% $CO_2$.

5. One day after transduction, remove the medium containing lentiviral particles from wells. Wash the cells with 3 ml of 1 X PBS three times and add 3 ml of human fibroblast medium.

6. Four days after transduction, plate $5.5 \times 10^5$ MEFs in MEF medium on a 10-cm dish coated with 0.1% gelatin. Incubate until the next day.

7. Five days after transduction, incubate the infected fibroblasts with 1 ml 0.05% trypsin/EDTA for 3 min at 37°C. Stop the trypsinization with 11 ml human fibroblast medium and place all cells into one 10-cm dish plated with MEFs (prepared at step 6).

8. Seven days after infection remove human fibroblast medium and add 10 ml hESC medium (containing KOSR and 10 ng/ml of βFGF).

9. Replenish the cells with 10 ml hESC medium every day and observe the cells for any sign of colony formation.

Typically, sometime around three weeks after infection, various types of colonies will appear.

10. Under a dissection microscope and using a 20-μl pipette, pick a colony that shows morphology similar to hES cells and put the colony into one well of a 24-well plate that has been preplated with MEFs at a density of $1 \times 10^4$ cells/$cm^2$ on 0.1% gelatin-coated wells.

11. Follow standard human ES cells culture procedure to expand the colonies.

**Note:**
SureENTRY Transduction Reagent enhances transduction of most cells, however, some cells like primary neurons are sensitive to the SureENTRY Transduction Reagent. Do not add SureENTRY Transduction Reagent to these types of cells. If working with a cell type for the first time, a SureENTRY Transduction Reagent control only well should be used to determine cell sensitivity.

# V. Frequently Asked Questions:

**1. Which reprogramming system should I chose, plasmids or lentiviral particles?**

Both systems work for iPSC induction. Plasmids would not integrate into the host genome therefore is the better choice if the genome integrity is your first concern. Lentiviral particles generally have higher expression of the genes and is the better choice if the iPSC induction rate if your first concern.

**2. I see DYKDDDDK on the vector maps. What is it?**

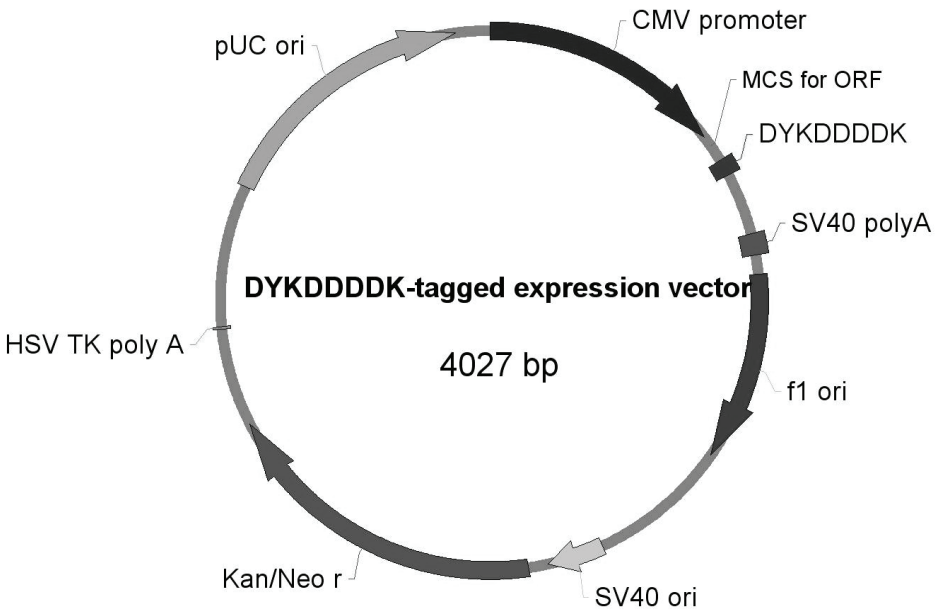
DYKDDDDK is the epitope tag that is also referred to as the FLAG® epitope tag.

**3. What is MOI?**
MOI is an abbreviation for Multiplicity of Infection or the number of viral particles exposed to a cell.

**4. Where I can get more information about the plasmids or lentiviral particles?**
More information can be found at:
http://www.sabiosciences.com/support_faq.php?target=reporter

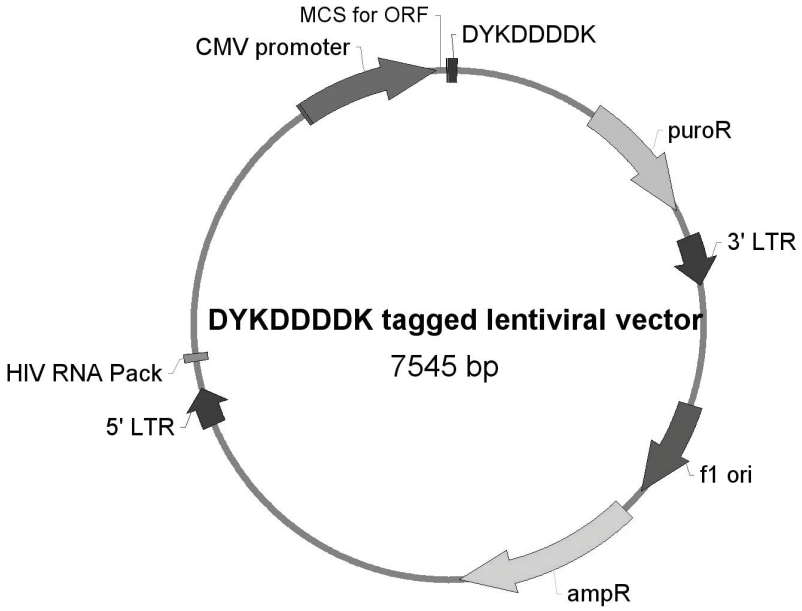
# Appendix: Vector Maps

1. Vector map of reprogramming factor expression plasmid:




MCS: Multiple cloning site; ORF: open reading frame

2. Vector map of lentiviral plasmid expressing reprogramming factors:




MCS: Multiple cloning site; ORF: open reading frame

# Ordering Information

| Product | Contents | Cat. no. |
|---|---|---|
| qBiomarker iPSC Expression Lentivirus | $\geq 0.8 \times 10^7$ TU/ml ready-to-transduce lentiviral particles | 337402 |
| qBiomarker iPSC Expression Plasmid | 10 µg ready-to-transfect expression vector | 337401 |

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**Notes**

## Notes

## Notes

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