Generating stable cell lines with quantifiable protein production using CRISPR/Cas9-mediated knock-in

Chiu-An Lo[1,2], Alexander W. Greben[1,2], and Brian Edwin Chen[1,2]
[1]*Centre for Research in Neuroscience, Research Institute of the McGill University Health Centre and*
[2]*Departments of Medicine and Neurology and Neurosurgery, McGill University, Montréal, Quebec, Canada*

BioTechniques 62:165-174 (April 2017) doi 10.2144/000114534
Keywords: protein quantitation reporter (PQR); CRISPR/Cas9; protein production; recombinant protein; stable cell line

Supplementary material for this article is available at www.BioTechniques.com/article/114534.

Cell lines expressing foreign genes have been widely used to produce a variety of recombinant proteins. However, generating recombinant protein–expressing cell lines is usually a lengthy process and the resulting protein expression levels are often inconsistent. Here, we describe an efficient method for making stable cell lines expressing any recombinant protein of interest in a controllable and quantifiable manner. We integrate transgenes into specific genomic loci using CRISPR/Cas9 such that transgene expression is driven by endogenous promoters to ensure consistent and predictable expression of the recombinant protein. Expression levels can be predetermined by selecting promoters from genes with the desired level of expression. To quantify recombinant protein expression, a protein quantitation reporter (PQR) is incorporated between the endogenous and foreign genes. The PQR allows equimolar production of the endogenous protein, the recombinant protein, and a fluorescent reporter. As a result, expression levels of both the endogenous and recombinant proteins can be continuously monitored using fluorescence.

Stable cell lines are able to express a foreign gene that has been inserted into the cell's genome. Expression of the exogenous gene is stable because its DNA is not floating outside of the chromosomes transiently but is instead permanently expressed within the genome. Stable cell lines are a powerful tool for expressing specific protein products on a production scale and have wide utility in molecular biology and biotechnology applications, including reporter gene assays (1–3), cytokine assays (4), antibody production (5), and cellular screening for chemical compounds (1) or RNAi (6). Mammalian cell lines such as Chinese hamster ovary (CHO), baby hamster kidney (BHK), and human embryonic kidney (HEK293T) cells, have become the dominant systems (60%–70% proteins used) for producing recombinant protein pharmaceuticals for clinical use because they allow correct folding, assembly, and post-translational modifications of the recombinant proteins (7).

To create a cell line that stably expresses a specific recombinant protein, an expression vector encoding the recombinant gene is typically transfected into a wild-type cell line, which then integrates, often randomly, into the host cell genome. To confer a selective advantage to the transgenic cells, another gene encoding a drug resistance protein or an essential metabolic enzyme is also introduced, either through co-transfection with another plasmid or placed together with the gene of interest (GOI) in the same vector using a dual-promoter or bicistronic construct. After transfection, selection pressure (e.g., antibiotics or essential amino acid–deficient medium) is applied to eliminate all untransfected wild-type cells. As a result, only the cells expressing the selectable marker gene (often wrongly assumed to be co-expressed effectively with the recombinant GOI) survive and grow into colonies in the culture vessel. Transgenes that are expressed transiently or integrated in multiple copies can lead to inconsistent and heterogeneous expression levels across cells. Thus, multiple rounds of single-cell isolation and expansion are required to ensure that a cell population stably expressing the transgene is obtained. Different independent cell clones need to be further verified through genotyping, sequencing, western blotting, and activity assays to ensure proper expression of a functional protein of interest.

Although these widely employed protocols are well-established and straightforward, they have numerous shortcomings. First, expression of the randomly integrated transgene can be difficult to control as it is often subject to the differential influences of its genomic surroundings, an issue known as position effect (8–10). Second,

METHOD SUMMARY

A method for creating stable cell lines with built-in quantification of protein production is described. Using CRISPR/Cas9 genome editing, a gene of interest (GOI), along with a protein quantification reporter, is inserted into a specific genomic site. This preserves the host cell's genome integrity and facilitates the process of single cell clonal isolation while allowing the expression levels of the protein of interest to be monitored in single cells over time.

the copy number of the integrated gene is often indeterminate and can vary from cell to cell, which often results in a heterogeneous cell population. Third, continuous selection pressure is required to maintain the transgene, as cells tend to remove randomly inserted foreign genetic elements that affect genome integrity. Some selective agents, such as antibiotics, cannot be fully neutralized by the encoded resistance markers, leading to accumulated mutations in the host cells (11). These selection agents can be harmful and need to be removed from the purified recombinant proteins before being used in other applications. Such removal processes can be time-consuming and costly. Finally, the use of strong exogenous promoters, such as pCMV, often drives the expression of the recombinant gene at an elevated and non-physiological level (12). Overexpression of recombinant proteins can place a considerable burden on host cells, especially when multiple copies of the transgene are inserted, which can then affect proper protein production.

Here, we present a new protocol for creating stable cell lines with locus-specific integration of transgenes, physiological and controllable expression of recombinant proteins, and the ability to quantify recombinant protein expression. Locus-specific integration and physiological expression can be achieved by placing the GOI under the control of an endogenous gene promoter through the use of the CRISPR/Cas9 gene-editing technology. In addition, to allow for quantification of recombinant protein expression, a protein quantitation reporter (PQR) (13) is added to the transgene to enable equimolar production of the protein of interest and a fluorescent reporter molecule. Therefore, the fluorescence intensity of a cell can be used to quantify the expression of the protein of interest (13).

A multicistronic gene cassette, *PQR-RFPnols-PQR-GOI-PQR-Zeocin*^{R} (*nols* denotes nucleolar localization signal), was constructed and flanked by locus-specific homologous sequences in such a way that it can be incorporated into the target genomic locus through homologous recombination facilitated by CRISPR/Cas9. Moreover, only one copy of the gene cassette is inserted, under the control of an endogenous promoter while preserving all of the auxiliary regulatory elements, thus regulating gene expression through the targeted genomic loci. The preservation of the native genomic integrity and the physiological level of transgene expression minimize the burden imposed on the host cell, thereby ensuring permanent maintenance of the integrated transgene without the need for continuous selection pressure (Figure 1A).

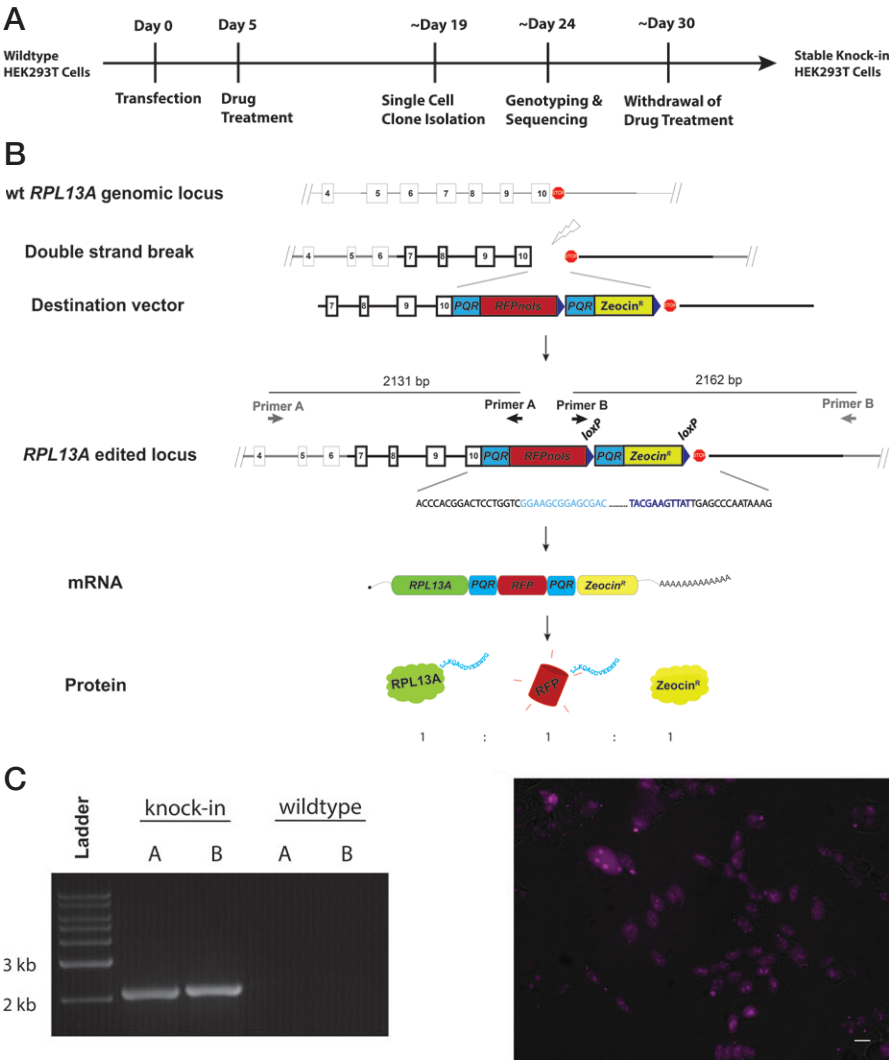

Figure 1. Creation of *RPL13A* stable cell lines. (A) The experimental timeline of the protocol for stable cell line creation. (B) Schematic of the insertion of a multicistronic gene cassette into the endogenous *RPL13A* gene locus through CRISPR/Cas9-mediated homology directed recombination. The wild-type (wt) *RPL13A* gene has 10 exons, represented as boxes. CRISPR/Cas9 induces a double-strand break targeted before the stop codon (lightning bolt). The *RPL13A* destination vector contains the multicistronic gene cassette flanked by 1.0 kb homology arms denoted by bold lines. Homologous recombination between the destination vector and the broken genomic strand results in integration of the gene cassette into the *RPL13A* locus. Two sets of PCR primer pairs (A and B), with one recognizing the gene cassette (black arrow) and the other outside the homology arm (gray arrow), were used to verify the correct knock-in. Colored nucleotide sequences represent sequencing results of a stably maintained cell clone with insertion of the gene cassette at the *RPL13A* locus. (C) The integrated gene cassette in an isolated stable cell line was detected by genomic PCR using Primer Sets A and B with fragment sizes of 2131 bp and 2162 bp, respectively. A reference DNA ladder is shown on the left. (D) A population of Zeocin-resistant cells expressing red fluorescent protein (RFP) in the nucleolus can be maintained indefinitely in Zeocin-free culture without the loss of red fluorescence. Scale bar: 100 µm.

Materials and methods

Construction of knock-in destination vectors

The genomic sequences of the human ribosomal protein L13A (*RPL13A*) and β actin (*ACTB*) genes were PCR-amplified from wild-type HEK293T cells. Human cell lines were chosen for validation in this study due to their broader applicability to other human cells, such as human induced pluripotent stem cells, but cell lines from other organisms could be used as well. The two protein quantitation reporters (PQRs) used here are optimized self-cleaving viral P2A peptides (13): P2A variant 2: 5′-GCGACGAATTTTAGTCTACTAAAACAAGCGGGT-

GATGTAGAAGAA AACCCTGGACCT-3′ and P2A variant 3: 5′-GCGACGAATTT-TAGTCTACTGAAACAAGC GGGAGAC-GTGGAGGAAAACCCTGGACCT-3′. These two P2A variants were demonstrated to have superior separation efficiency compared with the original P2A and other variants (13). Red fluorescent protein (RFP) and green fluorescent protein (GFP) were derived, respectively, from TagRFP-T and superfolder GFP for their superior photostability and fast maturation times (13). The Zeocin resistance gene (*Zeocin*^{R}), originating from *Streptoalloteichus hindustanus,* was synthesized at BioBasic (Markham, Ontario, Canada). Different gene fragments were assembled into two multicistronic gene cassettes, *PQR-RFPnols-PQR-loxP-Zeocin*^{R}*-loxP* and *PQR-RFP-PQR-GFPnols-PQR-loxP-Zeocin*^{R}*-loxP,* and placed between the homology arms of human *RPL13A* or *ACTB* to generate different destination vectors through the use of In-Fusion Cloning (Clonetech, Mountain View, CA). The homology arms did not include the *RPL13A* or *ACTB* promoter, thus preventing the expression of the transgene until the in-frame genomic integration at the correct locus occurred. The sizes of each of the homology arms for *RPL13A* and *ACTB* were 1.0 kb and 1.2 kb, respectively.

Preparation of Cas9 and sgRNA expression vectors

Four different single guide RNAs (sgRNAs) were designed to guide the Cas9 nuclease to make a double-strand break at the end of the coding region of each gene (*RPL13A* and *ACTB*). They were designed in a 20 bp DNA oligonucleotide format as previously described (14). Individual sgRNAs were cloned into the backbone of the dual promoter plasmid pX459-U6-Chimeric_BB-CBh-hSpCas9-2A-Puro (Addgene #62988; www.addgene.org), such that both the sgRNA and Cas9 were expressed from the same vector. Different sgRNA/Cas9 vectors were co-transfected along with a circular *RPL13A* or *ACTB* destination vector into HEK293T cells using Lipofectamine 3000 (Thermo Fisher Scientific Inc., Waltham, MA). The efficiency of sgRNA/Cas9-mediated integration of the destination vector into the genome was evaluated 5 days after transfection by cellular fluorescence and genotyping. The most efficient sgRNA sequences for *RPL13A* and *ACTB* are GCATTGTTGCCCTGGAATGTA and GACCTGGGCAGGTCGGCTGT, respectively.

Cell culture and optimal Zeocin concentration establishment

HEK293T cells were cultured at 37°C under 5% CO_2 in Dulbecco's Modified Eagle Medium, supplemented with 10% FBS and 1× penicillin-streptomycin (Wisent, St-Bruno, Quebec, Canada). To determine the optimal Zeocin (Thermo Fisher Scientific Inc., Waltham, MA) concentration for selection, wild-type HEK293T cells were treated with a series of 8 different Zeocin concentrations (0, 50, 100, 150, 200, 300, 400, and 500 µg/mL), and the effect on cytotoxicity was evaluated over the course of 1 week. Treatment with 200 µg/mL Zeocin resulted in a 75% reduction of cell growth after 1 week and was thus chosen for subsequent experiments. For genome editing experiments, cells at 30% confluency were transfected with 1 µg of CRISPR/Cas9 plasmid DNA and 1 µg of circular stable cell line destination vector in a 12-well plate. Two days after transfection, cells were expanded into 60 mm dishes and allowed to grow for another 3 days before 200 µg/mL Zeocin was added into the culture medium to eliminate all cells lacking expression of the GOI. Culture medium was changed every 2 or 3 days with occasional trypsinization of cells when

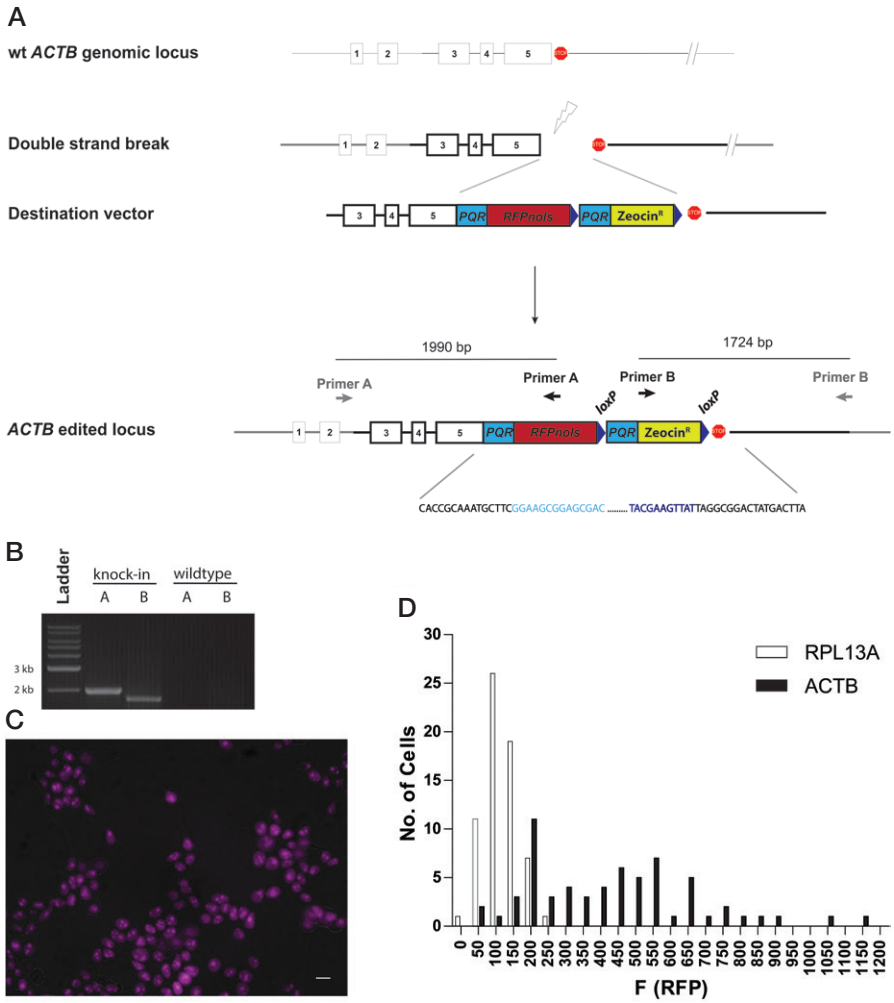

Figure 2. Creation of an *ACTB* stable cell line. (A) Schematic of the insertion of a multicistronic gene cassette into the endogenous *ACTB* gene through CRISPR/Cas9 knock-in. The wild-type *ACTB* gene has five exons, which are represented as boxes. CRISPR/Cas9 induces a site-specific double-strand break close to the stop codon (lightning bolt). The *ACTB* destination vector contains the gene cassette flanked by 1.2 kb homology arms, which are denoted by bold lines. Homologous recombination between the destination vector and the broken genomic strand results in the integration of the gene cassette into the *ACTB* locus. Two sets of PCR primer pairs (A and B), with one recognizing the gene cassette (black arrow) and the other outside of the homology arms (gray arrow), were used to verify the correct knock-in. Colored nucleotide sequences represent sequencing results of a stably maintained cell clone with the insertion of the gene cassette at the *ACTB* locus. (B) The integrated gene cassette in an isolated stable cell line was detected by genomic PCR genotyping using Primer Sets A and B with target amplicon sizes of 1990 bp and 1724 bp, respectively. A DNA ladder with respective fragment sizes is shown on the left. (C) A population of Zeocin-resistant cells expressing red fluorescent protein (RFP) in the nucleolus can be maintained indefinitely in Zeocin-free culture without loss of the red fluorescence. Scale bar: 100 µm. (D) Frequency distribution of red fluorescence intensities measured from single *RPL13A* and *ACTB* knock-in cells demonstrates moderate and tight expression levels of *RPL13A* compared with elevated and broad expression levels of *ACTB* among individual cells.

they reached 100% confluency to gradually remove dead or dying cells. After 2 weeks of Zeocin treatment, drug-resistant cells started to grow into individual colonies, and ~20% of the colonies were fluorescent. Individual fluorescent colonies were isolated using cloning discs (Sigma-Aldrich, St. Louis, MO) and then expanded into larger populations. The correct PQR plus transgene integrated cell lines were verified by genotyping using a primer outside of the homology arm and another primer recognizing inside the inserted gene cassette. The successfully amplified PCR products were further verified using Sanger sequencing (Supplementary Material).

Image acquisition and analysis

Fluorescence and brightfield images were taken using a Zeiss AxioScope A1 fluorescence microscope (Carl Zeiss Canada Ltd., North York, Ontatrio, Canada). All images were acquired at 1388 × 1040 pixels with a 40× water objective, N.A. 1.0 (epifluorescence) corresponding to a 215 × 160 µm field of view. Fluorescence was detected using a charge-coupled device camera. All image acquisition parameters were fixed for each imaging channel for exposure time, excitation intensity, gain, and voltage.

Images were selected for analysis on the basis of identification of single cells with normal morphology. Images in figures were adjusted for contrast and brightness for presentation. Average fluorescence pixel intensities were measured by choosing a region of interest that covered 70% of the nuclei or the entire cell using ImageJ. Fluorescence pixel intensities were background subtracted and presented in arbitrary units.

Results and discussion

Identification of genomic loci for integration

The first step of the protocol is to choose the appropriate genomic locus for integration (Figure 1B). The selection of genomic locus depends on several criteria: the intended level of expression, the required consistency of expression, and the preferred type of expression, such as inducible or constitutive. To express the protein of interest at easily detectable levels, we chose to express it from housekeeping genes.

Housekeeping genes are groups of genes that are responsible for the maintenance of cellular structure or homeostasis. Their expression levels are moderate to (physiologically) high and are often considered to be constant across experimental conditions and cell types. As a result, theses genes are widely used as reference genes in quantitative PCR and as loading controls in western blots. Numerous studies have been conducted to evaluate the consistency of expression of different housekeeping genes at the mRNA (15,16) and protein levels (13,17). However, the nature of their expression in individual cells is still unclear. Here, we targeted a multicistronic gene cassette into two housekeeping gene loci, human ribosomal protein L13A (*RPL13A*) and β actin (*ACTB*), and measured the expression of the protein of interest and the corresponding housekeeping protein in single living cells (Figures 1 and 2). *ACTB* has been shown to be the most highly expressed gene at both the mRNA and protein levels (17). It is also one of the most widely used loading controls for western blot analysis. Expressing the transgene from the *ACTB* locus would not only achieve the maximum expression level of a protein of interest that can be attained, but it also gives us the opportunity to assess the consistency of *ACTB* expression across single cells, even

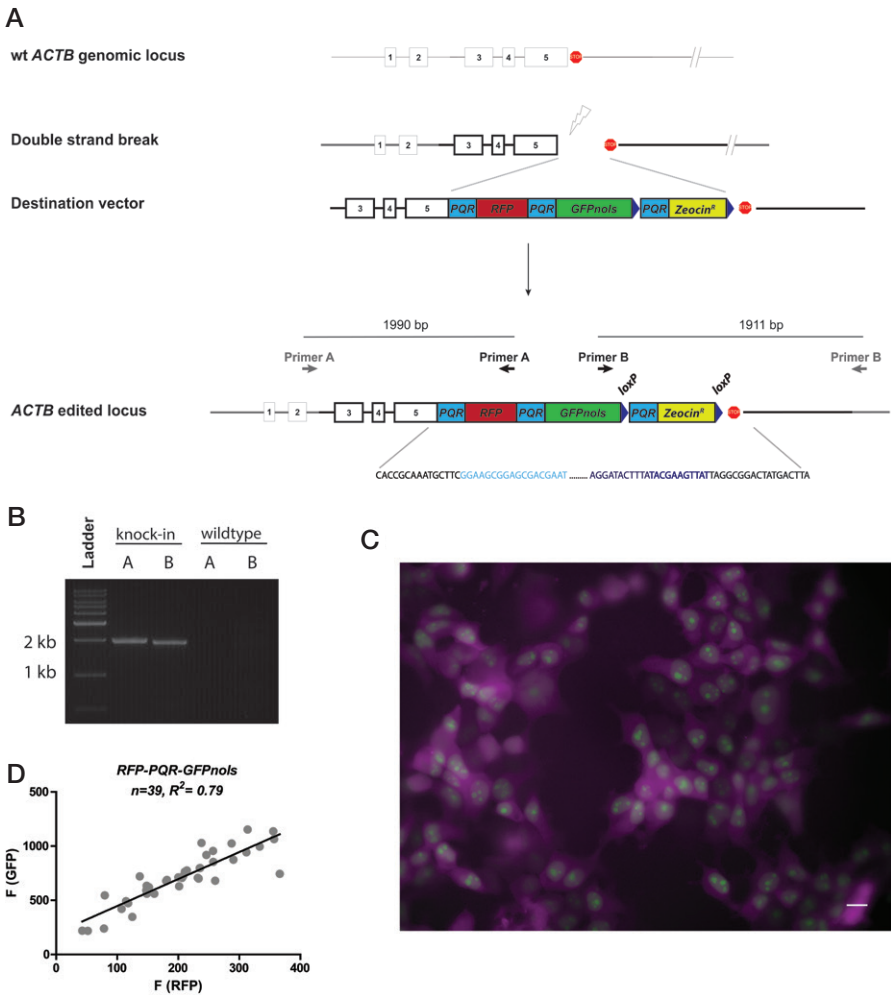

Figure 3. Creation of an *ACTB* stable cell line expressing a recombinant protein. (A) Schematic of the insertion of a multicistronic gene cassette with *GFPnols* as a representative recombinant gene into the endogenous *ACTB* gene locus through CRISPR/Cas9 knock-in. Homologous recombination between the destination vector and the broken genomic strand results in the integration of the recombinant gene cassette into the *ACTB* locus. Two sets of PCR primer pairs (A and B), one recognizing the gene cassette (black arrow) and the other outside of the homology arm (gray arrow), were used for verifying the correct knock-in. Colored nucleotide sequences represent sequencing results of a stably maintained recombinant stable cell line with the insertion of the gene cassette at the *ACTB* locus. (B) The integrated gene cassette in an isolated stable cell line was detected by genomic PCR genotyping using Primer Sets A and B with product sizes of 1990 bp and 1911 bp, respectively. A DNA ladder with respective fragment sizes is shown on the left. (C) A population of red cytoplasmic fluorescent, green nucleolar fluorescent, Zeocin-resistant cells can be maintained indefinitely in culture. Scale bar: 100 µm. (D) Red and green fluorescence intensities from different cellular compartments were linearly correlated with a coeffcient of determination (R^2) of 0.79 ($n = 39$ cells).

when the population is derived from a single common progenitor cell. On the other hand, *RPL13A* has a moderate to high expression level (2). As a ribosomal protein, the RPL13A level reflects the global transcription and translation level of a cell. In other words, RPL13A fluorescence intensity (Figure 1D) can indicate both the expression level of the recombinant protein and the global regulation of cellular homeostasis (18).

Construction of a PQR multicistronic knock-in vector

Previously, we created multiple PQR constructs to allow for quantification over time of the protein production in single mammalian and insect cells (13). The PQR genetic tag is transcribed with the GOI along with a fluorescent protein of choice. During protein synthesis, the protein of interest and the fluorescent protein are expressed in an equimolar ratio, and the two are immediately separated through self-cleavage of the PQR tag (13). The fluorescence intensity of the cell is then used to quantify relative protein amounts, because the number of molecules of fluorescent protein is equal to the number of molecules of the protein of interest.

We constructed two destination vectors based on either the human *RPL13A* (Figure

1) or *ACTB* (Figure 2) genomic sequences as homology arms flanking a multicistronic cassette, *PQR-RFPnols-PQR-loxP-Zeocin^R-loxP*. PQR and an RFP with a nucleolar localization signal (PQR-RFPnols) as the reporter is connected to a floxed Zeocin antibiotic resistant gene (Zeocin^R). Thus, red fluorescence intensity in the nucleolus is a measure of the expression levels of all the encoded proteins from either the *RPL13A* (Figure 1) or *ACTB* locus (Figure 2). Zeocin^R confers cell resistance to the antibiotic Zeocin, which induces cytotoxicity by binding to and cleaving DNA. The product of Zeocin^R binds to Zeocin in a one-to-one ratio and prevents the DNA cleavage. Zeocin^R was used as the selection marker owing to its relatively smaller size (375 bp versus 1 kb for the hygromycin resistance gene) and greater reliability in stable cell clone selection (19). PQR linkers were cloned in frame between all of the encoding genes to split them into discrete proteins. To prevent vector recombination, different PQR tags with different synonymous codon compositions were used (see Materials and methods section). The flanking *loxP* sites may be used to remove the selection marker after the establishment of the stable cell line.

Generation of a stable and pure HEK293T cell line expressing a fluorescent reporter and a drug resistance protein

The two destination vectors (*RPL13A-PQR-RFPnols-PQR-loxP-Zeocin^R-loxP* and *ACTB-PQR-RFPnols-PQR-loxP-Zeocin^R-loxP*) (Figure 1B and Figure 2A) and custom-designed CRISPR/Cas9 reagents were transfected into HEK293T cells. Five days after transfection, 200 µg/mL Zeocin was added into the medium to eliminate all untransfected cells. After 2 weeks of drug selection, Zeocin-resistant cells started to grow into individual colonies, and 20% of them contained red fluorescent nucleoli (RFP positive). Individual red fluorescent colonies were isolated and expanded (Figure 1A). The distribution of RFP fluorescence intensities in individual cells of the two cell lines demonstrated that the two housekeeping genes have distinct expression patterns (Figure 2D). *RPL13A* expressed at a lower but more consistent average level, whereas *ACTB* had a higher average expression level but showed greater expression heterogeneity among cells. The variability in expression levels of the two housekeeping genes among individual cells demonstrates the heterogeneous nature of single-cell gene expression. Genes expressed at high levels have larger variances in protein expression, for example in cells that secrete the immunoglobulin kappa light chain antibody protein (13) or even in cells from a monoclonal cell line. PQR can exploit this variability by selecting for a desired level of protein expression that can be identified using fluorescence intensity. These results suggest that for use as a reference gene for protein quantification across different cells, *RPL13A* has better accuracy for normalization. In contrast, if producing large quantities of recombinant protein is the primary goal, *ACTB* would be a better choice.

The correct stable knock-in cell clones were verified by PCR genotyping with one primer recognizing the genomic region outside the homology arm and the other primer within the *RFP* or *Zeocin^R* sequence (Figure 1C and Figure 2B). Amplified PCR products were verified by Sanger sequencing (Supplementary Material). These results suggest that RFPnols and Zeocin were correctly co-expressed and functionally active in the selected stable cell clones.

A stable cell line expressing a protein of interest at quantifiable levels

We demonstrated that the correct knock-in of *PQR-RFPnols-PQR-Zeocin^R* at both *RPL13A* and *ACTB* loci confers Zeocin resistance to the cells with red fluorescent nucleoli. Next, we wanted to demonstrate the ease and advantages of expressing proteins of interest using this method of stable cell line generation. As a proof of principle experiment, we chose GFP with a nucleolar localization signal (GFPnols) as a representative protein of interest because green fluorescence in the nucleoli is a simple and quantitative phenotype. An accompanying RFP reporter (cytoplasmic) was used to measure both ACTB and GFPnols expression. A destination vector with the recombinant gene, *PQR-RFP-PQR-GFPnols-PQR-loxP-Zeocin^R-loxP*, was constructed and transfected into HEK293T cells along with the *ACTB* CRISPR/Cas9 reagent (Figure 3A). Correlation between red and green fluorescence intensities was then used to validate the hypothesis that the expression of the recombinant protein is reliably measured using fluorescence (Figure 3D).

Following the same protocol, the *ACTB* stable cell line expressing the GFPnols was created within the same amount of time as the previous experiments (Figure 1A). RFP and GFP were expressed and localized to their respective cellular compartments (Figure 3C), and the red and green fluorescence signals were correlated with a coefficient of determination (R^2) of 0.79 (Figure 3D). This confirms that the production of any protein of interest can be quantified on the basis of the fluorescent reporter output of a cell and that any resulting cellular phenotype can be correlated as a function of protein concentration (13). This also demonstrates that four proteins: the endogenous protein (ACTB), the fluorescent reporter (RFP), the protein of interest (GFPnols), and the drug resistance protein (Zeocin^R), can be effectively expressed without compromising their native functions (Figure 3C).

Compared with the traditional protocol for the creation of stable cell lines, our new protocol has numerous advantages. First, integration of the transgene into a predetermined locus so that it is driven by an endogenous promoter provides the opportunity to predict and control the level of recombinant protein expression. This is impossible when the recombinant gene is driven by an exogenous promoter and is integrated randomly into the genome in multiple copies. Second, the precise targeting of the transgene to the intended locus through CRISPR/Cas9 can obviate the time-consuming characterization of the isolated cell clones, shortening the selection process to 2–3 weeks as opposed to several months when a traditional random integration–based method is used (Figure 1A). Third, the selected homogeneous knock-in cell lines can be maintained without continuous selection pressure. This is particularly beneficial since Zeocin cannot be fully neutralized by the product of the *Zeocin^R* gene (11). Chronic exposure to Zeocin during a prolonged selection process has been shown to cause mutagenesis in the established recombinant cell lines (11). We verified that all cells maintained their fluorescence intensities for >15 passages after removal of the Zeocin selection. Fourth, the obtained knock-in HEK293T cells have comparable growth rates and morphologies as their wild-type counterparts, suggesting that the physiological levels of expression of RFP and GFP as well as Zeocin^R do not exert too much stress on the host cells. The preservation of native cellular phenotypes and molecular processes is particularly beneficial, as any impairment of normal cellular physiology would be considered undesirable when it comes to experiments such as drug screening, gene function studies, and other applications in which a normal homeostatic cellular milieu is needed. In contrast, when the transgene is driven by an exogenous promoter and integrated into the genome in multiple copies, overexpression of the recombinant protein or disruption of the genome can cause unwanted side effects, leading to incorrect interpretation of the experimental results. Finally, the accompanying RFP or other PQR fluorescent reporter provides the opportunity to quantify recombinant protein expression under conditions in which the level of *RPL13A* or *ACTB* housekeeping gene expression can be stable or experimentally manipulated.

Author contributions

C.-A.L. designed and performed the experiments, analyzed data, and wrote the first

draft of the manuscript. A.G. performed experiments. B.E.C. supervised the study, established the primary objective of the manuscript, and edited the manuscript.

Acknowledgments

We thank members of Brian E. Chen's laboratory for their assistance with experiments.

Competing interests

The authors declare no competing interests.

References

1. **Lemaire, G., G. De Sousa, and R. Rahmani.** 2004. A PXR reporter gene assay in a stable cell culture system: CYP3A4 and CYP2B6 induction by pesticides. Biochem. Pharmacol. *68*:2347-2358.
2. **Lai, C., X. Jiang, and X. Li.** 2006. Development of luciferase reporter-based cell assays. Assay Drug Dev. Technol. *4*:307-315.
3. **Xu, Z.-L., H. Gao, K.-Q. Ou-Yang, S.-X. Cai, and Y.-H. Hu.** 2004. Establishment of a cell-based assay to screen regulators for Klotho gene promoter. Acta Pharmacol. Sin. *25*:1165-1170.
4. **Hu, J., Y. Yu, H. Han, F. Civoli, Y. Zhuang, J. Thomas, S. Swanson, S. Jing, and S. Gupta.** 2014. Development of a Novel BAFF Responsive Cell Line Suitable for Detecting Bioactive BAFF and Neutralizing Antibodies against BAFF-Pathway Inhibiting Therapeutics. Cells *3*:79-91.
5. **Li, F., N. Vijayasankaran, A. Shen, R. Kiss, and A. Amanullah.** 2010. Cell culture processes for monoclonal antibody production. MAbs *2*:466-479.
6. **Rangasamy, D., D.J. Tremethick, and I.K. Greaves.** 2008. Gene knockdown by ecdysone-based inducible RNAi in stable mammalian cell lines. Nat. Protoc. *3*:79-88.
7. **Wurm, F.M.** 2004. Production of recombinant protein therapeutics in cultivated mammalian cells. Nat. Biotechnol. *22*:1393-1398.
8. **Dobzhansky, T.** 1936. Position effects on genes. Biol. Rev. Camb. Philos. Soc. *11*:364-384.
9. **Akhtar, W., J. De Jong, A.V. Pindyurin, L. Pagie, W. Meuleman, J. De Ridder, A. Berns, L.F.A. Wessels, et al.** 2013. Chromatin position effects assayed by thousands of reporters integrated in parallel. Cell *154*:914-927.
10. **Chen, M., K. Licon, R. Otsuka, L. Pillus, and T. Ideker.** 2013. Decoupling Epigenetic and Genetic Effects through Systematic Analysis of Gene Position. Cell Reports *3*:128-137.
11. **Oliva-Trastoy, M., M.O. Trastoy, M. Defais, and F. Larminat.** 2005. Resistance to the antibiotic Zeocin by stable expression of the Sh ble gene does not fully suppress Zeocin-induced DNA cleavage in human cells. Mutagenesis *20*:111-114.
12. **Xia, W., P. Bringmann, J. McClary, P.P. Jones, W. Manzana, Y. Zhu, S. Wang, Y. Liu, et al.** 2006. High levels of protein expression using different mammalian CMV promoters in several cell lines. Protein Expr. Purif. *45*:115-124.
13. **Lo, C.A., I. Kays, F. Emran, T.J. Lin, V. Cvetkovska, and B.E. Chen.** 2015. Quantification of Protein Levels in Single Living Cells. Cell Reports *13*:2634-2644.
14. **Ran F.A., P.D. Hsu, J. Wright, V. Agarwala, D.A. Scott, and F. Zhang.** 2013. Genome engineering using the CRISPR/Cas9 system. Nat. Protoc. *8*:2281-2308.
15. **Mane, V.P., M.A. Heuer, P. Hillyer, M.B. Navarro, and R.L. Rabin.** 2008. Systematic method for determining an ideal housekeeping gene for real-time PCR analysis. J. Biomol. Tech. *19*:342-347.
16. **Ragni, E., M. Viganò, P. Rebulla, R. Giordano, and L. Lazzari.** 2013. What is beyond a qRT-PCR study on mesenchymal stem cell differentiation properties: How to choose the most reliable housekeeping genes. J. Cell. Mol. Med. *17*:168-180.
17. **Schwanhäusser, B., D. Busse, N. Li, G. Dittmar, J. Schuchhardt, J. Wolf, W. Chen, and M. Selbach.** 2011. Global quantification of mammalian gene expression control. Nature *473*:337-342.
18. **Lindström, M.S.** 2009. Emerging functions of ribosomal proteins in gene-specific transcription and translation. Biochem. Biophys. Res. Commun. *379*:167-170.
19. **Lanza, A.M., D.S. Kim, and H.S. Alper.** 2013. Evaluating the influence of selection markers on obtaining selected pools and stable cell lines in human cells. Biotechnol. J. *8*:811-821.

Received 20 September 2016; accepted 17 February 2017.

Address correspondence to Brian Edwin Chen, The Research Institute of the McGill University Health Centre, Montreal General Hospital, 1650 Cedar Ave., Bureau L7 224, Pavilion Livingston, Montréal (Québec) Canada H3G 1A4. E-mail: brian.chen@mcgill.ca