Genes to Cells

REVIEW

# Experimental design for stable genetic manipulation in mammalian cell lines: lentivirus and alternatives

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**The use of third-generation lentiviral vectors is now commonplace in most areas of basic biology. These systems provide a fast, efficient means for modulating gene expression, but experimental design needs to be carefully considered to minimize potential artefacts arising from off-target effects and other confounding factors. This review offers a starting point for those new to lentiviral-based vector systems, addressing the main issues involved with the use of lentiviral systems *in vitro* and outlines considerations which should be taken into account during experimental design. Factors such as selecting an appropriate system and controls, and practical titration of viral transduction are important considerations for experimental design. We also briefly describe some of the more recent advances in genome editing technology. TALENs and CRISPRs offer an alternative to lentivirus, providing endogenous gene editing with reduced off-target effects often at the expense of efficiency.**

## Introduction

Many viruses have evolved to be highly efficient at integrating their own genome into that of a host organism, effectively turning the host organism into a factory for propagation of the virus. This feature can be easily and safely exploited for genetic modification of mammalian cells for basic research. Lentiviral vectors can infect nondividing cells with high efficiency and are commonly used for this purpose; however, caution must be taken to avoid introducing phenotypic artefacts due to inadvertent open reading frame (ORF) disruption or gene activation. Careful experimental design can minimize potential artefacts and allow lentiviral delivery systems to be used to their full potential; however, newer technologies such as Tal effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats (CRISPRs) offer even further reduction of artefacts and their use is increasing rapidly.

Below, we address basic design principles and common potential roadblocks of lentiviral use in basic cell biology with the aim of minimizing artefacts that may confound experimental data. We also briefly address new technology for modification of endogenous genetic sequence to provide an updated guide for those new to this fundamental tool of molecular cell biology.

## Lentiviral vectors and mammalian cells in basic biology

Retroviral vectors are an efficient means of stable introduction of synthetic DNA constructs; however, they are limited in that they only infect cells actively dividing, which greatly limits efficiency (Reiser *et al.* 1996). Lentiviral vectors make up a subset of retroviral vectors, which will transduce regardless of cell-cycle stage. These vectors are often constructed using HIV-1 provirus pseudotyped with a VSV-G protein coat, which is highly effective at transducing a vast majority of mammalian cells due to its binding mechanism (Schlegel *et al.* 1983; Akkina *et al.* 1996; Naldini *et al.* 1996; Reiser *et al.* 1996). The typical time frame of a pooled lentiviral infection takes approximately two weeks from generation of virus to expansion of the genetically modified cell line.

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Most areas of biomedical research have embraced lentiviral vectors because third-generation vectors have made viral transduction safe for the use by biologists with minimal specialist training. Early lentiviral vectors (now referred to as first and second generation) are now rarely used for basic biology and as such will not be addressed in this review. The high level of biosafety of third-generation lentiviral vectors comes from the physical separation of the minimal genetic elements of HIV-1 onto three accessory plasmid DNA constructs (see Fig. 1A), whereas a separate transfer vector carries the transgene (Dull *et al.* 1998; Barde *et al.* 2010). Production of replication competent virus would require multiple complex and specific recombination reactions to occur during the transduction process, which although unlikely can be easily detected by PCR analysis. A further inherit feature of third-generation vectors is self-inactivation. This occurs due to a deletion in the long terminal repeat (LTR) sequence resulting in a loss of proviral enhancer sequence on integration (Yu *et al.* 1986).

Lentiviral systems have been instrumental in enabling functional genomics screens in various disease contexts using arrayed or pooled short-hairpin RNA (shRNA) and complementary DNA (cDNA) expression libraries. Large-scale loss-of-function screens have been enabled by the availability of RNAi libraries, which are now in widespread use

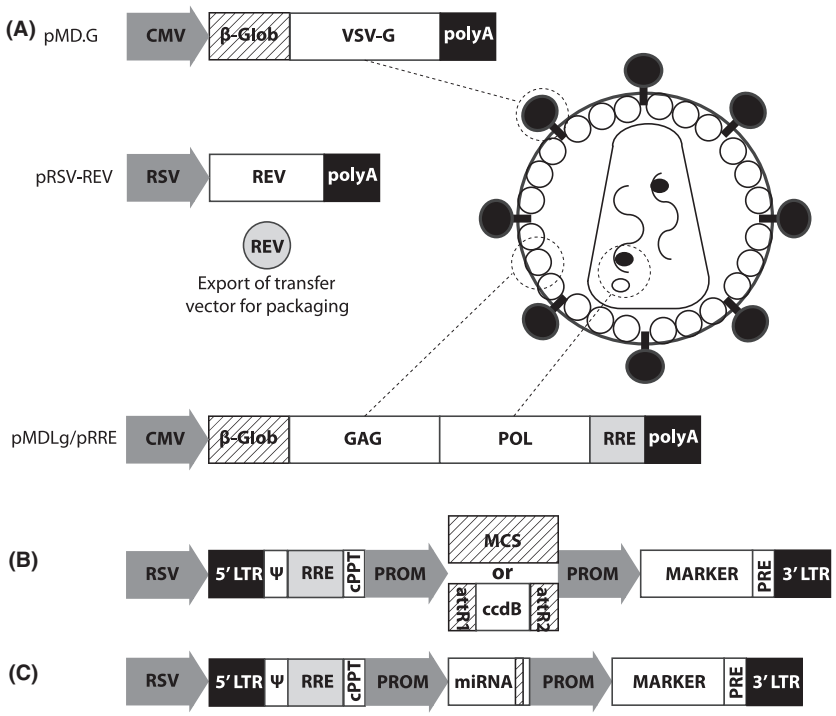


**Figure 1** (A). Third-generation HIV-1-based lentiviral vector structure. Essential viral genes are split amongst three separate packaging vectors to maximize biosafety (Dull *et al.* 1998). pMD.G encodes the pseudotyped VSV-G protein coat responsible for the amphitropic nature of the lentiviral system. pRSV-Rev encodes REV which is essential for nuclear export of the proviral RNA, by binding the Rev-responsive element (RRE). pMDLg/pRRE encodes Gag and Pol polyproteins which are processed to form structural proteins and downstream viral integration enzymes (reverse transcriptase and integrase), respectively. (B). General structure of a transfer vector for expression of cDNA. The gene of interest can be cloned into the transfer vector by conventional cloning at a multiple cloning site (MCS) or by recombination-based cloning such as Gateway. Note that the separate promoter shown for the marker gene is often replaced by use of an IRES or polyprotein cleavage sequence, which allows expression of the marker from the same transcript, or can be omitted completely if the marker is expressed as a fusion protein with the gene of interest. Other general transfer vector features depicted include the long terminal repeat sequences (LTR), which are involved with transcription of the expression cassette and integration with the host cell genome (Yu *et al.* 1986). The central polypurine tract (cPPT) and the post-transcriptional regulatory element (PRE) significantly increase transduction efficiency (Barry *et al.* 2001). The packaging signal known as Psi (Ψ) provides selective packaging of viral RNA (Rulli *et al.* 2007). (C). General structure of a transfer vector for expression of shRNA. The target-specific hairpin is often cloned into existing miRNA structure by restriction cloning.

(Root *et al.* 2006; Blakely *et al.* 2011). Similarly, large-scale over-expression studies have also been made possible by the publicly available human ORFeome, which can be obtained cloned into lentiviral expression vectors or in Gateway® Entry vectors (Yang *et al.* 2011). For example, a genome-wide ORFeome library was used to screen for genes involved with breast cancer tumorigenesis (Skalamera *et al.* 2011).

## Choosing a lentiviral platform

Numerous factors should be considered when selecting the most appropriate vector system to be used. Key considerations include host cell, promoter, selection marker, fusion tag and length of ORF to be used.

## Over-expression

Figure 1B shows the general structure of a typical cDNA/ORF transfer vector and a selection of commonly used vectors and their key features are described in Table 1. First and foremost a suitable mammalian host cell must be selected. Along with disease relevance, the type of cell used should be checked for expression of the gene of interest. For example, if the protein is already expressed at relatively high levels, over-expression may not contribute to an observable phenotype. We strongly recommend that the ORF should be sequenced to detect any mutations which may confound experiments involving over-expression of wild-type protein.

The size of the transfer vector has significant influence on the efficiency of viral titer. The effective size of the cassette is the base pair distance between the 5′ and 3′ LTRs. This distance represents the provirus which will ultimately integrate into the host cell genome. Lentivirus is generally considered to have an upper proviral length limit of 10–12 kbp with efficiency generally increasing as kpb length is reduced. However, successfully packaged lentiviral particles have been generated with proviral length exceeding 18 kpb (Kumar *et al.* 2001).

An effective strategy for mitigating nonspecific effects on viral transduction is the generation of appropriate control cell lines alongside experimental lines, as driving ORF over-expression requires cellular resources and as such will add metabolic burden to the host cells (Rowe & Summers 1999). Furthermore, over-expressed protein that is prone to forming aggregates may disrupt the ubiquitin-proteasome system causing cellular toxicity (Bence *et al.* 2001). Correctly functioning molecular chaperones and the ubiquitin-proteome system are a requirement of healthy cells, and higher protein expression levels may increase aggregation potential (Stefani & Dobson 2003).

Generally, a cell line expressing a phenotypically inert ORF (e.g., GFP) makes for a suitable control; however, expression of active-site mutant ORF (if available) is preferable. Expression of GFP not only controls for off-target effects of ORF over-expression, but also will allow for an easy assay to test the effectiveness of the promoter/enhancer elements in the construct. The use of inducible expression sys-

**Table 1** Commonly used third-generation lentiviral transfer vectors for expression of cDNA and shRNA knockdown

| cDNA Transfer Vector | Promoter | Cloning | Selection | Fusion tag | (Original paper) Citations |
|---|---|---|---|---|---|
| pLenti6.3/TO-DEST-V5 | CMV(TO) | Gateway | Blasticidin S | C-terminal V5 | (Dull *et al.* 1998) 402⋆ |
| pLV-eGFP | CMV | MCS | EGFP fusion | C-terminal EGFP | (Dull *et al.* 1998) 402⋆ |
| FUGW | CMV | MCS | hUbC-EGFP | n/a | (Lois *et al.* 2002) 407 |
| pLJM1 | CMV | MCS | PGK-puro | C-terminal EGFP | (Sancak *et al.* 2008) 276 |

| shRNA Transfer Vector | Promoter | Cloning | Selection | (Original paper) Citations |
|---|---|---|---|---|
| pLKO.1-puro | hU6 (TRE variant available) | AgeI/EcoRI | Puro | (Stewart *et al.* 2003; Moffat *et al.* 2006) 139 |
| pLKO.1-TRC | hU6 | AgeI/EcoRI | Puro | (Moffat *et al.* 2006) 336 |
| pSICO | CMV (Cre inducible) | HpaI/XhoI | CMV-EGFP | (Ventura *et al.* 2004) 148 |
| pSLIK | TRE | BfuAI/Gateway | UbC-Neo/Venus | (Shin *et al.* 2006) 54 |

⋆pLV vectors are based on the pLenti backbone.

tems may also be effective in mitigating potential toxic effects.

## Attenuating gene expression (i.e. 'knockdown')

Lentiviral vectors are also very effective for introducing stable RNA interference (RNAi) cassettes into mammalian cells to attenuate the expression of a specific gene. Many vector systems using simple cloning techniques allow for rapid production of cell lines with constitutive expression of RNAi. Figure 1C shows general structure of a typical shRNA transfer vector, and some commonly used vectors are described in Table 1. Constitutive or stable knockdown systems are ideal for large-scale selection-based loss-of-function screens, which require longer-term attenuation of expression than can be provided by short-interfering RNA (siRNA), and are much cheaper and faster than knockout models.

Many stable knockdown systems use miR30a architecture for optimal shRNA function, and the specific hairpin sequence required can be easily subcloned. Online resources such as the RNAi Codex (Olson *et al.* 2006) provide searchable databases of existing hairpins targeting a wide range of genes, and genome-scale shRNA libraries are available in a number of different vector formats (Silva *et al.* 2005; Root *et al.* 2006).

Appropriate controls for knockdown studies are crucial, as shRNA is known to have off-target effects (Jackson & Linsley 2010). Driving over-expression of shRNA hairpin can also stress cells in much the same way as the over-expression of a cDNA, potentially introducing phenotypic artefacts.

## Stable integration and selectable markers

Enriching for a population of transduced cells requires either a marker to sort positive cells (such as a fluorescent protein) or antibiotic resistance for negative selection against nontransduced cells. The selection marker may be co-expressed with the transfer vector insert using an internal ribosome entry site (IRES) sequence and may be expressed alongside the gene of interest as a polyprotein complex using either self-cleaving 2A peptide (Ryan *et al.* 1991; Kim *et al.* 2011) or even fused to the protein of interest. Often the marker will be expressed from a separate promoter from the gene of interest. Fluorescent markers allow for simple sorting of transduced cells by flow cytometry. However, if downstream studies include protein localization by immunofluorescence, a fluorescent marker occupies a channel, which could otherwise be used for costaining.

Antibiotic selection markers offer a simpler means of enriching for transduced cells; however, some drawbacks exist with this approach. Natural variations in susceptibility and sometimes completely resistant subpopulations exist within *in vitro* cultures (Gillet & Gottesman 2010). Therefore, susceptibility to a particular antibiotic should be determined empirically by titration prior to transduction.

## Constitutive promoters

The promoter used should be tested in the mammalian host before transduction, which can be achieved by transient transfection and immunoblot and commercial kits are now available which allow for rapid testing of a panel of promoters in cell lines of interest. Cytomegalovirus (CMV) or elongation-factor 1 alpha (EF1$\alpha$) promoters are commonly used to drive high-level constitutive expression (Thomsen *et al.* 1984). Promoters that drive constitutive over-expression should not be used if the protein under their control is associated with toxicity or suppression of proliferation.

Cultured mammalian cells are capable of silencing stable expression constructs over time. Although the exact mechanism is unclear, a recent study showed transgene silencing by CpG methylation in embryonic and somatic stem cells after lentiviral transduction (Herbst *et al.* 2012). Silencing can be particularly problematic if the desired experiment requires large quantities of cells, necessitating multiple passages as the culture is expanded. This problem can be largely avoided using a conditional or inducible expression system, which allows a transduced cell population to be expanded before exposing the cells to any possible stress associated with over-expression.

## Inducible and conditional promoters

Control of gene activation in mammalian expression systems can be achieved through the conditional tetracycline-based (Tet-On) system (Gossen & Bujard 1992). The basis of this system is a tetracycline-responsive promoter element, which is not activated by a tetracycline transactivator (tTA) factor in the absence of the antibiotic tetracycline, or the more commonly used doxycycline. Once tetracycline or doxycycline is added to the culture media, a conformational change allows the tTA to bind the promoter

and drive transgene expression. An alternative form of this system (Tet-Off) uses a repressor (tetR), which remains bound to the tetracycline-responsive promoter until tetracycline or doxycycline binds tetR to relieve repression and permit transcription (Yao *et al.* 1998).

Inducible expression systems allow time for selection of an enriched population of transduced cells and culture expansion without possible selective changes occurring to the expression system should protein over-expression confer negative selection. It is strongly recommended to freeze many aliquots of the enriched, transduced culture after expansion and then induce expression to test how long over-expression/knockdown is maintained, allowing more precise, rational experimental.

There are, however, some potential drawbacks to using inducible systems. The effects of doxycycline on cell lines *in vitro* are debatable. For example, doxycycline has been shown to partially inhibit the growth of human prostate cancer cells (Fife *et al.* 1998). However, this study used experimental concentrations at least five times higher than the 1 μg/mL concentration commonly recommended for inducible gene expression. A more recent study has shown that human cell lines have altered metabolic profiles and decreased cellular proliferation in the presence of doxycycline at concentrations commonly used for induction (Ahler *et al.* 2013). Potential side effects of doxycycline can be minimized by careful design of the inducible system, and titration to minimize doxycycline dose required for effective expression. The required dosage of doxycycline should be carefully titrated not just to minimize the effect of doxycycline on cell growth, but also to ensure the level of expression is suitable for the desired experiment. A simple immunoblot of lysates taken from host cells treated with increment concentrations of doxycycline can achieve this quickly, minimizing transduction artefacts.

The use of inducible systems can complicate experiments by increasing the need for extra controls to ensure the induction of the expression machinery itself is not contributing to a phenotype. Use of an internal control of 'no induction' (i.e., without doxycycline) is common; however, this does not take into account any nonspecific effect of driving over-expression of cDNA or an RNAi cassette (as discussed above). More appropriate controls are to generate separate cell lines expressing a 'benign' insert (usually an expression marker such as GFP), or a nontargeting hairpin for over-expression or knockdown experiments, respectively.

## Potential drawbacks to lentiviral use

A number of potential drawbacks exist with the use of lentiviral transduction in cell biology. The primary concern is endogenous gene disruption. Lentiviral systems work by reverse transcribing viral RNA into double-stranded DNA, which is then inserted into the host organism's genomic DNA by nonhomologous recombination, which is essentially a random process (reviewed in (Ramezani & Hawley 2002)). This nonspecific mechanism can result in the DNA cassette being inserted within the open reading frame or regulatory regions of a gene, resulting in 'off-target' effects. These random events may confound experimental data through modulating expression, mutation or even truncation of nontargeted genes.

The effects of random lentiviral cassette insertion can be mitigated by the use of a pooled population of transduced cells, which will have a heterogenous pattern of cassette insertion. However, over multiple passages *in vitro*, the heterogeneous population may suffer 'drift' due to selection of subclones which exist naturally within *in vitro* cultures (Martinez *et al.* 1978; Thompson & Holliday 1983). Another way to mitigate off-target effects is the use of internal controls within infected cell lines. This can be achieved using inducible expression systems, where the uninduced cells act as a negative control. Uninduced negative control cells will contain the same genetic abberations resulting from the viral transduction; however, this approach requires careful design and can be compromised by expression system 'leak' (Zabala *et al.* 2004).

It should be noted that there are targeted methods of introduced exogenous DNA into the genome of mammalian cells. For example, the Rosa26 locus is commonly targeted for cDNA insertion by homologous recombination in human/mouse cells, as it supports constitutive expression without any known ORF disruption (Friedrich & Soriano 1991; Irion *et al.* 2007). Another targeted method of gene insertion is using PhiC31 integrase, which catalyzes insertion of a cassette into a previously inserted 'docking' site of known genomic location (Michael *et al.* 2012). Although these tools work with high efficiency, they rely on additional steps, including transient transfection of donor vector constructs.

Ideally, minimal levels of lentiviral transduction sufficient to achieve gene expression should be used. However, as lentiviral transduction is often highly efficient, multiple insertions per cell are common and can cause off-target effects due to insertional mutagenesis (Connolly 2002). This undesirable outcome can be

mitigated in a number of ways. The standard method of controlling the multiplicity of infection (MOI) is to determine the number of infectious units of virus per milliliter of viral supernatant, often expressed as transducing units per milliliter of viral supernatant (TU/mL). This can then be used to deduce the amount of virus which should be used to obtain a certain MOI. Viral titer can also be determined by analyzing viral stocks by qPCR or ELISA to determine viral RNA copy number or amount of viral proteins, respectively, by analyzing the DNA of transduced cells for the extent of transduction events or by quantifying expression of virally encoded fluorescent markers (see Fig. 2A). A clear standout method for all applications has not been conclusively determined, but analyzing copy number has been suggested as the most reliable method, as it is less likely to be influenced by nonfunctional virus (Sastry *et al.* 2002; Geraerts *et al.* 2006).

Calculation of capable transducing units in a viral supernatant is not likely to equivocate with actual transduction events, as the efficiency of transduction is not considered (i.e., it assumes each viral particle successfully transduces a target cell). A simpler method is empirical titration of the amount of virus required for an acceptable transduction efficiency using serial dilution. The titration approach is ideal for viral constructs with a fluorescent marker, as the infected population can be easily observed (see Fig. 2B). This can also be achieved using antibiotic selection, ensuring appropriate controls are in place and the selection process is monitored very closely (see Fig. 2B).

As multiple integrations increase the chance of nontargetted gene disruption, the most desirable outcome is a single integration event per cell in culture to minimize off-target effects. An easier method of achieving a low MOI is to perform multiple transductions in a serial dilution fashion. This will allow for selection of a minimal viral concentration which gives an acceptable proportion of transduced cells.

Some inducible expression systems allow for transduction of regulatory elements independent of the expression cassette (i.e., bi-cistronic). For example, a viral transfer vector encoding tetracycline-based inducer/repressor elements separate to the transfer vector encoding the inducible transgene can be independently titrated to achieve various levels of transgene activation (see Fig. 2C). An advantage of this system is that it can be used to make cell lines with various base levels of repression for relatively comparable over-expression of multiple transgenes.

Infectious units will naturally vary between batches of artificially packaged lentivirus. Suboptimal packaging due to fluctuations in vector DNA quantity and time of harvest will affect viral titer (Logan *et al.* 2004). The general condition of packaging cells and passage number will also affect viral titer (Thorsen *et al.* 1997). For this reason, care must be taken when performing transductions using different batches of packaged lentivirus. Separate viral batches are not necessarily comparable, which can complicate experiments requiring empty vector or nontargeting hairpin controls.

## Alternatives to lentiviral transduction

As discussed above, one of the limitations of lentiviral transduction is the potential for off-target effects from ORF disruption. Recent developments in single-strand-nicking-based genome editing provide an alternative route to the modulation of gene expression and the introduction of exogenous DNA sequences. Although the end result of gene expression modulation is the same for these two techniques, a direct comparison is difficult as genome editing technology goes far beyond the capabilities of lentiviral gene delivery in an *in vitro* context. Generally, use of newer genome editing techniques offers reduced off-target effects at the cost of efficiency, and this approach should be considered during experimental design.

## Zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs)

ZFNs couple a *FokI* cleavage domain with a zinc finger DNA-binding domain to target a restriction enzyme to a limited set of DNA sequences specified by the assembly of zinc finger modules. Endogenous DNA repair machinery is exploited for silencing or modification of genes (Bibikova *et al.* 2003). TALENs are similar to zinc finger nucleases in that they cleave double-stranded DNA using a *FokI* domain; however, the DNA-recognition domain is made up of tandem repeat sequences, each with a repeat-variable di-residue (RVD), which specifies an individual nucleotide target. For this reason, TALENs can be engineered to target virtually any sequence.

Double-stranded DNA breaks induced by TALEN cleavage are repaired by one of two mechanisms. Endogenous nonhomologous end joining (NHEJ) will often introduce a small insertion or deletion,

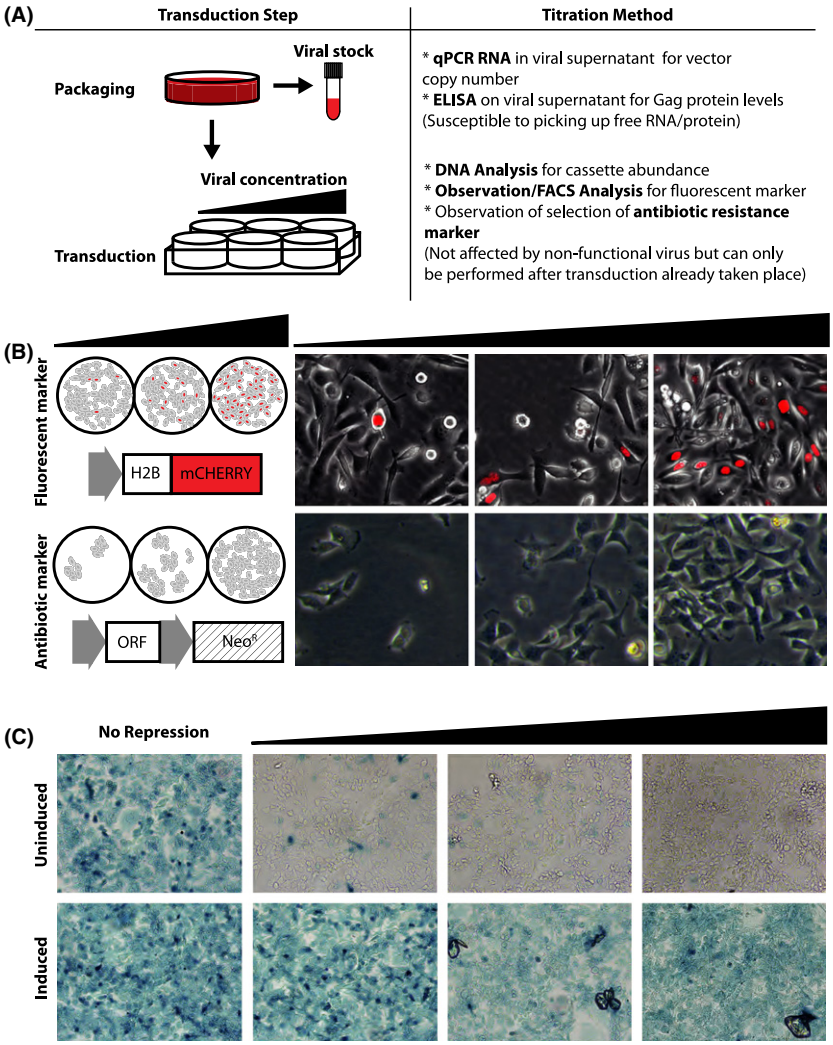


**Figure 2** Viral titration and controlling transduction *in vitro*. (A). Commonly used methods to determine viral titer at each step of the viral transduction process. (B). Examples of limiting dilution of virus *in vitro* to determine transduction efficiency. (Top-row) PC-3 cells with stably integrated construct expressing nuclear localizing mCHERRY fluorescent protein, 48 h post-transduction. Phase-contrast images with overlayed mCHERRY epifluorescence. Transduction efficiency can be estimated by observing transduced cells as a proportion of total cells in the field of view. (Bottom-row) MDA-MB-231 cells with stably integrated construct expressing resistance to G418, 4 days postaddition of 1 mg/mL G418 antibiotic. Phase-contrast images. Transduction efficiency can be estimated by observing colony formation after transduction. Bars indicate increasing viral concentration. (C). Repression of transgene expression (B-Gal, blue) using the tet repressor (TR) using a two-vector expression system (A11144, Invitrogen). MDA-MB-231 cells stably transduced with a β-galactosidase expression construct also transduced with increasing levels of TR construct. Bar indicates increasing level of TR element construct. Bright field images taken after 2 h X-gal stain, 48 h postinduction of expression using 1 μg/mL doxycycline.

causing a frameshift useful for gene knockout studies. Alternatively, homology-based repair may be used to repair TALEN cleaved double-stranded breaks. Exogenous DNA cotransfected with the TALEN machinery can undergo homologous recombination during break repair (Miller *et al.* 2011), which can be used to introduce specific mutations.

An advantage to using this method over lentivirus is reduced off-target effects on the cells, due to the targeted nature of genome editing. Furthermore, apart from toxicity of transfection reagents, TALENs are thought to be tolerated quite well by transfected cells (Miller *et al.* 2011). A drawback to using this method is the relatively low mutation frequency

compared with the high efficiency of viral transduction, which may result in mosaic cell populations. However, this drawback can be overcome using a selectable marker.

Another setback of using this system is the complex nature of the cloning strategy in building the DNA-binding domain of the TALEN. Fortunately, a simplified method has been established in the, which uses recombination cloning of an available plasmid set to build TALENS (Cermak *et al.* 2011).

## Clustered regularly interspaced short palindromic repeats (CRISPRs)

A more recent development in genome editing technology uses another naturally occurring system observed in bacteria and archea as a form of adaptive immunity used to defend against pathogenic exogenous DNA (Wiedenheft *et al.* 2012). This system has been adapted to function in mammalian cells using guide RNA (gRNA) coupled with a humanized form of Cas9, which can effectively target and cleave short genomic sequences (Mali *et al.* 2013). Although multiple systems have been engineered, the most commonly used system originates from *Streptococcus pyogenes*. DNA cleavage is directed to specific target sequence followed directly by the protospacer adjacent motif (PAM) in the format NGG (Jinek *et al.* 2012). This method is rapid, and as with TALENs can use homologous recombination to introduce small genome modifications from donor DNA (Mali *et al.* 2013).

Although the CRISPR-Cas system is targeted to defined loci, off-target effects have been observed recently in a study targeting the beta-globin and CCR5 genes (Cradick *et al.* 2013), possibly due to mismatch binding, as only a single DNA strand is targeted. Methods for detection of off-target CRISPR activity are already available (Sander *et al.* 2013). TALENs require dimerization of two separate motifs, which increases specificity at the expense of efficiency. Another issue with genome editing is the tedious design of DNA-targeting constructs. A CRISPR gRNA design platform has been published along with a database for depositing gene-specific gRNA sequence (Ma *et al.* 2013).

## Summary

Use of lentiviral vectors has become standard practice in most areas of biomedical science, providing a powerful and adaptable platform for modulation of gene expression across diverse cell types. However, caution needs to be taken with experimental design to mitigate or limit off-target effects, and issues with viral integration which may introduce artefacts into the model system. With careful planning and appropriate titration of the viral vector, one can avoid introducing an unacceptable level of experimental artefacts into the model system, although the artificial nature of lentiviral expression systems must be considered when drawing conclusions from experimental data.

This review covers general considerations for transgene over-expression and attenuation or 'knock-down' of gene expression while identifying some potential issues with the systems examined. Although only a few systems are discussed, the general structure and mechanism of this type of vector are largely the same, with promoters and expression conditions often the only variation. Hence, these effects are likely to be relatively consistent across different vector systems.

Recent developments in gene editing technology provide new alternatives to lentiviral transduction, which help reduce off-target effects at the expense of efficiency. The use of gene editing technologies such as TALENs and CRISPRs should be considered on a case-by-case basis, as the required efficiency and the need to reduce off-target effects will vary between studies. As the initial setup of these transgenic systems can be time consuming and expensive, considerable time should be taken in selecting an appropriate platform to avoid wasted time and cost.

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