Improving Reproducibility of Large-Scale Experiments by Using a Pipette+ System

Karsten Krey[1] (karsten.krey@tum.de) and Andreas Pichlmair[1,2] (andreas.pichlmair@tum.de)
1: School of Medicine, Institute of Virology, Technical University of Munich, 81675 Munich, Germany
2: German Center for Infection Research (DZIF), Munich Partner Site, Munich, Germany

Funding information: European Research Council: ProDAP (817798), Deutsche Forschungsgemeinschaft:
PI 1084/3, PI 1084/4, PI 1084/5, TRR179, TRR237, Bundesministerium für Wissenschaft und Forschung: COVINET

Abstract

The studies of biological systems demand well-controlled experiments and high reproducibility. Methodological aspects and the experimental setup should address and control for external factors that could alter the outcome of the experiment. Only the factors directly studied or manipulated should affect the readout. If the experimental design cannot address these external factors, results might be misleading and challenging to interpret. Over the last few years, reproducibility became an increasingly pronounced problem in the field of life sciences. Among the reasons for non-reproducible data, not only do experimental settings contribute as a potential source of error, but also the experimentalist.

One aim of the experimental setup is to avoid interference during an experiment. However, identifying those factors is of equal importance. Pilot studies are conducted to identify any aspects that could interfere with the experiment and that could be utilized to fine-tune the experimental setup. Hence, traceability becomes an effective way of narrowing down the connection between cause and an unwanted effect. To enhance both reproducibility and traceability, many steps in the experiment workflow can be automated by using programmable devices for liquid handling. Fully automatic robots provide optimal reproducibility and complete traceability. However, those machines often present a considerable financial obstacle and demand extensive training of the user. The integration of a semi- automatic liquid handling platform, e.g. programmable pipettes into existing workflows results in fewer user-related errors. Using the Pipette+ system of Andrew Alliance together with the cloud-native solution OneLab, experiments can be quickly designed, operated, and subsequently, fully traced back. Different screening platforms exist to study the function of specific genes in a focused or genome-wide manner, both, however, demand a large-scale experimental setup. Such experiments require a low sample-to-sample deviation with regard to sample preparation and handling. The lower the level of noise introduced by handling samples, the more sensitive the readout of the screen will be. Using programmable devices, e.g. programmable pipettes, can result in better reproducibility and fewer variations between samples compared to a manual workflow. At the same time, error-rates can be decreased, and traceability increased. This poster describes work carried out demonstrating a time saving of >80% using the Pipette+ smart guided pipetting system (vs fully manual pipetting).

Materials & Methods

• Transfer plasmid (e.g. lentiCRISPRv2, Addgene #52961)
• Equipment for molecular cloning to insert a guide sequence specific for the target gene
• 2nd Generation Lentivirus Production system • psPAX2 (Addgene #12260)
• MD2-G (Addgene #12259) • Pipette+ pipettes and stand • OneLab Software

There are diverse ways to deliver the target-specific sgRNA and Cas9 to the target cells. However, using a lentivirus- based system allows for easy workflows, straightforward, reproducible protocols and homogenous transduction efficacy (Figure 1). To achieve similar titers of lentiviruses and thus, similar transduction and genome editing efficiencies, the amounts of individually transfected components of the lentiviral vector system have to be highly similar between samples [1]. At least three plasmids — a transfer plasmid with the desired gene for delivery into target cells, and two plasmids encoding the lentiviral envelope (e.g. pMD2.G) and packaging components (e.g. psPAX2) — have to be mixed to reach a certain, empirically determined ratio of e.g. 4:2:1 [2]. Commonly the transfer plasmid is prepared by molecular cloning techniques and then amplified in a suitable bacterial strain [3,4]. Subsequently, the concentration of the transfer plasmids needs to be normalized to enable rapid workflows. By manual pipetting, this task demands a focused mind over a long period of time, which quickly becomes a limiting factor regarding sample throughout and work accuracy.

This issue can be overcome by using the Pipette+ system during normalization of the transfer plasmid concentrations and combining them with the envelope and packaging plasmids. Once the procedure is set-up using the cloud- native OneLab software platform, the preparation of a defined mix of three plasmids (transfer, envelope, and packaging plasmid) can be easily achieved within 1 hour for 96 samples, compared to over 4.5 hours by manual pipetting (4.6x faster). Using the Pipette+ system, the software guides the user through each step, avoiding user-related errors in pipetting.

In this example, variable amounts of deionized water are added onto a 96-well plate. These volumes were calculated to achieve the same concentration of all transfer plasmids by adding a fixed volume of each plasmid with a multi- channel pipette. From this plate, equal amounts of transfer plasmids are transferred onto a new 96-well plate on which deionized water and a master mix of the envelope and packaging plasmid was added beforehand. Every well then contains a target-specific transfer plasmid and an envelope and packaging plasmid in the desired ratio. From this mix, HEK293T cells can then be transfected using a suitable transfection reagent, e.g. polyethylenimine (PEI).

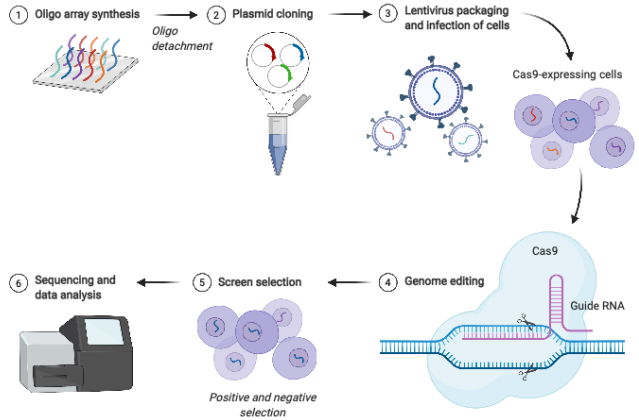

Figure 1: Workflow of a Pooled, Lentivirus-Mediated CRISPR/Cas9 Knockout Screen

A set of oligo-nucleotides has to be generated with a sequence complementary to the targeted gene. (2) The oligonucleotides are then cloned into a transfer vector, compatible with a 2nd generation plasmid system for the production of lentivirus particles. (3) A mixture of the transfer vector, a packaging plasmid, and an envelope plasmid are then transfected into a cell line suitable for lentivirus production, e.g. HEK 293T cells (ATCC® CRL-3216TM). Eventually, generated lentiviruses can be harvested, now containing all necessary factors to transduce target cells in which the sgRNA and Cas9 can then be expressed. Usually, positive or negative selection is applied to select for interesting candidates, which can then be identified by different readouts, e.g. sequencing. Created with BioRender.com.

Discussion

To use all features of the Pipette+ system, the cloud- native virtual lab bench OneLab can be utilized (Figure 2). As described above, the normalization of the transfer plasmids can easily be achieved this way. After cloning and amplification of different sequence-specific transfer plasmids (stored on a 96-well plate), an aliquot of the plasmids is transferred onto a new 96-well plate, with previously added deionized water. This way, the plate contains variable amounts of diluted plasmids, all with the same concentration. Using OneLab, all vessels containing liquids are checked for sufficient amounts to complete all pipetting steps or are automatically determined. Subsequently, the mixture of all three plasmids (concentration-normalized transfer plasmid, packaging and envelope plasmid) needs to be prepared. By using a master mix of the packaging and envelope plasmid, more reproducible results are obtained. This master mix is, together with corresponding amounts of deionized water, added onto the destination plate. In the last step, a fixed volume of the transfer plasmid is added to this plate. Eventually, all three plasmids required for transecting a suitable cell line to generate lentiviral particles are represented at the desired ratio.

Conclusion

Using the Pipette+ system in this scenario allows for the rapid execution of the above-described workflow. As the total time can be reduced by around 4.6 times, this task is less demanding and less strenuous for the user, while at the same time increasing overall efficiency and data quality. As the user is aided through each pipetting step, the frequency of user-related errors is minimized, thus increasing reproducibility. If errors occur, they are logged and traceable. To sum up, the Pipette+ system in concert with OneLab is a handy advancement to conduct large-scale experiments whilst unburdening the user for higher level tasks.

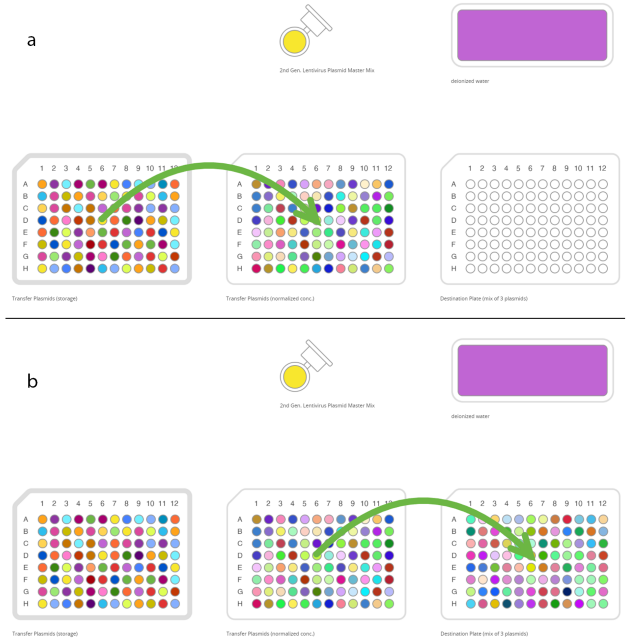

Figure 2: Workflow overview using OneLab

The concentration of the transfer plasmids (e.g. lentiCRISPRv2 with target-specific sequence integrated) is normalized by adding variable amounts of deionized water and fixed amounts of plasmids from the storage plate (a). To obtain a mix of all three plasmids (transfer plasmid, packaging, and envelope plasmid), a master mix, containing the packaging and envelope plasmid, is prepared in advance and a corresponding amount is added onto the destination plate together with deionized water. In the last step, the concentration-normalized plasmids are added onto this plate, now containing all three plasmids in the desired ratio (b).

References

1. Addgene. Lentivirus Production. https://www.addgene.org/protocols/lentivirus-production/.
2. Shalem, O.; Sanjana, N.E.; Hartenian, E.; Shi, X.; Scott, D.A.; Mikkelson, T.; Heckl, D.; Ebert, B.L.; Root, D.E.; Doench, J.G.; et al. Genome-scale CRISPR-Cas9 knockout screening in human cells.
3. Science 2014, 343, 84–87, doi:10.1126/science.1247005.
4. Sanjana, N.E.; Shalem, O.; Zhang, F. Improved vectors and genome-wide libraries for CRISPR screening. *Nat. Methods* 2014, 11, 783–784, doi:10.1038/nmeth.3047.