To further increase viral titer, we also cloned a two-vector system, in which Cas9 (lentiCas9-Blast) and sgRNA (lentiGuide-Puro) are delivered using separate viral vectors with distinct antibiotic selection markers (**Fig. 1a**). lentiGuide-Puro had an ~100-fold increase in functional viral titer over that of the original lentiCRISPRv1 (**Fig. 1b**). Both the single- and dual-vector systems mediated efficient knockout of a genomically integrated copy of EGFP in human cells (**Supplementary Fig. 1**). Whereas the dual-vector system enables generation of Cas9-expressing cell lines that can be subsequently used for screens using lentiGuide-Puro, the single-vector lentiCRISPRv2 may be better suited for *in vivo* or primary-cell screening applications.

We also designed and synthesized new human and mouse GeCKOv2 sgRNA libraries (**Supplementary Methods**) with several improvements (**Table 1**). First, for both human and mouse libraries, to target all genes with a uniform number of sgRNAs, we selected six sgRNAs per gene distributed over three or four constitutively expressed exons. Second, to further minimize off-target genome modification, we improved the calculation of off-target scores on the basis of specificity analysis[5]. Third, to inactivate microRNAs (miRNAs), which play a key role in transcriptional regulation, we added sgRNAs that direct mutations to the pre-miRNA hairpin structure[6]. Finally, we targeted ~1,000 genes not included in the original GeCKO library.

Each library, mouse and human, is divided into two sublibraries, each containing three sgRNAs targeting every gene as well as 1,000 nontargeting control sgRNAs. Screens can be performed by combining both sublibraries, yielding six sgRNAs per gene. Alternatively, individual sublibraries can be used in situations in which cell numbers are limiting (for example, with primary cells or *in vivo* screens). We cloned both human and mouse libraries into lentiCRISPRv2 and lentiGuide-Puro and sequenced them to ensure uniform representation (**Supplementary Figs. 2** and **3**). These new lentiviral vectors (see **Supplementary Data** for full sequences) and libraries further expand the GeCKO toolbox for diverse screening applications. Reagents are available to the academic community through Addgene (lentiCRISPRv2: 52961; lentiCas9-Blast: 52962; lentiGuide-Puro: 52963; human GeCKOv2 in lentiCRISPRv2: 1000000048; human GeCKOv2 in lentiGuide-Puro: 1000000049; mouse GeCKOv2 in lentiCRISPRv2: 1000000052; mouse GeCKOv2 in lentiGuide-Puro: 1000000053). Associated protocols, support forums and computational tools are available at http://www.genome-engineering.org/.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper (doi:10.1038/nmeth.3047).

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AUTHOR CONTRIBUTIONS

N.E.S., O.S. and F.Z. conceived of and designed the experiments. N.E.S. and O.S. performed the experiments and analyzed the data. N.S., O.S. and F.Z. wrote the manuscript.

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The authors declare competing financial interests: details are available in the online version of the paper (doi:10.1038/nmeth.3047).

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iPipet: sample handling using a tablet

To the Editor: Biological experiments increasingly involve large numbers of specimens, making liquid handling in these experiments a challenge. We and other groups previously devised high-throughput experimental designs using combinatorial pooling schemes that reduce experiment costs but require complex pipetting steps according to mathematical patterns[1–3]. We used a liquid-handling robot to execute experiments with bacteria[4], but we found that using a robot with sensitive human samples has several caveats and inherent limitations, such as occasional robotic failures, dead volume (inability to aspirate liquid close to the bottom of the well) and bending or clogging of tips owing to plate septum piercing that risked finite samples. In addition, liquid-handling robots are quite expensive and require trained personnel to operate them.

Several devices offer semi-automated solutions for pipetting complex protocols that mainly consist of a programmable LED panel with lights under the wells of microtiter plates that guide pipetting (**Supplementary Table 1**). But these devices support a relatively narrow set of designs, have minimal visual cues and do not display volumes. In addition, their price range is about $1,000–$2,000.

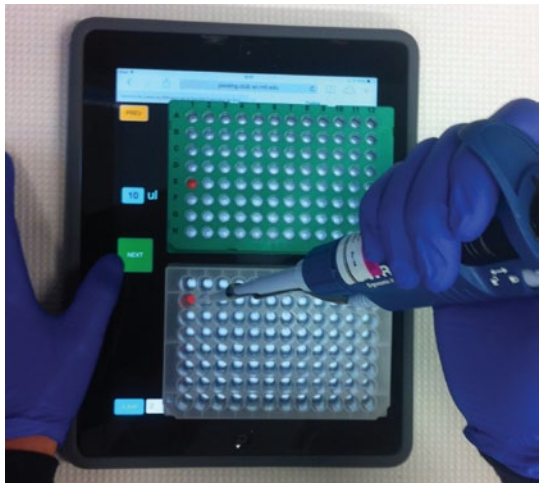

Figure 1 | A bird's-eye view of the iPipet run screen with 96-well plates.

Here we present iPipet, a highly adaptable solution for semi-automated liquid handling using a tablet computer (**Fig. 1** and **Supplementary Fig. 1**). Our solution is a free web service that allows users to upload and execute complex pipetting patterns in 96-well or 384-well plates with either a single-channel or multichannel pipet. The application is hosted on our public lab website (http://ipipet.teamerlich.org/) and requires only a CSV file detailing the experimental design. The website includes several examples and a video to demonstrate how to use iPipet. The website also allows users to publicly share pipetting designs, which can enhance experiment reproducibility and create easy-to-follow protocols. The entire iPipet project is freely available for further development by the community on GitHub under a GNU General Public License (GPLv3).

A simple solution for fixing the plates on the tablet is to cover the screen with cling wrap. For more frequent users, we also created and tested an adaptor to hold the plates on the screen (**Supplementary Fig. 2**). The design files are freely available on the iPipet website under a Creative Commons Attribution–Share-alike license. Users can create their own adaptors using a three-dimensional (3D) printer for less than $50.

We tested iPipet using a standard iPad in several experiments involving complex liquid-handling patterns. (**Supplementary Note**) With fixed-volume pipetting, 2,880 pipetting steps took ~7 h. After considerable optimization to minimize variation in pipetting, a Tecan Evo liquid-handling robot with a 4-tip cherry-picking arm accomplished only half of the pipetting steps in the same amount of time. In addition, similar to previous results[5], we found that the precision of the pipetting volumes of the robot, with similar pipetting conditions, was lower than that with the manual design (**Supplementary Data**). As such, iPipet is an easily accessible assistive technology that can facilitate reproducible and accurate experiments.

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