Plasmids: shopping in the age of plenty

Vivien Marx

Managing, sharing and controlling the quality of plasmids can be simple. But many techniques to disseminate them do not scale, which leads nonprofits and companies to explore new options.

There are many ways to 'shop' for plasmids, the extrachromosomal, circular DNA found in bacteria and some eukaryotic cells. Plasmids accept and easily replicate snippets of foreign DNA, making them versatile vectors for cloning and manipulating genes in the lab.

The do-it-yourself route to getting a desired gene into a plasmid is to reverse transcribe its corresponding mRNA and clone it into a plasmid vector. But researchers can also obtain a plasmid containing a gene or other DNA element from colleagues or from nonprofit or commercial repositories. In the hunt for plasmids or plasmid libraries, scientists encounter a few headaches. The same is true when managing, controlling the quality of and sharing plasmids. Here some scientists share their experiences and recommendations for addressing some challenges, especially those related to large plasmid collections.

Matthew Coussens/Sigma-Aldrich

Elise Eckels, a researcher at Sigma-Aldrich, chooses a plate from the TRC shRNA collection. She trains scientists on the collection's use and maintenance.

Finding what you need

One 'go-to' repository is the nonprofit organization Addgene, which has just celebrated its ten-year anniversary and offers more than 30,000 plasmids from almost 2,000 labs around the world. Depending on the number ordered, each plasmid costs between $45 and $65, plus shipping.

It is free to deposit plasmids with Addgene, which keeps triplicate samples of each plasmid, one at a separate site. Scientists at nonprofit institutions and in academia can order from the entire collection; commercial customers can choose from part of it. In 2013, Addgene shipped 90,000 plasmids and is on track to ship more than 110,000 this year. In 2013, more than 12,000 of the shipped plasmids were for gene-editing experiments using CRISPR (clustered regularly interspaced short palindromic DNA repeats) and the nuclease Cas9.

Neuroscientist Thomas Knöpfel, who creates optogenetics tools at Imperial College London, praises Addgene as "great" and says it is where he deposits his published plasmids. He also sends plasmids directly from his lab to users—before publication, for example, or when he hopes other groups might find additional applications for them. In his view, "plasmid sharing is as important as open access of publications." He also thinks that funding agencies should mandate sharing and that deposition needs to be mandatory when papers are accepted for publication. (Many publishers, including Nature Publishing Group, require plasmid deposition.)

Nicolas Casadei, a molecular biologist at the University of Tübingen, could not find the two small plasmids he needed in Addgene's collection, so he went hunting for them. He received three for the first vector—one from an academic lab and two from companies. For the second vector, he received one from a university and one from a company. Of the five, only one vector—from a commercial source—passed the Sanger sequencing quality-control (QC) step, he says. He made the missing gene fragment by reverse transcription and then inserted it into the plasmid, which was not difficult but cost him time he had been trying to save.

Casadei received a refund for one of the commercial orders; the other lacked a warranty. The experience led him to do everything by reverse transcription and to order long constructs such as bacterial artificial chromosomes or yeast artificial chromosomes only when they have an explicit warranty.

Catherine Seiler, who runs the DNASU plasmid repository at Arizona State University, has had a similar experience. "I ordered a plasmid and received a completely different gene than what I expected,"

David Fox Photography/Addgene

Addgene, where scientists deposit plasmids for free, has just celebrated its ten-year anniversary with a gala.

she says. The lesson is that sequence verification is an "unavoidable" step for researchers and avoids later headaches. Melina Fan, Addgene's outreach scientist, agrees and says the QC sequencing step is "way shorter than the actual experiment."

Plasmid identity issues can occur in many ways; labels can go missing between the time that plasmids are put in the freezer and the time months or years later when a request for it comes in to a lab. Joanne Kamens, director of Addgene, says that the organization routinely 'spot sequences' plasmids with a few primers to confirm the identity of the plasmid and features of its insert such as the presence of mutations, reporter gene fusions or immune tag sequences. And she recommends that scientists practice careful science and verify their plasmids with, for example, restriction digest analysis.

The majority of plasmids in the DNASU repository are at least partially sequence verified. Typically, only part of the plasmid—the gene insert—is sequenced. DNASU also stores materials used to solve a particular protein structure generated by the Protein Structure Initiative (PSI), and those plasmids are sequenced in full so that they will be verified, she says. Seiler says sequencing and automation help to reduce human error when managing plasmids at DNASU. For the 248,000 plasmids distributed thus far, fewer than 1% have generated complaints, and in almost all of these cases the issues were resolved and the researchers ultimately received the correct plasmid.

Although errors can happen in a depositor's lab, both Addgene and DNASU say that most depositors are diligent when preparing their plasmids and offer detailed information so that it will be clear in the database and colleagues who want to use their plasmids can start experiments quickly.

At DNASU, plasmid stocks are stored as bacteria in a glycerol solution at –80 °C. Each plasmid is stored in a tube bearing a unique barcode that is linked to all of the information about that plasmid—such as gene name, sequence, growth conditions and links to external experimental data—in the database. "This information is nearly as important as the plasmid itself, Seiler says, adding, "what would a collection of wine be worth if the labels on the bottles were removed?" When a researcher places an order, the freezer's automated robots use the barcode to 'pick' each plasmid. The final part of QC is to grow bacteria from the glycerol stock to make sure the cells are still viable and grow as expected.

The Addgene team has similar procedures in place and curates the information associated with each plasmid. That task can take sleuthing in some cases, such as when a plasmid was made by a postdoctoral fellow who is no longer at the lab that is depositing the material.

Plasmid collections

Addgene is currently processing a large deposit from a lab that develops fluorescent proteins, and its staff will collaborate with any lab seeking to share a plasmid library. "We work very closely with them to bring in that data," says Fan. "The earlier we can work with them, the better," says Kamens.

Addgene has mid-sized plasmid libraries, such as a kinase library with around 600 plasmids. These libraries may include different plasmids arrayed in a 96-well format, or they might be pooled libraries, with many plasmids in one tube. Researchers choose which one works best for their experiment type.

QC for pooled libraries, which may include tens of thousands of plasmids, is a challenge, says Kamens. Labs that do not have a lot of equipment at their disposal can use pooled libraries for screening studies and then validate 'hits' with sequencing, rather than try to sequence the entire library, she says. Addgene sequences a subset of each plasmid library to ensure that there is diversity of gene sets and to make sure that the types of genes that are expected are actually represented in the library.

In addition to the PSI plasmids, DNASU has human gene sets from the ORFeome Collaboration, which are full open-reading-frame cDNA clones. Other sets include plasmids containing genes thought to be involved in breast cancer and whole-genome collections from yeast and various pathogens.

The RNAi Consortium (TRC), headquartered at the Broad Institute, is a public-private venture tasked with creating RNA interference (RNAi) constructs to target all human and mouse genes[1]. It includes other institutions such as Massachusetts General Hospital, the Whitehead Institute for Biomedical Research, the Dana-Farber Cancer Institute and the Ontario Institute for Cancer Research and companies such as Johnson & Johnson, Bristol-Myers Squibb and Sigma-Aldrich.

The TRC's first project involved creating a lentiviral short hairpin RNA (shRNA) library, and other efforts have ensued. The TRC collection contains 300,000 different shRNA clones, which target approximately 20,000 genes each in the human and mouse genomes.

David Root, who directs the TRC, is on Addgene's board of directors, and his expertise is helpful to Addgene as high-throughput experiments become more common, says Kamens. Quality control for libraries at the Broad Institute is handled with second-generation sequencing, which allows scaled-up experiments.

Sigma-Aldrich provides the TRC collection to academic, nonprofit and commercial customers, the majority of whom order slices of the collection that pertain to genes of interest for a given experiment. Another company that distributed an early subset of the TRC collection is Open Biosystems. The company is now owned by the Dharmacon division of GE Healthcare Life Sciences, which distributes bacterial glycerol stocks of the Open Biosystems subset of TRC clones. Handling the TRC collection has involved much organization and automation, says Shawn Shafer, a molecular biologist at Sigma-Aldrich.

Shafer has supervised the preparation, manufacture and QC of the TRC collection for the company and now manages the product line, the Mission TRC shRNA collection. Sigma-Aldrich distributes the entire TRC collection of 300,000 shRNAs and many other TRC reagents. It is sold in three different forms: glycerol stocks of bacteria carrying the plasmid that expresses the shRNA; extracted, purified DNA; or DNA packaged into virus.

The company created a dedicated lab for these tasks and has developed methods along the way, says Shafer. Culturally, offering the collection has been a meeting of different worlds. The Broad Institute took on a large manufacturing challenge to produce around 300,000 individual shRNA clones. And Sigma-Aldrich, a chemical manufacturer, became part of an academic and biology-focused enterprise to handle clones, which are not "Legos in a dish" but growing, living entities, says Shafer. In addition to the threat of clones dying is that of cross-contamination.

Sigma-Aldrich joined the TRC because the company held lentivirus and shRNA patents and wanted to offer the technology. "It was right in our wheelhouse," Shafer says. The TRC library has evolved in terms of data annotation and biology. For example, he says, the latest version allows for more possibilities to target a single isoform of a gene transcript, to study that isoform's role in a disease.

DNASU

At DNASU, the BioStore freezer is equipped with a robotic arm to retrieve, track and 'cherry-pick' samples from its plasmid collection.

In readying the library for distribution, the Sigma-Aldrich team developed small- and large-scale techniques to minimize cross-contamination. "All it takes is one bacterium to move to the neighboring well to cross-contaminate it," says Shafer. But thousands of plates needed to be opened, and bacteria can be aerosolized immediately upon removal of a plate's heat seal. The team made it a habit to turn plates upside down to remove their heat seals. This step directs any aerosol downward, Shafer says.

On a larger scale, all plates and tubes were barcoded and scanned at each step, tracking each removal from and return to the freezer. To further minimize the chance of cross-contamination, plates were replenished from stock after ten freeze-thaw cycles.

Shafer now guides scientists who buy and use the library, which can cost a six-figure sum. That guidance includes interacting with researchers in their darker hours. One large university bought a library of 1,200 plates. "They called us about six months later in a panic," he says, and that was when he learned that the scientists had not heeded the company's recommendations. The researchers had put the library in the freezer and opened its use to all the lab's scientists and students.

When the principal investigator checked on the library, some plates were missing the heat seal and in others liquid in the wells had moved around, cross-contaminating wells. "It was literally destroyed," says Shafer about the library, which the company helped the researchers replace. He has heard that the researchers have put constraints in place, such as limiting access to the library and instituting plate-tracking systems.

Handling libraries is as much a scientific challenge as an organizational one, he says. The Sigma-Aldrich staff help scientists troubleshoot issues that come up in handling libraries, such as clones not growing or low DNA yields. Lab experiences and recommendations flow back to the company, and staff members continually update the guidelines that are distributed with the library.

Meghan Moore, Megpix/Addgene

Addgene will ship more than 110,000 plasmids this year. Last year, more than 12,000 shipped plasmids were for CRISPR-based gene-editing experiments.

Different distribution

Plasmids obtained from companies tend to carry a label that prohibits sharing. That is not a policy set by Sigma-Aldrich, says Shafer, but it is usually connected to the license and royalty provisions regarding that plasmid or library, which may have originated in an academic lab. Collaborating institutions can obtain licenses to allow sharing among the partners, he says.

Although he appreciates the reasons for limiting sharing, it creates a vicious cycle, says Jiwu Wang, CEO of Allele Biotechnology and Pharmaceuticals, which develops molecular biology reagents and offers advice to scientists for their experiments. He believes that commercial distributors know scientists will violate the request to not share. In anticipation of this behavior, companies raise the plasmid price, motivating violation of the policy by researchers who dislike the price. Wang has launched a different kind of distribution model encouraging scientists to "share as broadly as they possibly can," he says.

The vectors are not sold but licensed at around one- to two-thirds of the sales price from a traditional distributor, says Wang. The price depends on whether a license includes all vector formats and ready-made fluorescent fusions. If multiple labs are using the plasmids in collaboration, each lab needs a license.

Wang says this approach allows the company to encourage sharing but not 'cannibalize' its own innovation in the process. The company has sold nearly 100 licenses for the fluorescent protein mNeonGreen, developed by Allele researcher Nathan Shaner and his group, who previously developed the fluorescent proteins mCherry and tdTomato. Allele's distribution model delivers a revenue stream so the company can continue funding research and tool development by Shaner's team.

Sustained growth

DNASU's Seiler says she hopes that many of the resources connected to the PSI program will continue to receive funding through other mechanisms after the current PSI funding soon ends, because the value of such large programs is difficult to recreate once they are dismantled.

DNASU's PSI-related materials repository includes nearly 85,000 plasmids made by PSI researchers. To be sustainable, DNASU charges users a small fee for plasmids, which covers technician salaries and consumables and allows DNASU to archive and distribute plasmids, including those from the PSI, after the end of this grant period.

Biodesign Institute, Arizona State University

Sequence verification of plasmids is an unavoidable step that avoids later headaches, says Catherine Seiler.

But the PSI grant has been funding DNASU's sequence verification of newly deposited plasmids, clone archiving, management and maintenance, freezer storage system and website. Seiler says that she and her team will be looking for other sources of funding to support the growth of DNASU.

In the United States and Europe, grant-funding agencies are withdrawing funding for the "maintenance stage" of repositories, says Kamens. "Many of them are scrambling to figure out how they're going to keep going forward."

Small repositories generally have difficulty securing funding to maintain their collections. When finances are tight, scaling and growing becomes harder, says Kamens. These repositories can also run into logistical challenges, such as those involved in shipping materials to other countries. Kamens is in contact with many repositories to help them explore how they might use Addgene's self-sustaining model. In the case of older repositories there is reticence on the part of users to pay for services, she says, but "the money has to come from somewhere." One nascent idea she and others are exploring is to create a consortium of repositories that use Addgene's distribution model to maintain them and keep costs low.

Addgene has just opened an office in London to better serve European scientists, and it also has distributors in China and Japan to help with logistics such as customs. All materials are shipped from Addgene's headquarters in the United States, but having a London office will increase Addgene's visibility in Europe, says Fan. Offering support to scientists on the phone or in person is important in Europe, where around one-quarter of requests for Addgene materials originate.

The volume of requests from around the world to Addgene is "increasing dramatically," says Kamens, and the nonprofit has been hiring additional staff to address users' science-related and logistical questions. "We're not about storing things, we're about sharing them," she says. Sharing plasmids in an age of plenty can help scientists with many new experiments, as long as QC and management approaches scale with the demand.

1. Moffat, J. *et al. Cell* **124**, 1283–1298 (2006).

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