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Open Sharing During COVID-19: CRISPR-Based Detection Tools

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Addgene is facilitating the availability and open sharing of plasmids and resources for COVID-19 research and detection of SARS-CoV-2 RNA

The Coronavirus Disease 2019 (COVID-19) pandemic is yet another reminder of the need for rapid and open sharing of scientific information. While the pandemic threatens to overcrowd hospitals and strain our diagnostic capabilities, scientists are working to understand the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus and advance diagnostics and treatment. Within weeks of COVID-19 spreading in the United States, two CRISPR labs were poised to develop CRISPR diagnostic tools for COVID-19 detection.

The Start of CRISPR-based Diagnostics

These labs—one led by Feng Zhang at the Broad Institute, the other by Jennifer Doudna at University of California Berkeley—independently developed CRISPR-based nucleic acid detection tools in 2017[1] and 2018.[2] The ultimate goal of these tools is fast, point-of-care diagnostics that do not require specialized equipment. Other labs soon followed, developing related CRISPR-based assays made possible through open sharing of materials and protocols.

The discovery of the indiscriminate nucleic acid cutting ability of Cas13[1] and Cas12a[2] led these teams to develop detection and diagnostic tools. Cas12a and Cas13 become activated when they bind their target nucleic acids, DNA and RNA, respectively. Upon binding the target, the proteins begin cutting other nearby nucleic acids—a perfect opportunity to use guide RNAs that target pathogen-specific nucleic acids to unleash cutting of a quenched fluorescent probe resulting in fluorescence. Zhang's lab named their Cas13-based method SHERLOCK (Specific High Sensitivity Enzymatic Reporter UnLOCKING), while the Doudna lab named their Cas12a-based method DETECTR (DNA Endonuclease Targeted CRISPR Trans Reporter).

Both labs shared these tools with the research community through Addgene, the nonprofit plasmid repository. Reagent sharing to speed scientific progress is at the heart of Addgene's mission and has been instrumental to the democratization of CRISPR.[3] Without such a resource, labs such as Zhang's would otherwise be swamped fulfilling requests.[4] Plasmids from SHERLOCK and DETECTR have been requested hundreds of times by the scientific community. Overall, as of April 2020, Addgene has distributed more than 180,000 CRISPR reagents to 4,200 institutions in 87 different countries.

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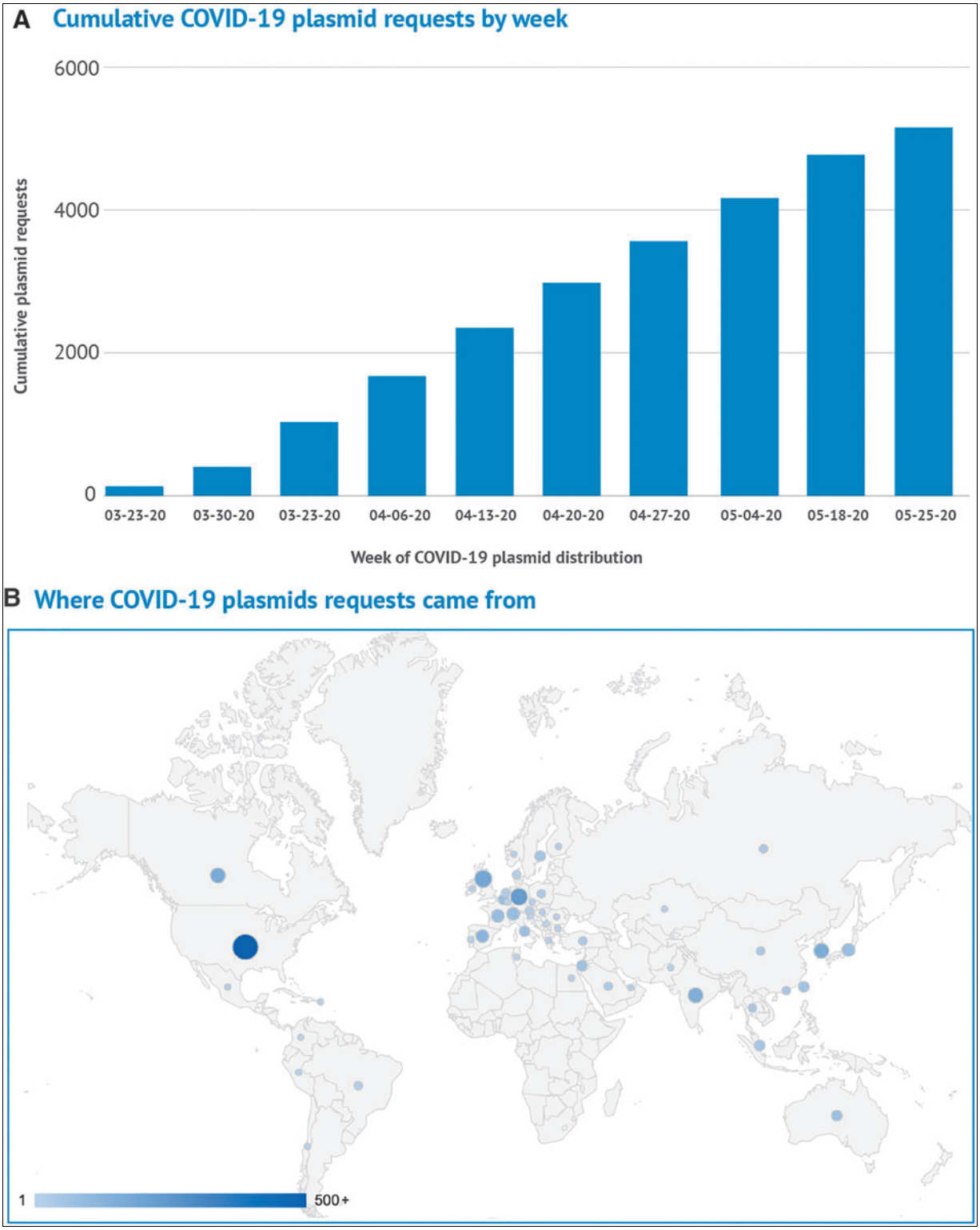


Fig. 1. Global reach: The distribution of plasmid requests via Addgene for COVID-19 research. **(A)** Cumulative COVID-19 plasmid requests by week since March 23, 2020. **(B)** Countries that have requested COVID-19 plasmids. These numbers include requests that have not yet been shipped due to worldwide lab closures (~10%). The United States accounted for ~3,000 of total requests.

Building an Arsenal of COVID-19 CRISPR Diagnostic Tools

In the midst of the COVID-19 pandemic, we are gratified to see an explosion of new CRISPR diagnostic tools built upon Cas12 and Cas13. Early on, the Zhang lab developed a SHERLOCK-based protocol applicable to purified RNA[5] with a paper strip readout for fast diagnostics. The lab later optimized the SHERLOCK-based method into a one-pot format for SARS-CoV-2 detection called STOP (SHERLOCK Testing in One Pot) Covid.[6] Starter kits for SHERLOCK and STOPCovid are available through the lab for scientists interested in developing and deploying the assays (see the accompanying ''First Cut'' by Keith Robison).

Scientists around the globe have adapted these Cas12- and Cas13-based tools for SARS-CoV-2 detection, trending towards assays that are low cost, quick, and require no special equipment. For example:

- Changchun Liu's lab (University of Connecticut Health Center) developed an all-in-one dual CRISPR-Cas12a assay,[7] where detection can be read under no excitation light, under UV light, or under LED blue light.
- A team from CASPR Biotech and University of Buenos Aires, Argentina, developed a Cas12-based detection method where the sample comes from saliva[8] and not a nasal or throat swab.
- Using equipment created from the DIY-bio movement, University of California Santa Barbara scientists developed a Cas13a-based assay called CREST (Cas13-based, Rugged, Equitable, Scalable Testing).[9]

The COVID-19 pandemic is not the first time that scientists have built upon Cas12- and Cas13-based detection systems for disease diagnosis. Open sharing has helped scientists develop CRISPR diagnostics for many infectious disease outbreaks. Using the DETECTR plasmids deposited at Addgene, an international research team developed a point-of-care microfluidic device using Cas13a to rapidly detect Ebola virus RNA[10] within 5 minutes. That same year, scientists also developed an on-site avian influenza A H7N9 nucleic acid detection[11] based on DETECTR plasmids.

Plasmid Sharing During COVID-19

As a major facilitator of community sharing of molecular biology reagents, Addgene has built up a collection of plasmids for SARS-CoV-2 research in recent months. Less than 2 weeks after COVID-19 was declared a pandemic by the World Health Organization, Addgene published a COVID-19 resources page, highlighting plasmids for COVID-19 research and related CRISPR tools.

As of May 31, 2020, Addgene had received approximately 2,100 plasmids to be shared openly for COVID-19 research. Eleven of these plasmids are CRISPR nucleic acid detection tools deposited within the past few years, while a dozen are gRNA plasmids that target host genes. Addgene has also increased outreach efforts and streamlined the intake and quality control processes to quickly bring in new, high-quality SARS-CoV-2 plasmids (the average turnaround time for COVID-19 plasmids is two weeks from receipt to availability for distribution).

To speed research further, a subset of these plasmids is available to scientists working in both academia and industry. Of the 11 CRISPR detection plasmids, nine are available to industry scientists. The COVID-19 pandemic has spurred institutions to use expanded sharing agreements for COVID-19 research, increasing Addgene's ability to send plasmids to all researchers working on new solutions during this crisis.

As in the early days of CRISPR, Addgene has been instrumental in disseminating these resources globally. From March 23 to May 31, 2020, Addgene received about 5,300 COVID-19 plasmid requests (Fig. 1A) from ~50 countries (Fig. 1B). Of these, 94 plasmid requests (from 27 countries) are for CRISPR plasmids that are part of the COVID-19 collection. As with the widespread sharing of CRISPR tools in previous years, it's likely that as labs reopen, many will adopt these tools to study coronaviruses and build new diagnostic tools using the reagents from Addgene's COVID-19 collection.

The COVID-19 pandemic reminds us that CRISPR tools are fast and easily adaptable to many systems. The flexibility of CRISPR tools combined with the rapid dissemination through centralized repositories and other sharing measures has contributed to the swift development of CRISPR detection methods during the COVID-19 pandemic. Like many CRISPR tools, the reach of these detection tools is broad and has the power to transform many areas of disease detection and diagnostics.

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