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Use of the Singer ROTOR+ for Chlamydomonas Reinhardtii Cell Culture

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INTRODUCTION

The ROTOR+ was initially designed primarily for use in budding yeast *(Saccharomyces cerevisiae)* genetics. We are carrying out broad genetic screens in the green alga *Chlamydomonas reinhardtii*, for which the ROTOR+ technology would be very useful. However, *Chlamydomonas* differs from budding yeast in cell size, growth rate, colony morphology and adhesiveness, and light responsiveness. Therefore, it was important to test specifically if the ROTOR+ technology would work with *Chlamydomonas*.

In this Applications Note I address the following issues:

1. *Are cells reliably picked up from liquid or solid medium onto the RePads (replica-plating pads for the ROTOR+); are the cell numbers/density reasonably reproducible between pins, between pads and between plates?*

   Reproducible pickup and dispensing in terms of number of cells will depend on surface properties of the cells; how clumpy or sticky they are, how much they stick to (or are repelled by) the transferring surface, etc. *Chlamydomonas* is different from yeast in these properties. For example, in replica-plating using velvets (standard method for yeast), *Chlamydomonas* colonies have a strong tendency to either stay entirely on the plate or transfer entirely to the velvet.

2. *Do cells retain high viability through this procedure?*

   Independent of number of cells transferred, it is obviously desirable for high viability to be retained in the transferred cells. It is clear from our initial work that in many respects *Chlamydomonas* is more susceptible to damage during some operations than yeast or *E. coli*. For example, when picked from agar onto a glass or stainless steel needle, *Chlamydomonas* will lose viability almost completely after ~40 seconds in the air.

3. *Is there significant cross-contamination between pins or across the source or target plates?*

   Depending again on properties of the organism, it could easily be imagined that a spray of viable cells could be spread by ROTOR+ replicating operations, resulting in cross-contamination and ruined experiments.

4. *What density of colonies is the maximum that can reliably be plated and distinguished using the ROTOR+?*

   The maximum density that the colonies can be plated is dependent on the size and the growth rate of the organism, and will affect the throughput of the screens that can be performed on the organism.

APPLICATION: LIQUID-TO-AGAR TRANSFER

I used Singer PlusPlates©, filled with 50 ml of TAP agar (standard Chlamydomonas medium) supplemented with 50 µg/ml ampicillin to suppress bacterial contamination. Because *Chlamydomonas* is highly motile in liquid medium and also highly light-responsive, we removed the interior lights from the ROTOR+, and kept the cover closed during operations to the extent possible. Without this precaution, the algae strongly aggregated in different regions of the source plate, obviously confounding reproducibility of spotting across the plate.

Using an open plate filled with 25 ml of liquid TAP medium, containing wet *Chlamydomonas* (cc-124 background) at ~10^6 cells/ml, we made liquid-to-agar transfers using the long-pin 384 RePad©, in a 384 -> 1536 arraying pattern (revisiting the source between pinnings). Plates were incubated for 3 days at 33°C under strong illumination *(Figure 1A)*, and were examined microscopically at intervals.

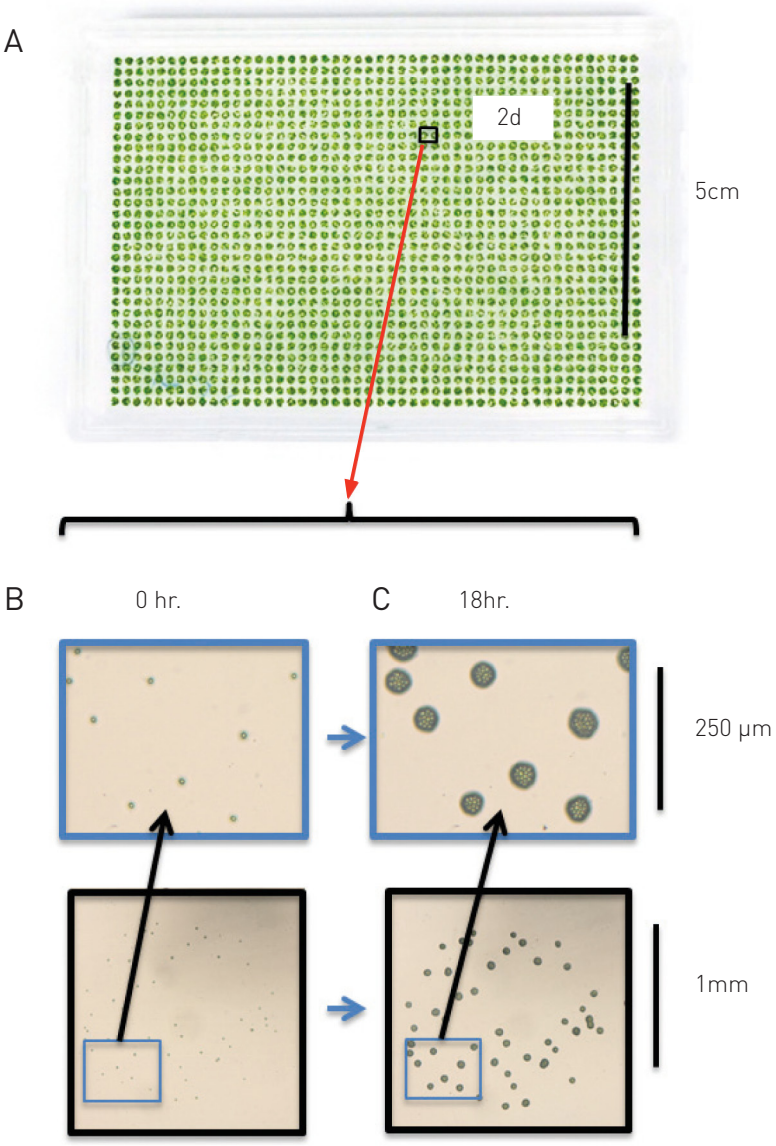

Figure 1: Liquid-to-agar transfer.
(A) Photograph of a plate showing 1536 60 nl drops of *Chlamydomonas* suspension spotted with 384-long RePad© after 2 days incubation. The photograph shows uniformity across spots.

B) Micrograph of region in 1 of 1536 drops after plating (0 hr.) at high- (top) and low- (bottom) magnification.

(C) Same region after 18 hours incubation (right) at high- (top) and low- (bottom) magnification. Viability is >95% (all cells form microcolonies).

Microscopic examination showed that the pinned spots were approximately 1.5mm in diameter, with fairly random distribution of cells across the spot *(Figure 1B)*. Microscopic counts of cells per spot plated indicated 62 +/- 11 cells per spot (mean +/- sd; n=56). The error is greater than predicted for a Poisson distribution, implying variability in actual volume transferred; a rough calculation suggests a volume distribution of ~60 +/- 10 nl, with occasional outliers.

High viability is evident since essentially every cell plated formed a microcolony by 18 hrs. *(Figure 1C)*. A macroscopic image of the plate demonstrates reproducibility across the pinned area. Good reproducibility between pinned plates was also observed (data not shown).

At this pinning density there was clear spatial separation between spots, with no evidence of contaminating colonies in between.

In other experiments we have observed sporadic occurrences of 'heavy' pinning events, where a volume of ~120 nl is transferred. When this occurs, it is plate-wide, but specific to an individual pinning event. Because of the sporadic nature of these occurrences we have been unable to determine how they come about. It is not reproducible for a given RePad©, source or target plate.

The ROTOR+ requires ~5-10 sec between pickup and plating. To determine viability of *Chlamydomonas* held in air on the RePad©, I paused operation after pickup and before plating for an additional 0, 10, 20, and 40 seconds. The number of cells transferred and their viability on incubation remained essentially unchanged.

I tested liquid-to-agar transfer from a Singer plate containing 50 ml of cell suspension at the same density rather than 25. Results were essentially identical, indicating that the effective drop size is largely independent of the depth of liquid into which the pin is inserted.

APPLICATION: AGAR-TO-AGAR TRANSFER

A lawn of cc-124 background *Chlamydomonas* on a Singer PlusPlate© containing TAP agar was used as a source. A 384 long-pin RePad© was used to pick up and transfer cells to a fresh plate, pinning 16 consecutive spots without a revisit to the source. This results in 6144 spots, in sets of 16 that progressively dilute the inoculum. These plates were examined microscopically, and incubated to grow up colonies.

Figure 2 shows images of the spots. Spot diameter is ~1 mm. The sequential pinnings result in progressive diminution of the number of cells transferred, although this effect is quantitatively irregular; thus, this method can be used to reduce inoculum density down to isolated single cells, although the dilution factor per strike is variable. Cell viability upon agar-to-agar pinning is generally high, probably comparable to liquid-to-agar pinning.

In general, both individual cells and resulting microcolonies and colonies stay within spatial borders dictated by the pin geometry, because *Chlamydomonas* is non-motile on plates, and the procedures are accurate with little effective spray or aerosol. Therefore, these procedures are suitable for effective parallel transfer of large numbers of individual *Chlamydomonas* cultures, in a very space- and materials-efficient format.

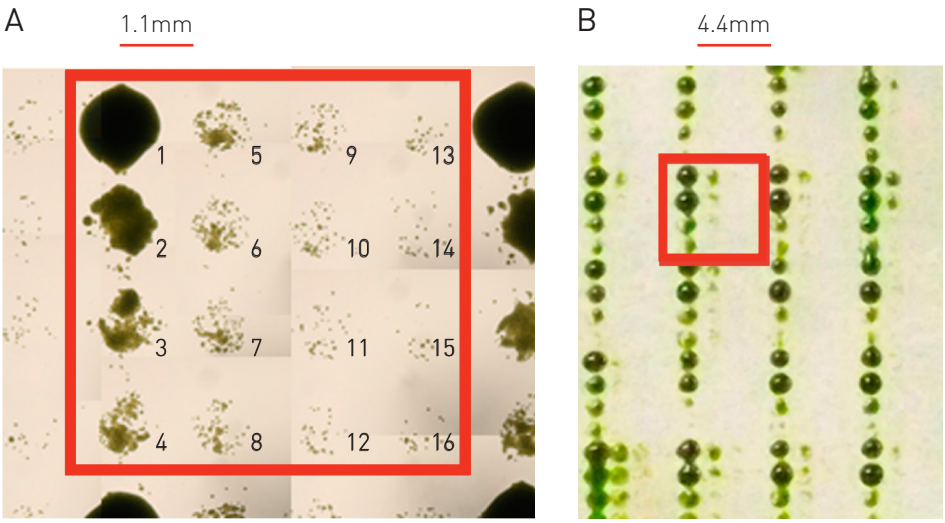

Figure 2: Agar-to-agar transfer. A lawn of *Chlamydomonas* was pinned with a 384-long RePad, and cells were pinned to a fresh plate 16 times without a revisit.

(A) Micrograph (montage) of cells immediately after pinning; progressive dilution can be seen.

(B) A region of the plate after 2 days incubation. The effects of dilution are detectable as differential amounts of growth.

CHLAMYDOMONAS CAN BE CONSISTENTLY AND RELIABLY PINNED AT 6144 DENSITY

We are interested in the maximum density at which parallel cultures of *Chlamydomonas* can be maintained using the ROTOR+ technology. A *Chlamydomonas* lawn was used as a source for pinning with the 6144-density RePad©. This resulted in quite even transfer of small inocula onto the target plate, with no evidence of contamination between spots *(Figure 3A)*. These 6144 individual cultures grew well into well-separated 0.5 mm diameter colonies *(Figure 3B)*. The grown-up 6144 array was used as a source for a subsequent pinning 1 day later, and excellent alignment of the grown array and the subsequent pin-strikes was observed *(Figure 3C)*.

Overall, the 6144-density is usable with *Chlamydomonas*, and promising for future work; probably the main modification compared to work at 96 to 1536-density is the need for flat plates (without the usual meniscus at the edges and corners, and also lacking any other irregularity in the surface) since at 6144-density this can seriously perturb transfer either across a region, or in individual spots.

Figure 3D shows the result of 16-fold pinning (with revisit) of a 384-array of randomly selected *Chlamydomonas* mutants (UV mutagenesis). Strong differences in growth rate are obvious among the mutants, and these differences are highly reproducible across the 16 replicates. This suggests that 6144-density might be usable with a highly complex assembly of different genotypes, on one plate.

6144-density is, in my opinion, completely unworkable with standard microbiological methodologies (velvets, toothpicks, etc) because the spatial resolution of these methods (even for expert workers) is seldom at the sub-mm resolution needed at this high density. Therefore, the ability to work at this density is a unique advantage of the ROTOR+.

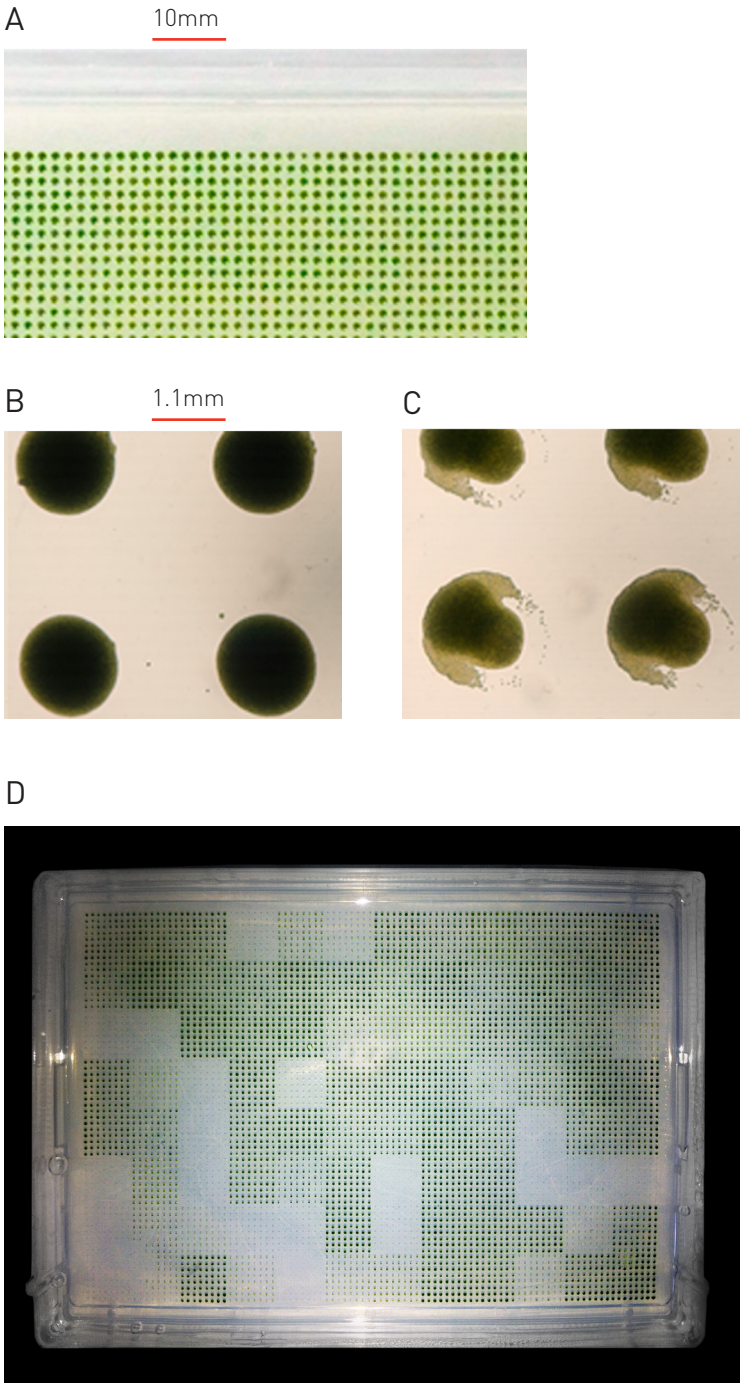

Figure 3: Agar-to-agar transfer at 6144 density.
A lawn of *Chlamydomonas* was pinned to a fresh plate using the 6144 RePad.

(A) An area of the plate after 2 days incubation. Growth is even for all inocula across the field.

(B) Micrograph of higher regular resulting microcolonies obtained after growth.

(C) Reproducibility of pin-strikes. A 6144 array of colonies was used as a source for a second pinning with a 6144 RePad. The second pin-strike was well within center-to-center spacing of the first one, carried out with a different pad 1 day previously.

(D) A collection of 384 randomly UV-mutagenized *Chlamydomonas* clones was replicated 16x (with revisit) and grown up for 2 days. Note high reproducibility between the 16 replicates of growth rate variability associated with each mutant.

CONCLUSION

Chlamydomonas reinhardtii is fully compatible with the Singer ROTOR+ for liquid-to-agar, agar-to-agar or agar-to-liquid transfers. In general, viability and quantitative reproducibility of transferred cells appears to be high. The 6144 array density is likely workable, although demanding in terms of agar plate quality, and could be an efficient format for high-throughput genetic assays. It can likely be integrated effectively with direct microscopic examination for phenotypic classification, thus in some systems eliminating the need for any macroscopic testing or culturing.

Using the ROTOR™ to investigate SARS-CoV-2 viral-host protein interactions

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SUMMARY

COVID-19, the respiratory syndrome caused by infection with the SARS-CoV-2 coronavirus has resulted in greater than 1.2 million deaths world-wide causing a strain on medical care facilities in many countries and a significant economic impact worldwide [1-3]. The SARS-CoV-2 virus is a zoonotic coronavirus that crossed from bats to humans in a form that could spread rapidly via respiratory droplets and aerosols. Two other major respiratory syndromes caused by coronaviruses, SARS and MERS, have emerged within the last two decades [4, 5]. Thus novel coronavirus infections are likely to be a continuing threat to human health.

THE SCIENCE

Coronaviruses are enveloped positive strand RNA viruses in the order *Nidovirales*. All coronaviruses contain a spike protein that spans the envelope membrane, it is important for host receptor binding and infection, and gives these viruses their crown-like appearance in electron micrographs [6]. Coronaviruses contain the largest genomes of any positive strand RNA viruses - about 30 Kb - in part due to encoding an RNA exonuclease that acts as a proofreading mechanism during genome replication [7]. The first two thirds of the viral genome is translated into two large polyproteins that are processed into individual non-structural proteins through the action of viral-encoded proteases. These non-structural proteins rearrange membrane structures in the host and form the viral replicase. The later third of the genome encodes the structural proteins nucleocapsid (N), spike (S), envelope protein (E) and M protein as well as several open reading frames that vary widely among coronaviruses [6].

Although much has been learned about the SARS-CoV-2 virus through intensive study in the last 10 months, much more needs to be understood to combat this virus as well as likely novel emerging infectious coronaviruses. Yeast can serve as a flexible experimental platform to help study SARS-CoV-2 safely. Although positive strand RNA viruses have not been found in yeast, several positive strand RNA viral replication systems have been constructed by expressing the viral RNAs from yeast promoters [8-10]. Individual viral proteins from SARS-CoV-2 have also been expressed in yeast to identify genetic interactions that are common to eukaryotes [11]. In addition, viral proteins have been expressed in yeast as a way of identifying enzymatic activities of viral proteins as well as to evaluate inhibitors of those activities [12].

We are using the Singer Instruments ROTOR™ to screen the yeast gene disruption library for virus-host genetic interactions. Our method, Selective Ploidy Ablation (SPA), provides a high throughput, mating-based approach to move expression plasmids into an arrayed yeast library [13]. The method relies on a donor strain in which all sixteen yeast chromosomes contain a centromere-proximal counter-selectable marker (URA3) and the galactose-inducible promoter from the GAL1 gene (Figure 1). Growth on galactose induces expression and disrupts centromere function in these strains leading to chromosome loss during mitotic growth. In a haploid context, this strain dies when grown on galactose. In a diploid heterozygous for the

CEN-marked chromosomes, growth on galactose leads to specific loss of the CEN-marked chromosomes and all the accompanying genetic information. Thus a diploid heterozygous for all sixteen chromosomes can become a haploid simply by mitotic growth on galactose.

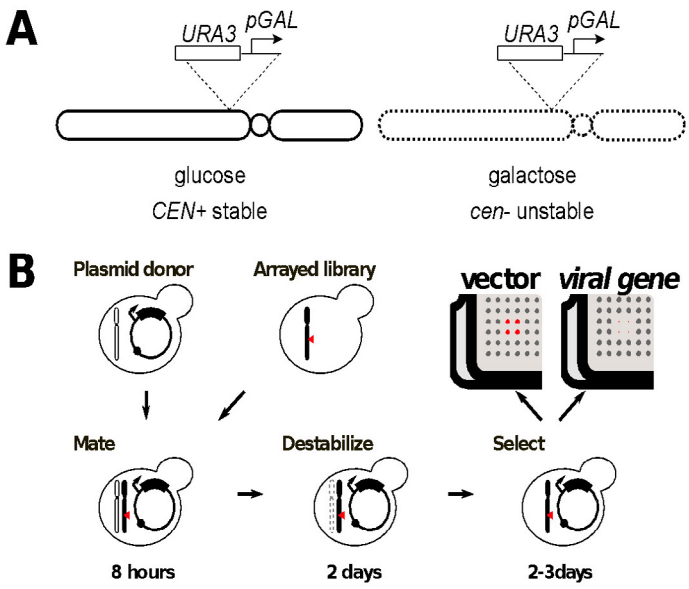

Figure 1. Genome-wide screens of the yeast gene disruption library by selective ploidy ablation (SPA). **A.** Depiction of yeast chromosomes in the universal SPA donor strain. Each chromosome contains an integration of the *GAL1* promoter and a *URA3* marker proximal to its conserved centromere sequence. Activation of transcription from the *GAL1* promoter inactivates the centromere leading to its destabilization during mitotic growth. **B.** Plasmid transfer from the universal donor to a recipient strain or library of strains is achieved by mating the plasmid-containing universal donor strain to the recipient for 8 hours. Strains are then transferred to galactose-containing dropout medium for 2 days to select for the plasmid and destabilization of the donor chromosomes. Strains are then transferred to 5-FOA dropout medium for 2-3 days to counterselect any remaining donor chromosomes, while maintaining selection for the transferred plasmid. Negative synthetic genetic interactions are identified by colonies that grow with the empty vector control (red in cartoon) but fail to grow when a viral gene is expressed.

With the Singer Instruments ROTOR™, the SPA procedure can be used to perform plasmid transfer into a library of strains. The SPA donor strain is first transformed with an expression plasmid containing the viral gene of interest using LEU2 as the selectable marker. Transformant cultures are grown in dropout medium overnight then spread onto YPD medium in Singer PlusPlates™ to form a confluent lawn. A compatible yeast library of opposite mating type is freshly pinned onto YPD and both sets of plates are grown overnight. The following day, the donor lawn and the library array are pinned together for mating, grown for 6-8 hours on YPD to allow mating, then pinned to -LEU dropout medium containing galactose as a carbon source. Plates are incubated at 30°C for two days on galactose to destabilize the donor strain chromosomes, then pinned to -LEU dropout containing galactose and 5-fluoro-orotic acid (5-FOA) to counterselect any remaining strains that still contain URA3-containing donor chromosomes. Plates are incubated 2-3 days on 5-FOA for haploid growth before imaging. Colonies containing viral expression plasmids are compared to an empty vector control to determine fitness effects in a library of yeast mutants.

The SPA screening procedure is efficient due to the mating-based transfer, and rapid because it does not require sporulation and haploid selection. The genome-wide screens can be accomplished in six days. Imaging and colony size measurement are performed using an R package version of ScreenMill package [14] (https://github.com/EricEdwardBryant/screenmill). This package provides R functions for processing multiple types of plate images to generate colony size measurements. The package also allows for processing of time-series images suitable for visualizing growth curves (Figure 2).

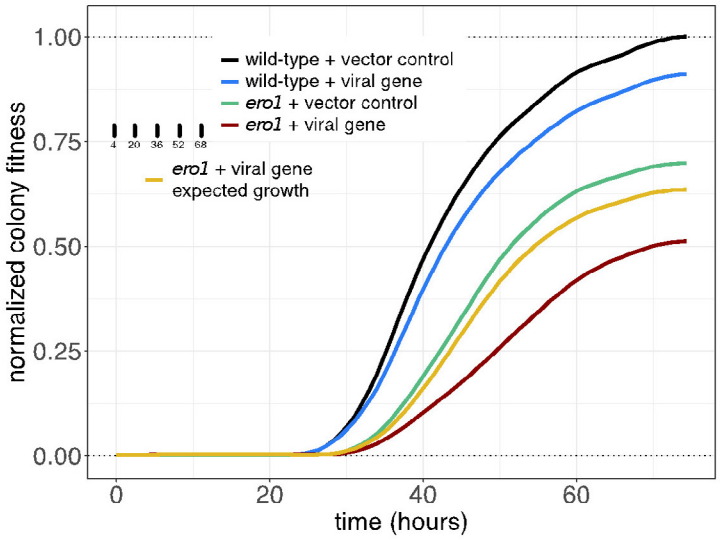

Figure 2. Yeast cultures expressing the empty vector or the viral gene clone are equalized by OD_{600} and spotted onto Singer PlusPlates™. The plates are incubated and are scanned once an hour over three days to monitor growth. Image density at each colony position is measured using the *ScreenMill* R package. Growth curves are plotted from measurements of colony density (selected colony timepoint in inset). Confidence intervals for mean growth values are indicated by shading. All values are normalized to maximal growth of the wild-type strain containing the empty vector (black line). Expected growth of the viral gene in the *ero1* strain (yellow line) is calculated from the product of the wild-type strain expressing the viral gene (blue line) and the mutant strain expressing the vector control (green line) at each time point. Observed growth of the *ero1* mutant containing the viral gene (red line) is less than the expected growth, indicating a negative genetic interaction. Since the confidence intervals of these curves do not overlap, the difference is significant.

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Bacteria-genetic-screening – E. coli Synthetic Genetic Array (eSGA)

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SUMMARY

E. coli Synthetic Genetic Array (eSGA) analysis involves the manipulation of high-density bacteria arrays to monitor bacterial genetic interactions. In a typical eSGA screen, a query strain is systematically conjugated to a genome-wide arrayed collection of single gene mutants. After rounds of robotic pinning and duel marker selection, *E. coli* strains harboring both gene mutations can be assayed for growth defects. Systematic and quantitative identification of genetic interactions allows the construction of large genetic networks, revealing functional dependency and pathway redundancy in *E. coli*.

PRINCIPLES OF ESGA

The generation of double mutants in eSGA relies on the principle of conjugation, which is the horizontal transfer of genes between bacteria [1].

In conjugation, a high frequency of recombination (Hfr) donor strain that contains the integrated fertility (F) factor can transfer genetic materials to an F^- recipient strain in a unidirectional manner. Once inside the recipient strain, the donor DNA can be integrated into the recipient genome by homologous recombination [2].

Based on this principle, eSGA is developed using the following steps [3]

1. Donor mutant is constructed in the *E. coli* Hfr strain by replacing the gene of interest with a chloramphenicol (Cm)-resistance marker.
2. The recipient mutant arrays are constructed in the *E. coli* F^- strain containing ~4000 single gene deletion mutants marked with a

THEORY BEHIND GENETIC INTERACTIONS

If two genes were functionally unrelated, the double mutant containing these two gene mutations would exhibit a phenotype that is approximately the product of the phenotypes of the individual mutants.

However, when mutations in two gene products produce a phenotype that deviates from each gene's individual effect, these two genes exhibit a genetic interaction. There are two general categories of genetic interactions: aggravating interactions and alleviating interactions (Figure 2A).

An aggravating interaction occurs when a double mutant exhibits a phenotype that is more severe than expected from the phenotypes of individual mutants. This type of interaction indicates that the gene products function in redundant parallel pathways, and highlights the robustness of the molecular network in tolerating genetic variations [4].

An alleviating interaction occurs when a double mutant exhibits a phenotype that is less severe than expected from the phenotypes of individual mutants. This type of interaction indicates that the gene products operate in concert or in series within the same pathway [4].

Genes that function in the same pathway tend to have similar genetic interaction profiles. Therefore, identifying genetic interactions can reveal functional relationships between genes and biological pathways [4].

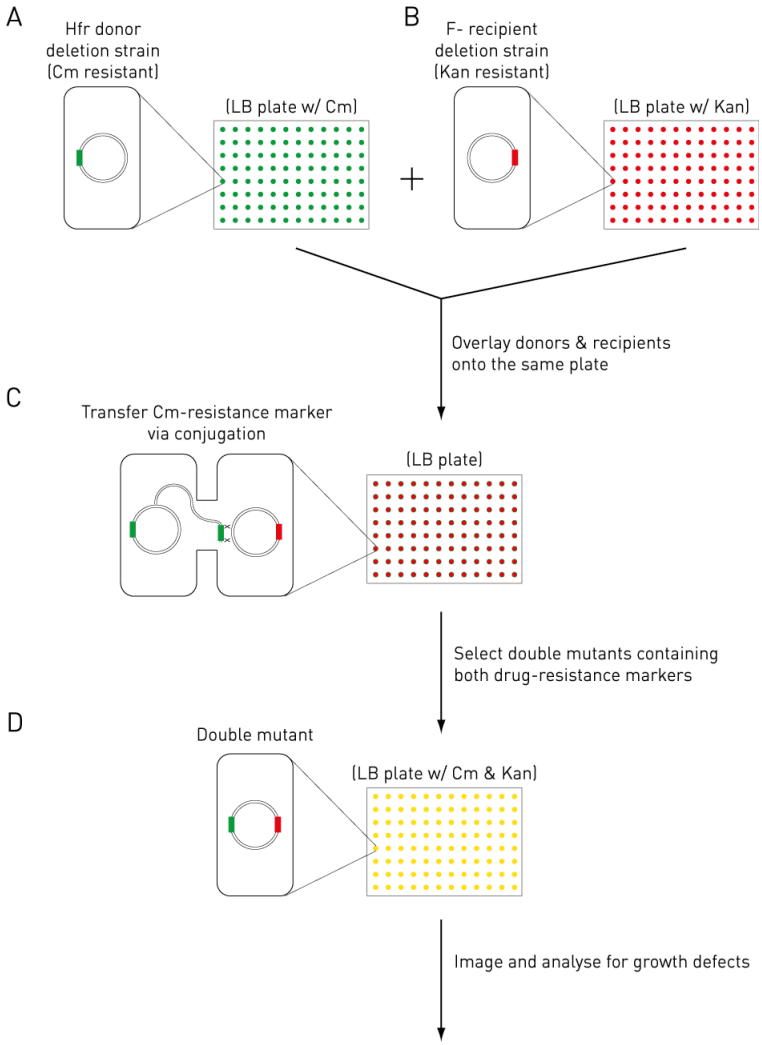

FIGURE 1: ESGA PRINCIPLES AND STEPS.

Aggravating interactions are shown in red, while alleviating interactions are shown in green. See text for details.

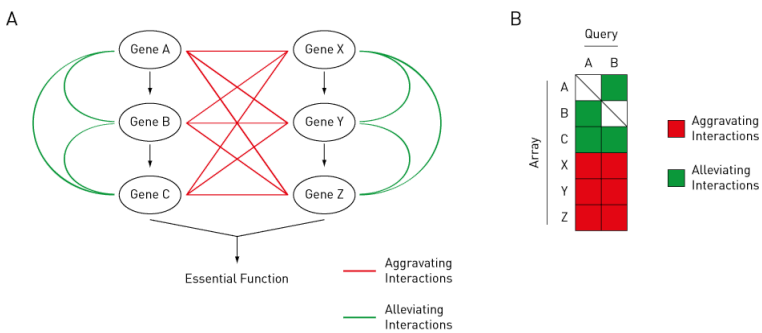

FIGURE 2: THEORY BEHIND GENETIC INTERACTIONS.

Aggravating interactions are shownin red, while alleviating interactions are shown in green.

(A) Pathway ABC and XYZ both contribute to an essential function in a cell.

BUILDING GENETIC INTERACTIONS INTO BIOLOGICAL NETWORKS

Genes in the same pathway often display aggravating interactions with genes in parallel and functionally redundant pathways. On the other hand, genes often display alleviating interactions with one another when their products function in the same pathway or protein complex [5]. Based on these two principles, constructing two-dimensional clusters of all pair-wise genetic interactions could reveal roles of individual genes in various cellular processes and identify components of a protein complex, or those within a specific pathway [4].

APPLYING ESGA TO OTHER BACTERIAL SYSTEMS

Because conjugation is a widespread phenomenon in prokaryotes [1], the eSGA technique can be applied to study genetic interactions in other bacterial systems. Indeed, *E. coli* has been shown to efficiently transfer non-replicating plasmids to other bacterial species [6]. Therefore, with various recipient gene deletion mutant collections being available [7,8], it is possible to apply eSGA to systematically analyze pair-wise genetic interactions in other prokaryotes.

WORKING WITH LIBRARIES

High-throughput eSGA screening can be done quickly and easily using automated robotics, such as the Singer ROTOR+ HDA, which can rapidly pin high-density arrays of bacteria and facilitate strain conjugation and selection. In addition, the ROTOR screening robot provides an automated platform to simultaneously produce multiple experimental replicates.

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BACTERIA GENETIC SCREENING APPLICATIONS

High-throughput, quantitative analyses of genetic interactions in E. coli

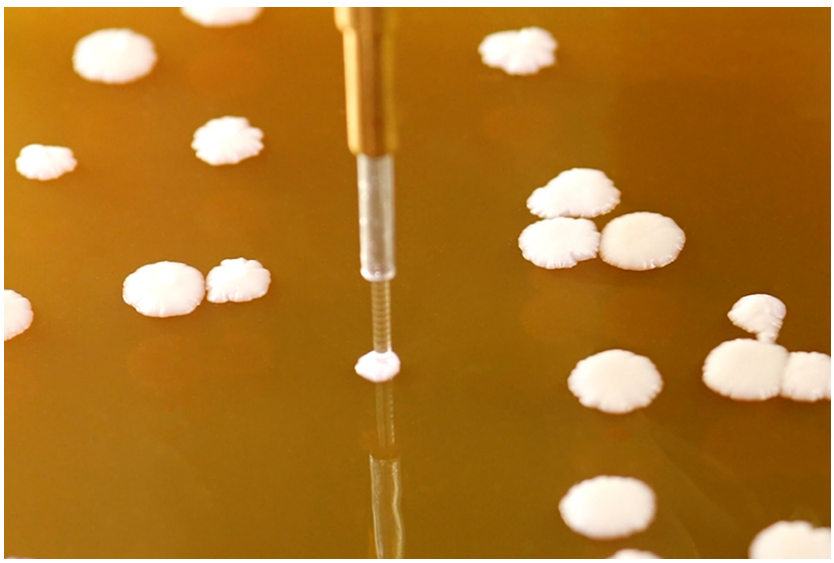
Abstract Typas A, Nichols RJ, Siegele DA, Shales M, Collins SR, Lim B, Braberg H, Yamamoto N, Takeuchi R, Wanner BL, Mori H, Weissman JS, Krogan NJ, Gross CA

Large-scale genetic interaction studies provide the basis for defining gene function and pathway architecture. Recent advances in the ability to generate double mutants en masse in Saccharomyces cerevisiae have dramatically accelerated the acquisition of genetic interaction information and the biological inferences that follow. Here we describe a method based on F factor-driven conjugation, which allows for high-throughput generation of double mutants in Escherichia coli. This method termed genetic interaction analysis technology for E. coli (GIANT-coli), permits us to systematically generate and array double-mutant cells on solid media in high-density arrays. We show that colony size provides a robust and quantitative output of cellular fitness and that GIANT-coli can recapitulate known synthetic interactions and identify previously unidentified negative (synthetic sickness or lethality) and positive (suppressive or epistatic) relationships. Finally, we describe a complementary strategy for genome-wide suppressor-mutant identification. Together, these methods permit rapid, large-scale genetic interaction studies in E. coli.

Synthesising a designer yeast chromosome with the help of PIXL

Introduction

The Cai lab is at the forefront of synthetic genomics, DNA synthesis automation, and applying synthetic biology approaches to antimicrobial drug discovery. Most notably, they are one of the teams tackling the Sc2.0 project, an ambitious, global effort to re-design and synthesise an entire yeast genome, to which Patrick and his team will contribute the design and synthesis of at least one of the constituent neochromosomes.



“We do a lot of combinatorial DNA assemblies and molecular cloning in E. coli which constantly results in the need to pick many candidate colonies, for example, for plasmid extractions.”

“Our lab is also building synthetic chromosomes and large pathways in yeast, so we have to pick and characterise multiple isolates of 96 or more candidates. For this example, I used PIXL to do a stability assay to see if the neochromosome is lost between multiple rounds of growth without selection pressure.

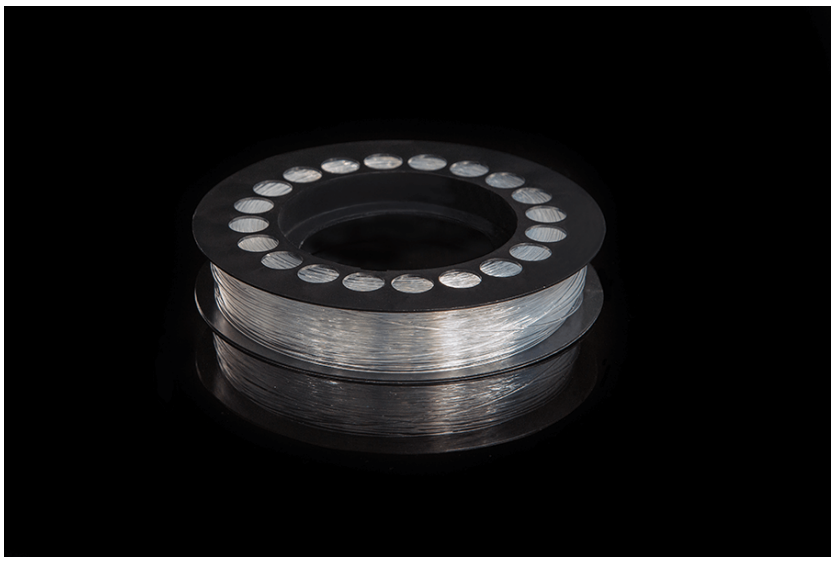
PIXL automates imaging, colony recognition, and selection, as well as picking and pinning, allowing Daniel to achieve the level of throughput necessary for confirming genetic stability in thousands of colonies per day. “The most valuable part of PIXL is picking to high-density arrays. When we do high-throughput assays, it’s really important to get an accurate array. which we can use on other machines or with imaging software,” Daniel explained. “That’s the most important feature besides, maybe, the re-arraying function: if I have a certain pattern, and I want to re-array them into a new format, that’s really helpful.”

When asked why manual colony picking wasn’t an option for this type of work, Daniel laughed, “That’s obvious, right? It’s challenging to generate arrayed plates of 384 colonies manually, even 96. I picked in one experimental setup up to, in total, 12 x 384 colonies per day over a time course of multiple days, so manual picking is impossible at this stage. No one would be able to pick colonies for eight hours every day, but PIXL can.”

Aside from producing the high-density arrays required for these PIXL TESTIMONIAL experiments, PIXL frees up more time for Daniel to focus on other areas of his research, due to both PIXL’s picking accuracy and it not requiring supervision. “PIXL saves time by being able to perform other experiments while PIXL does its job, so I am more productive. It also reduces the potential error rate, which reduces a lot of downstream troubleshooting time.”

Through the adoption of PIXL, the Cai lab has also benefited from a reduction in expenditure on consumables: “The exclusion of manual errors saves a lot

picks, eliminating the need for washing cycles, which can be a common source of microbial or ethanol contamination. *"PIXL uses a sterilised filament to pick individual colonies. For each step, the filament is cut so you have a fresh tip. That means you always transfer a colony with a sterile tip. For us, this is a good concept.*"



PIXL's user-friendly interface may also help to further reduce experimental downtime. "The user experience is quite simple and the interface is really easy to handle. You don't need a lot of training- people can start straight away," Daniel confirms. *"The maintenance is really low, even changing the filament is a piece of cake. Everyone can use the machine."*

Tens of thousands of engineering test cycles are performed to ensure PIXL is as reliable as possible and behaves as intended, though there is still service and support available in case issues arise. "Singer's customer support is outstanding, I think so far the best I have been in touch with. Support and turnaround times are quick and problems are usually solved very quickly: whenever we ran into a problem, we got a response immediately. The team is very responsive and helpful," Daniel recalled.

"That was always a good experience for me, that the company is really caring about the machine and our input was valued. In general, it was a very positive experience."

"It's likely that I would consider Singer Instruments to purchase more machines and I would recommend them to suitable colleagues and

“I am now moving on to the Max Planck Institute for Terrestrial Microbiology to build my own group. Hopefully, I will have the chance to include some Singer products in my setup.”

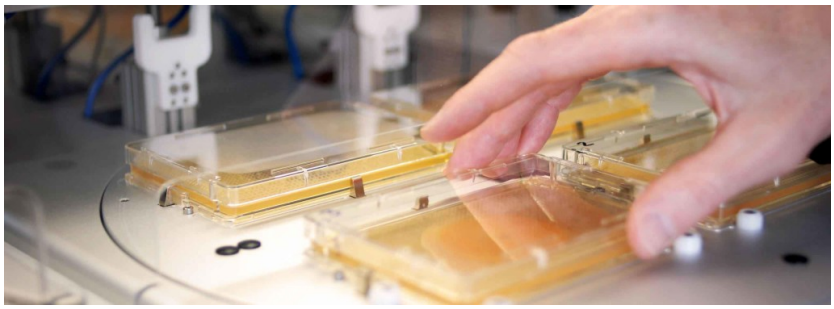
Patrick agreed: *“I would recommend PIXL to anyone looking to pick colonies. My lab finds PIXL really useful for synthetic biology.”*

Tackling superbugs and the antibiotic crisis with ROTOR+

Eric Brown McMaster University

It's really in the last 100 years or less, that we've have had the upper hand on infectious pathogens like bacteria. We've been able to just eradicate infection at will, it's a real blip in our timeline as human beings. However, bugs have a very clever survival strategy which involves relatively high rates of mutation and the acquisition of foreign DNA that allows them to adapt and survive in the presence of a variety of drugs. When this process carries on through multiple exposures to many different drugs, you wind up with a superbug, which is resistant to all kinds of drugs.

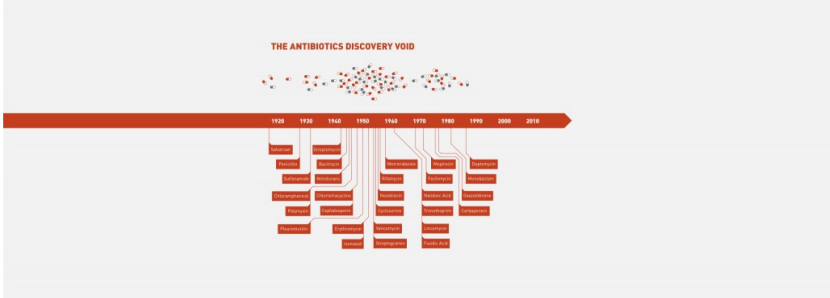
In fact, there are situations, such as *Pseudomonas* infections, where clinicians are really challenged in their ability to use any antibiotics with any effect. So, you end up with patients who are just helpless to infection. It's going to take some real diligence, and I dare say vigilance, to make sure that we stay a step ahead of the microbes.



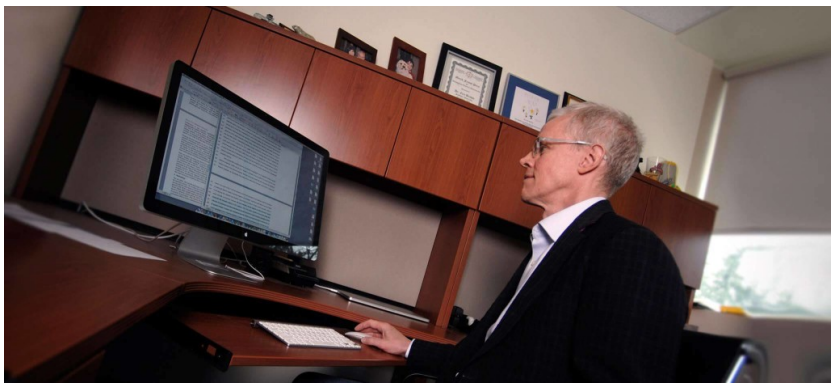
The antibiotics crisis is a tremendously important problem. If you look back to the history of antibiotics, not many new ef cacious antibiotics have been discovered. In fact, the last mechanistic class to be discovered was really daptomycin, which was in the late 80s. Despite the incredible advances in biomedical research in the last 10-15 years, there's been a slump in the area of antibacterial drug discovery. I think there's certainly lots of room for innovation there.

Being on the lake in my sailboat is certainly one of my favourite ways to spend summer days. There is nothing to do but just optimize your point of sail, and feel the wind in your face. The time on a sailboat is premium thinking time for me.

In my group we are really concerned with how antibiotics are discovered. Our focus is to unravel the complex biology of microbes with the ultimate aim of trying to elucidate systems we can manipulate to the detriment of drug resistant superbugs. The overriding idea is that can we come up with new agents or probes that not only allow us to understand the biology, but may also be leads for new antibacterial drugs.



Certainly we're not interested in treading into territory where lots of other people have been. We are known for having crazy and unthinkable ideas. We try to do things and look into places that pharmaceuticals haven't.



The idea of using drug synergy to ght superbugs probably came about over a beer. The idea for drug synergy is that a non-antibacterial drug can potentiate the effect of an existing antibiotic when applied simultaneously. Take *Pseudomonas* infection for example, which are resistant to many antibiotics. We looked into a collection of more than 1,000 off-patented drugs for molecules that would potentiate Minocycline, which is the tetracycline molecule, a relatively poor antibiotic against *Pseudomonas* infection. We discovered that Imodium would act synergistically with Minocycline against *Pseudomonas* infection.

Science is so incremental and so frustratingly slow, but it really does feel as though we are pushing things forward in the right direction.

There is an infectious disease clinician in California who has been prescribing tetracycline along with Imodium for years to patients who have gastroenteritis. Suddenly our work has helped him make sense of it.

Our work certainly attracts interests in the pharmaceutical sector. They seem to follow our work extremely closely, the way people follow baseball in North America. I think that's really where the rubber hits the road. We'd like to know that ultimately our stuff is pushing things ahead, and it certainly feels as though we are.

There is no other instrument out there that can pin colonies like this thing can pin, which allows us to do chemical genomics just like nobody's

It is a pleasure to work at the Michael DeGroote Institute for Infectious Disease Research (IIDR). It's a multi-disciplinary team of more than 400 researchers from different backgrounds. In particular, the IIDR has incorporated lots of infectious disease clinicians. Having clinicians on board is a key touch-down for me because I get to meet regularly with clinicians and make sense of what the medical need is. Some of our best work has been done in collaboration with other groups here at IIDR.

We've built some infrastructure at IIDR for high-throughput screens, which is unlike what you would see in the small biotech companies. We've got a really robust platform of pinning deletion or expression clones. We're monitoring the growth of those pinned strains at as high as 6144 density using the ROTOR+. I just love the fact that my students are hands-on with the robot, which makes them more in touch with the experiments they are doing.

I am fortunate to work with an extraordinary group of young researchers who do some of the most amazing work. They have a great deal of pride in what we produce as a group. I'm tremendously interested in their careers and their success when they move on, which just makes for a really amazing work environment. It really is a privilege that I constantly have fresh and bright young people coming in with new ideas.

Being a scientist is the greatest job in the world.

SINGER INSTRUMENTS

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