Towards optimization of yeast colony array phenotyping

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Saccharomyces cerevisiae has proven to be a very valuable tool for genome-wide experimental techniques. Since this is one of the simplest eukaryotic organisms, but at the same time evolutionary conserved pathways are present in mammalian cells, it played a significant role in development of whole-genome approaches. In our work, we use agar-media based platform to perform chemical genomics, synthetic genetic array and synthetic dosage lethality analyses. With the help of a Slovenian engineering company, we designed and constructed a robotic manipulator, which helps us with the manipulation of the cells on the solid media. Single deletion mutant collection and the manipulator form a powerful combination for performing high-throughput experiments and this combination is widely used elsewhere. To exploit its full potential, a suitable method for acquisition and quantitative analysis is needed. Most methods used today estimate colony finesses by measuring area in contact with agar. Since a colony is a 3D formation, variance in height between individual colonies could contribute to less accurate results. In addition, older colonies exhibit morphological changes that can be seen on the side of the colonies but do not affect the contact area. Thus, we proposed a method for colony volume measuring. After exploring different possibilities, the best option for this purpose turned out to be transmissive-mode scanning. When the light from the scanner illuminates the matter, recorded intensity correlates with the thickness of colony. This approach is powerful enough to provide reliable colony volume measurements and at the same time 3D morphological reconstructions. With implementing a solution for automated scanning in arbitrary time intervals, dynamics of growth over the plate can be observed. We took advantage of this and started with a study of (non)uniformity of behavior that colonies exhibit. Since the aim of the experiments is to shed some light on the global behavior of solid-media based experiments, the equivalent approach as in chemical genomics was followed. First, range of different indicators was tested and bromcresol green was found to be suitable as a test substance for this purpose. It is a pH indicator that changes color at pH range 5.4-3.8 from blue to yellow, which is easily observable. Besides, bromcresol green possesses another applicable characteristic. It migrates into viability impaired cells inside of the colony and the coloration is visible by naked eye. Showing changes in pH and incorporating dye into the certain types of cells enable us to observe changes in micro-environment, i.e. how every individual colony acidifies its surrounding, what is the delay of acidification start between individual colonies and how this is connected to the indicator uptake. With automated scanning we track those changes over the time. The results showed that at the positions on the plate, where more cells were transferred, acidification starts first and those colonies also stayed bigger throughout the experiment. The coloration appeared just above the bottom of the colonies and it spread towards the top, although top itself remained uncolored. This implies that viable colonies are mostly on the sides and on the top of the colonies. Observed pattern is homogeneous through the plate and consistent among repetitions. To get better understanding of the described processes, more dyes and conditions will be tested to determine if observable effects are constant or may vary with environmental changes.