Protocol for Automated Dissection Chip (AD-Chip)

I. Sample Preparation

1. Culture yeast cells overnight. On next morning, transfer 50µL of cultured cells to 1mL of filtered YPD medium (**NOTE #1**). Keep on ice until experiment.

   NOTE #1: Actual amount of overnight culture added can vary according to cell growth rate and other properties but is best kept between OD600 of 0.1 to 0.4. The preferred cell count for loading is 1 x 10^6 – 10^7 cells/mL. Cultures with higher cell counts need to be diluted before loading. The channel may be hard to fill at a desired proportion if too little cells were added, and too much cells may lead to blockage.

II. Setting Up Experiments

1. Fill 20mL syringe with liquid YPD medium or other medium of choice (**NOTE #2**). Dispose of any air bubbles in the syringe. Connect the medium-filled syringe with luer-stub adapter and non-DEHP medical grade tubing with a hollow pin, then install onto syringe pump for supplying medium. Turn on the syringe pump at a flow rate of 10µL/min and make sure liquid flow is not obstructed.

   NOTE #2: Any liquid medium used in experiments should be filter-sterilized instead of autoclave, as unfiltered medium may contain crystals and other insoluble components which could block the channels. The medium should be at least room temperature, using cold medium will interfere with cell growth and cause complications on experiments.

2. Connect the medium-filled syringe with the inlet of the AD-Chip once medium flow is stable (**NOTE #3**). Multiple syringes can be installed at same time if using a multi-syringe pump.

NOTE #3: All components of experimental system should be connected in an air-tight fashion and be void of any air-bubbles. If stalling of liquid flow is observed, disassemble the system and make sure no air bubble is present.

3. Check the AD-Chip under microscope and wait until the channel is fully primed with the medium (**NOTE #4**) and the medium comes out from the outlet tubing.

   NOTE #4: Cells should only be loaded after the medium comes out from the outlet tubing to ensure all air is pushed out from the channel and the tubing. All air bubbles on the traps should be washed away during this period.

III. Loading Cells into AD-Chip

1. Thoroughly vortex diluted yeast medium and fill 1mL syringe (**NOTE #5**). Dispose of any air bubbles and connect with luer-stub adapter and non-DEHP medical grade tubing with hollow pin. Install the cell suspension-filled syringe on the syringe pump for loading cells, turn on the syringe pump and confirm the cell suspension flow comes out from the tubing.

   NOTE #5: Vortex culture thoroughly before filling. The experiment steps after filling up the syringe should not take too long, or cells will start to settle on bottom of the syringe, making them hard to come out.

2. Gently remove the tubing connected to the medium-filled syringe from the inlet, connect the cell suspension-filled syringe to the inlet, and set the flow rate of the syringe pump at 1-2μL/min (**NOTE #6**). Alternatively, load the cells by gently pressing on the plunger of the cell suspension-filled syringe by hand instead of using syringe pump. Should be careful that too much pressure by hand could damage the bonding of the chip, resulting in leakage from the channel.

   NOTE #6: The cell filling rate is determined empirically, and may vary drastically when using different pumping system, chip design or cell concentration. We suggest performing pilot experiments to determine appropriate speed. Start with a lower filling rate and increase if not enough.

3. Closely monitor channel loading status under microscope.

4. When traps are filled with cells, remove the tubing connected to the cell suspension-filled syringe from the inlet, and reconnect the medium-filled syringe to the inlet.

5. Set the medium flow rate of the syringe pump at 4μL/min.

IV. Time-lapse Imaging

1. Set up the beacons for imaging. The magnitudes should not be less than 20x.

2. After setting up beacons, set onstage incubator with temperature to 30°C and humidity to off. Set experimental time as desired.

3. For determination of replicative lifespan, image cells every 10 min. When using fluorescence microscopy, it is preferred to image the cells less frequently (e.g. every 20 min) to avoid phototoxic effects.