

# Growing Paramecia Under Sterile Conditions

(Source: W. Driever and C. Boggs from [Zebrafish Book 5th Edition](#))

## Culture of Paramecia

1. Grow paramecia cultures under "cell culture" conditions. Make passages in a sterile tissue culture hood.
2. Use 500 ml or 1000 ml media bottles (e.g., with orange caps) and culture at 28°C in the dark, for example in a cabinet in your fish room.
3. Dilute the starter culture (a clear suspension of paramecia) 1:1 with fresh (milky) medium and incubate with the cap loosened one half turn to provide air. After 23 days, the culture will clear and you should be able to observe clouds of paramecia.
4. Expand the culture by diluting it 1:1 with fresh medium. If you add twice the volume of fresh medium, it takes about 3 days to clear. Use clear cultures for harvesting paramecia or store for 3 to 5 days at about 20°C.

## Long-term storage of paramecia

Use Dryls solution (1 volume clear culture to 5 volumes Dryls) to prepare a backup stock in case the original culture becomes contaminated. Paramecia live about 10 days in this solution in the dark at 1820°C. Prepare the solution from autoclaved components and add the CaCl<sub>2</sub> last or it will precipitate.

## Long-term Paramecium Storage Medium Storage Medium

Component	Stock	Amount (ml)
NaH <sub>2</sub> PO <sub>4</sub>	0.01M	10
NaH <sub>2</sub> PO <sub>4</sub>	0.01M	10
Sodium citrate	0.1M	20
CaCl <sub>2</sub>	0.1M	15
dH <sub>2</sub> O		945

## Harvesting paramecia from clear cultures

### Materials:

- large funnel (BelArt) with grooves
- cotton handkerchief
- 24 cm fluted filter paper circles (Fischer 0979014G)
- 24 cm Whatman No. 1 filter paper circles.

1. Choose bottles of cleared paramecia cultures for harvest. Approximately 4050 ml of culture will feed up to 100 larval fish per day.
2. Place the funnel in a flask and filter the paramecia stock through the folded cotton handkerchief to remove precipitates of the Schönefeld's medium.
3. Filter the liquid through fluted circles until the flow decreases to a slow drip.
4. Rinse the paramecia off the filter paper into a flask using embryo medium. Repeat until all the stock is filtered.
5. Refilter the paramecia through fluted circles rinsing with embryo medium until most of the yellow color has disappeared.
6. Collect the paramecia in a clean bottle making sure that the yellow color is gone. Rinse again if necessary. Some paramecia pass through the fluted filters. If they are needed for feeding, they can be collected by filtering through the Whatman No. 1 circular filter. However, filtering through a Whatman 1 is very slow.
7. Adjust the volume with embryo medium to equal ½ of the original culture volume. Store in darkness at 20°C until needed for feeding.