



Development of *in vitro* sponge cell culture

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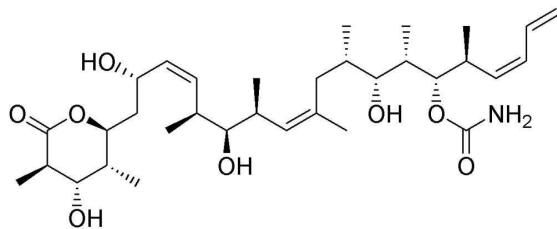
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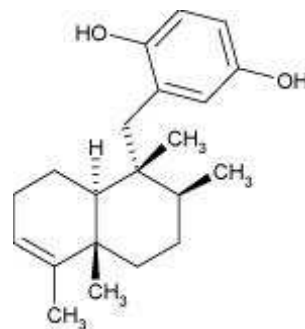
Why sponges?

Sponges are a source for bioactive compounds with pharmaceutical interest

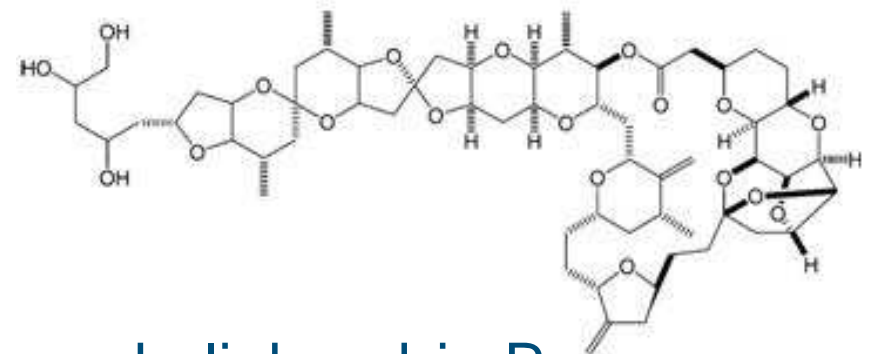
antibiotic, antifouling, antiviral,
anti-inflammatory, cytotoxic, antitumor



discodermalide
antitumor

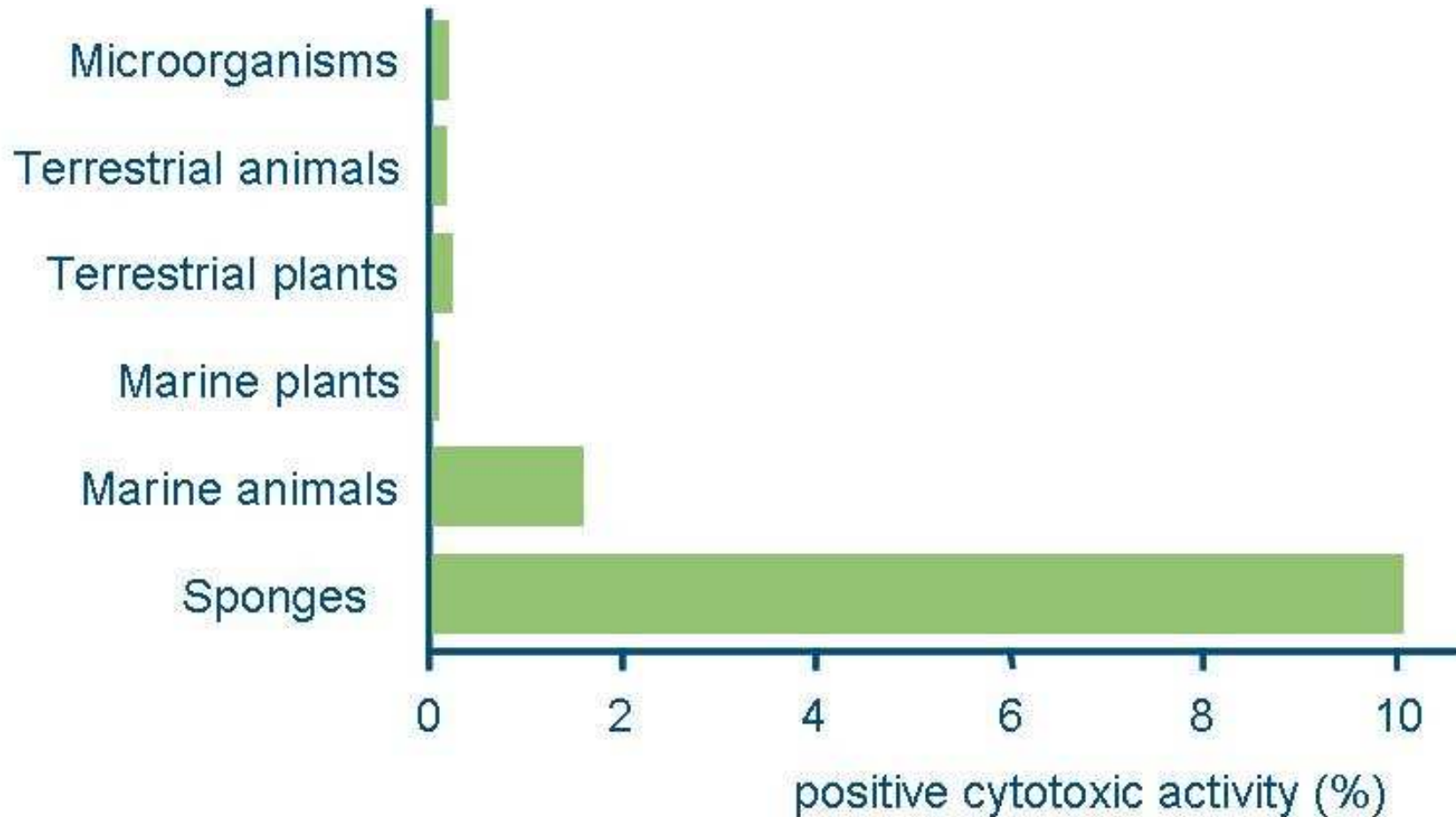


avarol
anti HIV



halichondrin B
anticancer

Why sponges?



From: M.J. Garson in *Sponges in time and space* (1993)

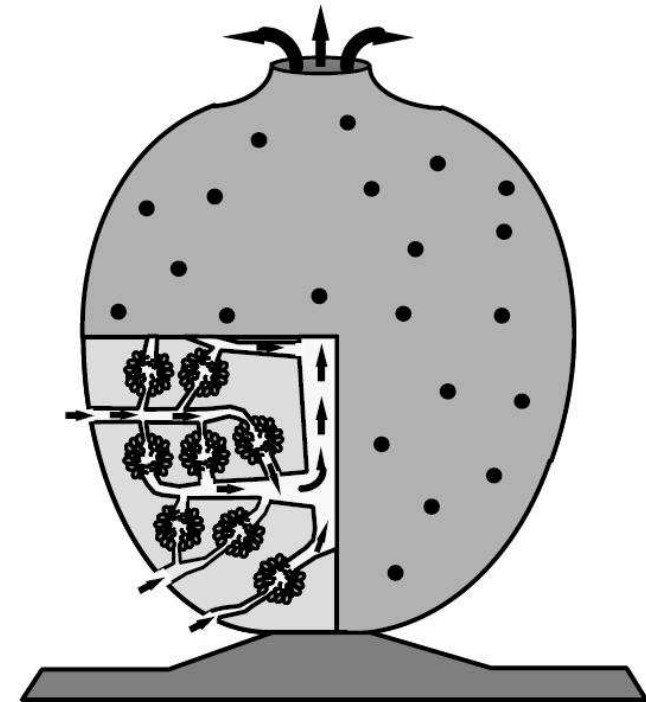
What are sponges?

Animal

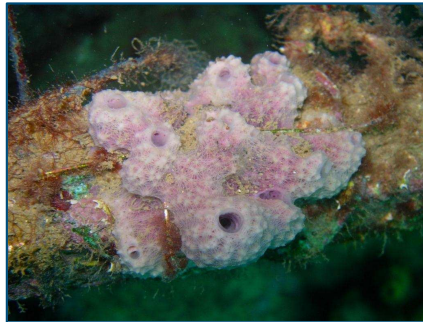
Sessile, benthic organism

Invertebrate

Filter feeder



How to make a sponge cell culture?



Dysidea avara

Ca²⁺/Mg²⁺ free seawater,
cell strainer 70 μm, centrifugation



Supernatant
contains bacteria

Pellet contains
sponge cells

Cryopreserve

Dissolve in
sponge cell
culture medium

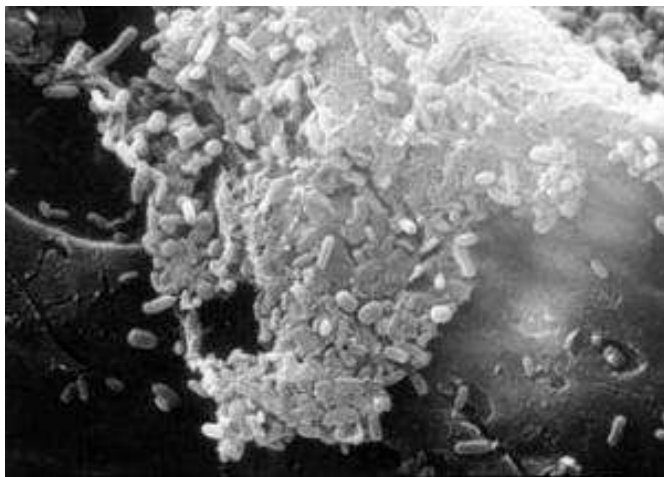
10⁵-10⁶ cells/ml
and measure
viability

Ready for
experiments

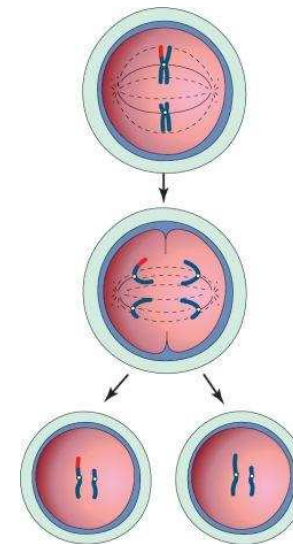
In vitro sponge cell culture

major bottlenecks

contamination by
symbionts

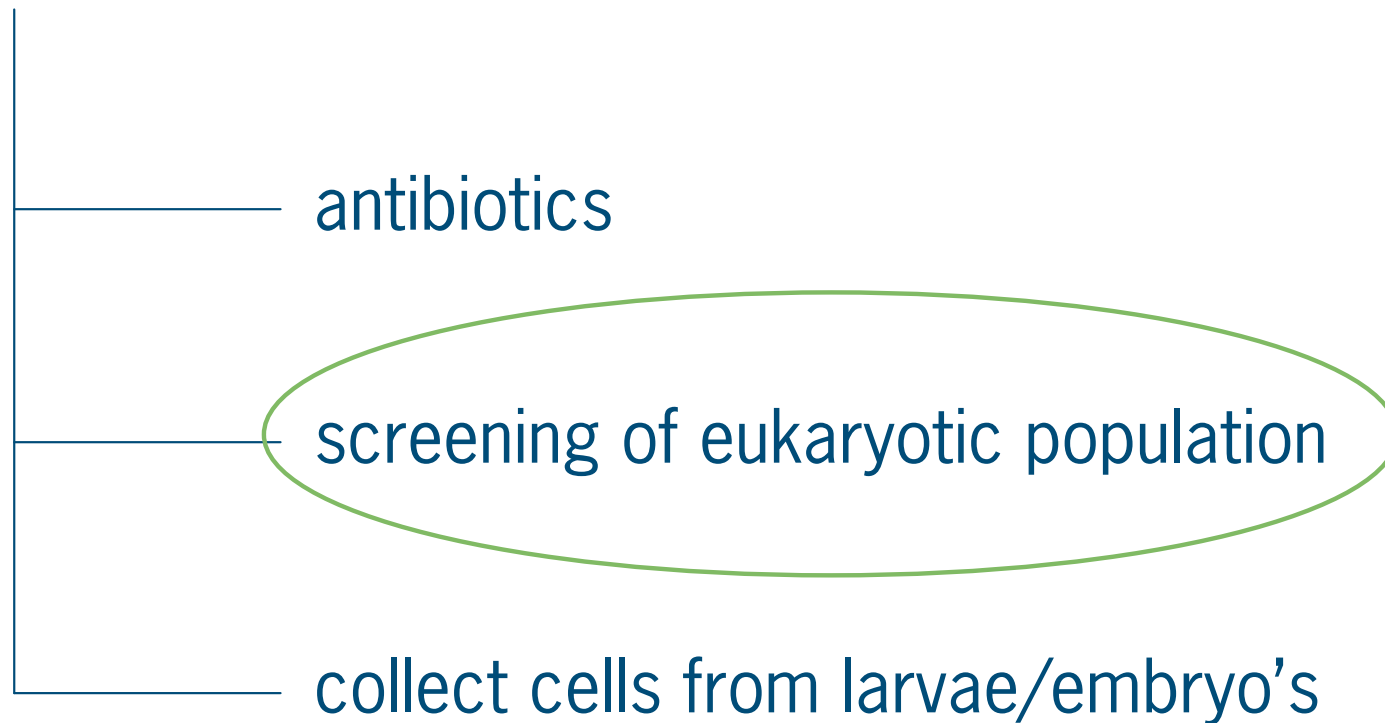


lack of proliferation

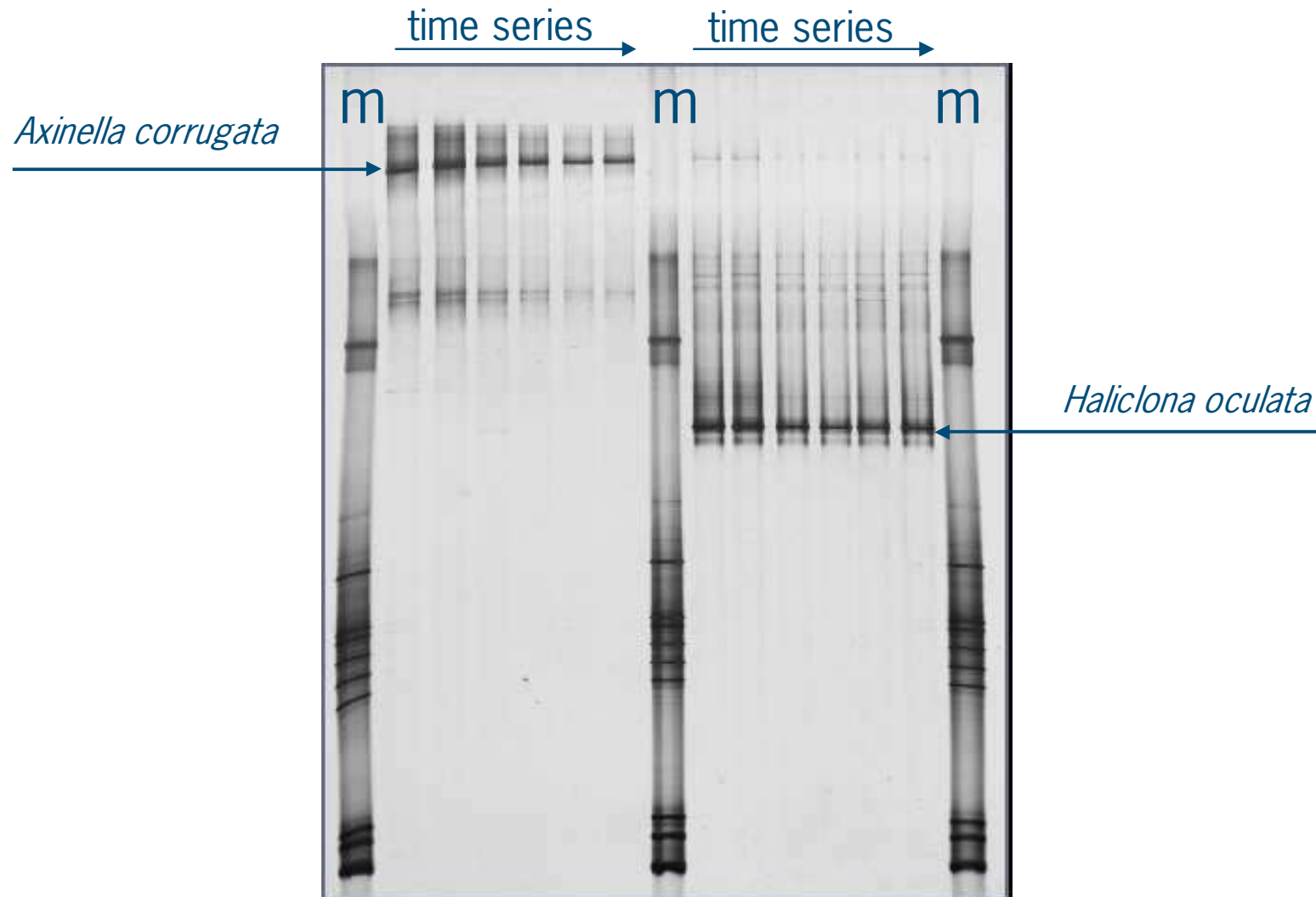


Contamination by symbionts

■ What to do?

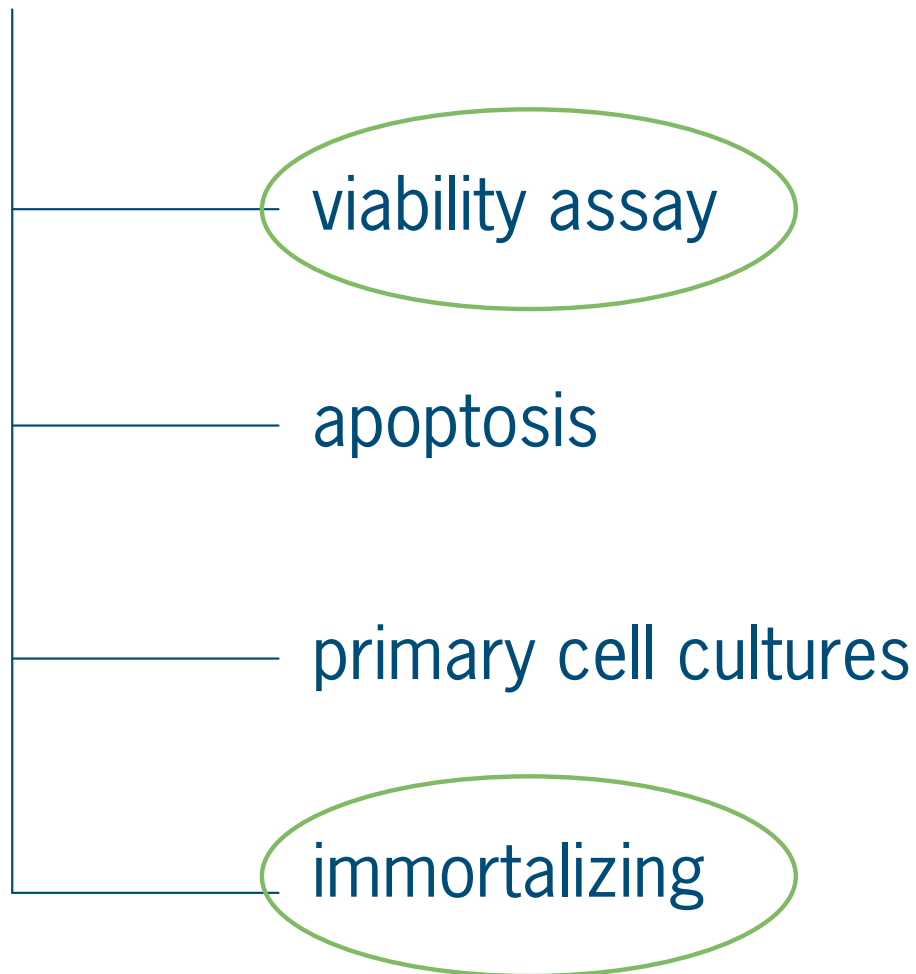


Screening: 18S rDNA-PCR DGGE

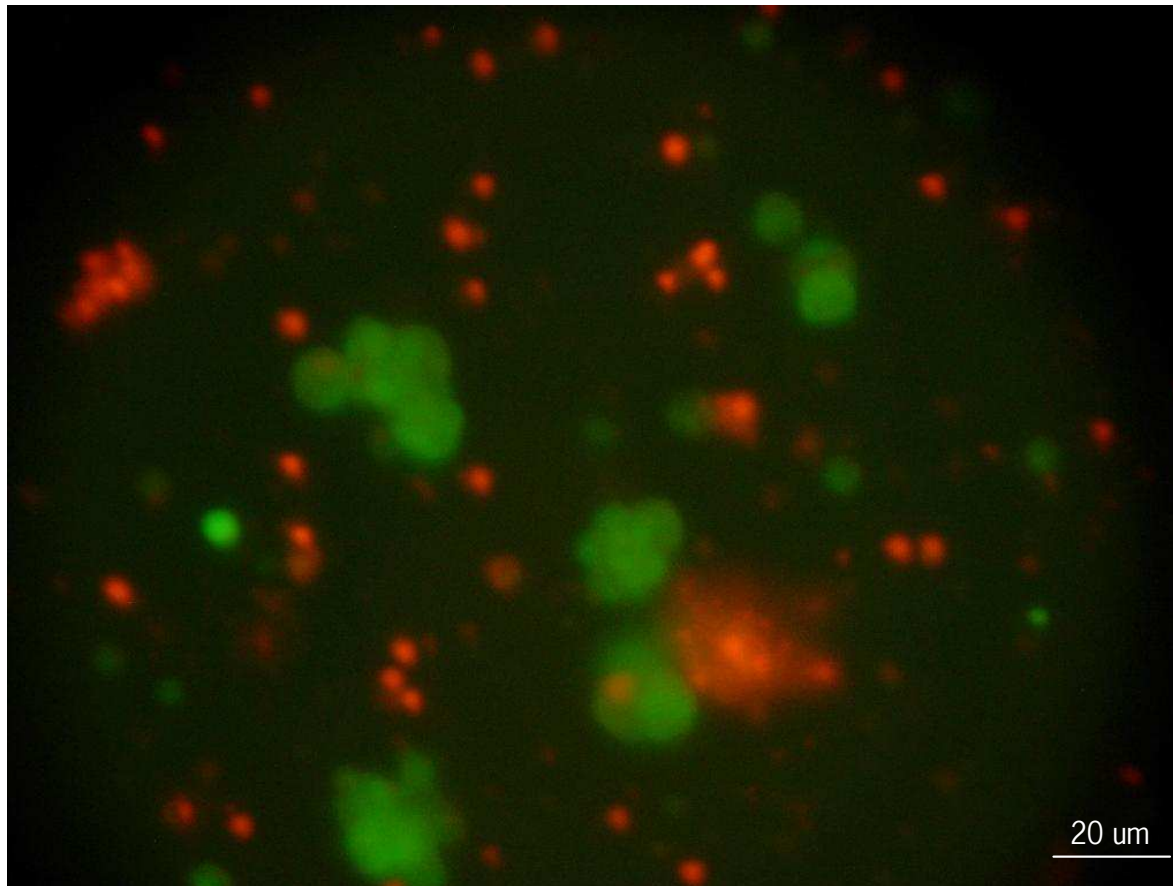


Lack of proliferation

■ What to do?



Viability assay



Dysidea avara

Fluorescence microscope

FDA → green → alive

PI → red → dead

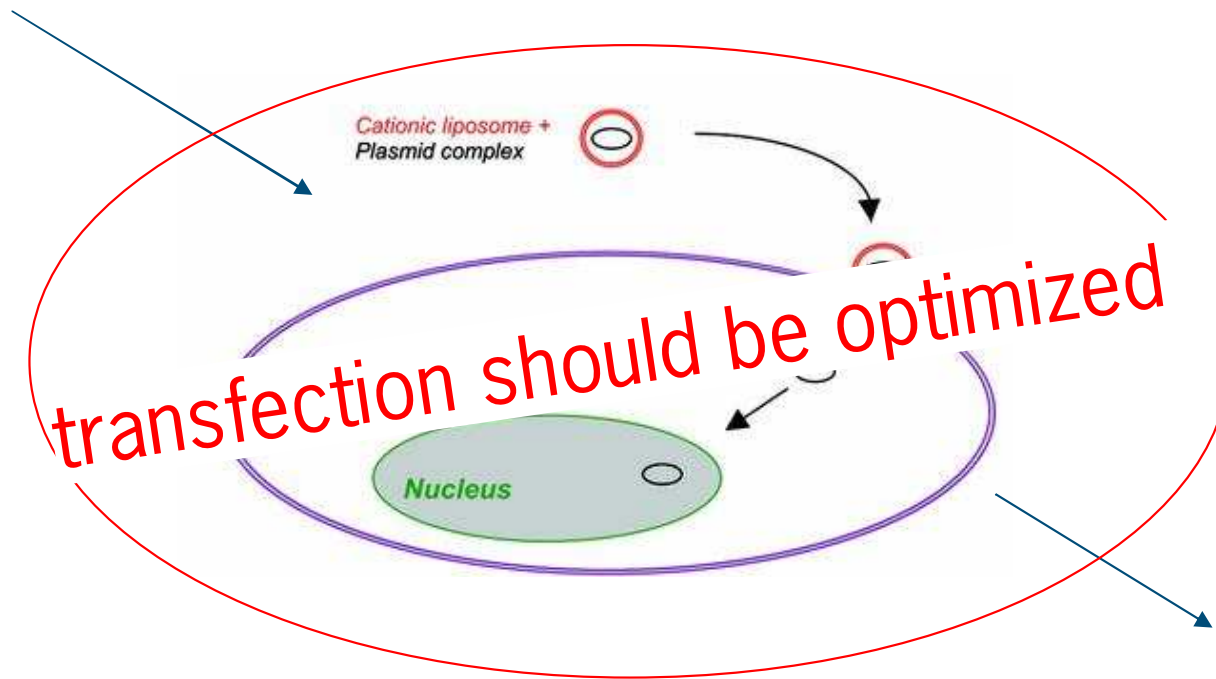
Immortalizing

Immortalizing genes:

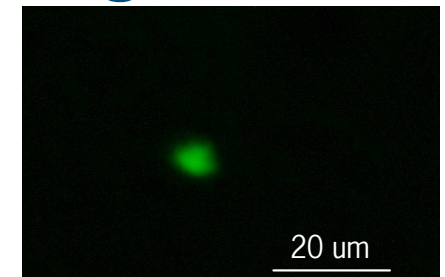
hTERT

SV40

EBV



expression
of gene



GFP in *Haliclonia oculata*

Transfection optimization

■ Is DNA taken up by cell ?

lipofection with
fluorescent
labeled DNA



■ Is gene being expressed?

- promotor
- metabolic active



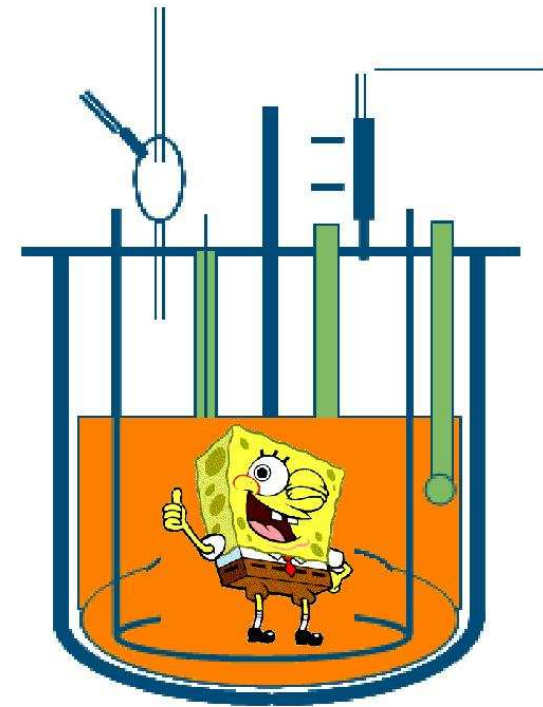


Future

- Proof apoptosis and proliferation in cell cultures
- Develop proliferating primary cell culture
- Develop transfection method in sponge cells
- Immortalize sponge cells

Summary

- Create immortalized sponge cell culture
- Produce bioactive compound
- Cultivation in bioreactor?





Thank you for your attention

Questions?

