Ginrei Lab

Horizontal Co-culture plate ICCP

Troubleshooting

Summary

- For the passage of liquid factor through the filter, fill the passage (filter surface) with enough solution.

- The recommended way to preprocess filters is washing with pure water and PBS after 1 minute with 100% ethanol and setting the filter to ICCP

Thank you for your purchase. This document is for use in experiments.
I have stated the points that I would like you to pay particular attention to.

Troubleshooting casebook

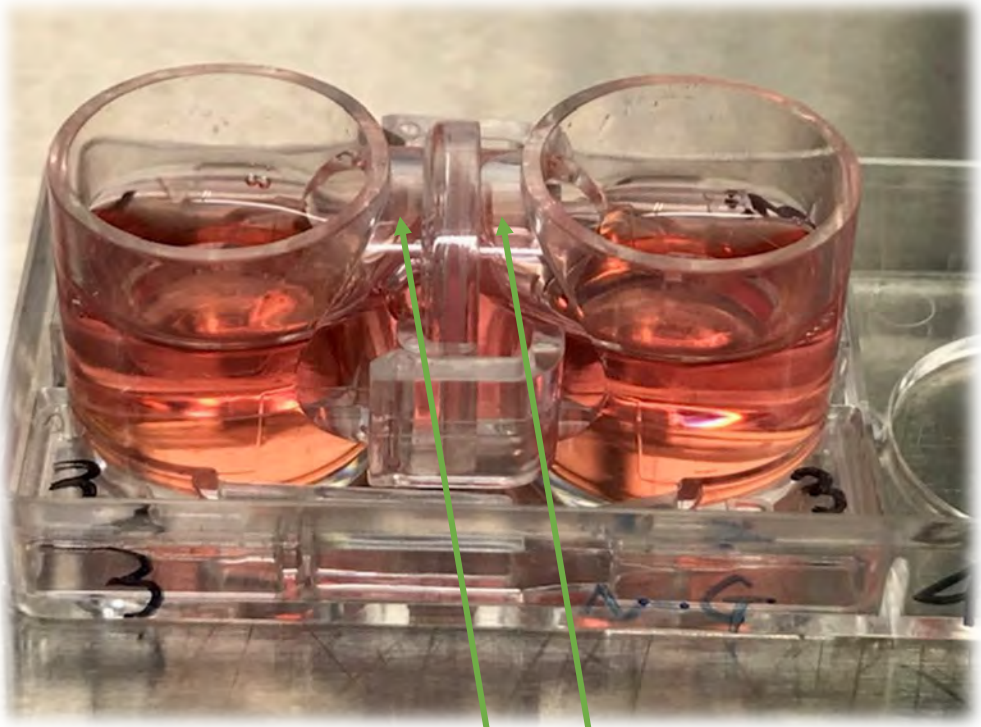
1. In the experiment with the filter, there is little passage of liquid factor.

Factors such as proteins and exosomes attach to filters, containers and extraction kits. Therefore, the more processes there are, the smaller the amount of recovery. If you can collect enough positive control samples (if there are no problems with the extraction process), first check the culture conditions and filter treatment. In most cases, the culture may be low or the filter may not be fully degassed. If the culture volume is small, the area of the filter contacting the culture solution will be small, and the co-culture effect may be significantly reduced.
In addition, air remains in the pores of the filter, so sufficient degassing is required before use. Please refer to the following pages for the specific amount, status, and filtering.

Troubleshooting cases Culture volume

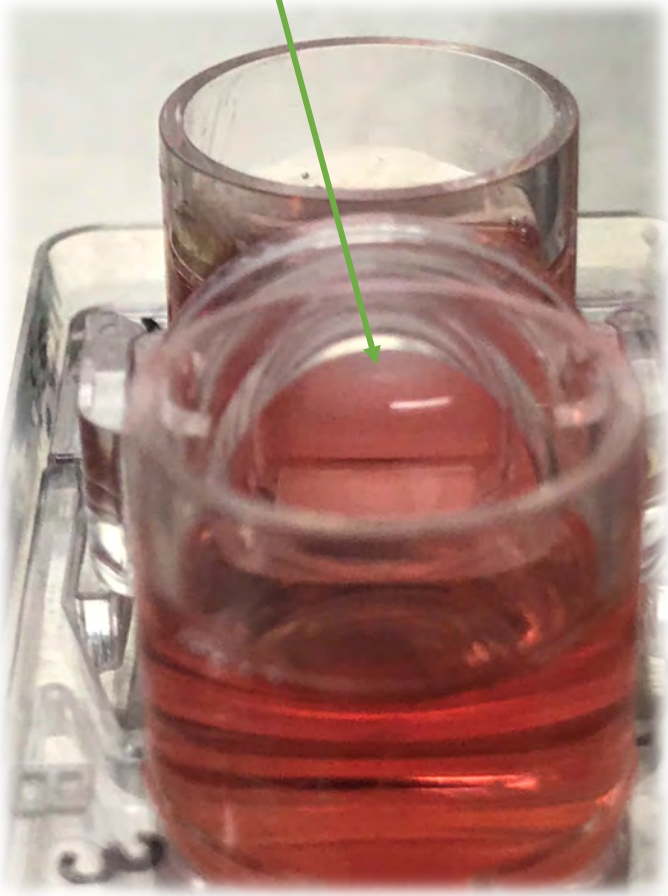
Bad condition

When there is a shortage of Medium (1 ml)



The passage is not covered by the liquid surface.
In such cases, the co-culture effect may be significantly reduced.

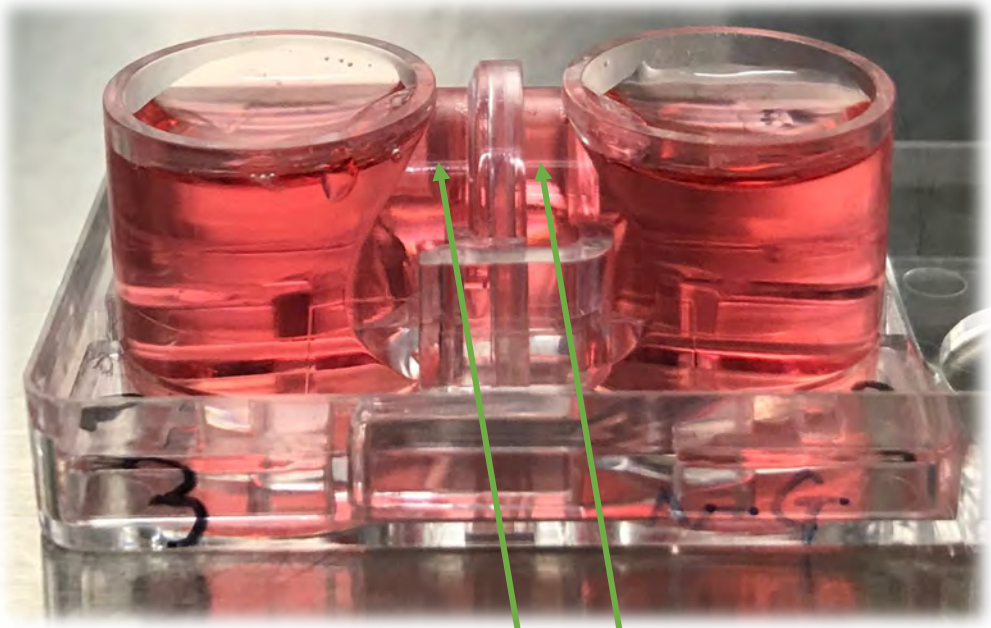
The filter is not completely covered by Medium.



Troubleshooting cases Culture volume

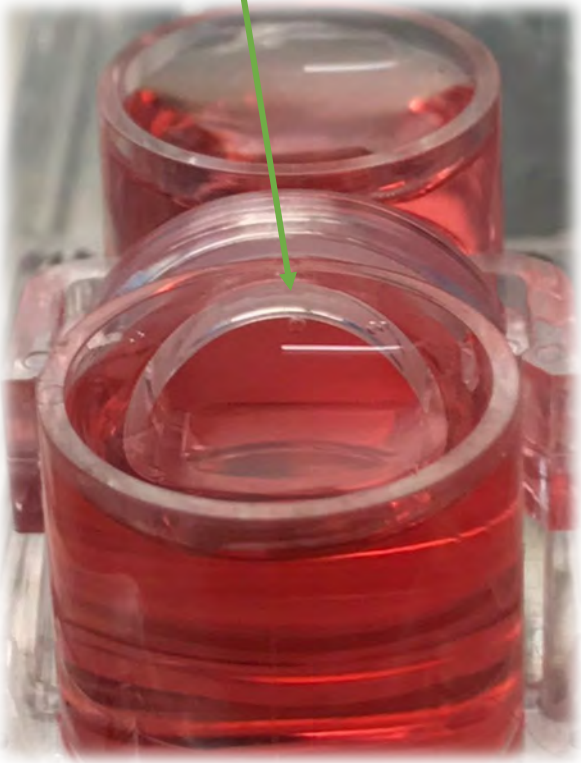
Good condition

When the amount of Medium is sufficient
(approximately 1.5 ml)



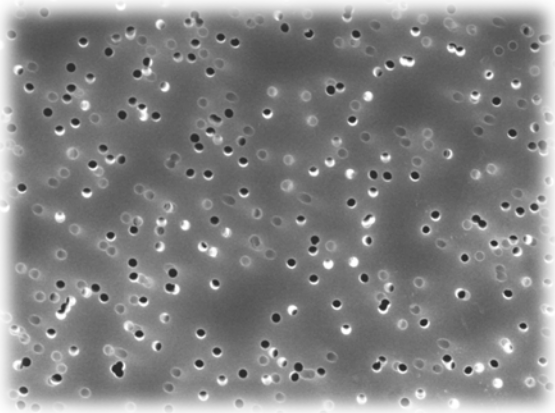
The passage is covered with liquid.
Please do co-culture in this state.
If the medium is filled more than this, the upper lid and liquid surface will adhere when the upper lid is fitted, and surface tension may cause the medium to overflow.

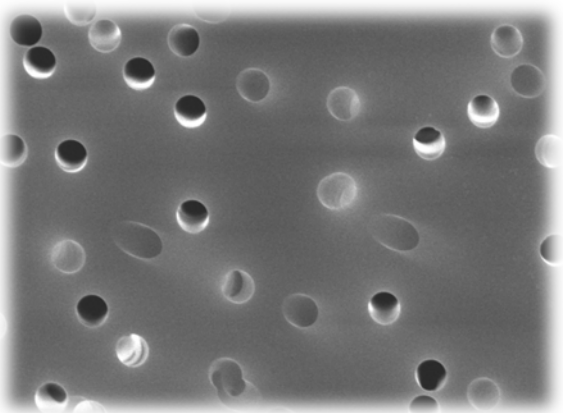
The filter is completely covered with Medium.

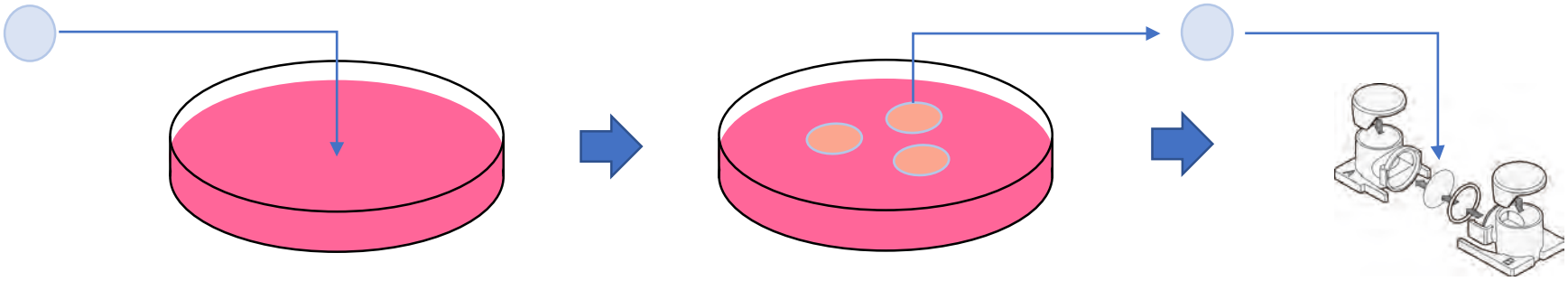


Troubleshooting casebook filter

Filter treatment is important to see the co-culture effect.
The filter has fine holes, which contain air.
If you do not degas sufficiently before use, air may remain and clog the pores, which may reduce the co-culture effect.



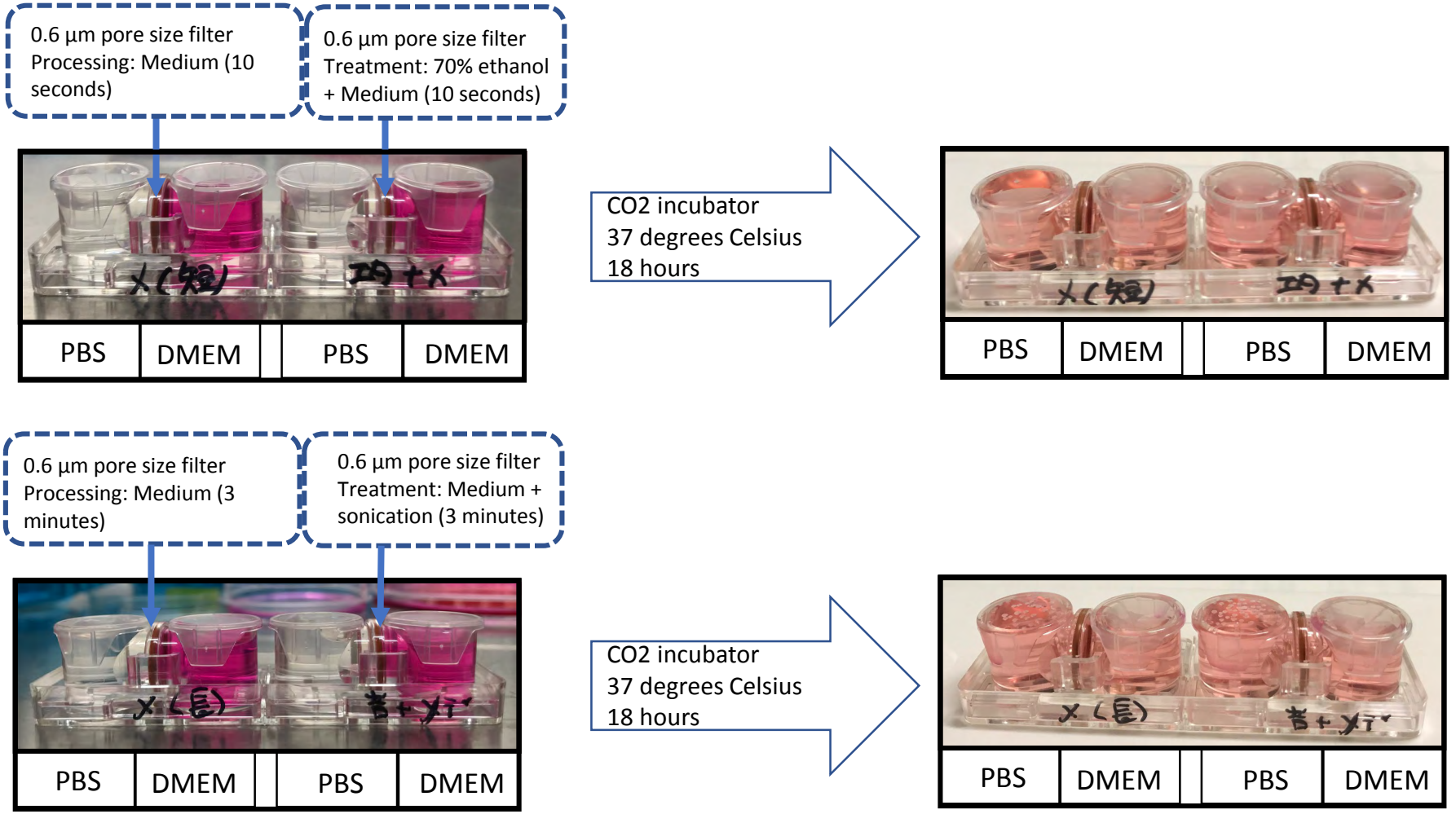




Recommended) Dip in medium to use for about 5 minutes.
Pinch the filter with tweezers, rinse well and take out.
Further recommended) 1minutes treatment with 100% ethanol before immersion in medium

Troubleshooting case study Transfer of culture medium

About the difference by filter pretreatment

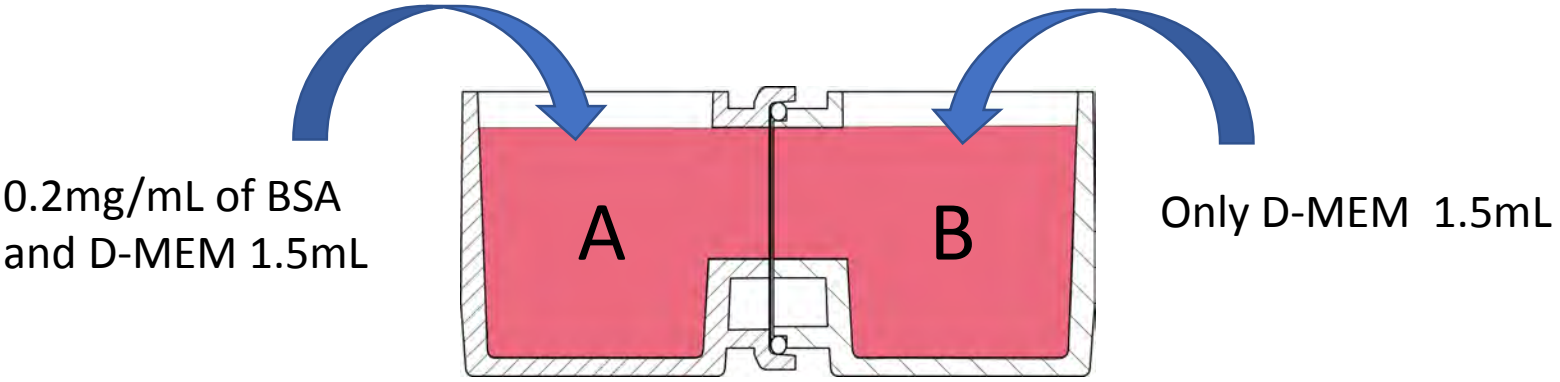


The mobility of phenol red was not different.

Troubleshooting case study BSA passage level

Study on the difference in protein pretreatment by Bovine Serum Albumin (BSA)

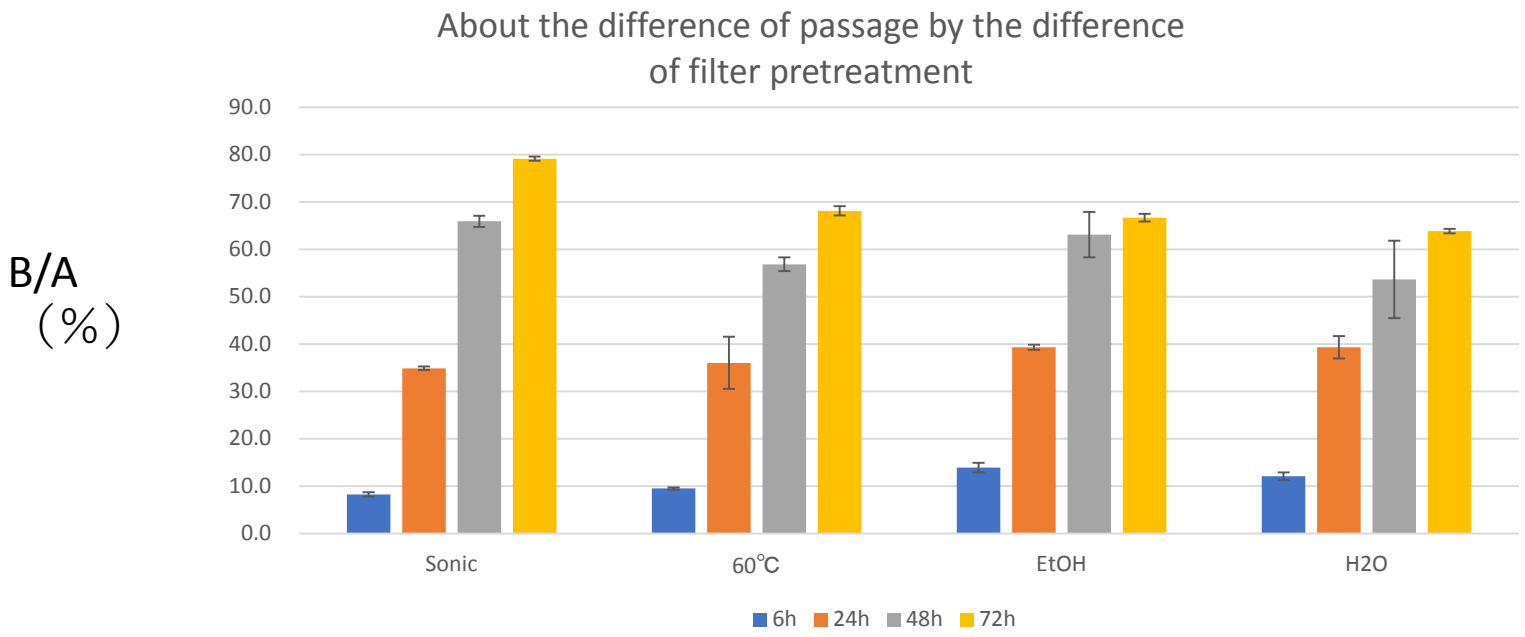
Use filter of 0.6µm pore.
0.2 mg / mL BSA and 1.5 mL D-MEM for A
D-MEM only 1.5mL for B
After docking 6 h, 24 h, 48 h, 72 h and collect 100 µL each from the A and B sides,
That amount was replenished every time. The collected sample was subjected to protein quantification, and the transfer rate over time was calculated.



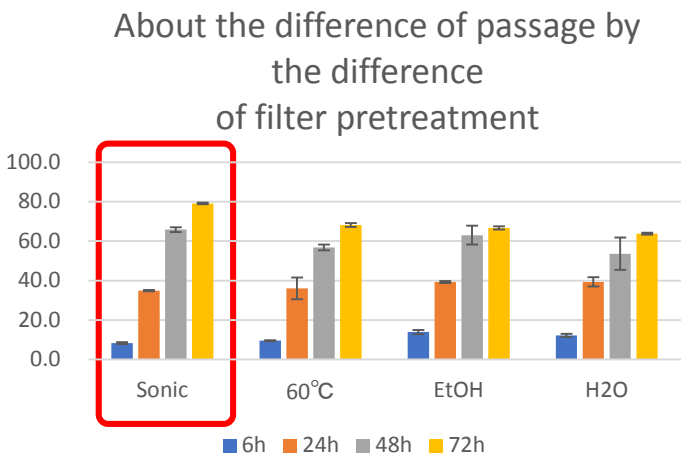
After 6 h, 24 h, 48, 72 h, quantify the amount of BSA on the left and right A, B
(The percentage (%) was measured for mobility to B.)

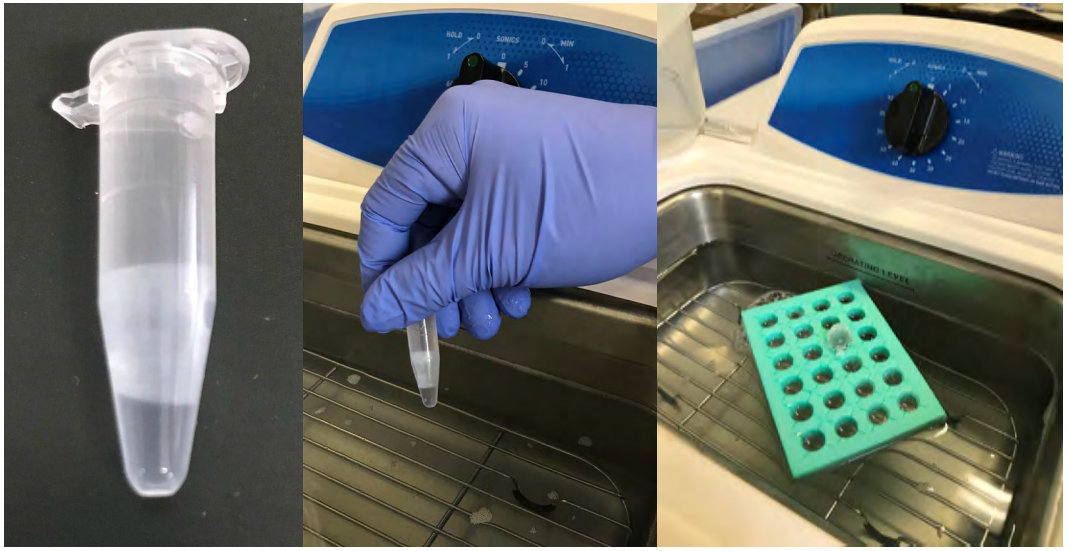
Troubleshooting case study BSA passage level

Process name	Processing method	⇒	⇒	⇒	⇒
Sonic	The tube was filled with Filter, filled with H_2O, and sonicated for 5 min.	Soak both sides of filter into EtOH	Soak both sides of filter into H2O quickly	Soak both sides of filter into PBS	Attach to ICCP and add Medium before drying
60℃	The tube was filled with Filter, filled with H 2 O heated to 60 ° C., and heated at 60 ° C. for 5 minutes				
EtOH	Immersed in EtOH for 1 min				
H2O	Immersed in H2O for 1 min				



Troubleshooting case study BSA passage level





Overall, there seems to be no major difference due to differences in pretreatment, but the addition of sonication resulted in a slightly better migration of BSA after 72 h.
However, at this point, we did not confirm the effect of ultrasonic waves on the filter holes, and we did not confirm the presence of tears in the holes due to vibration, so we decided that we could not recommend it. We will report if we can confirm the results in the future. It is also possible that 60-degree warm water treatment may be more effective than room temperature water, but it was judged that ethanol pretreatment was sufficient, considering that there was no difference in passability and time and effort. The recommendation is ethanol treatment, as it was a result that the passing degree around 48 hours with ethanol treatment may be better than nothing. However, it seems that there is no problem with the way we used to do so by dipping in medium without doing anything. Even BSA takes some time to pass. In experiments where cells are placed in only one container, it takes time to pass the substances produced by the cells through the filter, so keep in mind that there may be a difference in concentration between the left and right.