

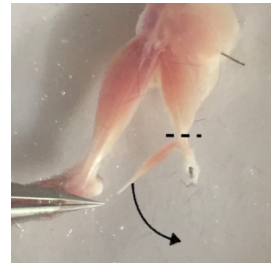
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Version 2

Dissection of mouse EDL and Soleus muscles V.2

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Optical Clearing of Tissue



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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol shows how to dissect the Extensor Digitorum Longus (EDL) muscle and Soleus muscle from a mouse leg.

Guidelines

Two muscles are dissected from a mouse leg: *Musculus Soleus* and *Musculus Extensor Digitorum Longus* (EDL). The dissecting process takes place in Ringer solution. The pictures show the right leg, so for the left leg.

Dissected legs of sacrificed mouse need to be stored in Ringer solution right after dissection and the tube containing the legs should be stored on ice at all times. This is very important because it helps to balance the water and electrolytes and stabilizes the pH value in the tissue.

Materials

MATERIALS

✕ 1X Dulbecco's Phosphate Buffered Saline (DPBS) **Thermo Fisher Scientific Catalog #14190094**

✕ Paraformaldehyd **Carl Roth Catalog #0335**

✕ NaCl

✕ KCl

✕ CaCl₂

✕ HEPES

✕ MgCl₂

✕ Glucose

✕ NaOH



Troubleshooting

Safety warnings

! Paraformaldehyde may cause sensitisation by skin contact and is harmful by inhalation and if swallowed.

Before start

Prepare Ringer solution:

	Reagent	Concentration
	NaCl	145 mM
	KCl	5 mM
	CaCl ₂	2,5 mM
	HEPES	10 mM
	MgCl ₂	1 mM
	Glucose	10 mM
	pH 7.4 with 1M NaOH	330 mosmol

Prepare 3.7% PFA/DPBS:

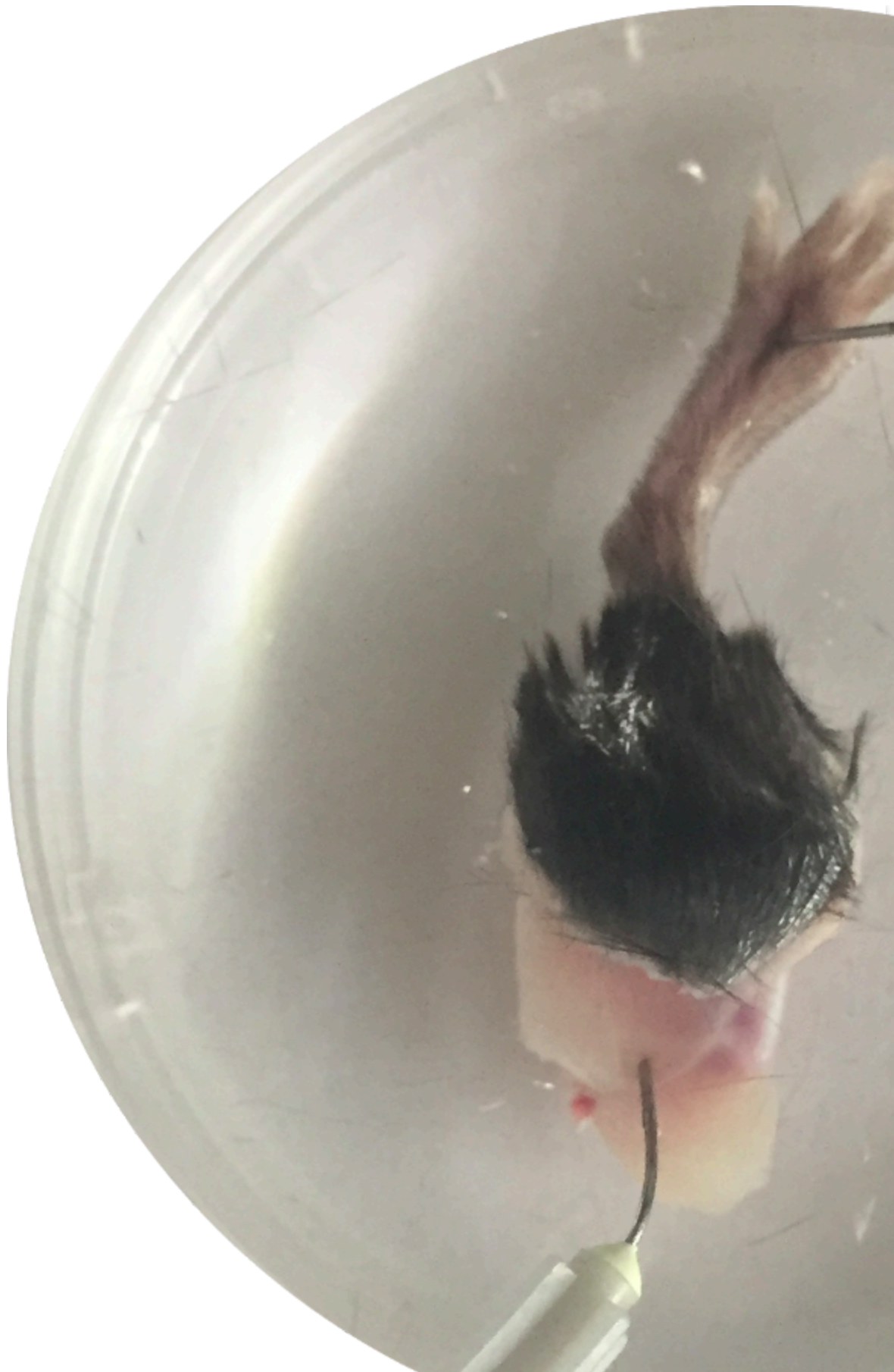
- Work under a fume hood!
- Use a magnetic stirrer and warm up to 55 °C
- Add a few drops of NaOH for better solubility
- Make sure all of the PFA is dissolved
- Adjust pH to ~7
- Freeze in portions till use

Additional materials:

- Dissecting instruments
- Dissecting dishes
- Dissecting needles
- Stereomicroscope
- Parafilm



- 1 Pin the leg in a flexed position to the dissecting dish and cover it with Ringer solution.





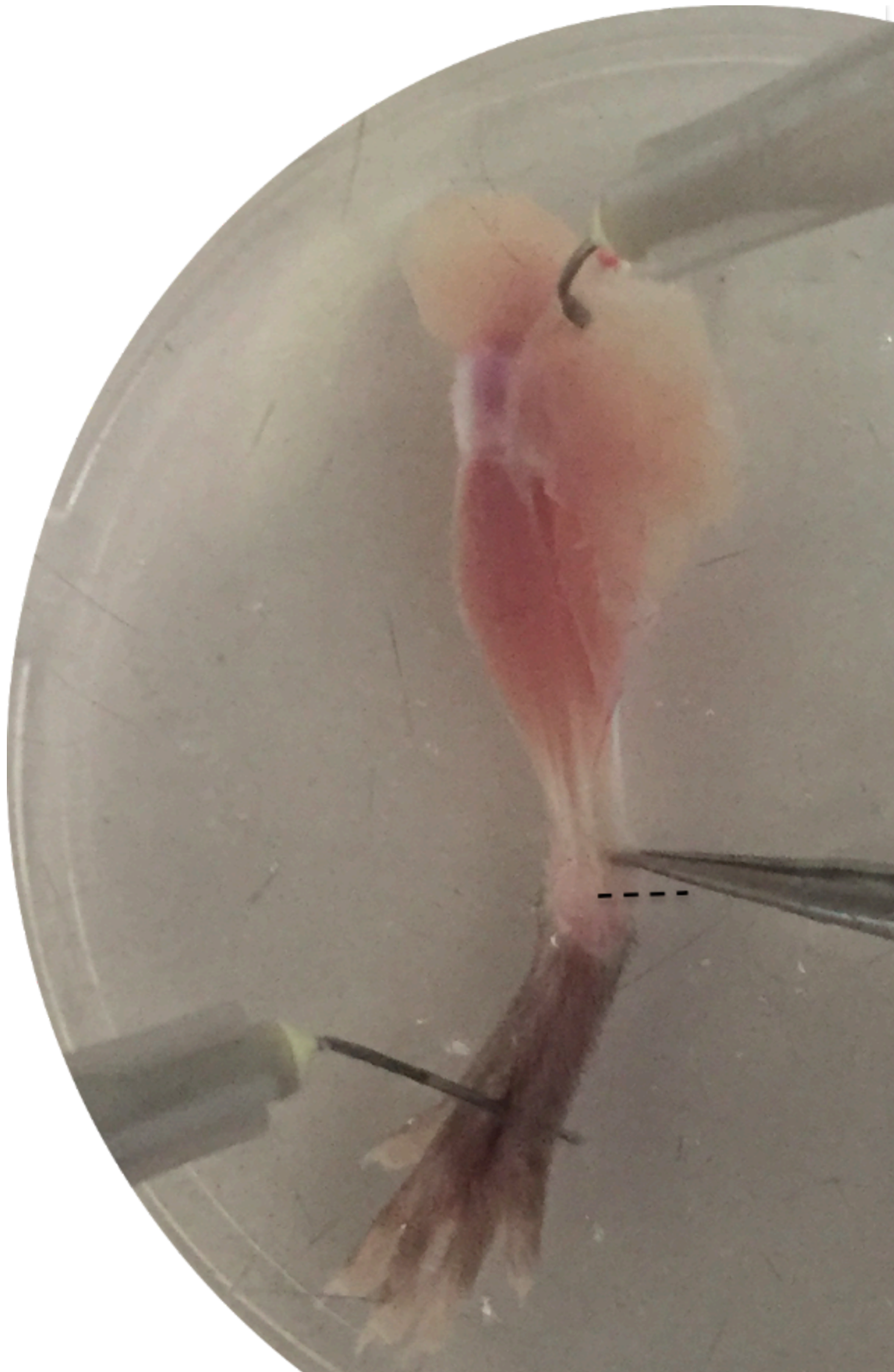
Soleus

- 2 For extraction of the Soleus, first, remove the skin of the leg. Be sure to remove all surrounding fascia as well.





- 3 Hold the Achilles tendon with the forceps. With the bodkin side of the scissors, carefully rub a gap open between the Achilles tendon and the other tendons. When the gap is visible, cut the Achilles tendon as close as possible to the foot.



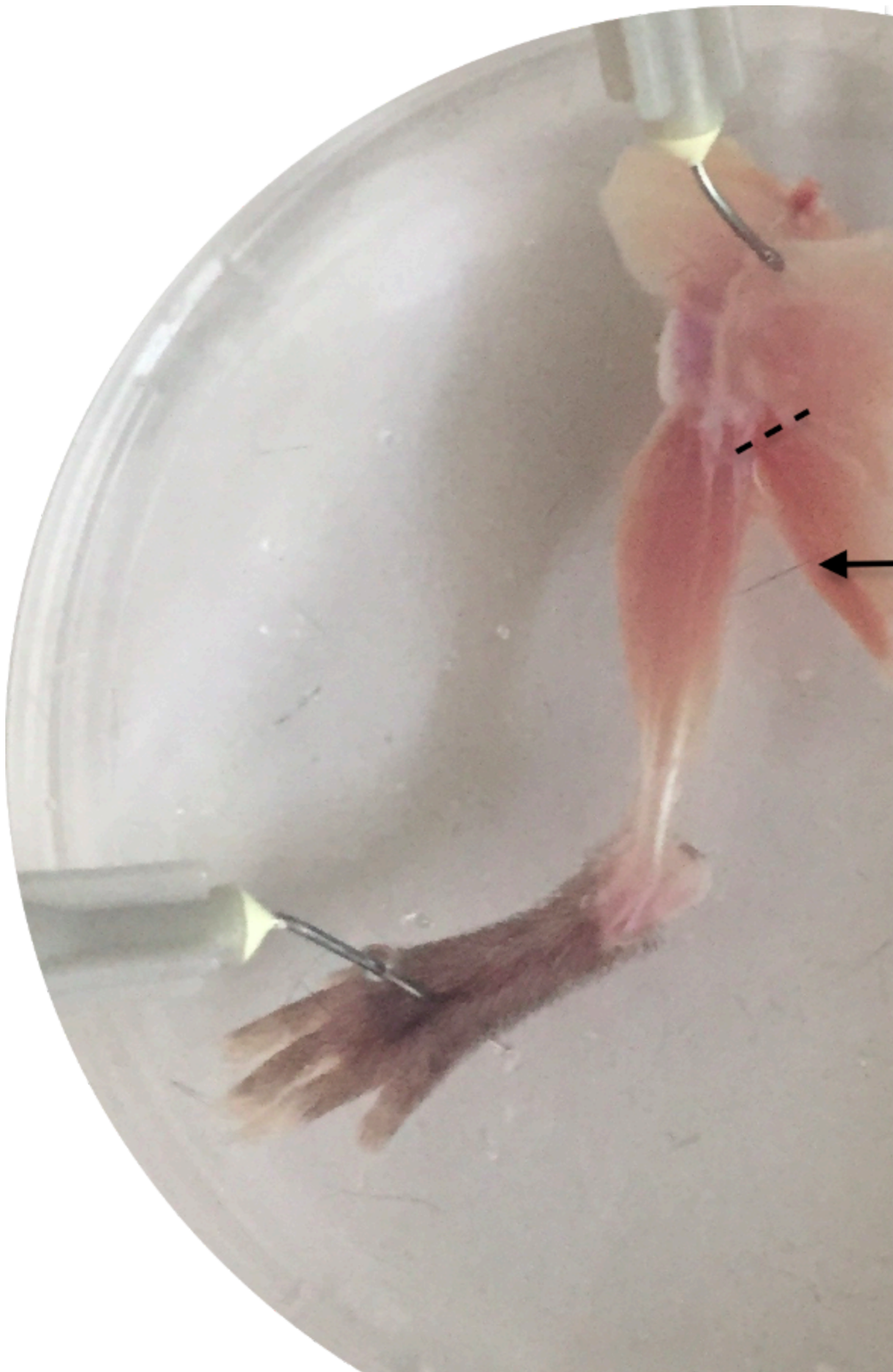


- 4 A gap should naturally start to open up. Carefully help that gap to open up. If surrounding fascia is still holding it together carefully cut it.



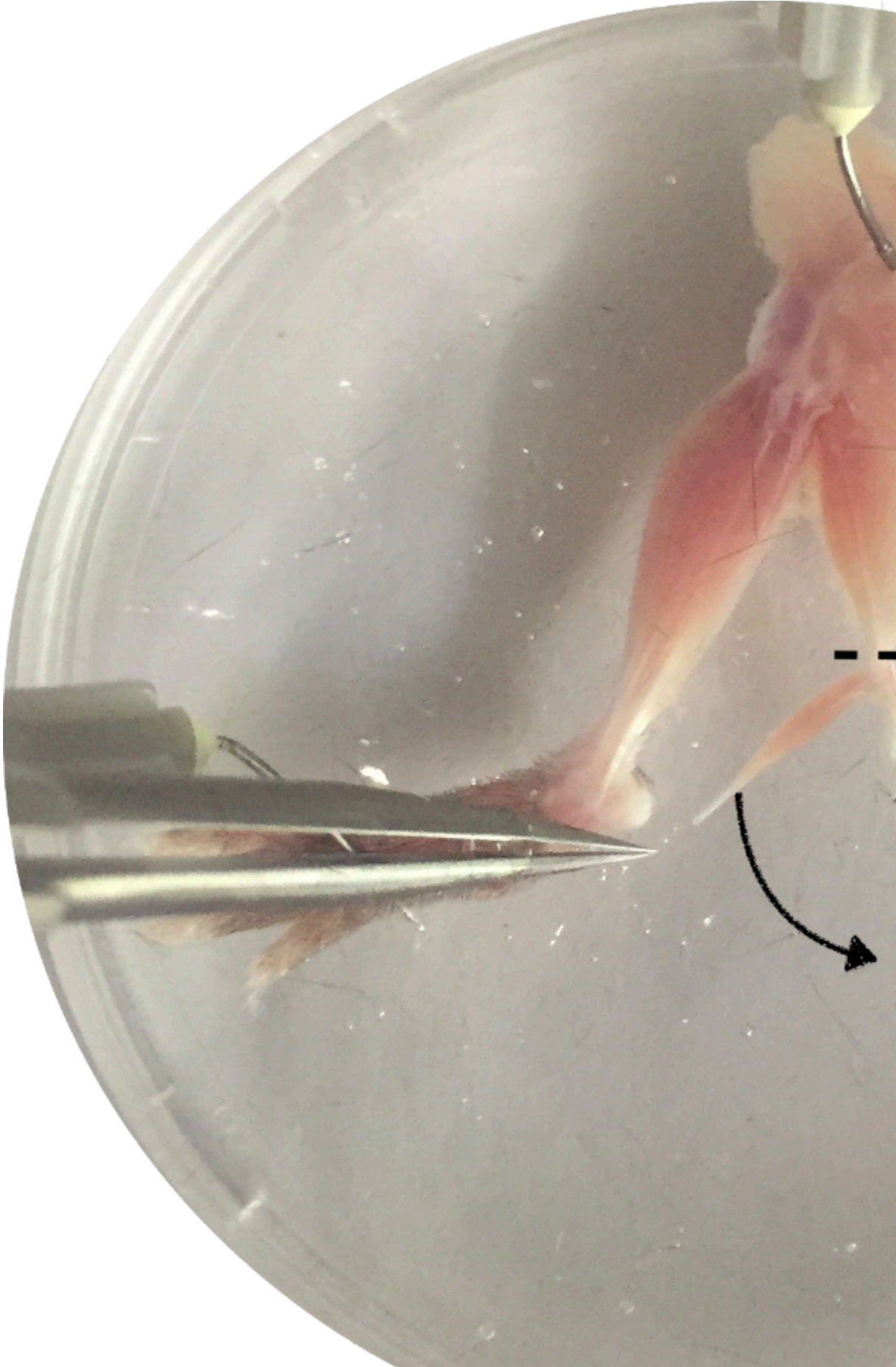


- 5 When the gap is open up to the knee the Soleus muscle is visible. You can recognize it by it's colour which is a darker pink. Carefully cut the tendon as close as possible to the knee.





- 6 Gently hold the Soleus muscle on its upper tendon with the forceps and pull down very slowly and careful while (if needed) cutting all surrounding fascia that's still holding it. After the whole muscle is freed from fascia make a cut at the lower tendon.





Note

It's ok to cut the Achilles tendon as well and clean it afterwards.

- 7 Separate the Soleus muscle from the leg and clean from all leftover fascia. For easier handling, you can move the muscle to a new dissecting dish, pin the muscle as shown in the picture, cover with Ringer solution, and store at 4 °C.





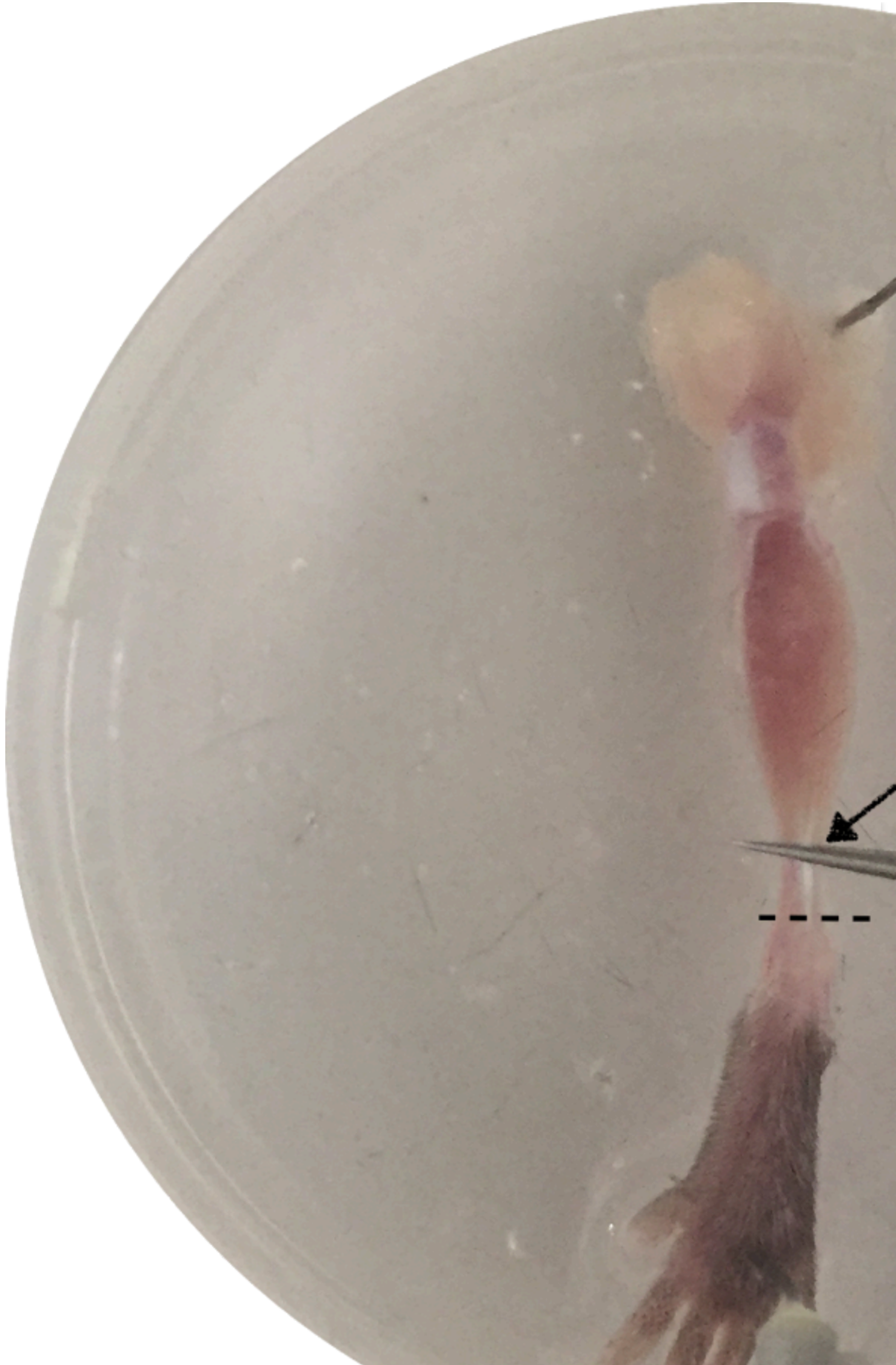
EDL extraction

- 8 For dissecting the EDL muscle, first, pin the leg into a stretched position. Then remove all fascia.



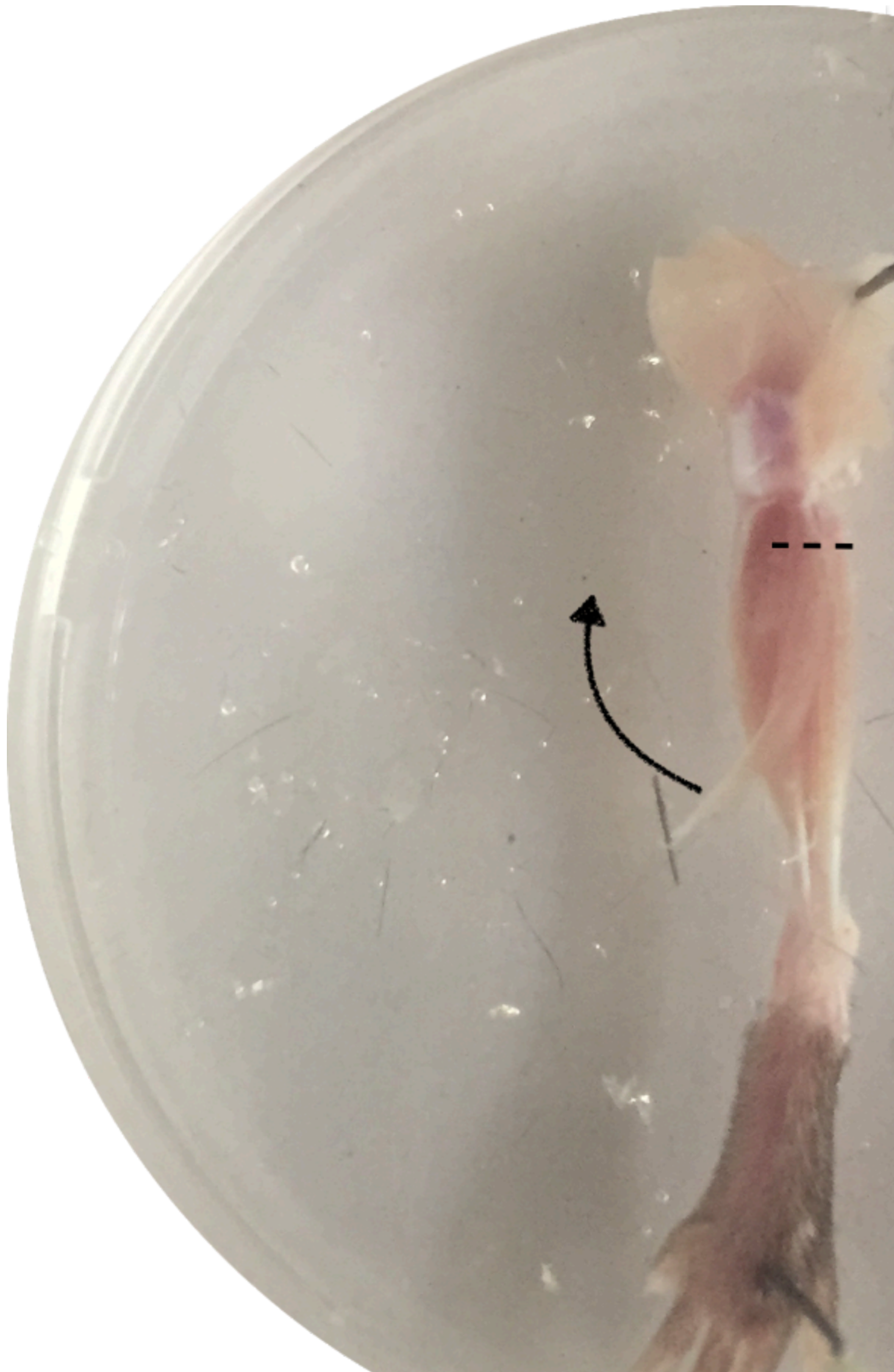


- 9 Rub the tendons on the lower legs free. The 4 EDL tendon ends are next to the V-looking Tibialis tendon. It can be checked if it's the EDL's by carefully lifting them up with the forceps. While lifting the tendon ends the toes of the paw should stretch. Cut the tendon ends as close as possible to the foot.





- 10 After the tendon ends are cut a 'pocket' naturally opens up. A few surrounding fascias might still need to be removed. Free the EDL muscle from the pocket till the upper tendon is visible. Then cut the upper tendon.





- 11 Separate the EDL muscle from the leg and free from all fascia and fat.





- 12 Move both dissected muscles to a clean dissecting dish. Pin muscles lightly stretched to the dish and cover with 3.7% PFA.
- 13 Cover the dish with parafilm and store at 4°C overnight.