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We are still developing and optimizing this protocol

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Passaging of Organoids in Matrigel

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ABSTRACT

This protocol is for passaging prostate cancer organoids in matrigel. Protocol modified from Drost et al. (see citation below)

Keywords: Organoids,
Prostate, Passaging, Matrigel

Before start

1 Prepare sufficient medium for passaging and prewarm immediately before use

2 Thaw Growth Factor Reduced (GFR) Matrigel® **Corning Catalog #354230** on ice o/n

3 Warm TrypLE™ Express Enzyme **Thermo Fisher Scientific Catalog #12604013** to 37°C before use

4 Coat 15 ml Falcons with 1% BSA o/n

5 Coat tips before use in 1% BSA by pipetting up and down a few times

6 Take pictures of the organoids to count organoids per well and measure the size of the organoids

> Note
>
> Add results from counting here:

Dissociate organoids

45m

7 Break up matrigel lense by pipetting up and down with a P1000 for approximately 10 times and transfer all content of a well to a coated 15 ml Falcon.
Repeat with all remaining wells

8 Once the content of all wells is transferred to the Falcon, pipette the whole suspension up and down a few more times to further break up the matrigel.

9 Add up to 10 mL of PBS

10 500 x g, Room temperature, 00:05:00 5m

The higher speed helps to pellet the cells below the matrigel layer. Otherwise they get trapped in the matrigel and it is easy to suck them up with the supernatant

11 Mark the top of the matrigel pellet. Remove as much supernatant as possible without disturbing the matrigel.

12 Add 5 mL PBS

13 500 x g, Room temperature, 00:05:00 5m

14 Remove supernatant as described above

15 Add 1-2 mL prewarmed TrypLE™ Express Enzyme **Thermo Fisher Scientific Catalog #12604013** and pipette up and down to make sure that the cell pellet is well resuspended.

16 Place falcon in incubator for 00:10:00 to 00:15:00 25m

17 Pipette up and down for 10 times every 5 min and check the progress under the microscope

18 When only small clusters and single cells remain, add 5 mL PBS

19 400 x g, Room temperature, 00:05:00 5m

20 Remove supernatant and resuspend pellet in another 5 mL PBS

21 400 x g, Room temperature, 00:05:00 5m

22 Remove as much supernatant as possible and keep the Falcon tube on ice until ready.

Seed cells in organoids 15m

23 Depending in the passaging ratio, decide how many wells you want to seed the cells into and calculate the necessary amount of matrigel.

	Plate size	Volume matrigel per well (ul)	Number of wells seeded	Split ratio	Total matrigel volume (ul)	Volume medium per well (ul)
	96-well					
	48-well	20	6	1:0.8	125	200
	24-well					
	12-well					
	6-well					

24 Add matrigel to the cell pellet and carefully resuspend cells. Avoid formation of bubbles.

25 Carefully add a matrigel drop to the center of the well.

26 Flip the plate and let the matrigel solidify at 37 °C in the incubator for 00:15:00 15m

27 Carefully add prewarmed medium on top of matrigel lense.

28 Incubate at 37 °C in the incubator to expand cells.