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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)





Protocol Citation: Annika Fendler 2023. Passaging of Organoids in Matrigel. protocols.io

https://protocols.io/view/pass aging-of-organoids-inmatrigel-cycexste

MANUSCRIPT CITATION:

Drost J, Karthaus WR, Gao D, Driehuis E, Sawyers CL, Chen Y, Clevers H. Organoid culture systems for prostate epithelial and cancer tissue. Nat Protoc. 2016 Feb;11(2):347-58. doi: 10.1038/nprot.2016.006. Epub 2016 Jan 21. PMID: 26797458; PMCID: PMC4793718. https://www.nature.com/articl es/nprot.2016.006

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Protocol status: In development We are still developing and optimizing this protocol

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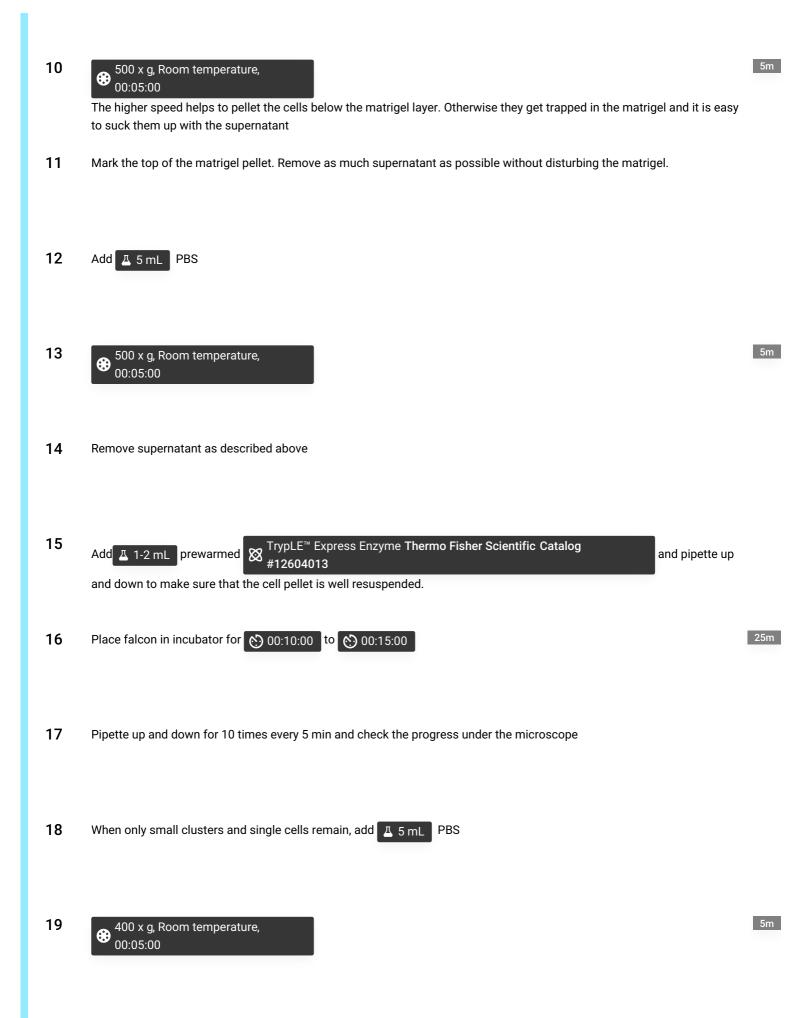
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PROTOCOL integer ID:

86118

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 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 A 10 mL of PBS	



20	Remove sup	pernatant and resuspend pelle	et in another 🔼 5 mL P	BS				
21	400 x g 00:05:0	, Room temperature, 0				5m		
22	Remove as	much supernatant as possible	e and keep the Falcon tub	e on ice u	ntil ready.			
	Seed cells in organoids							
23	Depending i amount of r	n the passaging ratio, decide in the passaging ratio in the pa	how many wells you wan	t to seed t	he cells into and calculate	the necessary		
	Plate size	Volume matrigel per well (ul)	Number of wells seeded	Split ratio	Total matricel volume (ul)	Volume medium per well (
	96-well	recurre manager per men (a.)			coa manger coame (a)	To a mountain por mon		
	48-well	20	6	1:0.8	125	200		
	24-well							
	12-well							
	6-well							
			,		,			

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

Carefully add prewarmed medium on top of matrigel lense.

Flip the plate and let the matrigel solidify at \$\mathbb{8}\$ 37 °C in the incubator for \bigode{\infty} 00:15:00

15m