Isolation and Expansion of Primary Tumour Cells from Cancer Tissue

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Introduction

Primary tumour-derived cells provide a physiologically relevant starting point for 3D culture models, including patient-derived organoids generated in LunaX™ ECM (see LX-APR-002). This protocol describes the isolation of viable tumour cells from biopsies or resected cancer tissue, and their expansion in 2D culture to approximately 80% confluence in preparation for 3D encapsulation.

Expected Results

Viable tumour cells typically attach and expand to approximately 80% confluence within 1–2 weeks, depending on tumour type and sample quality. Healthy expanding cultures show progressive monolayer formation with minimal floating debris by the end of the first week.

> ***Note:*** *Primary tumour samples do not reliably yield proliferative cultures in vitro. Success varies with tumour type, cellular composition, and sample quality. For breast cancer biopsies, successful in vitro expansion is achieved in approximately 50% of donor samples.*

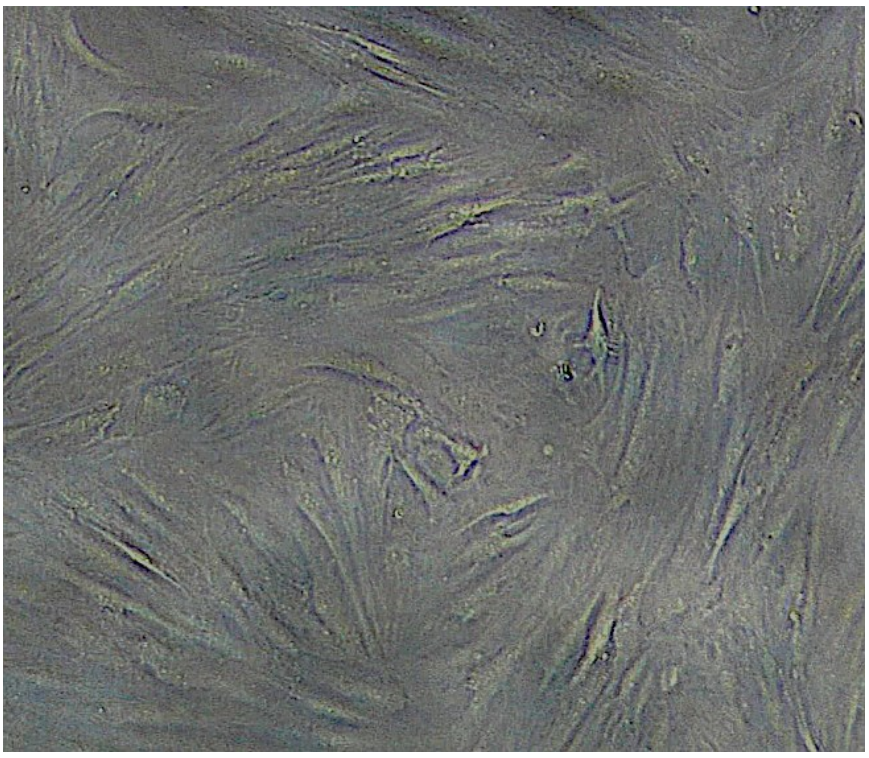

Figure 1. *Representative brightfield image of patient-derived 2D cell culture.*

Materials and Equipment

Reagent / Item	Specification / Concentration
Dulbecco's PBS (DPBS)	Sterile, calcium- and magnesium-free
Collagenase/Hyaluronidase 10X	STEMCELL Technologies, Cat. #07912 — dilute 1:10 in organoid medium before use
Trypan Blue	0.4% solution
Trypsin–EDTA	0.25% in DPBS
Fetal bovine serum (FBS)	Heat-inactivated; 10% v/v final concentration
Advanced DMEM/F12	Base medium
Penicillin–streptomycin	1% v/v
HEPES (1 M stock)	10 mM final concentration in medium
GlutaMAX	1×
Epidermal growth factor (EGF)	10 ng/mL final concentration in medium
Y-27632 (ROCK inhibitor)	5 μM
Hydrocortisone	1 μg/mL
Certified Class II biosafety cabinet	For aseptic handling of human-derived material
Orbital shaker or shaking incubator	~150 rpm
CO_2 incubator	37 °C, 5% CO_2, humidified
Centrifuge	Swing-bucket rotor
Automated or manual cell counter	For viability and density assessment
Cell strainers	100 μm and 37 μm, reversible
T75 cell culture flasks	Standard tissue-culture treated
Sterile Petri dishes, scalpels, and pipettes	For tissue mincing and handling

> ***Organoid Medium (also used for expansion):*** *Supplement Advanced DMEM/F12 with 10% FBS, 1% penicillin–streptomycin, HEPES, GlutaMAX, 10 ng/mL EGF, 5 μM Y-27632, and 1 μg/mL hydrocortisone. Prepare fresh and pre-warm to 37 °C before use.*

Procedure

1. Sample Receipt and Preparation

1. Receive fresh cancer tissue immediately in sterile medium on ice.
2. Transfer tissue to a sterile Petri dish inside the biosafety cabinet.
3. Using sterile scalpels, mince tissue into fragments of approximately 0.5 × 0.5 × 0.5 mm.
4. Transfer minced tissue into a sterile 50 mL centrifuge tube.

2. Enzymatic Digestion

1. Add 0.5 mL of Collagenase/Hyaluronidase 10X to 4.5 mL of pre-warmed organoid medium and mix to prepare a 1X working solution. Add this to the minced tissue. For large quantities of tissue, consider splitting across multiple centrifuge tubes.

2. Incubate at 37 °C, 5% CO_2 on an orbital shaker at ~150 rpm for 1–2 hours until the suspension appears partially digested.
3. Gently pipette up and down every 30 minutes to aid mechanical dissociation.

3. Filtration and Fraction Handling

1. Pass the digested suspension through a 100 μm strainer, followed by a 37 μm strainer, into a clean 50 mL tube.
2. Collect the cell suspension that passes through the strainers.
3. Wash once with DPBS and assess cell viability using Trypan Blue exclusion and count cells using a cell counter.
4. Optional: Transfer undigested fragments that are retained on the strainers to T75 flasks with organoid medium and culture as a separate fraction.

4. Initial Culture and Expansion

1. Seed cells into T75 flasks at an appropriate density (typically 1–3 × 10^6 cells/flask) in pre-warmed organoid medium.
2. Incubate at 37 °C, 5% CO_2 in a humidified atmosphere.
3. Replace medium twice weekly with fresh pre-warmed organoid medium.
4. Monitor cultures microscopically; cells should attach and expand to ~80% confluence within 1–2 weeks, depending on tumour type.

5. Cell Lifting and Sub-culture

1. Aspirate medium and rinse the monolayer once with DPBS.
2. Add sufficient pre-warmed 0.25% trypsin–EDTA to cover the cell layer.
3. Incubate at 37 °C until cells detach (typically 3–5 minutes).
4. Neutralise trypsin with an equal volume of organoid medium.
5. Collect the suspension, centrifuge at 300 × *g* for 5 minutes, and resuspend the pellet in fresh organoid medium for counting, reseeding, or downstream applications, such as encapsulation.

References

- LunaX ECM and LunaX Crosslinker manufacturer's instructions (Gelomics)
- LX-APR-002 – Cell Encapsulation to Generate Patient-Derived Organoids (PDOs) in LunaX ECM