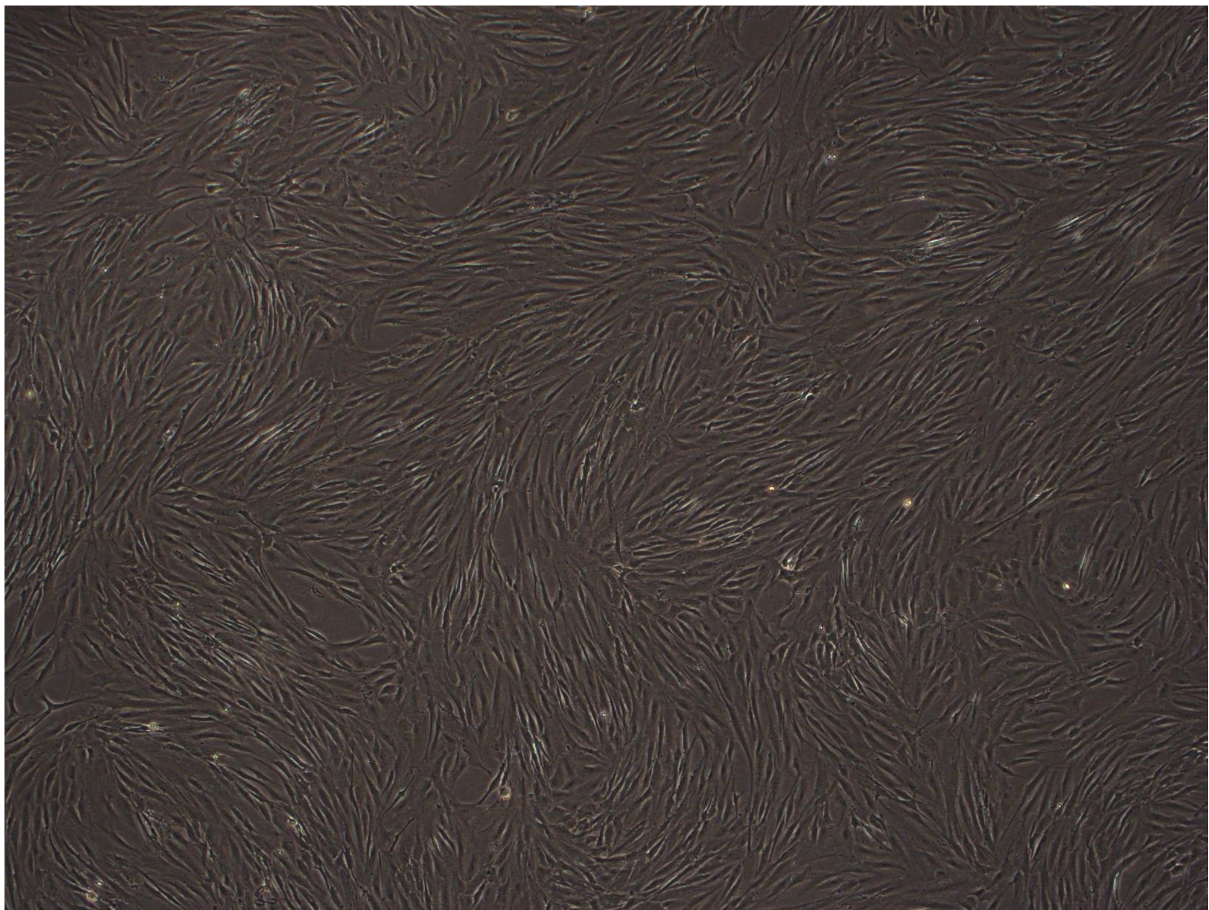
breast cancer now

Breast Cancer Now Cell Bank – Fibroblasts



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Breast Cancer Now Cell Bank- Fibroblasts

The Breast Cancer Now Tissue Bank takes tissue from patients with varying disease status. The tissue, with informed consent from the patient, is assessed by a pathologist who will ascertain what is required for diagnostics and supply the tissue bank with any excess material. We are able to provide fibroblasts from:

Normal reduction mammoplasty

Prophylactic mastectomy (removal of breast tissue due to family history and/or BRCA 1/2 genes)

Contralateral- prophylactic (following tumour in 1 breast, worried about spread or new tumour in other) or mastectomy (following removal of tumour in 1 breast, evening up for appearance).

Ductal carcinoma in-situ (DCIS)- pre-invasive cancer

Tumour- invasive cancer which can be of varying grade and hormone receptor status

Adjacent- from edge of tumour to 2 cm away

Surround- at least 5 cm away from tumour edge

Lymph Node- common place of tumour metastasis- only receive when obviously full of tumour alongside other lymph nodes going for pathology

Before the cells reach you:

Cell Isolation

All tissue on arrival is washed, once with 70% ethanol (if big enough) and 3 times with RPMI-1640 media plus 25mM Hepes, supplemented with 5% foetal bovine serum (FBS), penicillin (100U/ml), streptomycin (0.1mg/ml), amphotericin-B (5µg/ml) and Gentamicin (20 µg/ml). After washing the tissue is measured.

For normal, prophylactic mastectomy, contralaterals and, where there is sufficient material, DCIS, adjacent and surround the following method is used to isolate fibroblasts:

Tissue is chopped in to small pieces and is digested for 12 to 16 hours at 37˚C in RPMI-1640 medium plus 25mM Hepes, supplemented with 5% foetal bovine serum (FBS), penicillin (100U/ml), streptomycin (0.1mg/ml) and amphotericin-B (5µg/ml) containing 1mg/ml collagenase 1A and hyaluronidase on a rotary shaker. The digested tissue is then centrifuged at 380g for 20 minutes and washed in medium three times to remove enzymes. The tissue isolates are then sedimented three times at 1g for 30 mins to collect the denser organoids. The supernatants containing the fibroblasts are centrifuged (380g x 3 minutes) and the cell pellets re-suspended and cultured in DMEM:F12 supplemented with 10% FBS, hydrocortisone, transferrin, insulin, EGF, penicillin/streptomycin and amphotericin-B (Breast Culture Medium, BCM).

When there is sufficient tumour tissue and lymph node the following method is used:

Tissue is chopped in to small pieces and digested for 2-4 hours with 2mg/ml collagenase 1A and hyaluronidase or 12-18 hours with 1mg/ml collagenase 1A and hyaluronidase at 37˚C in RPMI-1640 medium plus 25mM Hepes, supplemented with 5% foetal bovine serum (FBS), penicillin (100U/ml), streptomycin (0.1mg/ml) and amphotericin-B (5µg/ml) on a rotary shaker. The digested tissue is

then centrifuged at 380g for 20 minutes and washed in medium three times to remove enzymes. After this the digested tumour tissue is filtered through a 40µm filter and the filtrate centrifuged and cultured as for the normal and surround cell isolates.

When only small pieces of tissue (explants) are available, for all tissue types:

Tissue is chopped into very small pieces (~2mm^3). These are allowed to attach to the surface of a flask with minimal media (BCM) for 1-4 hours. Once attached more media is added and the flasks containing these explants are separated from other ongoing cultures and left untouched for a week to avoid disruption and detachment. After this they are cultured as normal, with remaining explants gently removed at 1st passage.

All fibroblasts are grown for 2 passages in BCM and then a further passage in FM before being frozen at 1 million cells per vial to be given out. If the cells appear at this point to still have epithelial contamination further passaging will occur before giving out. Cells are then stored in liquid nitrogen before being sent to you on dry ice. If you are not culturing straight from arrival, cells should be kept in liquid nitrogen until use.

Each cell line is cultured separately and a laboratory management system is in place to ensure there is no cross-contamination. Previously, we have both stained via immunocytochemistry a number of our cultures, and probed via western blotting for various fibroblast markers including vimentin, fibronectin and α-SMA. Due to the consistency seen across these markers and in the cells grown we are confident in the cell type. The cells are also checked for mycoplasma by PCR of the cell culture media. Some fibroblasts have been RNA-sequenced by applicants to the bank and this has also given results consistent with fibroblasts.

Media Recipes

BCM

Media used for initial growth, the fibroblasts you receive sent shouldn't require this.

Make up 500ml of BCM by adding penicillin (100U/ml), streptomycin (0.1mg/ml) (pen/strep Sigma P4333), 2.5µg/ml amphotericin-B (Fungizone Sigma A2942), 50ml FBS, 0.5µg/ml hydrocortisone (H0888 Sigma), 10µg/ml apo-transferrin (T1147 Sigma), 10ng/ml EGF (E9644 Sigma), 5µg/ml insulin (I9278 Sigma) to 500ml DMEM:F12 (D8437 Sigma, already contains glutamine).
Full methods for reconstitution of additives is available should you require.

FM

We use this routinely in fibroblast experiments. Your cells have been in Fibroblast Medium (FM) prior to freezing.

Make up 500ml of FM by adding penicillin (100U/ml), streptomycin (0.1mg/ml) (pen/strep Sigma P4333), amphotericin-B (2.5µg/ml) (Fungizone Sigma A2942), 50ml FBS, to 500ml DMEM:F12 (D8437 Sigma, already contains glutamine).

FBS: we use Gibco Fetal Bovine Serum, qualified, heat inactivated, Brazil Catalog number: 10500056. We've previously experienced problems with serum causing cells to die with a marked difference in appearance with a large numbers of vesicles.

Methods for you

Reviving fibroblasts

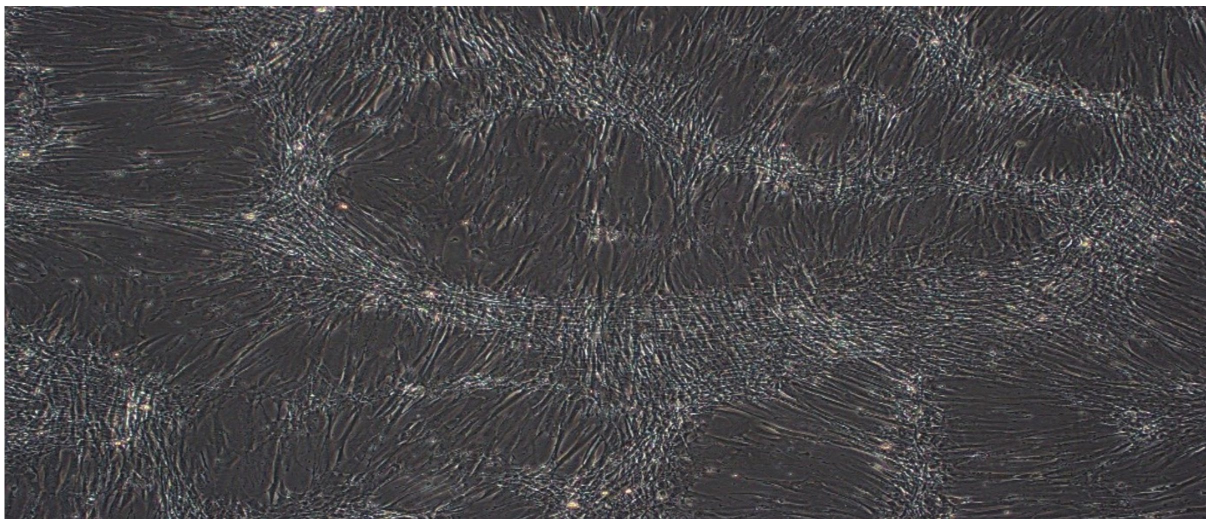
1. Keep the sample on dry ice until ready to thaw.
2. Thaw as quickly as possible using hands to warm the sample.
3. When just starting to melt, slowly add room temp medium dropwise to the sample.
4. Decant by pouring into tube containing 5mls of fresh medium.
5. Wash out the cryotube with medium to collect any cells left behind.
6. Spin the cells at 380g for 3mins.
7. Remove supernatant and re-suspend the cells in a small volume of FM medium, using a 200µl Gilson pipette. Viability and cell numbers can be checked at this point (fibroblasts are frozen at 1 million per vial and have consistently high viability after freezing).
8. Make cell volume up to 15mls with more medium and seed into a T75 (15ml medium) or T175 (25ml medium), depending on how quickly you want to use them. Our incubators are set at 5% CO_2.

Seeding density

This will depend on how soon you require the cells. Routinely:
T25 0.15 million cells
T75 0.5-1 million cells
T175 1-2 million cells
20,000/well for 24 well plate
50-100,000/well for 6 well plate

Culturing fibroblasts

Change medium twice a week. Leave a small amount of conditioned medium (2ml in T175) in the flask and make the volume back up with warmed FM.
Grow until almost confluent. These cells will compact as they become more dense and may also show a 'fish-bone' morphology (see picture below). The fibroblasts, if grown in FM, should not have any problems growing to full confluence without peeling.
In BCM the cells are more prone to rolling up and peeling off if over-confluent.
If you see anything that doesn't look as expected, e-mail the cell team and they'll attempt to advise. Adding a picture to this is particularly useful.



To passage

For fibroblasts growing in a T75 or T175 flask, wash the cell layer with 10mls PBS (without calcium and magnesium), remove and add 5mls 1 x trypsin/EDTA (see below). Incubate at 37°C until the cells lift off. Fibroblasts are very variable. Some come off easily (before 5 minutes) and some take a very long time (45 mins plus). To encourage cells to dislodge you can tap the flask, add a small amount of extra trypsin or scrape cells off using a cell scraper. In exceptional circumstances a low concentration of collagenase can be added to the trypsin to help dissociate. Cell counting can be a problem as the cells may be very clumpy. Use a 200µl pipette to triturate the sample in a small volume before diluting for counting. On first passage excess cells should be frozen to give your own stocks. Seed as in reviving (above).

The cells should passage at least 3 times once you've received them but can possibly go on for many more passages.

Trypsin/EDTA (Hyclone SV30031.01 Fisher Scientific HYC 020 010K)
0.25% trypsin plus 0.1% EDTA in PBS without calcium and magnesium.
Thaw and aliquot in 5ml aliquots. Store at -20°C.
Dilute this 1/5 in PBS w/o ca/mg (D8537 Sigma) to give a final concentration of 0.05% trypsin and 0.02% EDTA.

Freezing medium

FM with 10% FBS, Pen/Strep, Fungizone (final conc 2.5 µg/ml)
Add FBS to a final concentration of 40% and 6% DMSO (Sigma D2650)

Passage the cells as normal, count. Spin down again and re-suspend in Freezing Medium. We tend to freeze a million cells in 0.5ml aliquots.

Place in a freezing jar as soon as possible and place in a -80°C freezer overnight before storing in LN_2. When freezing the cell sample, it is best to try and minimise the time the samples sit at room temp in the DMSO.