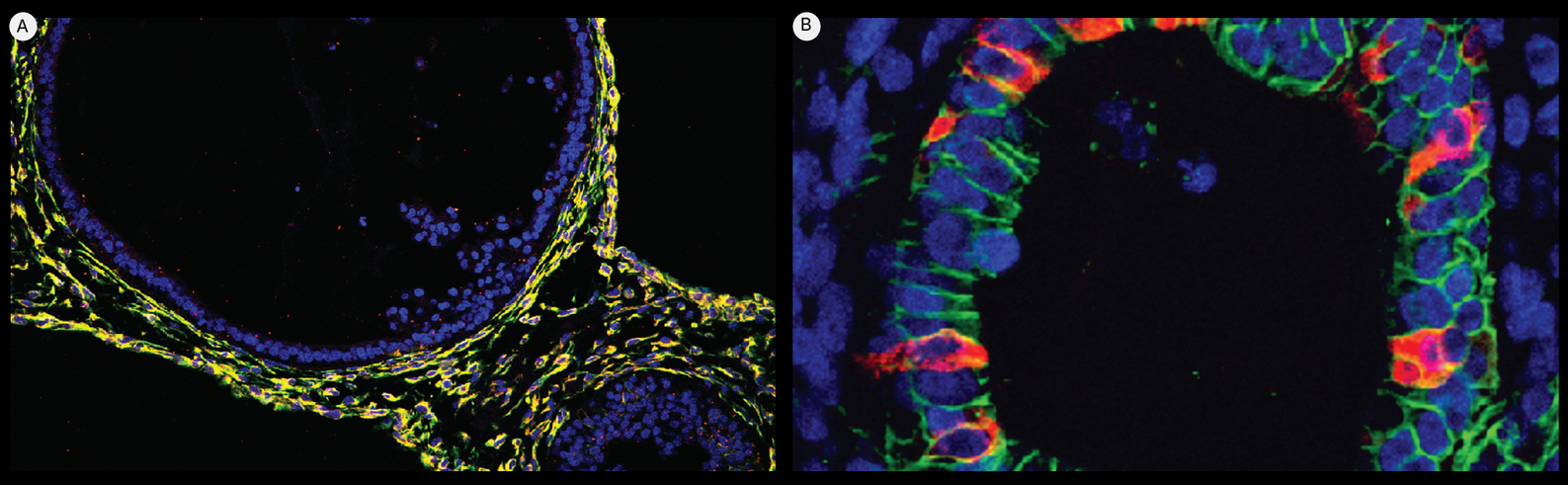
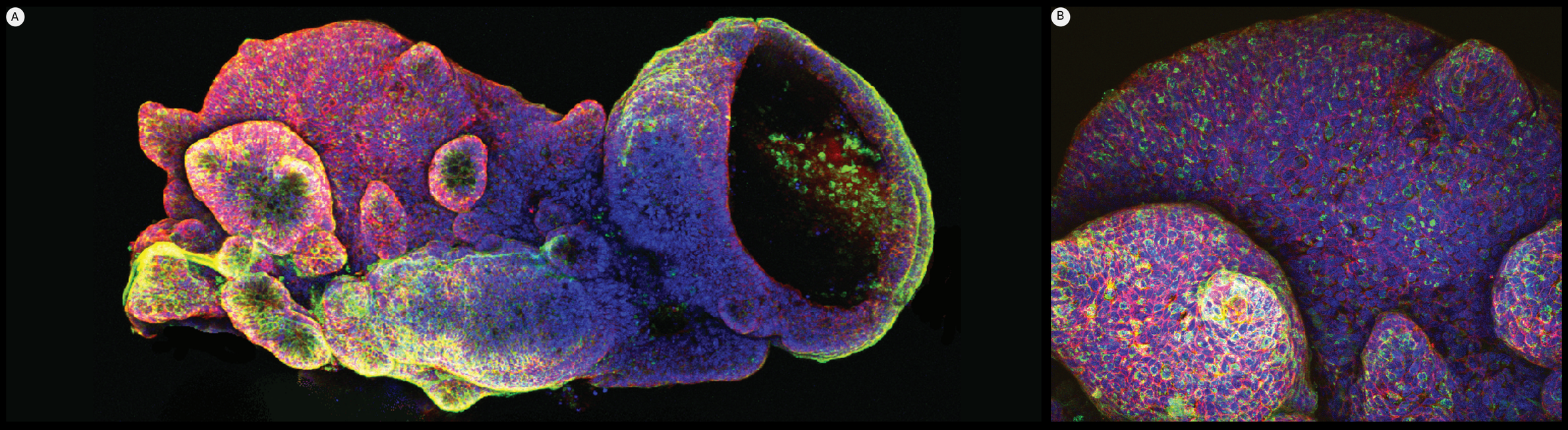
Organoids:
Model Systems of Tissue Development & Disease

Organoids and 3-D cell culture systems are emerging as powerful new platforms for exploring human organ development, modeling disease, screening for drug toxicity, and investigating personalized medicine. This poster shows some of the visually-stunning organoid images that have been generated by Cell and Gene Therapy scientists at Bio-Techne using the lists of reagents provided in the tables below.

Characterization of iPSC-derived Human Intestinal Organoids

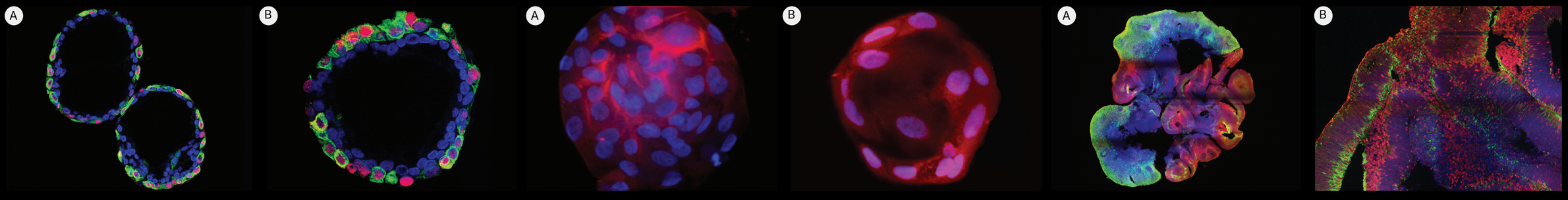
iPSC-derived human intestinal organoids were cultured using Cultrex™ UltiMatrix RGF Basement Membrane Extract (R&D Systems, Catalog # BME001-05) and the other reagents listed in the intestinal organoid culture recipe shown below. (A) Human intestinal organoids were stained using a Rat Anti-Human/Mouse Rat Vimentin Monoclonal Antibody (green; R&D Systems, Catalog # MAB2105) and a Goat Anti-Human/Mouse Desmin Antigen Affinity-purified Polyclonal Antibody (red; R&D Systems, Catalog # AF3844) to visualize myofibroblast cells and counterstained with DAPI (blue; R&D Systems, Catalog # 5748). (B) Human intestinal organoids were stained using a Goat Anti-Human/Mouse E-Cadherin Antigen Affinity-purified Polyclonal Antibody (green; R&D Systems, Catalog # AF748) and a Mouse Anti-Human MUC2 Monoclonal Antibody (red, Novus Biologicals, Catalog # NBP2-44431) and counterstained with DAPI (blue; R&D Systems, Catalog # 5748).





Characterization of Adult Stem Cell-derived Human Descending Colon Organoids

Adult stem cells isolated from human descending colon were embedded in Cultrex UltiMatrix RGF Basement Membrane Extract (R&D Systems, Catalog # BME001-05) and cultured in growth medium for 30 days. (A) Organoids were fixed and stained with a Mouse Anti-Human MUC2 Monoclonal Antibody (green; Novus Biologicals, Catalog # NBP2-44431), to visualize intestinal goblet cells, and counterstained with a Goat Anti-Human/Mouse E-Cadherin Antigen Affinity-purified Polyclonal Antibody (red; R&D Systems, Catalog # AF748) and DAPI (blue; R&D Systems, Catalog # 5748). The image shown was taken at 10x magnification. (B) Organoids were fixed and stained with a Mouse Anti-Human Chromogranin A Monoclonal Antibody (green; R&D Systems, Catalog # MAB90981) to visualize enteroendocrine cells, and counterstained with a Goat Anti-Human/Mouse E-Cadherin Antigen Affinity-purified Polyclonal Antibody (red; R&D Systems, Catalog # AF748) and DAPI (blue; R&D Systems, Catalog # 5748). The image shown was taken at 20x magnification.



Characterization of Human Lung Organoids

Adult stem cells isolated from human lung biopsy tissue were embedded in Cultrex UltiMatrix RGF Basement Membrane Extract (R&D Systems, Catalog # BME001-05) and cultured in lung organoid expansion medium for 20-60 days, where they were able to differentiate and exhibit key markers for various lung cell types. (A) Lung organoids were stained with a Hamster Anti-Mouse Podoplanin (PDPN) Monoclonal Antibody (green; Novus Biologicals, Catalog # NB600-1015) to visualize alveolar type I cells and a Goat Anti-Human p63/TP73L Polyclonal Antibody (red; R&D Systems, Catalog # AF1916) to visualize basal cells. (B) Lung organoids were stained with a Rabbit Anti-Human Cytokeratin 5 (KRT5) Monoclonal Antibody (green; Novus Biologicals, Catalog # NB110-56916) and a Goat Anti-Human p63/TP73L Polyclonal Antibody (red; R&D Systems, Catalog # AF1916) to visualize basal cells. All samples were counterstained with DAPI (blue; R&D Systems, Catalog # 5748).

Characterization of Adult Stem Cell-derived Human Liver Organoids

Adult stem cell-derived liver organoids were cultured using Cultrex UltiMatrix RGF Basement Membrane Extract (R&D Systems, Catalog # BME001-05) and the other reagents listed in the liver organoid culture recipe shown below. Differentiated human liver organoids were stained using a (A) Mouse Anti-Human Serum Albumin Monoclonal Antibody (red; R&D Systems, Catalog # MAB1455) and a (B) Goat Anti-Human HNF-3beta Antigen Affinity-purified Polyclonal Antibody (red; R&D Systems, Catalog # AF2400) and counterstained with DAPI (blue; R&D Systems, Catalog # 5748).

Characterization of iPSC-derived Cerebral Organoids

iPSC-derived cerebral organoids (day 45) were cultured using Cultrex UltiMatrix RGF Basement Membrane Extract (R&D Systems, Catalog # BME001-05) and the other reagents listed in the brain organoid culture recipe shown below. Cerebral organoids were stained for Syto6 (blue), Pax6 (red), and Vimentin (green). (A) Image taken at 4x magnification. (B) Image taken at 15x magnification. Images courtesy of LifeCanvas Technologies.

Intestinal Organoid Culture Reagents

Product Name	Supplier	Catalog #
Cultrex UltiMatrix Reduced Growth Factor Basement Membrane Extract or Cultrex Reduced Growth Factor Basement Membrane Extract, Type 2	R&D Systems	BME001-05 or 3533-005-02
GlutaminePlus	R&D Systems	B90210
HEPES	Tocris Bioscience	R35150
N21-MAX Supplement	R&D Systems	AR008
N-2 MAX Supplement	R&D Systems	AR009
N-Acetylcysteine	Tocris Bioscience	5619
Gastrin I (Human)	Tocris Bioscience	3006
Y-27632 dihydrochloride (Rho Kinase inhibitor)	Tocris Bioscience	1254
Recombinant Human EGF	R&D Systems	236-EG
Recombinant Human R-Spondin 1	R&D Systems	4645-RS
Recombinant Human Noggin	R&D Systems	6057-NG
Recombinant Human FGF-2	R&D Systems	3718-FB
A 83-01 (ALK5 inhibitor)	Tocris Bioscience	2939
CHIR 99021 (GSK-3 inhibitor)	Tocris Bioscience	4423
Recombinant Human Wnt-3a	R&D Systems	5036-WN

Lung Organoid Culture Reagents

Product Name	Supplier	Catalog #
A 83-01 (ALK5 inhibitor)	Tocris Bioscience	2939
Cultrex Organoid Harvesting Solution	R&D Systems	3700-100-01
Cultrex UltiMatrix Reduced Growth Factor Basement Membrane Extract or Cultrex Reduced Growth Factor Basement Membrane Extract, Type 2	R&D Systems	BME001-05 or 3533-005-02
Advanced DMEM/F-12 Cell Culture Medium		
GlutaminePlus	R&D Systems	B90210
HEPES	Tocris Bioscience	3173
N21-MAX Supplement	R&D Systems	AR008
N-Acetylcysteine	Tocris Bioscience	5619
Penicillin/Streptomycin	R&D Systems	B21210
SB 202190 (p38 MAPK inhibitor)	Tocris Bioscience	1264
Nicotinamide	Tocris Bioscience	4106
Y-27632 dihydrochloride (Rho Kinase inhibitor)	Tocris Bioscience	1254
Recombinant Human R-Spondin 1	R&D Systems	4645-RS
Recombinant Human Noggin	R&D Systems	6057-NG
Recombinant Human FGF-10	R&D Systems	345-FG
Recombinant Human FGF-7	R&D Systems	251-KG

Liver Organoid Culture Reagents

Product Name	Supplier	Catalog #
Cultrex Organoid Harvesting Solution	R&D Systems	3700-100-01
Cultrex UltiMatrix Reduced Growth Factor Basement Membrane Extract or Cultrex Reduced Growth Factor Basement Membrane Extract, Type 2	R&D Systems	BME001-05 or 3533-005-02
GlutaminePlus	R&D Systems	B90210
HEPES	Tocris Bioscience	3173
N21-MAX Supplement	R&D Systems	AR008
N-2 MAX Supplement	R&D Systems	AR009
N-Acetylcysteine	Tocris Bioscience	5619
Gastrin I (Human)	Tocris Bioscience	3006
Nicotinamide	Tocris Bioscience	4106
Y-27632 dihydrochloride (Rho Kinase inhibitor)	Tocris Bioscience	1254
Recombinant Human EGF	R&D Systems	236-EG
Recombinant Human R-Spondin 1	R&D Systems	4645-RS
Recombinant Human Noggin	R&D Systems	6057-NG
Recombinant Human FGF-10	R&D Systems	345-FG
Recombinant Human FGF-19	R&D Systems	969-FG
Recombinant Human BMP7	R&D Systems	354-BP
Recombinant Human HGF	R&D Systems	294-HG
Forskolin	Tocris Bioscience	1099
A 83-01 (ALK5 inhibitor)	Tocris Bioscience	2939
Recombinant Human Wnt-3a	R&D Systems	5036-WN
DAPT	Tocris Bioscience	2634
Dexamethasone	Tocris Bioscience	1126

Brain Organoid Culture Reagents

Product Name	Supplier	Catalog #
N-2 MAX Supplement	R&D Systems	AR009
N21-MAX Supplement	R&D Systems	AR008
N21-MAX Vitamin A Free Supplement	R&D Systems	AR012
Penicillin/Streptomycin	R&D Systems	B21210
GlutaminePlus	R&D Systems	B90210
Insulin		
2-mercaptoethanol		
Cultrex UltiMatrix Reduced Growth Factor Basement Membrane Extract or Cultrex Reduced Growth Factor Basement Membrane Extract, Type 2	R&D Systems	BME001-05 or 3533-005-02
Recombinant Human FGF basic	R&D Systems	3718-FB
Recombinant Human Noggin	R&D Systems	6057-NG
Y-27632 dihydrochloride (Rho Kinase inhibitor)	Tocris Bioscience	1254

NOTE: This poster conveys a general overview and should be considered neither comprehensive nor definitive. The details of this information are understood to be subject to interpretation.

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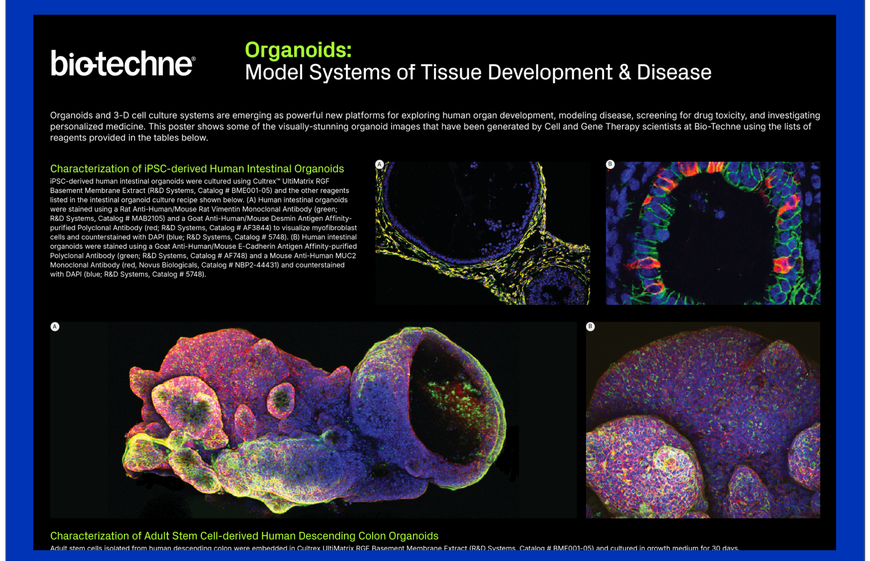

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