UNIVERSITY OF SURREY

Department for Environment Food & Rural Affairs

NC 3Rs National Centre for the Replacement Refinement & Reduction of Animals in Research

A bovine alveolus model to replace cattle in the study of host-pathogen interactions in bovine tuberculosis

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Bovine tuberculosis (bTB)

- *Mycobacterium bovis c*an infect almost all warm-blooded animals

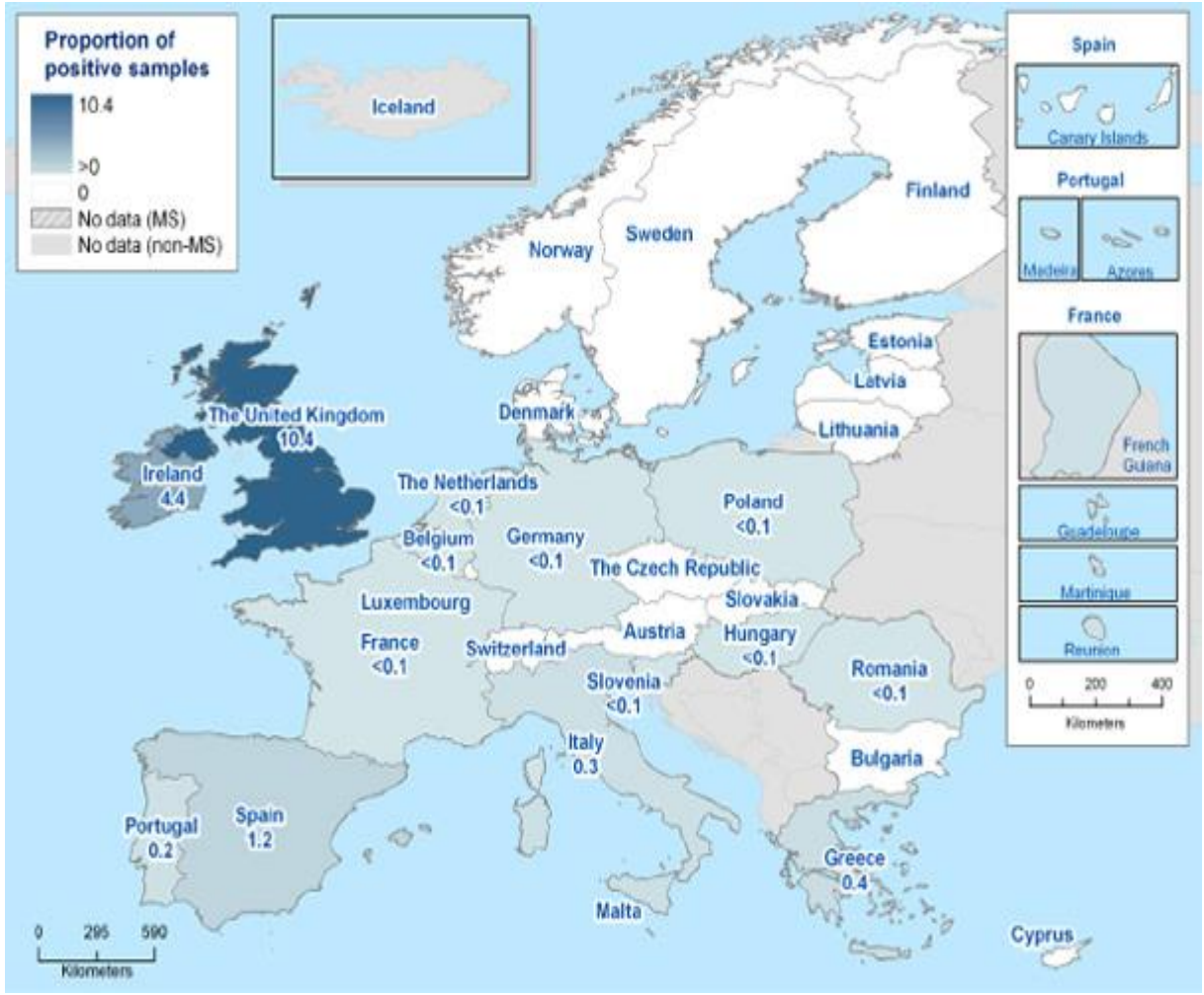
- 1900
  - 40% of British cattle suspected to be infected
  - *M. bovis* responsible for ~15% of all human deaths from TB

- 1950s
  - Pasteurisation of milk and ‘test-and-slaughter’ introduced

- 1970s
  - 0.22% of tests positive for bTB

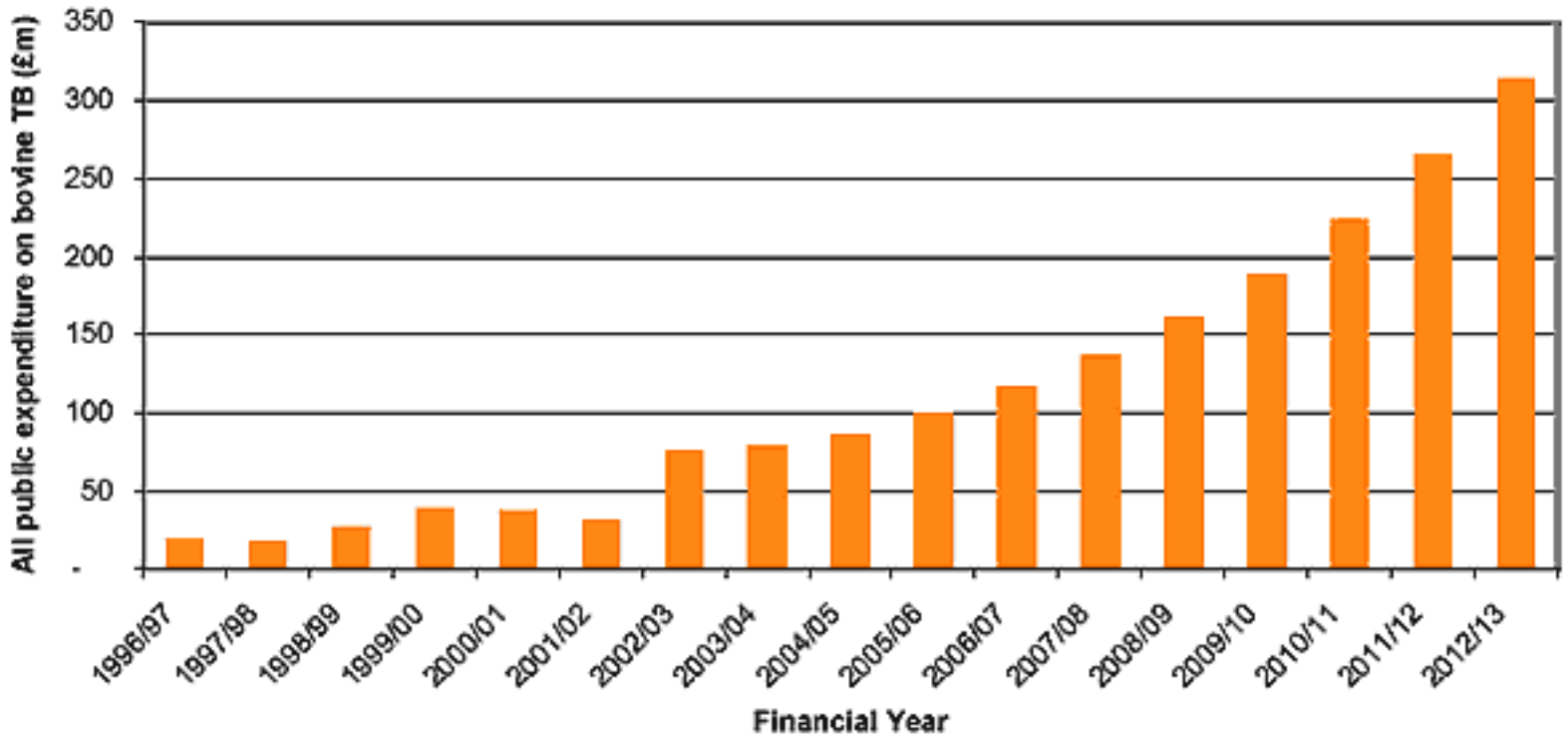
- 2016:
  - ~5%

Bovine tuberculosis (bTB)



Proportion of existing cattle herds infected with or positive for *M. bovis* country based-data, 2012. EFSA Journal 2014

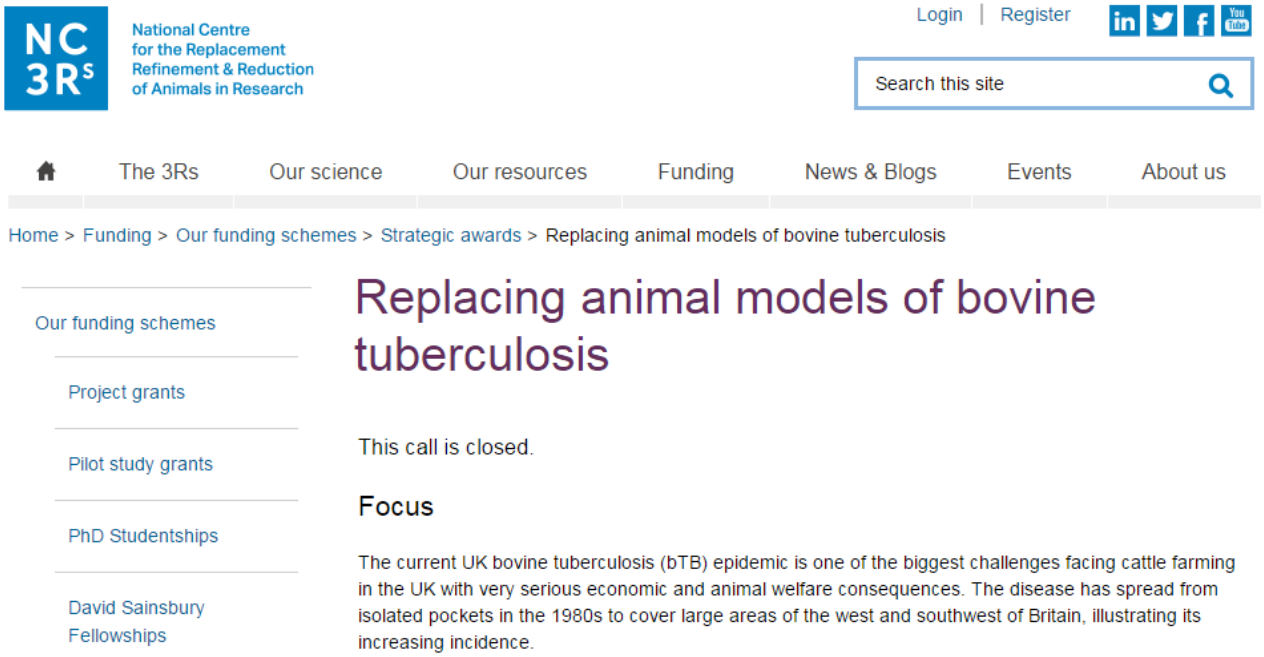
Bovine tuberculosis (bTB)



The Strategy for achieving Officially Bovine Tuberculosis Free status for England - 2014
https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/300447/pb14088-bovine-tb-strategy-140328.pdf

https://www.gov.uk/government/news/tb-strategy-ahead-of-schedule-as-england-set-to-apply-for-officially-tb-free-status-for-half-the-country
"Dealing with Bovine TB in England … required the culling of 28,000 cattle in 2015"

Bovine tuberculosis (bTB)



- Strategic award between NC3Rs and Defra (2015)
- Replace cattle for the study of the pathogenesis of bTB by providing a tissue culture model with which to study fundamental events following infection of the bovine lung with virulent mycobacteria that can't be conducted currently *in vitro*

Rationale for experimental design

- Using human lung epithelium-endothelium submerged bilayer models:
  - Epithelial cells are hosts for *M. tuberculosis* and allow virulent bacteria to access the deeper tissues – Birkness (1999)
  - Peripheral blood mononuclear cells (PBMCs) migrate from the basal layer to the apical layer during infection
  - Airway epithelial cells play an essential role in both innate and adaptive immune responses against *M. tuberculosis* – PRR expression

- A bilayer of human pulmonary artery endothelial cells and human alveolar epithelial cells at an air-liquid interface on opposing sides of a Transwell developed to study invasive pulmonary aspergillosis
  - Professor William Hope, University of Liverpool (NCR3Rs grant, G0700599)

Objectives of the project

- Objective 1
  - Isolate and immortalise bovine type II alveolar epithelial (B2AE) cell line
- Objective 2
  - Assemble a bovine alveolus (boAlv) culture model using bovine cells (adaptation of the Hope model)
- Objective 3
  - Demonstrate functional utility of the boAlv model by introducing PBMCs from cows expressing a strong or weak vaccine protection phenotype (VPP)

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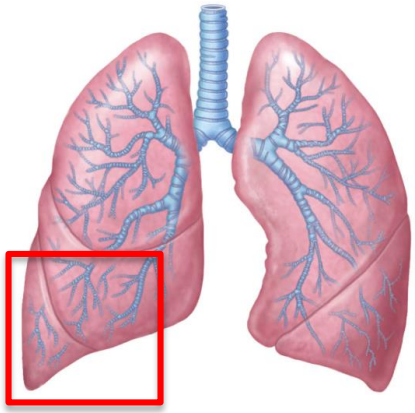
Objective 1 – Isolate and immortalise bovine type II alveolar epithelial (B2AE) cell line

Objective 1 (i) Devise and implement an isolation strategy



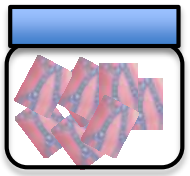
Dissection

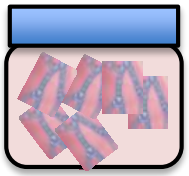
Wash
(PBS/Pen/Strep/EDTA)

Digestion
(Elastase/collagenase/Trypsin/DNAse I)

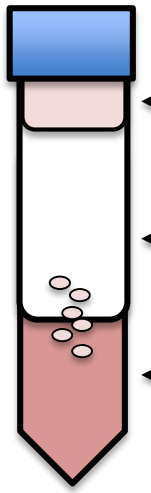
IgG ‘panning’

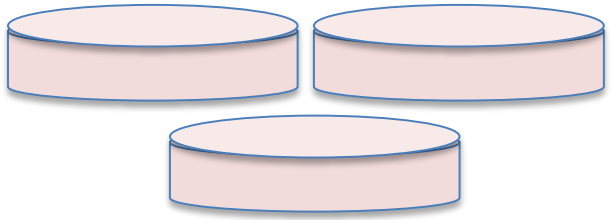
Percoll™ Gradient



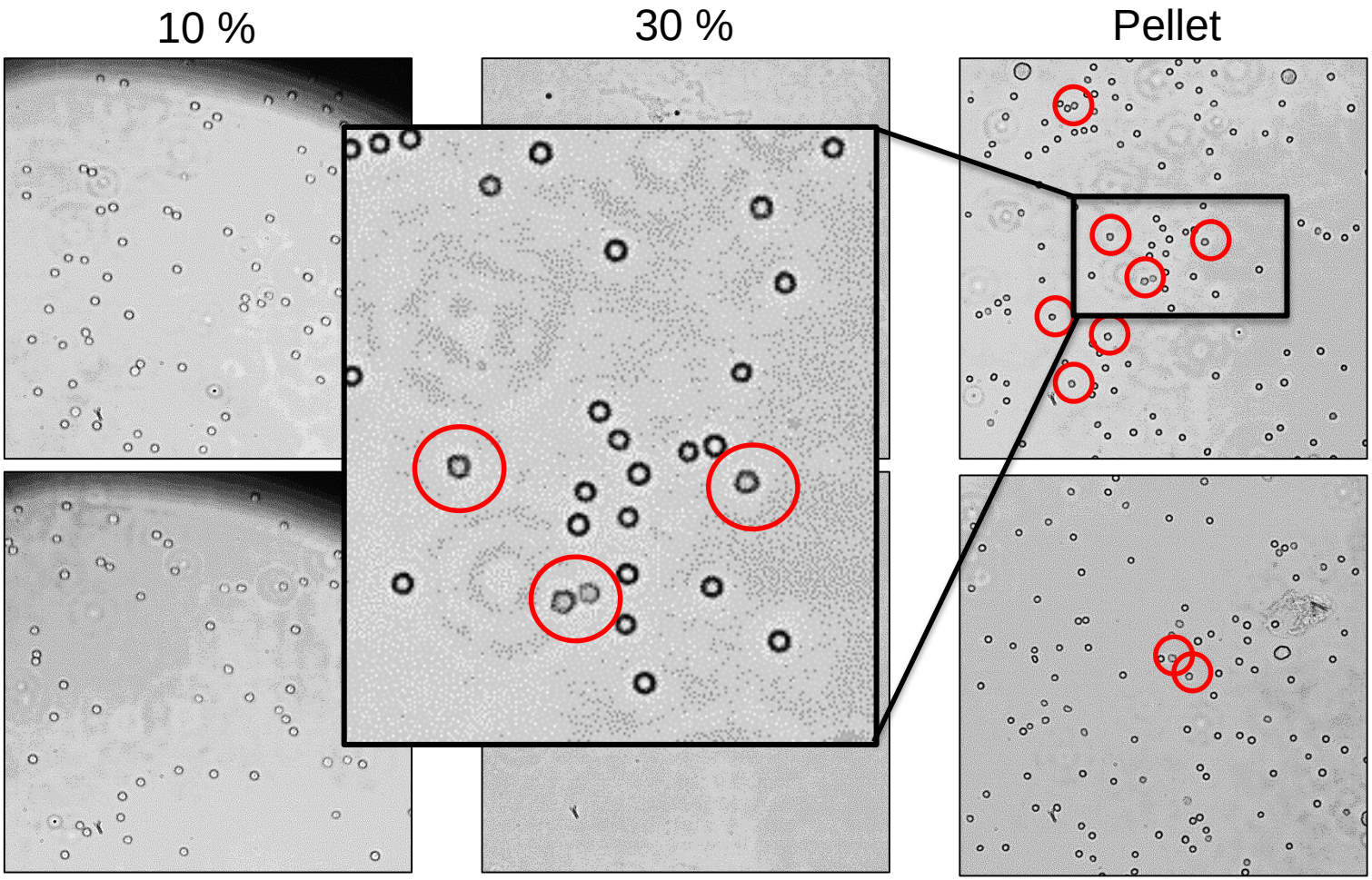


strained cells





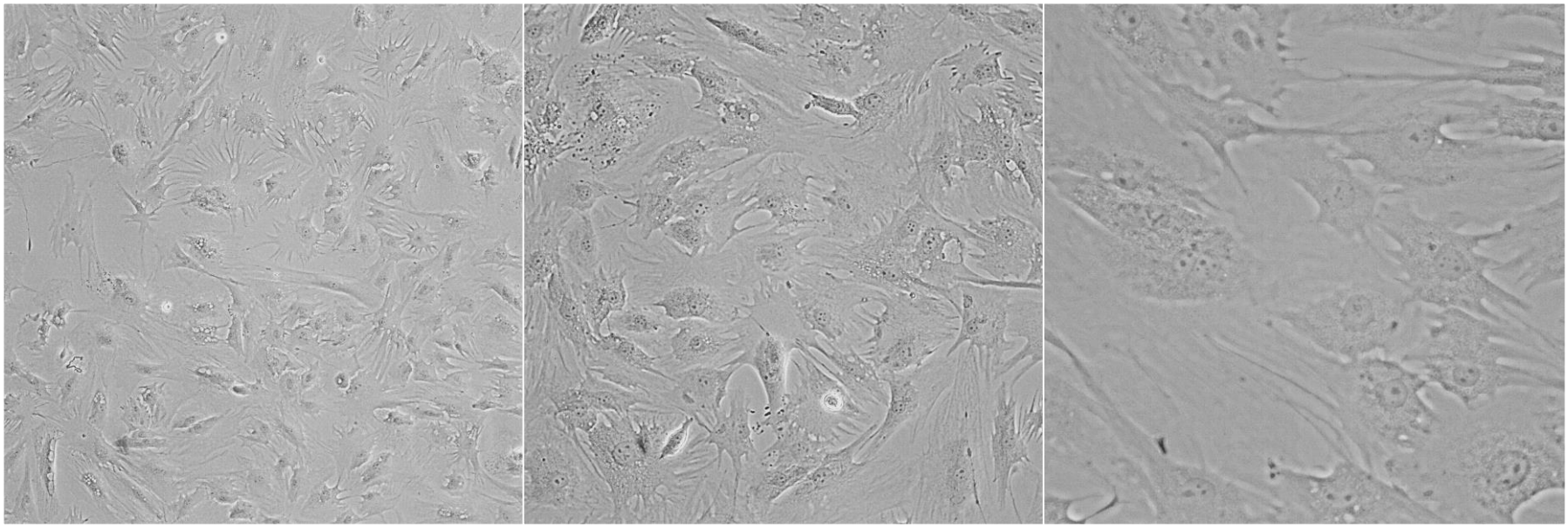
Objective 1 (i) Devise and implement an isolation strategy – results 1



Day 1 post-isolation

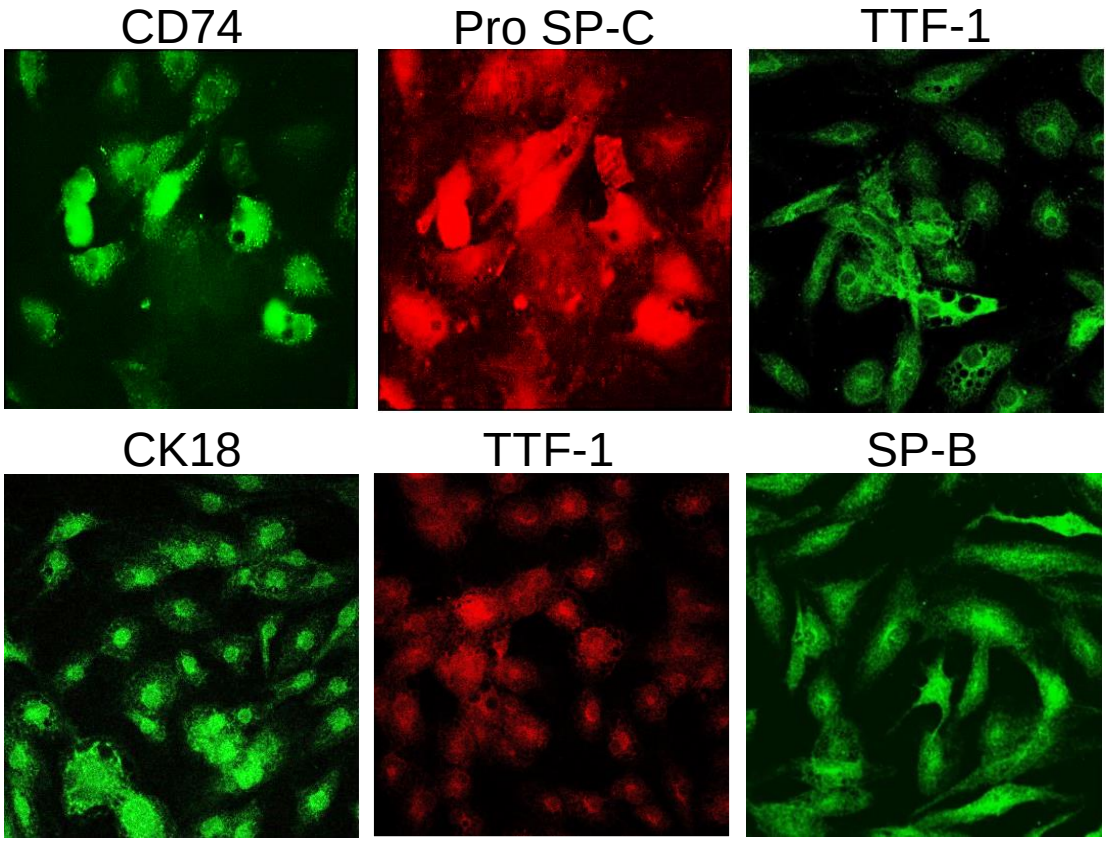
Objective 1 (i) Devise and implement an isolation strategy – results 2

10 x | 20 x | 40 x

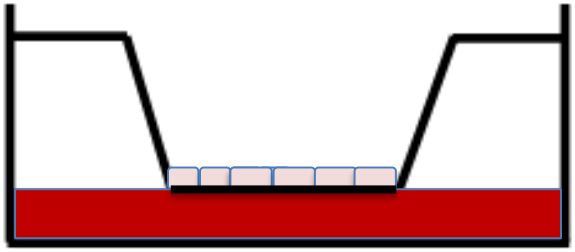


p2

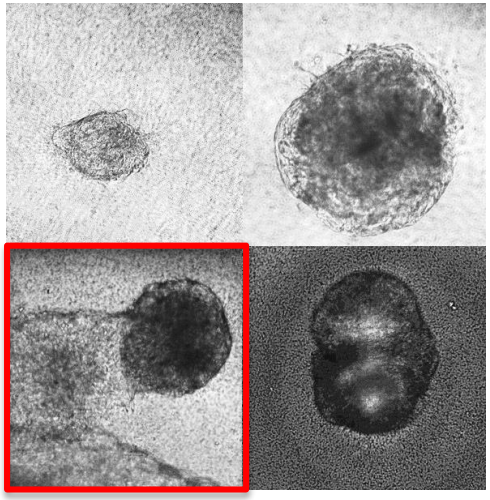
Objective 1 (i) Devise and implement an isolation strategy – results 3



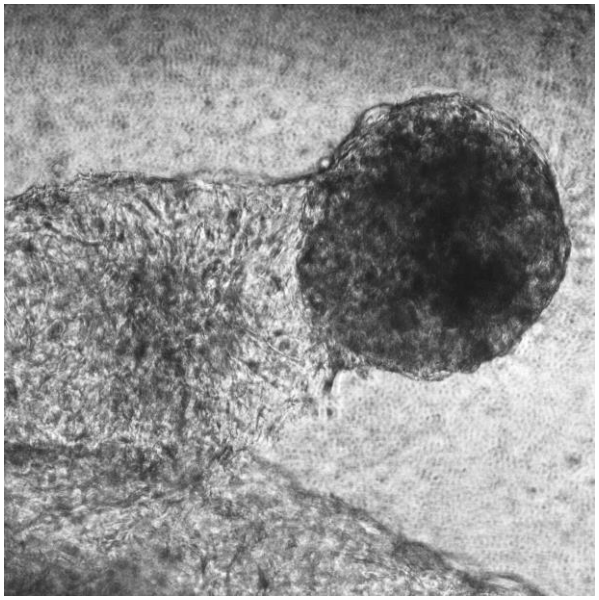
Objective 1 (i) Devise and implement an isolation strategy – results 4 - ALI cultures



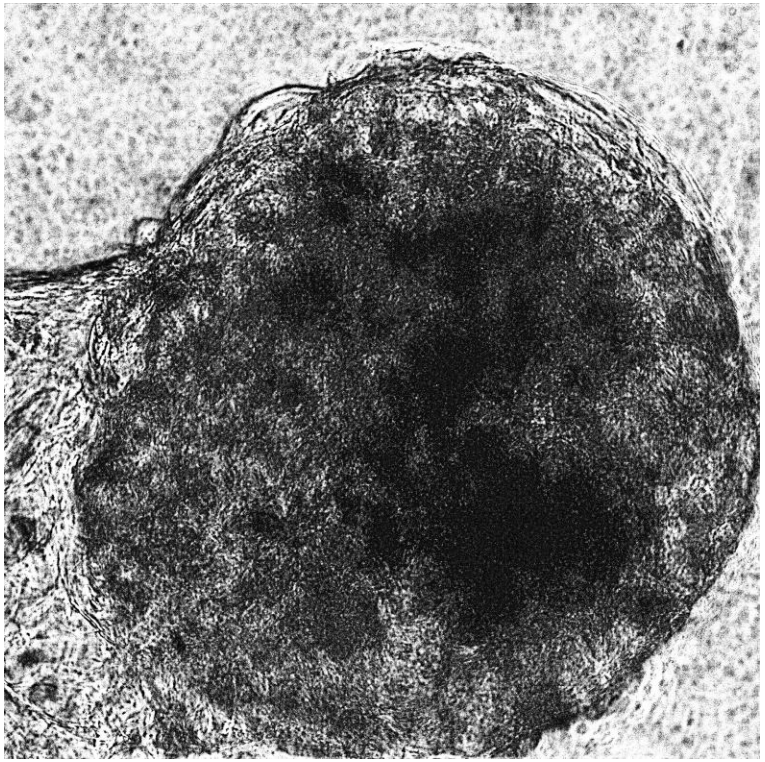
10 x



20 x

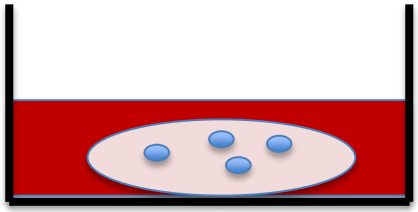


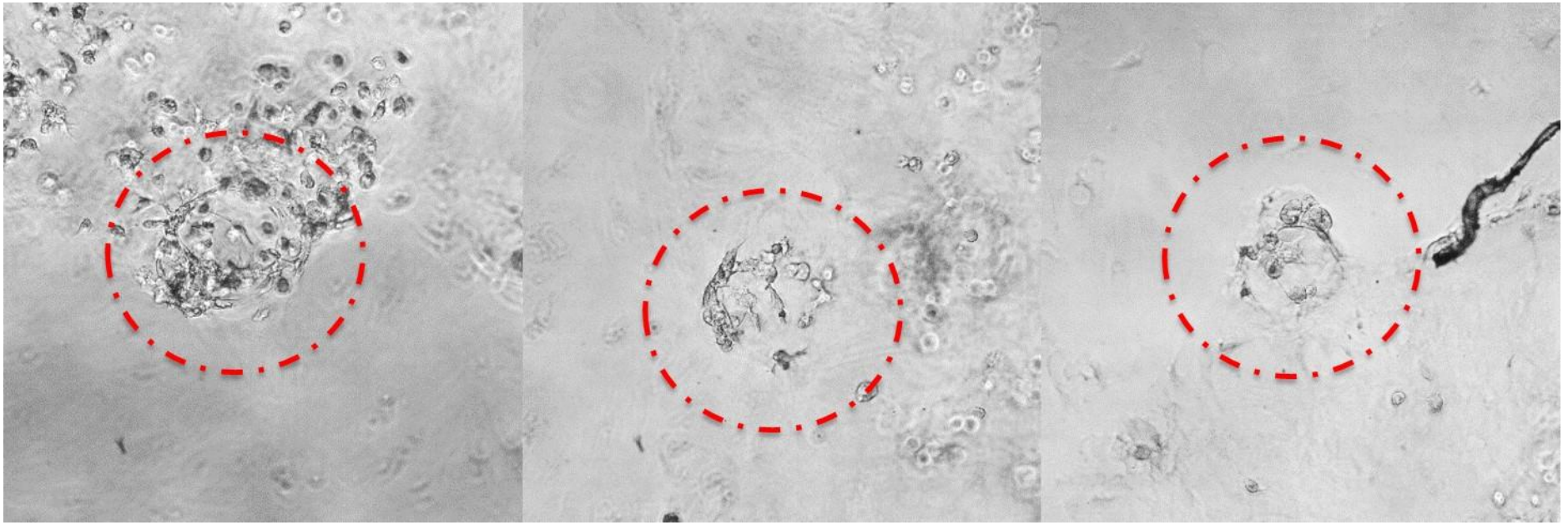
40 x



5 days on MG-coated Transwell-Clear 12 mm inserts; p5

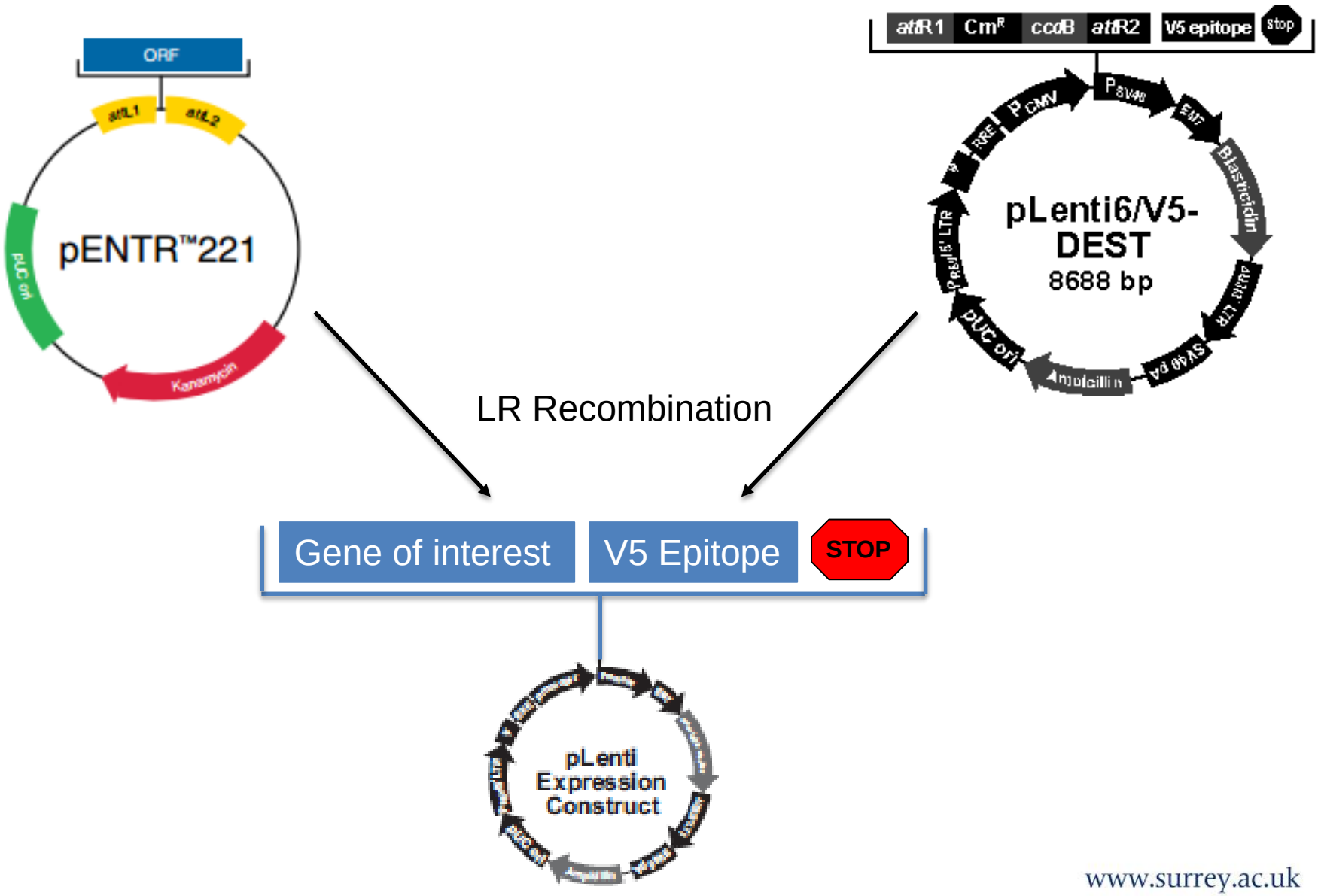
Objective 1 (i) Devise and implement an isolation strategy – results 5 – 3D ECM





10 days in 3D MG, submerged in SAGM; 10 x, p6

Objective 1 (ii) Immortalise isolated cells using Bmi1 and hTERT lentiviral constructs



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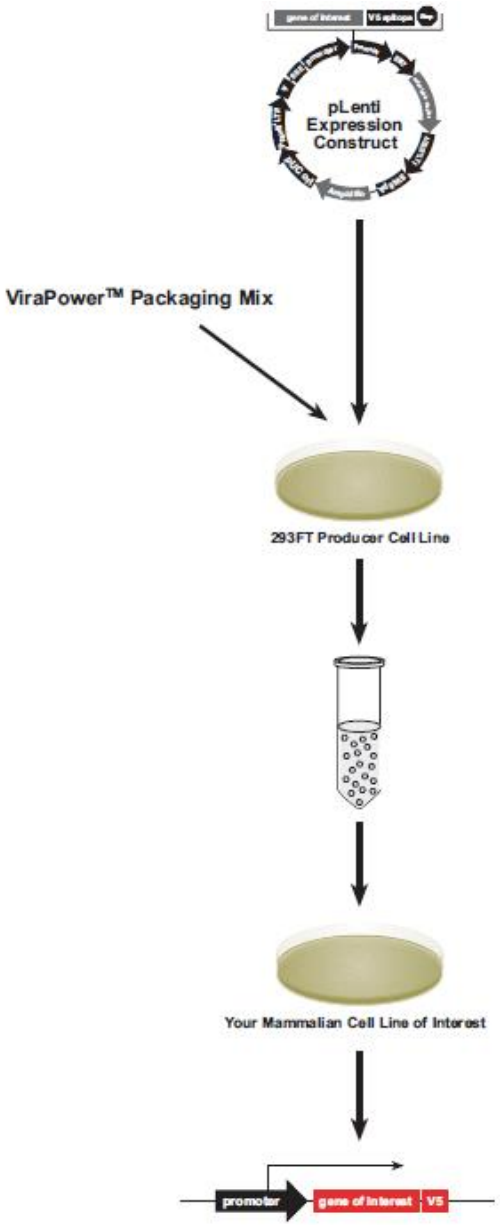


Co-transfect 293FT producer cell line with expression construct and packaging mix

Harvest viral supernatant and determine titre

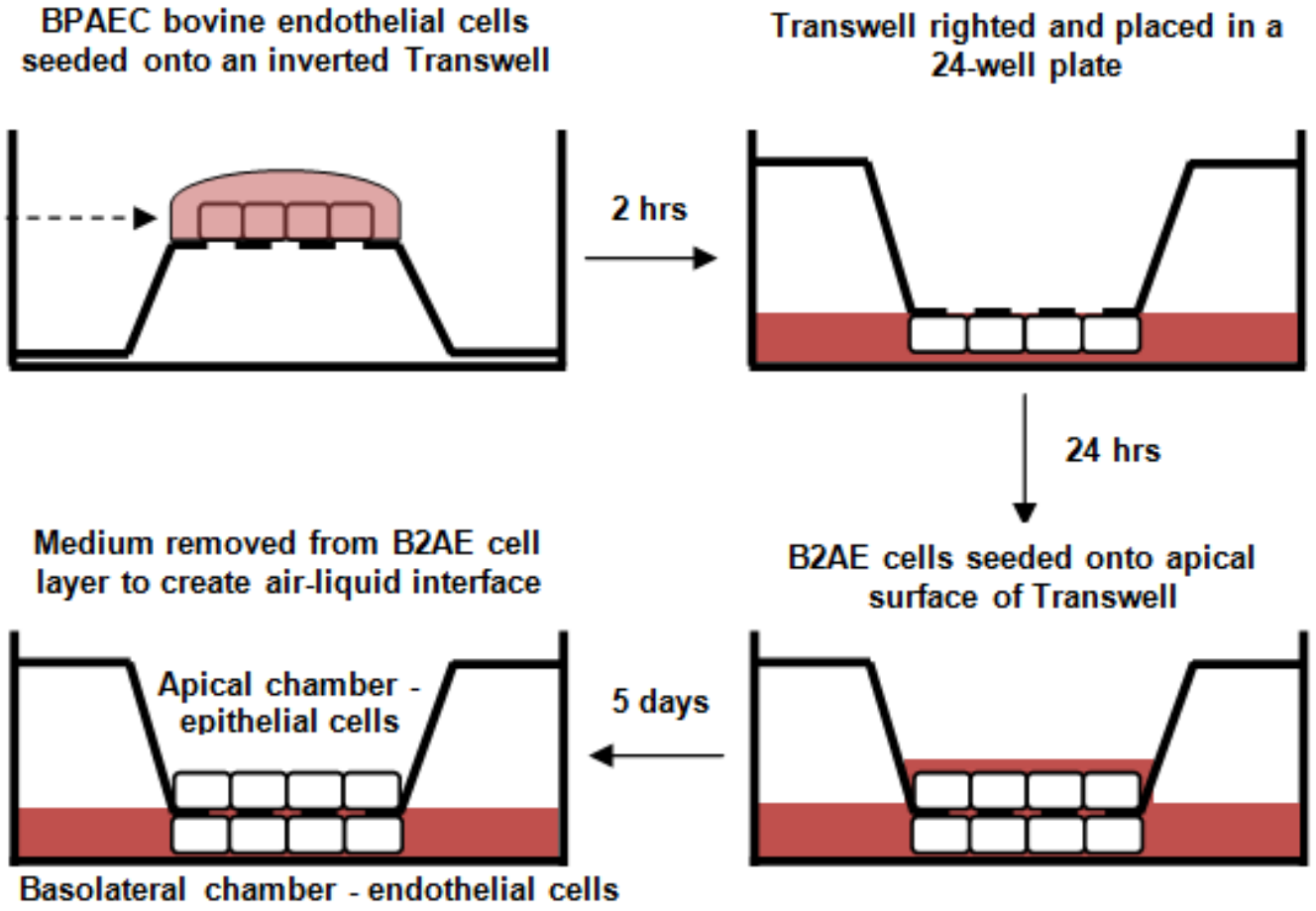
Transduce mammalian cells and select for clones

Objective 1 (ii) Immortalize isolated cells using Bmi1 and hTERT lentiviral constructs – Verification of expression clones

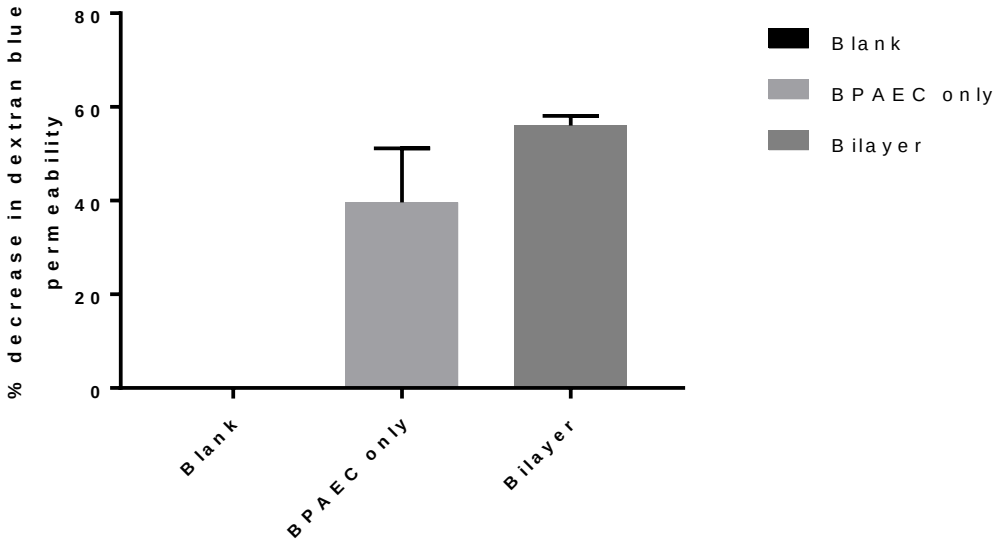
Criteria		Status
Digest of expression construct	(At miniprep and midiprep stages)	Digest verified at both stages
Sequencing (external)	(CMV and V5 C-term)	Sequence verified using diagnostic PCR and Sanger sequencing
Sequencing (internal)	(two sets of primers designed for hTERT)	Sequence verified using diagnostic PCR and Sanger sequencing
Blasticidin selection	(at titration and transduction stage)	Titrations obtained for both lentiviral preparations: $> 2 \times 10^7$ TU/mL

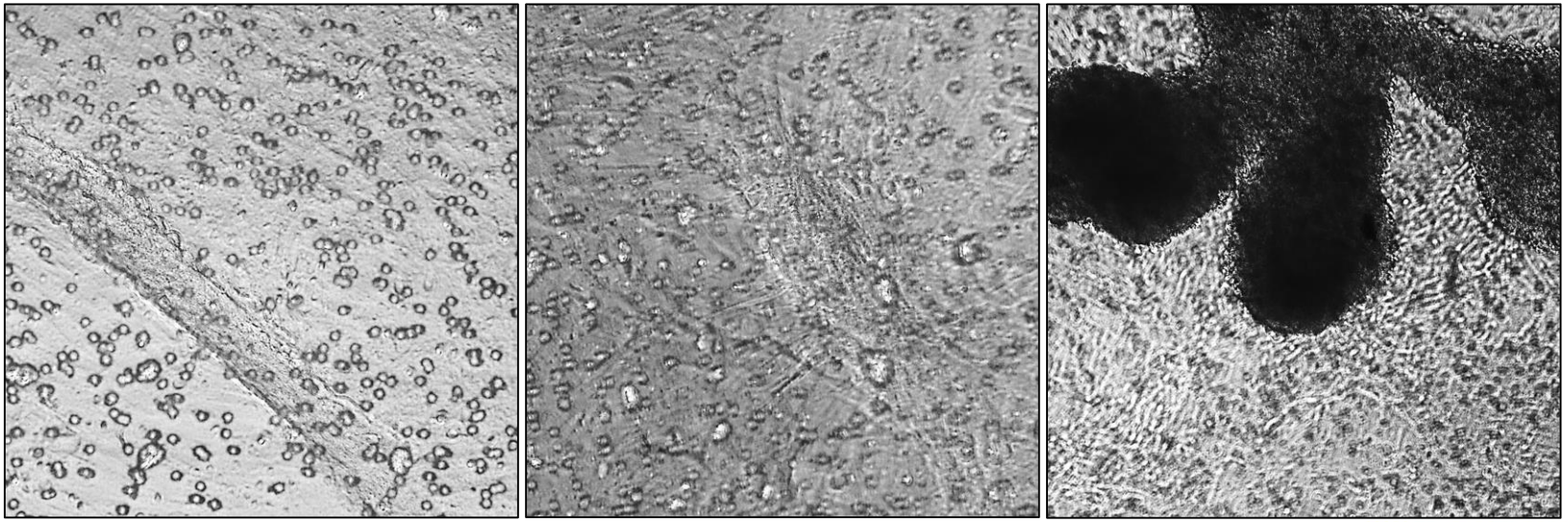
Objective 2 - Assemble a bovine alveolus (boAlv) bilayer culture model using bovine cells

Objective 2 - Assemble the boAlv culture model

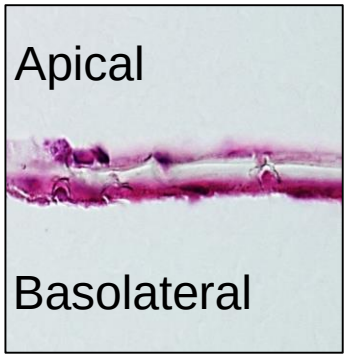


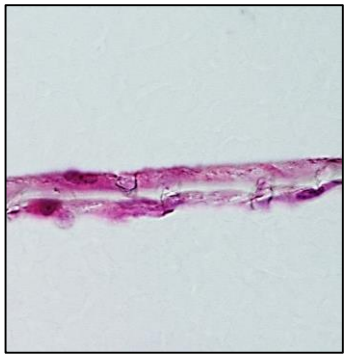
Objective 2 - Assemble the boAlv culture model – Results 1

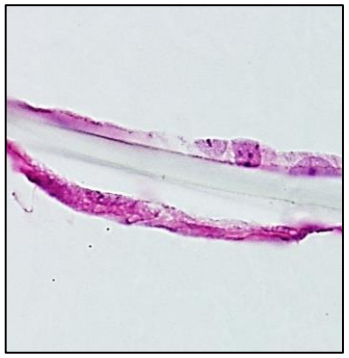


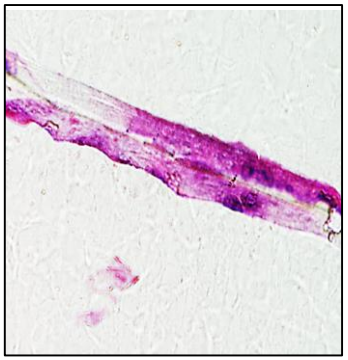


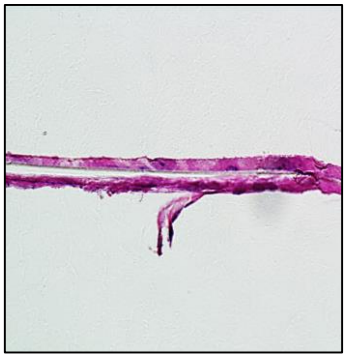
Objective 2 - Assemble the boAlv culture model – Results 2

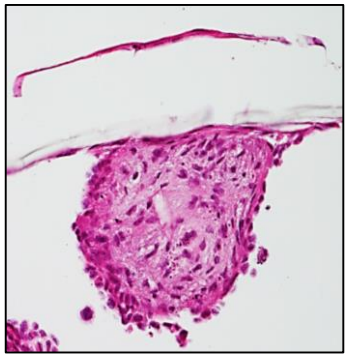


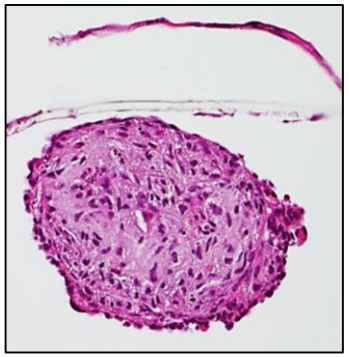


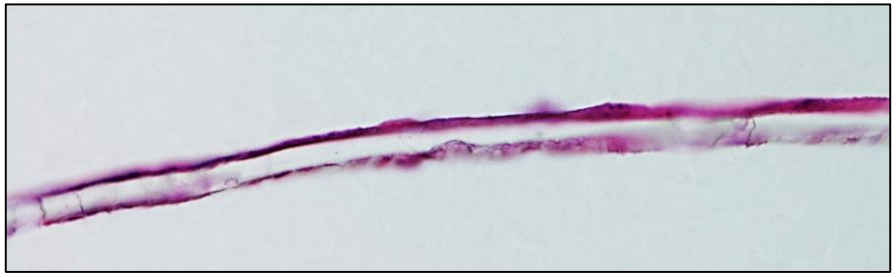












Ongoing and future work

- Perform fresh isolation of ATII cells from bovine lung and immortalise (B2AE cell line)

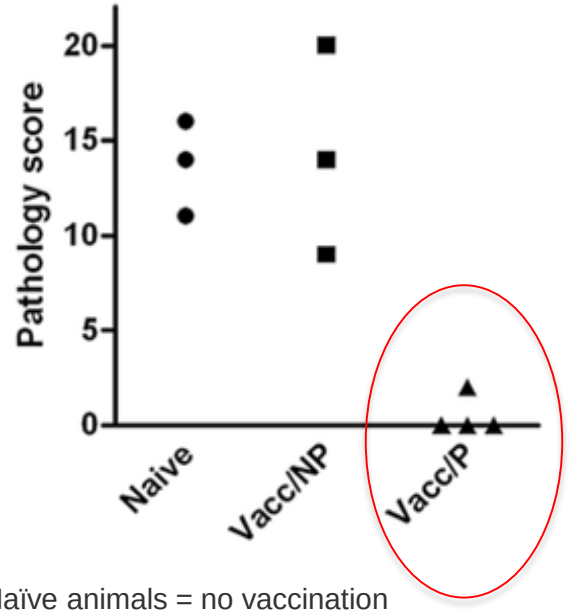
- Characterize clones in parallel with WT ATII
  - Bmi1/hTERT expression (qRT-PCR, WB)
  - Growth curve
  - Karyotype
  - ATII markers SP-B, SP-C, TFF1, CK18, CD74 (qRT-PCR and/or IF)

- Optimise bilayer model
  - seeding densities
  - culture period

Ongoing and future work: (Objective 3 - Demonstrate functional utility of the model)

- Evaluate at least 4 possible scenarios associated with vaccine protection, related to the speed and activity of the host response and the pathogen behaviour within the alveolus:
  - PBMCs from strong VPP animals restrict better the growth of mycobacteria;
  - PBMCs from strong VPP animals restrict better the migration of mycobacteria through the epithelium -> endothelium;
  - PBMCs from strong VPP animals migrate more efficiently and in greater numbers through the endothelium->epithelium;
  - PBMCs from strong VPP animals express and stimulate a greater ratio of IL-22/17

Distribution of vaccinated cattle into those that express a strong and weak VPP



Naïve animals = no vaccination
Vacc/NP = vaccinated calves that were not protected (weak VPP)
Vacc/P = vaccinated calves that were protected (strong VPP)
Taken from: Bhuju S et al. PLoS Pathog 8(12): e1003077.

With thanks to…

Prof. Mark Chambers
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Mr John Cooper

Ms Ella May
Mr Duncan Grainger
Mrs Gillian Wallis

Prof. William Hope
Ms Clara Negri

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References

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