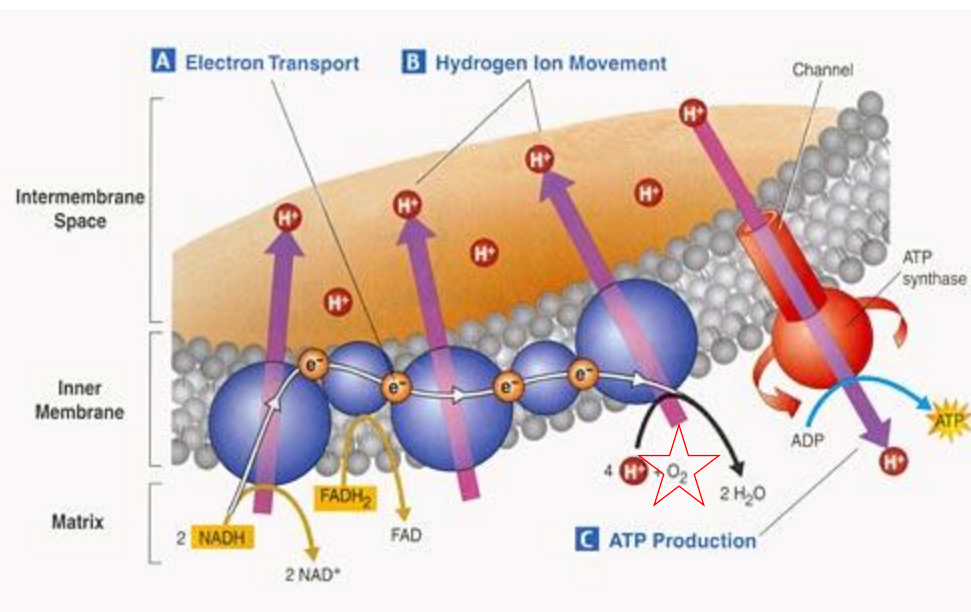


The Idea: Oxygen Sensing for Eukaryotes

Function of Oxygen in Eukaryotes

- Final electron acceptor in Cellular Respiration for Aerobic Metabolism
 - Determines the progression of respiration



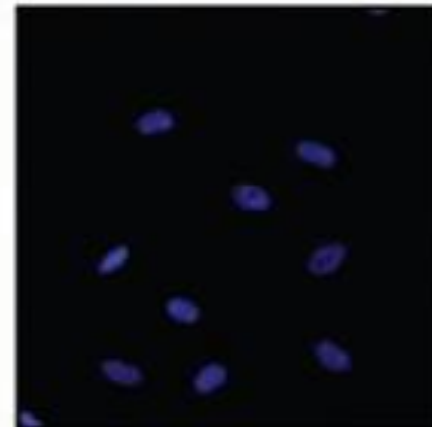
- Essential for harnessing energy in the cell
- Influences all energy-requiring reactions of the cell

Problems in Oxygen Availability

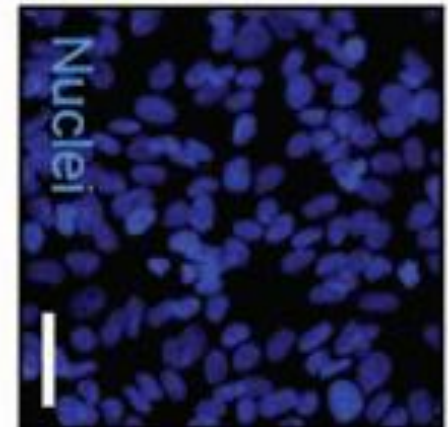
- Waterlogged plants and Anaerobic fermentation
- Energy Availability
- Effects on the plant
 - Senescence, chlorosis, stunting, death
- Mammalian cells – halts metabolism
- Molecular signaling cascade
- Effects on the mammal:
 - Cell death, fermentation, vasculogenesis/angiogenesis



Hypoxia



Normoxia



Objective: Develop Oxygen Sensitive Biological Devices

- Subsequent change in cellular activity
- Responsive to low oxygen levels
- Modular device

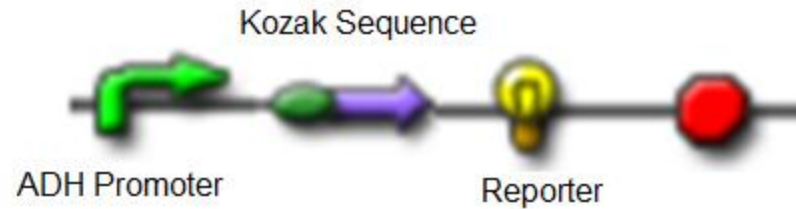


Problems in Developing Modular Oxygen Sensing Devices

- Incompatibility between plants and animals
- Evolutionary divergence
- Complex pathways
- Diverse responses to the same stimulus

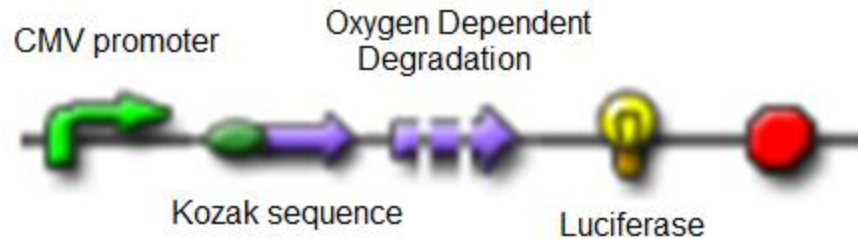
Analogous Devices for Oxygen Sensing in Plants and Animals

Plant Device



- Alcohol dehydrogenase (ADH) promoter
- Nuclear localization sequence, Kozak sequence
- Reporter protein (fluorescent protein, luminescent protein)

Mammalian Device



- Constitutive promoter (CMV)
- Nuclear localization sequence, Kozak sequence
- Protein domain – oxygen dependent degradation
- Reporter protein (luminescent)

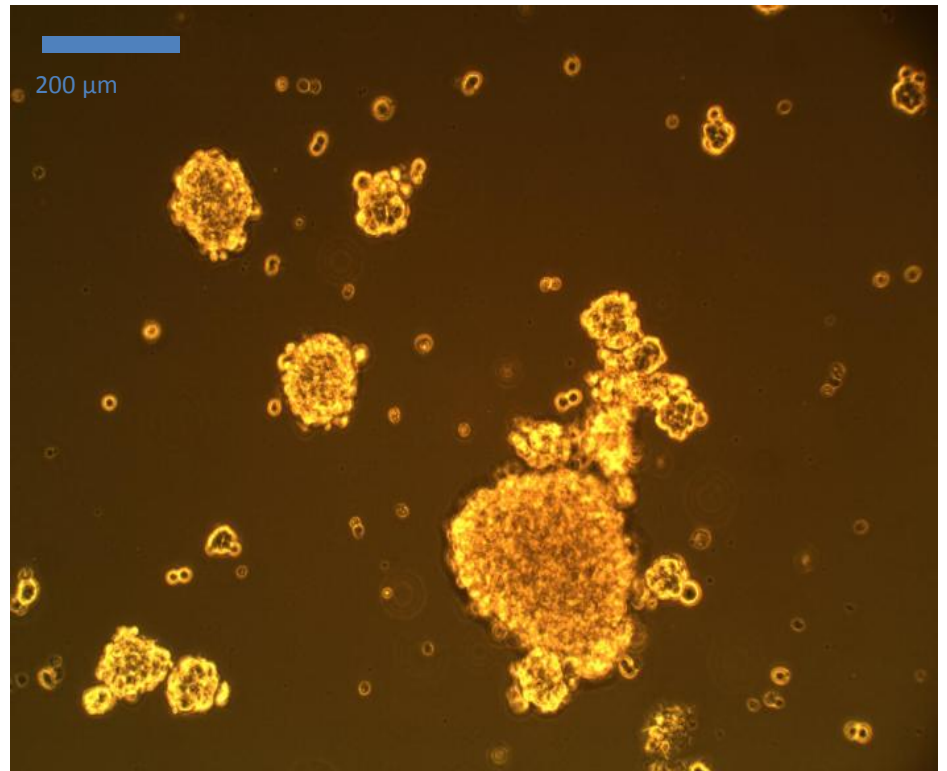
Divergence in Paths

Plants



Model : A. Thaliana

Mammals

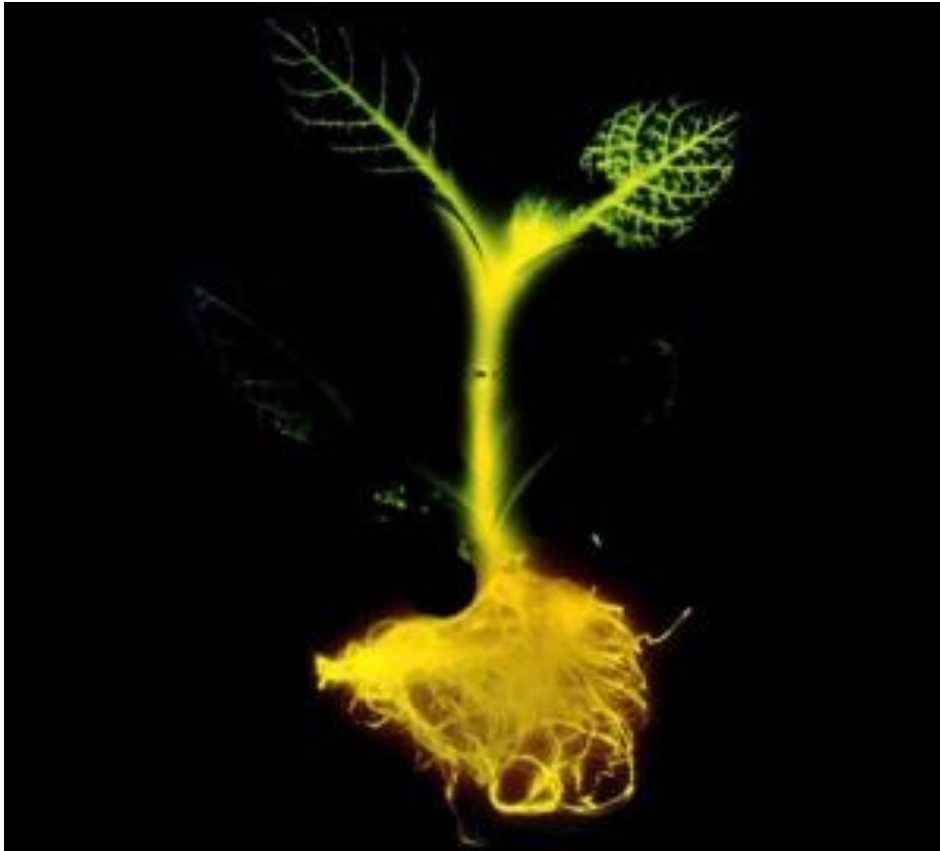


Model: Immortalized human GBAM1 cell line
(stem cell-like glioma cells)

Plants: Development Criteria

- Why ADH promoter?
 - Well-characterized in literature
 - Up-regulated in hypoxia
 - Directly involved in respiration pathway
 - Other options less feasible

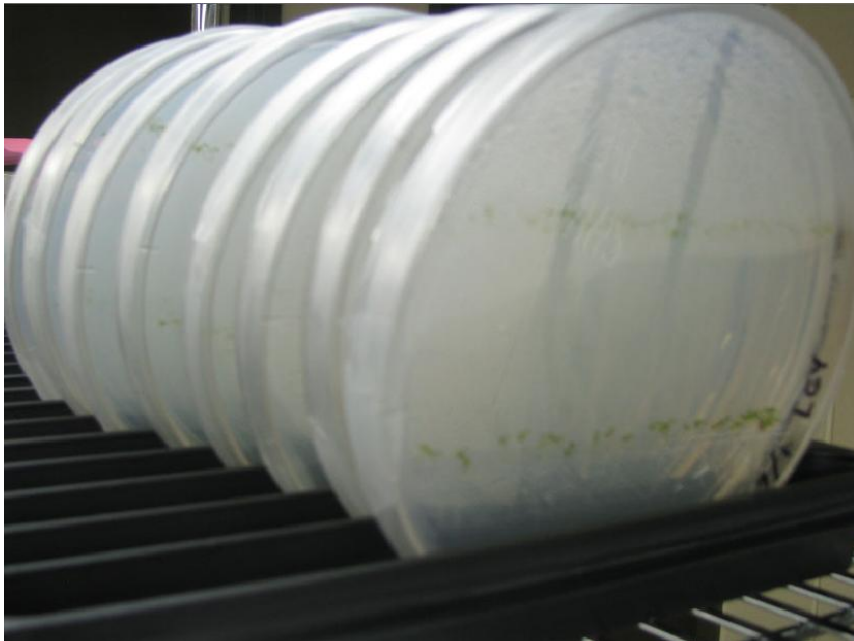
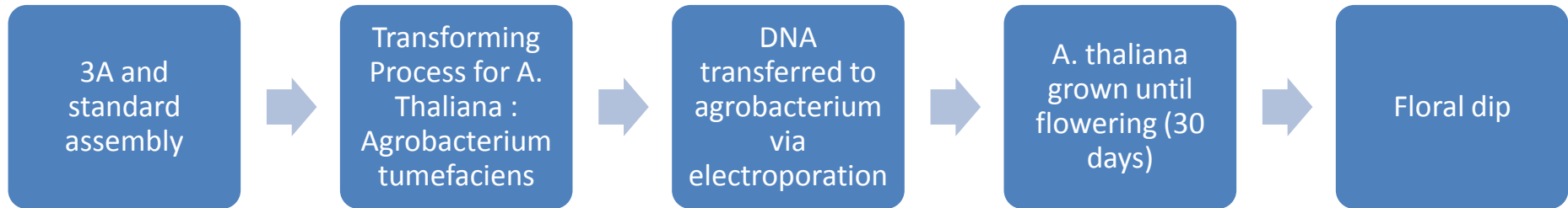
Plants: Indicating Oxygen Concentration



Choice of reporter protein:

- Fluorescent proteins – easily visualized in roots
- Luminescent proteins (luciferase) require luciferin substrate
- Practical quantification

Plants: Assembly and Insertion of Device



A. Thaliana grown in mist room pots



A. Thaliana cultured on agar plates

Plants: Determining Oxygen Sensitivity

- Experiments to include:
 - Plant response to low oxygen, varying time, concentration
 - Quantifying GFP production at different oxygen concentrations
 - Model development

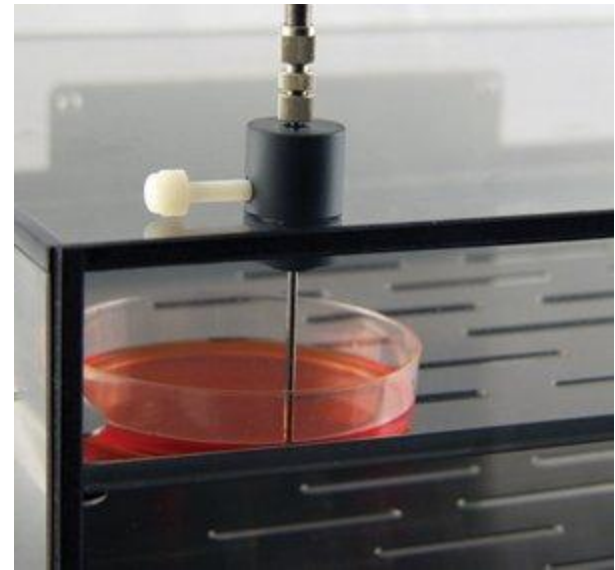


Image Source: biospherix.com

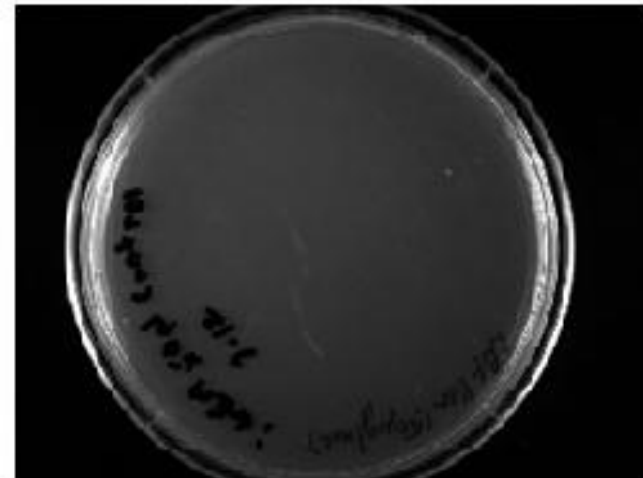
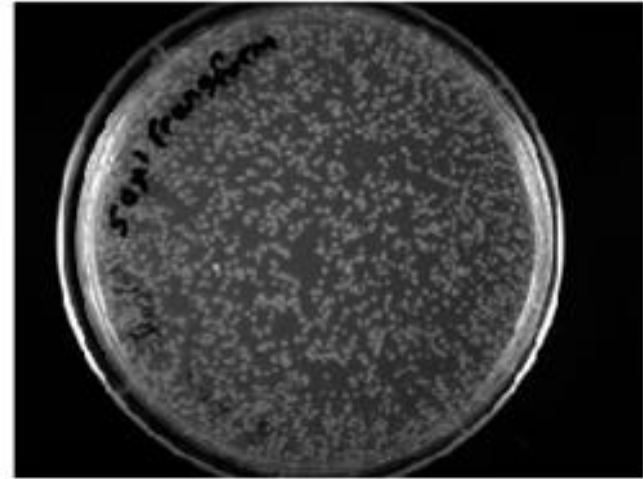
Mammals: Development Criteria

- Why a degradation domain?
 - Limits undiminished signal
 - Mimics the activity of proteins involved in hypoxia response
 - Constitutively up-regulates reporter
 - Terminates response in normoxia
 - Real time hypoxia sensor

Mammals: Indicating Oxygen Concentration

Successful transformation of luciferase from partsregistry

- Choice of reporter proteins
 - Bright, measurable signal
 - Luciferase (bioluminescence) - tissue penetration
 - Easily visualized
 - Luciferin in cell media

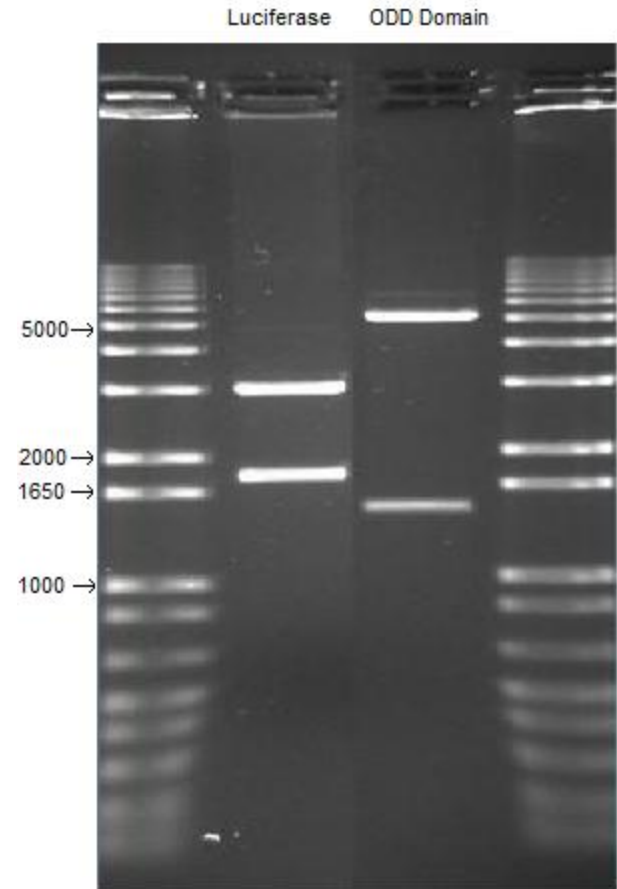


negative control

Preliminary transformations using luciferase

Mammals: Assembly and Insertion of Device

- Synthesized active sequence of oxygen dependent degradation (ODD) domain
- 3A assembly – modified with protein fusion
- For insertion into mammalian cells – transfection by electroporation



Confirmation of Parts
for assembly -

Mammals: Determining Oxygen Sensitivity

- Experiments to include:
 - Incubation at various oxygen concentrations
 - Expression in differentiated versus proliferative cells
 - Model to predict reporter activity
 - Modeling hypoxia in 3D solid tumor cultures

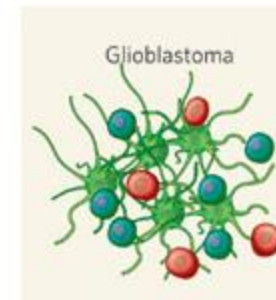
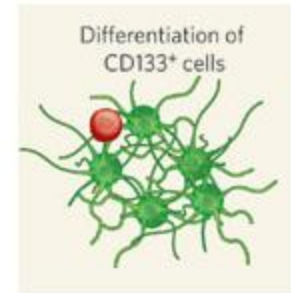


Image Sources: Dirks, Peter B. 2004

Applications

- Crop stabilization – food production
- Physiological conditions in plants
- Predictive models for cancer hypoxia
- Spatial models – tissue engineering



Ethical Review

- Weedy or invasive species
- Reductionist philosophy
- Homogenization of plant species
- Uncontrolled transfer of genetic material

- Binary vector systems
 - Formerly, transforming agrobacterium meant recovering, manipulating, and reincorporating the tumor-inducing plasmid
 - In a binary vector system, the plasmid containing the DNA of interest can be manipulated and copied in the more robust *E. coli*
 - The vector can then be transferred via electroporation to an agrobacterium with virulence genes on a separate plasmid

- Agrobacterium strain GV3101::pMP9
 - Disarmed strain; does not produce tumors
 - Expresses rifampicin resistance genes
 - Binary vector pCB302
 - Many advantages for plant transformation: Includes antibacterial (kanamycin) and herbicide (glufosinate ammonium) resistance for selection on appropriate media