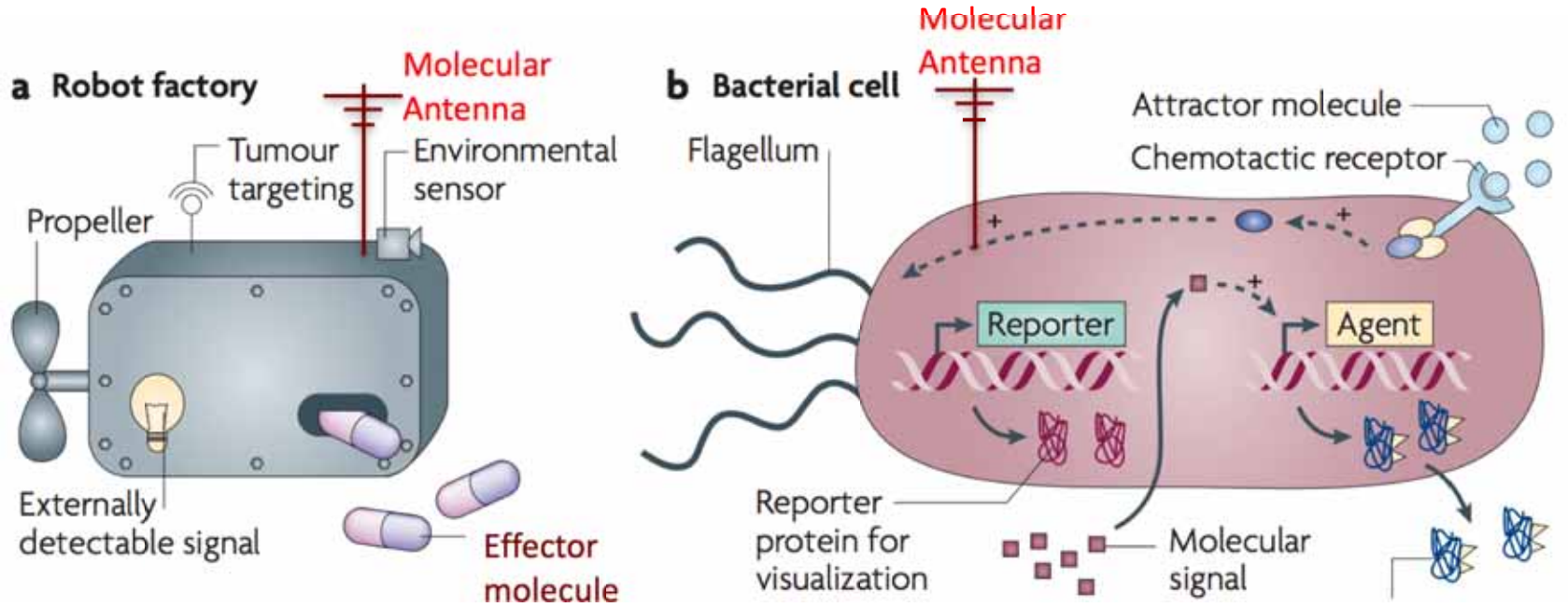
Magnetogenetic Cancer Therapy

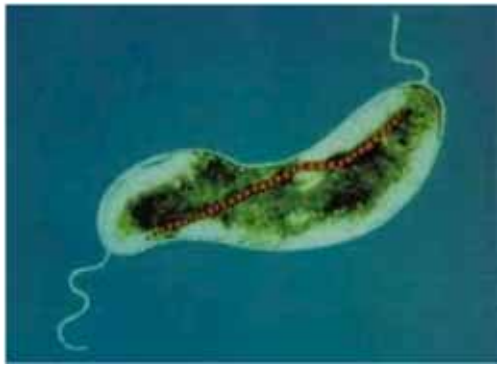
Ryan Spitler

SCIT Seminar

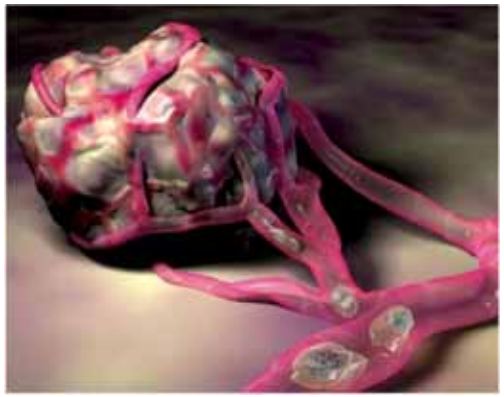
October 20, 2016

Engineering Bacteriotherapies and Artificial Endosymbionts





AMB-1 magnetotatic bacteria



cancer

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www.nature.com/reviews/cancer

Outline

- Synthetic Biology approach: cloning and gene expression profiling. (**Heat-inducible Molecular Switch**)
- Attachment of IONP onto the cell surface of *E. coli.* (**Heating**)
- MRI cell tracking using IONP and AMB-1 (**Tracking**)

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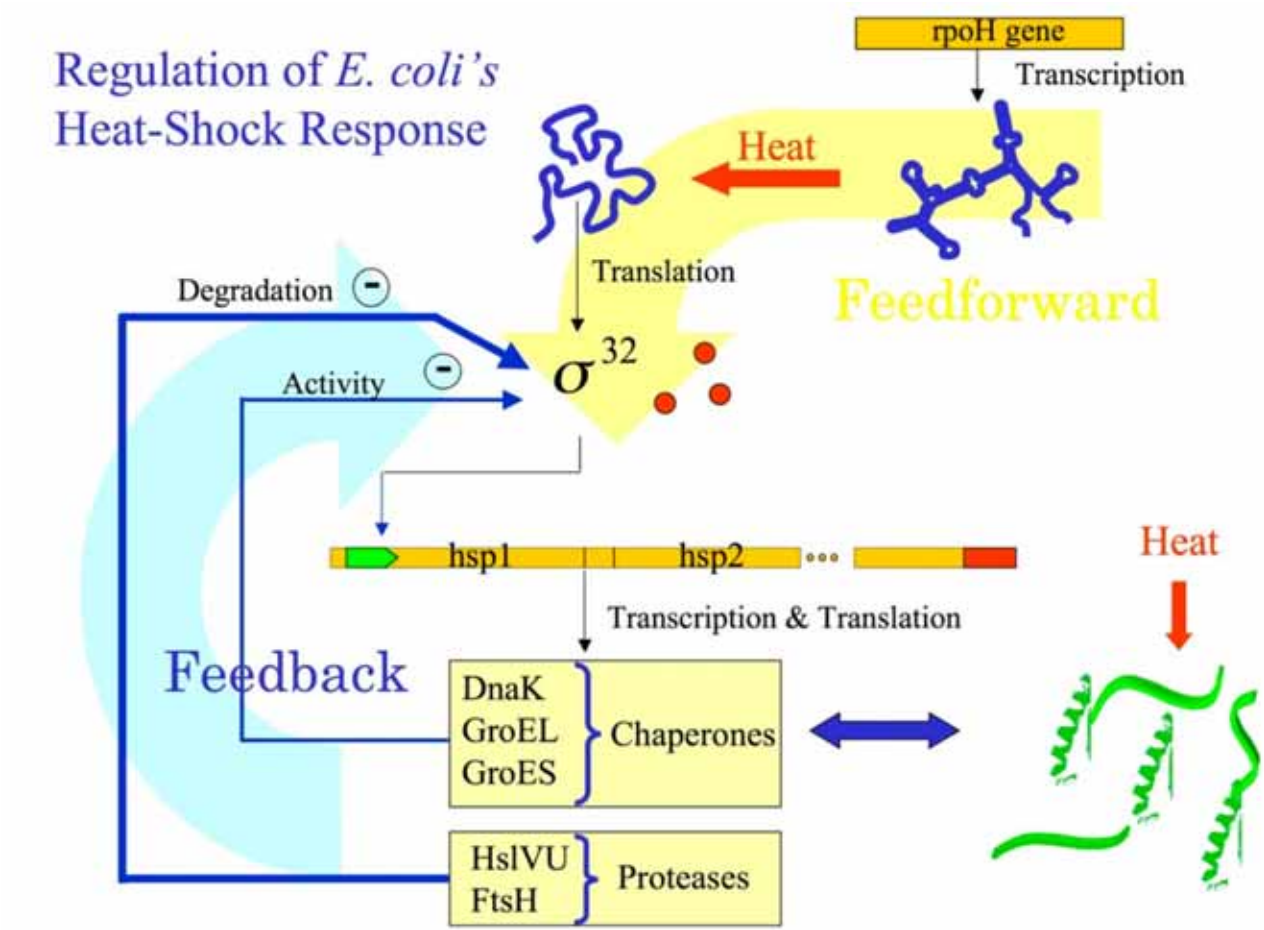
The Heat-Shock Response



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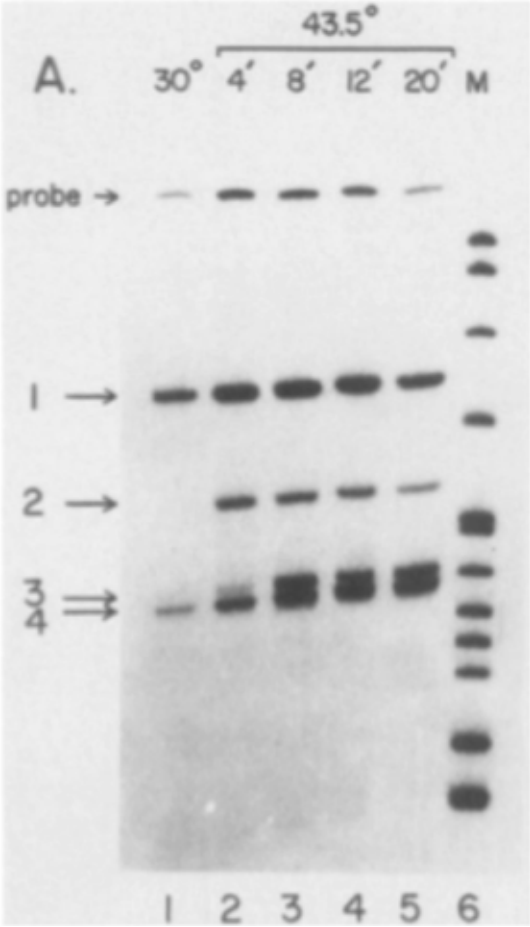
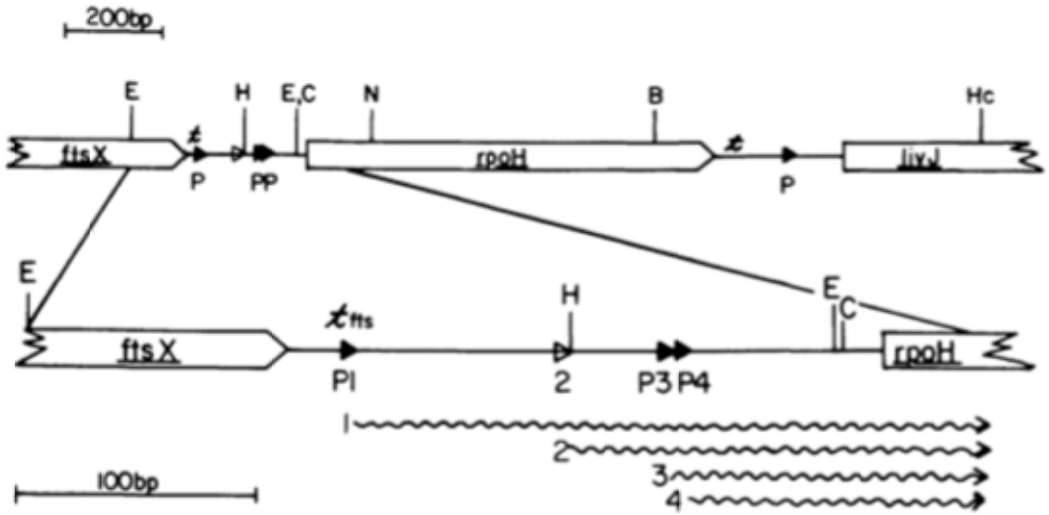
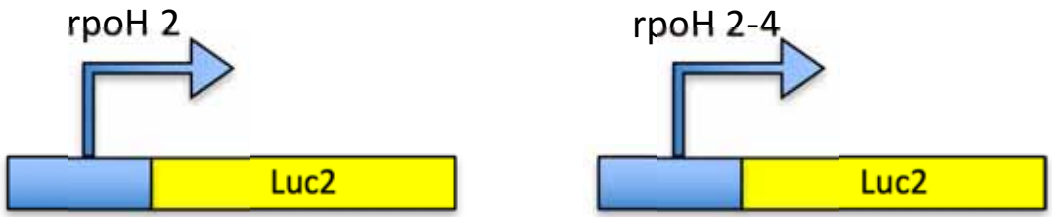
The σ^{32} gene (rpoH) UTR as a regulatory element







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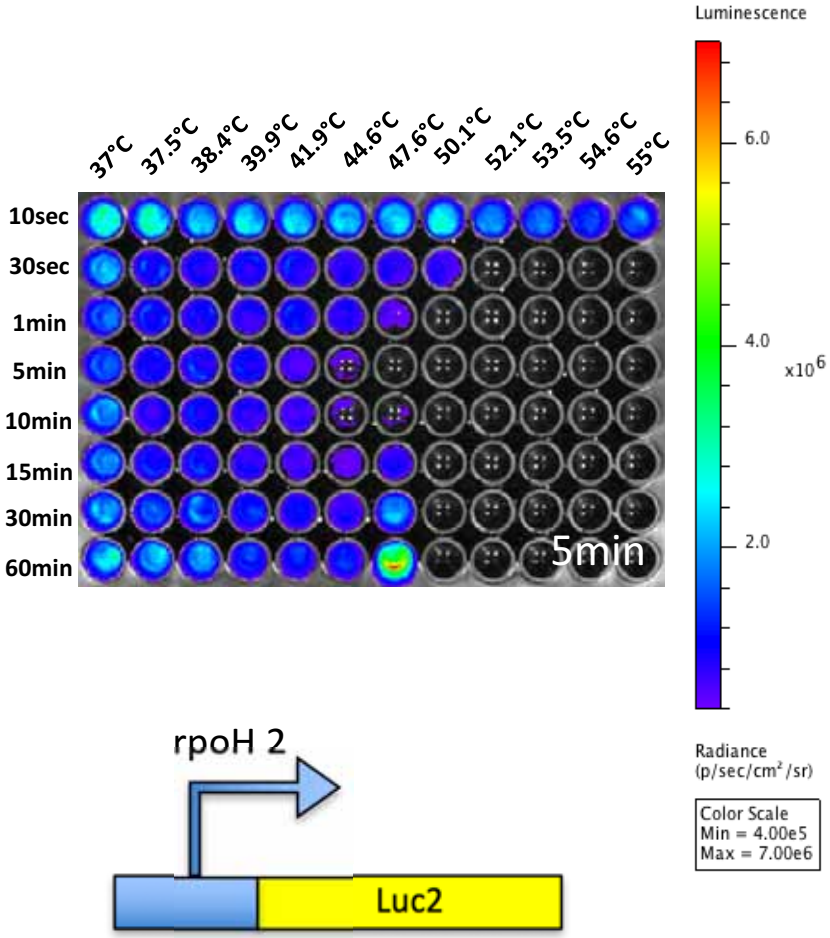
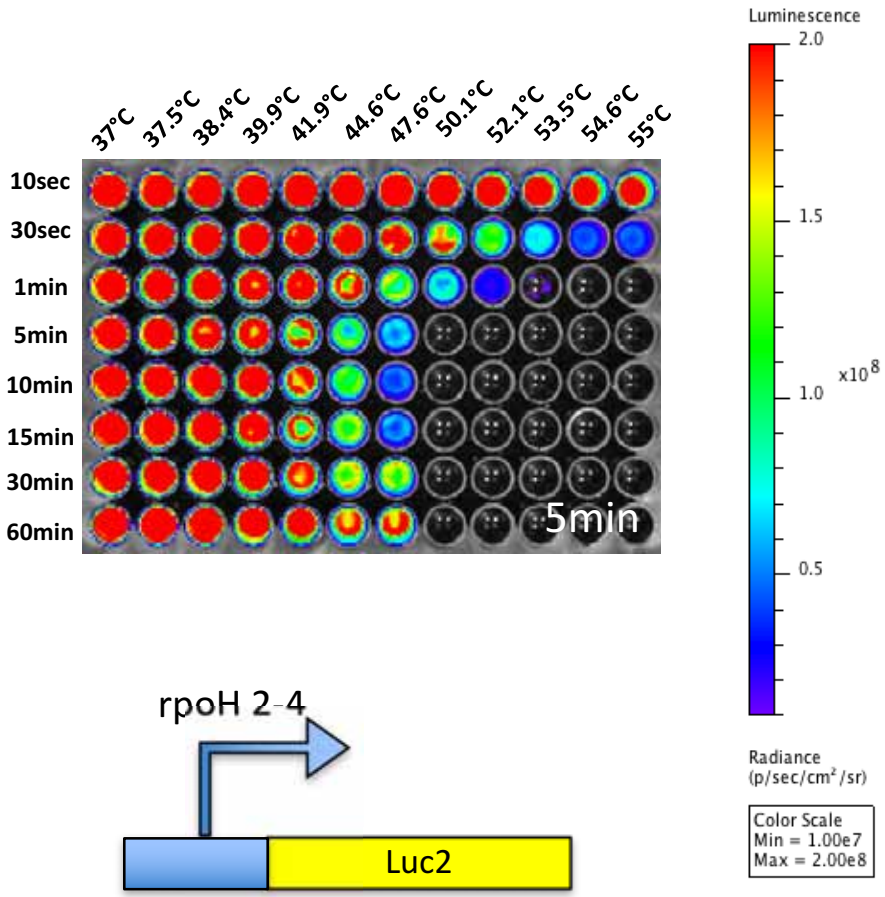
GENE5 • DEVELOPMENT 1:419-432

Comparison of rpoH Regulatory Elements





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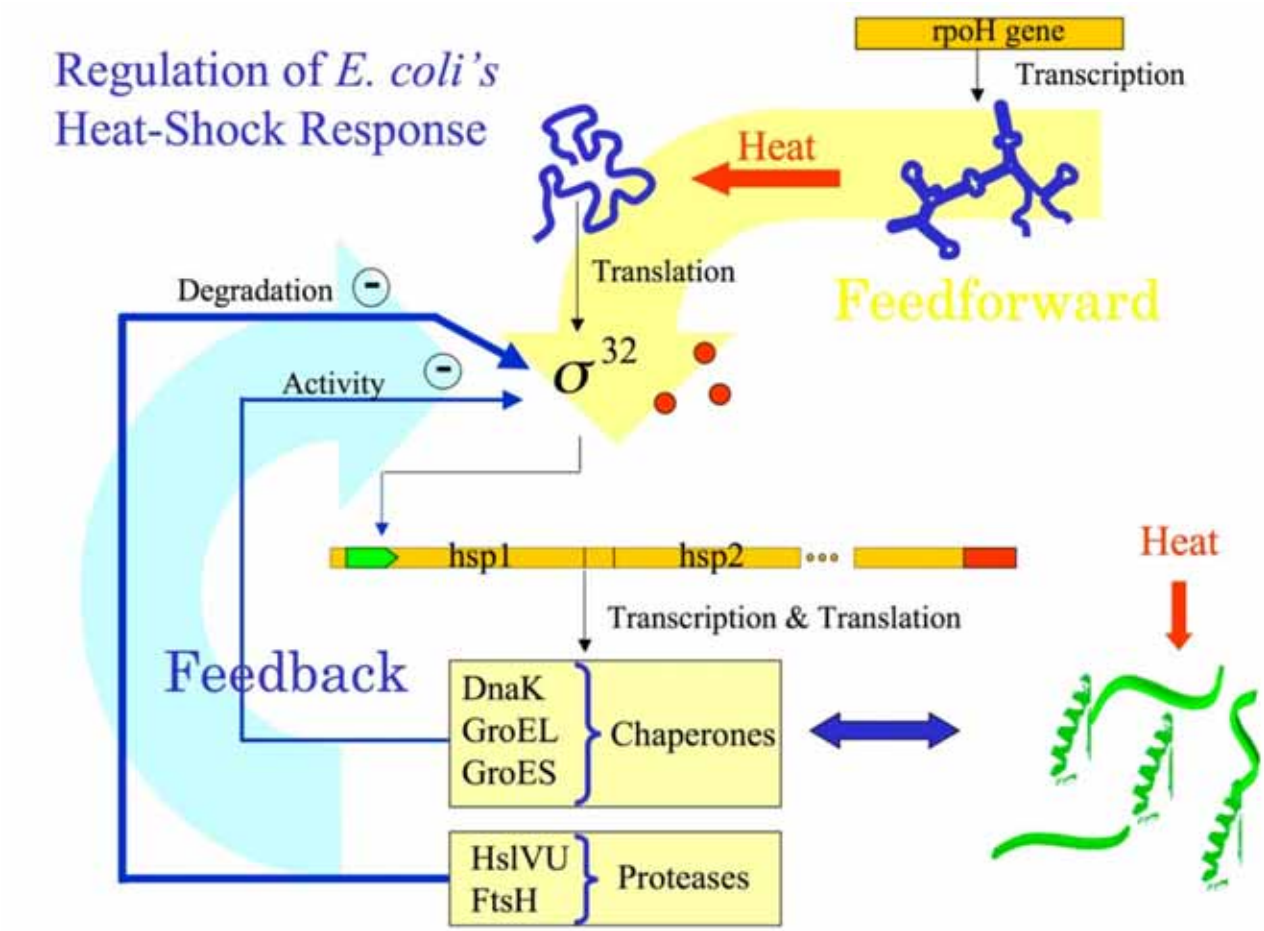
The Heat-Shock Response



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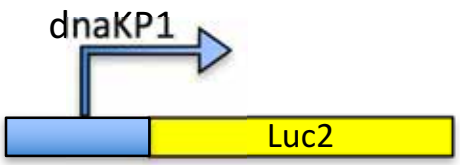
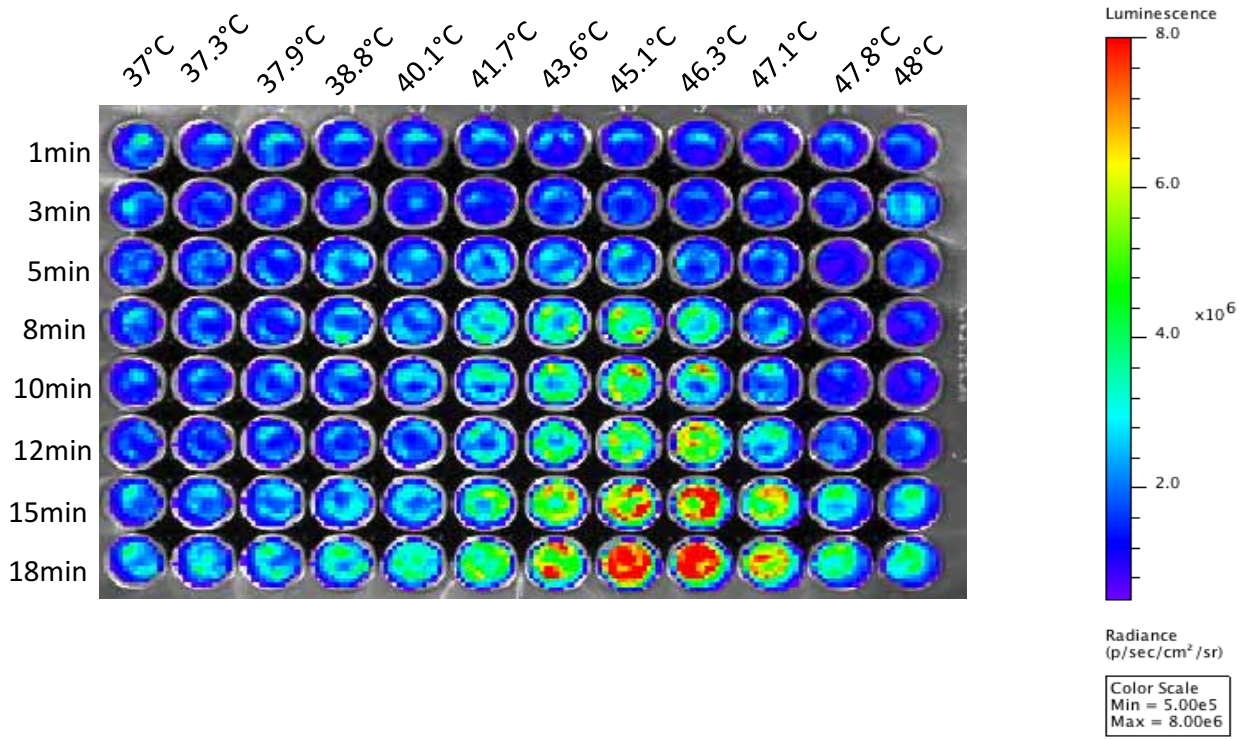
Optimized conditions for thermally induced gene expression using *E.coli* the dnak promoter





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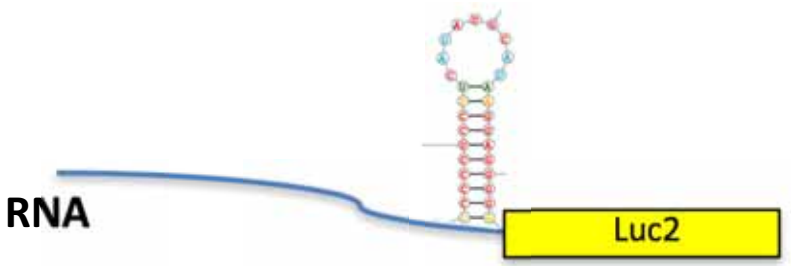
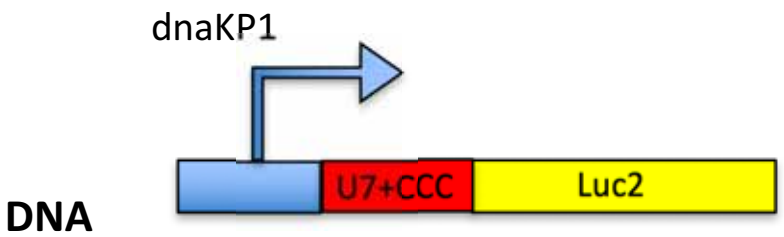
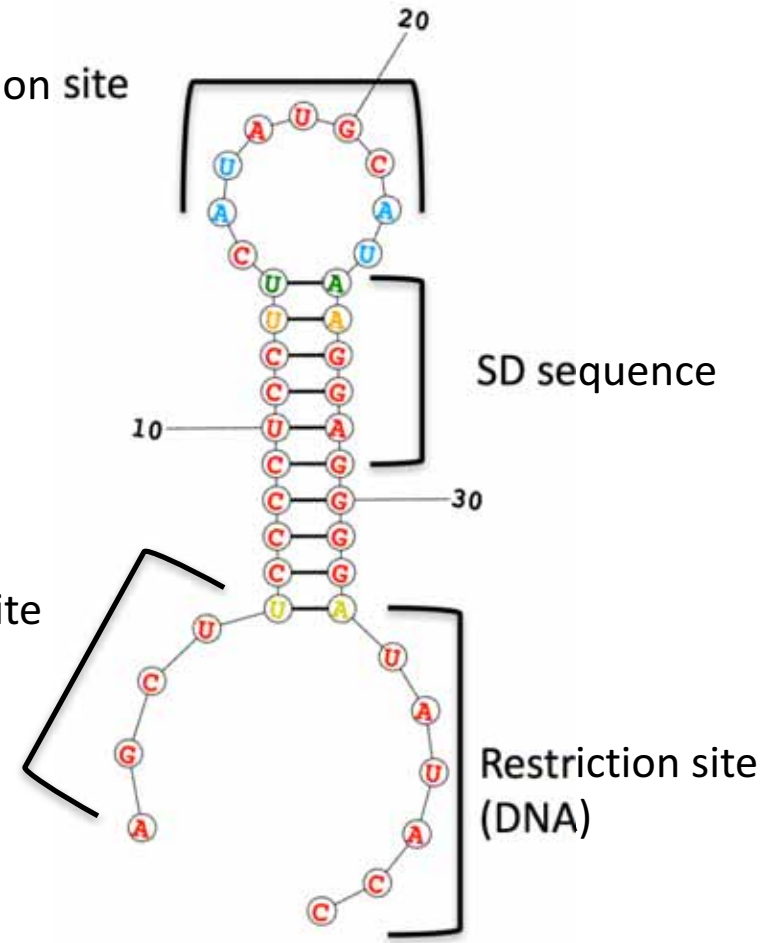
Construction of Ribothermometer Expression Vectors







U7+CCC RNA Structure

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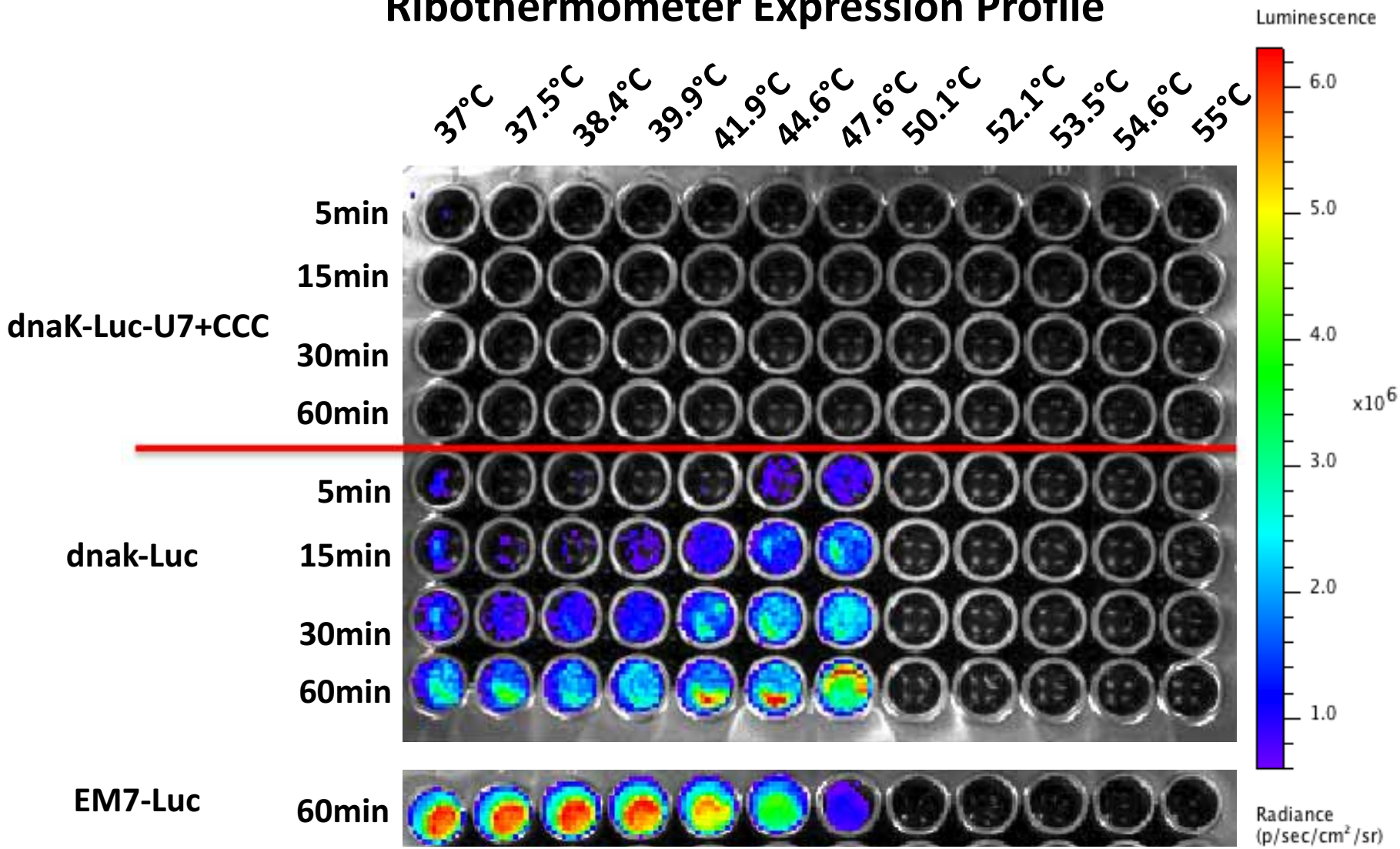
Ribothermometer Expression Profile



1 sec acquisition

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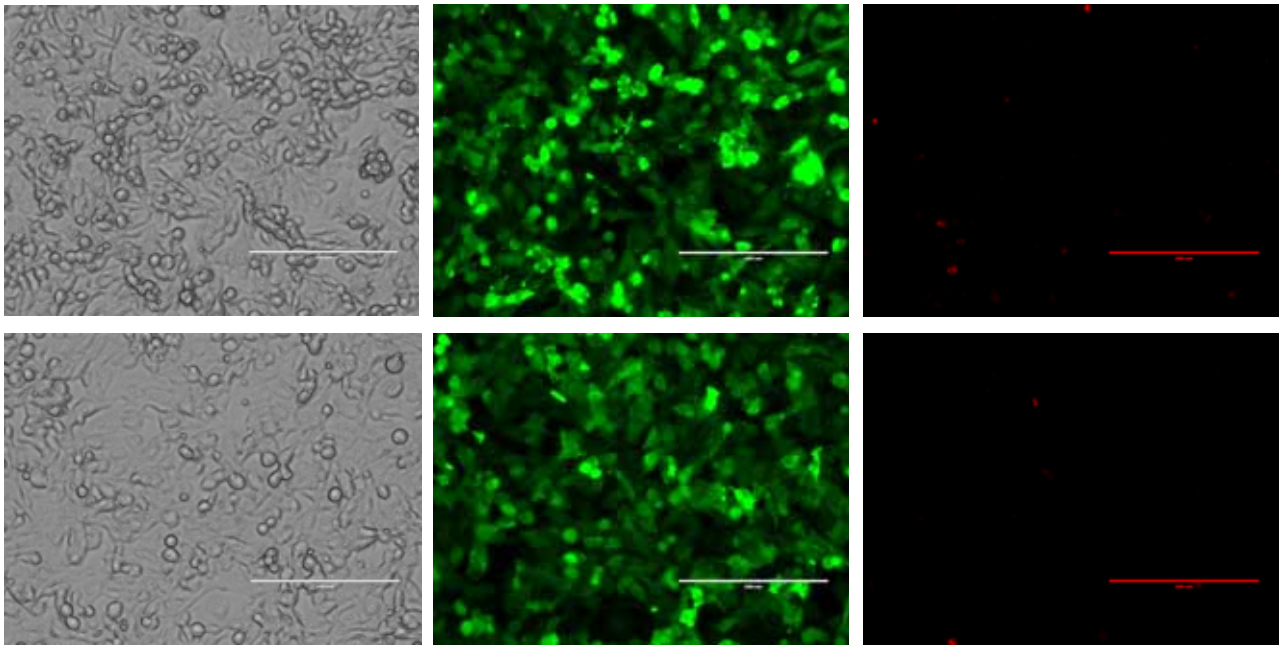
Magnetic Labeling of 231BR Cells

Phase | GFP | Propridium Iodide



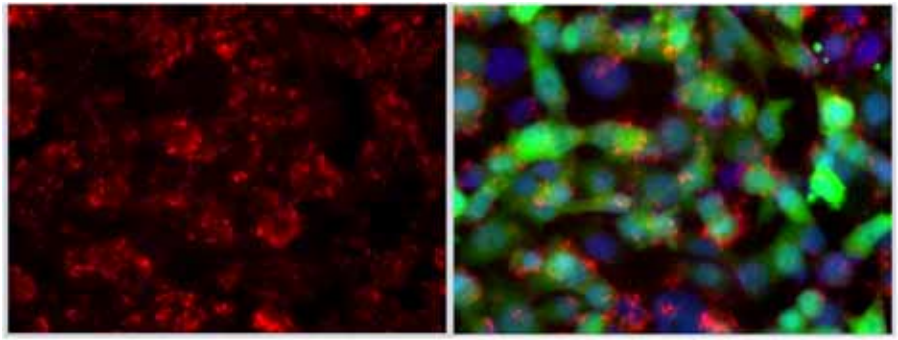
AMB-1 labeled

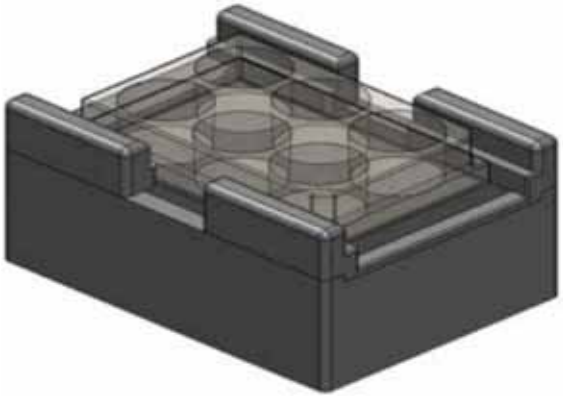
Control

Effective labeling
Of 231BR cells with
AMB-1

Scale bar = 200µm

AMB-1 | AMB-1 GFP DAPI





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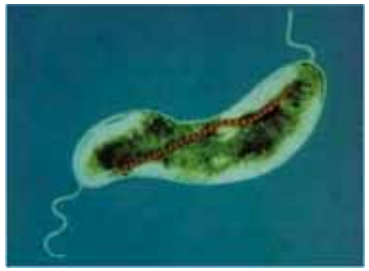
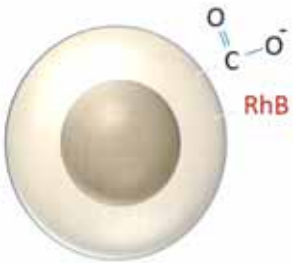
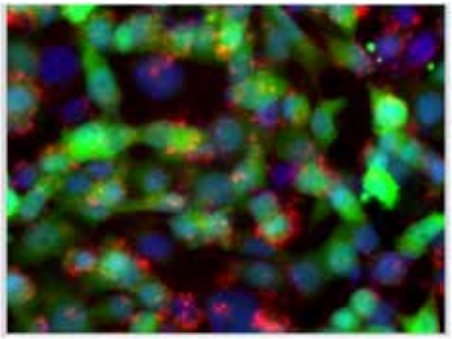
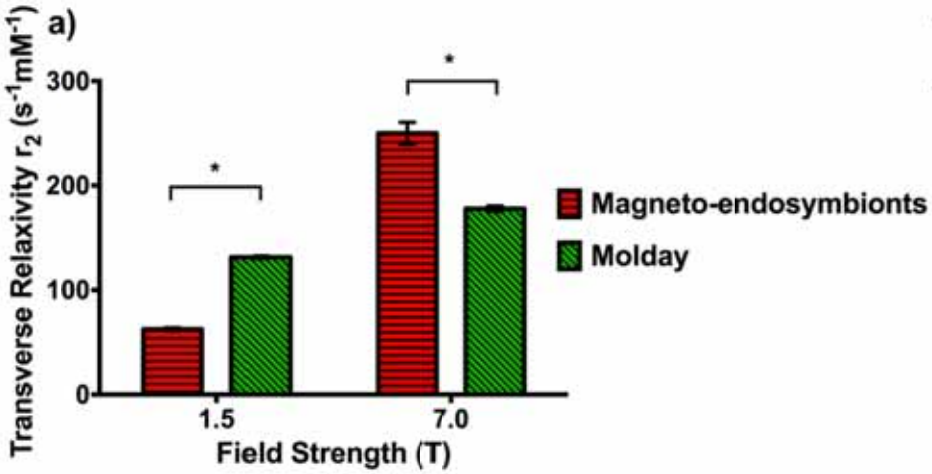
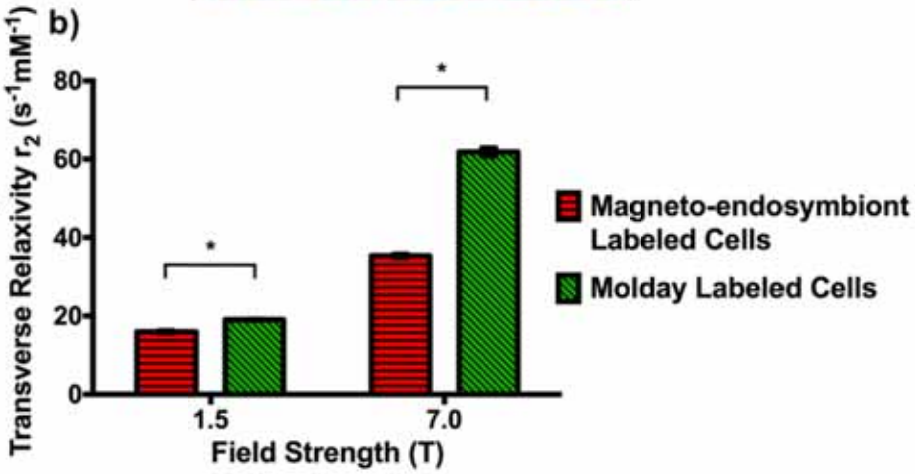
Relaxivity of MEs and Molday











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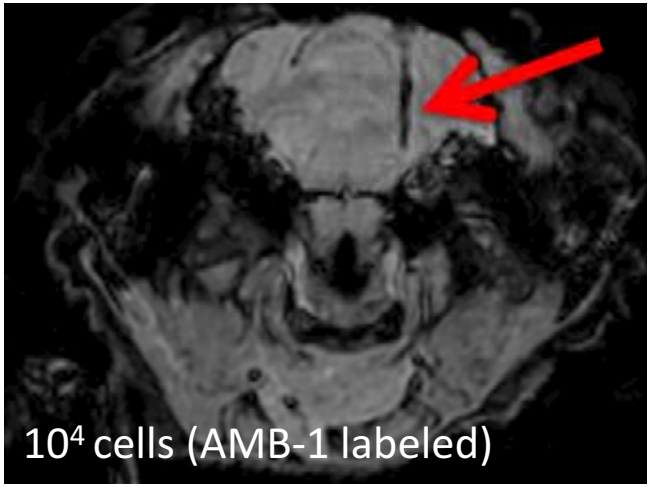
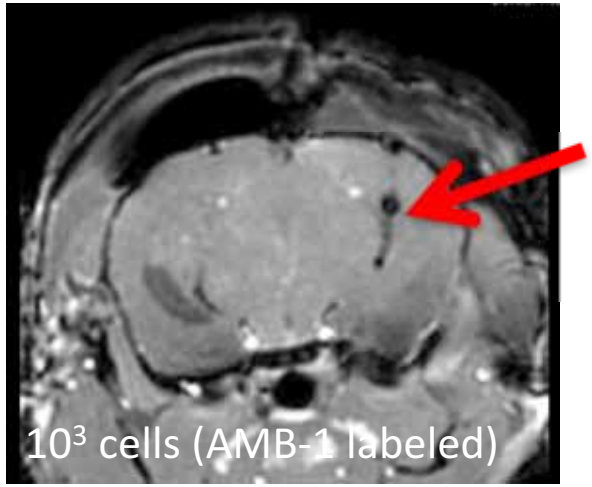
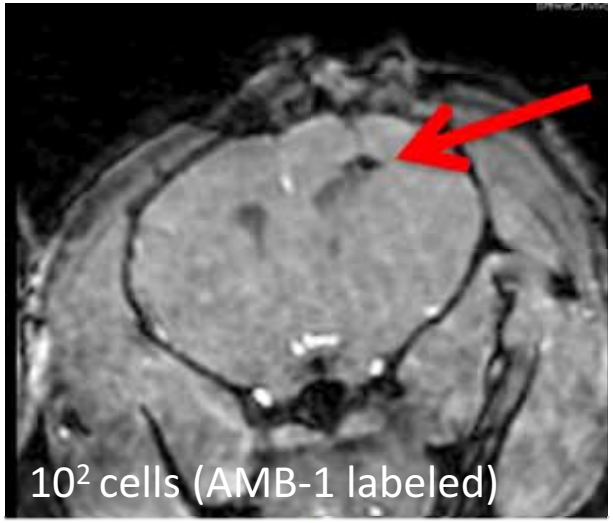
Spitler et al Submitted

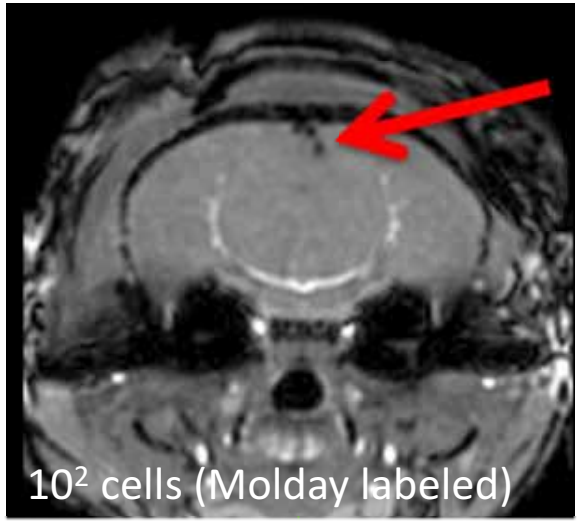
In vivo MRI – AMB-1-labeled 231BR cells





With Kim Brewer (Rutt Lab)





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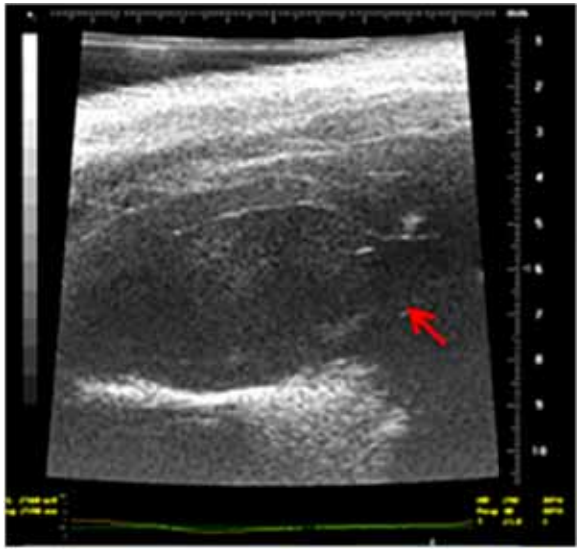
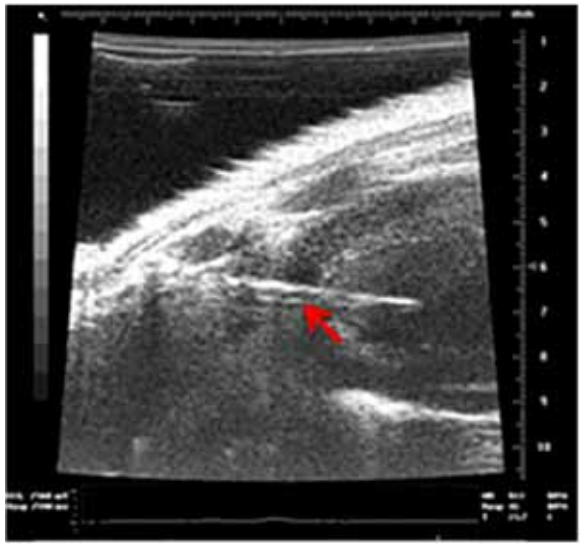
Spitler et al Submitted

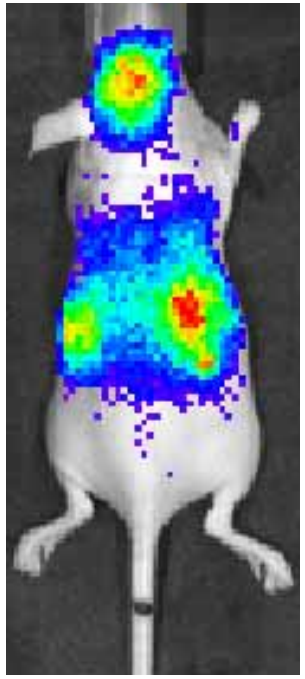
Single Cell Detection of 231BR Cells using MRI

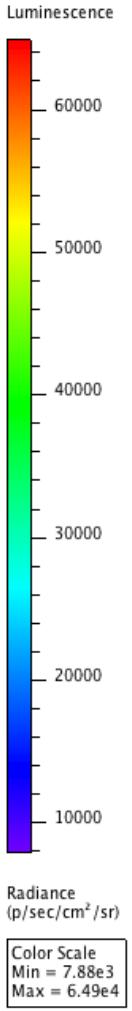
Ultrasound-Guided Intracardiac Injection





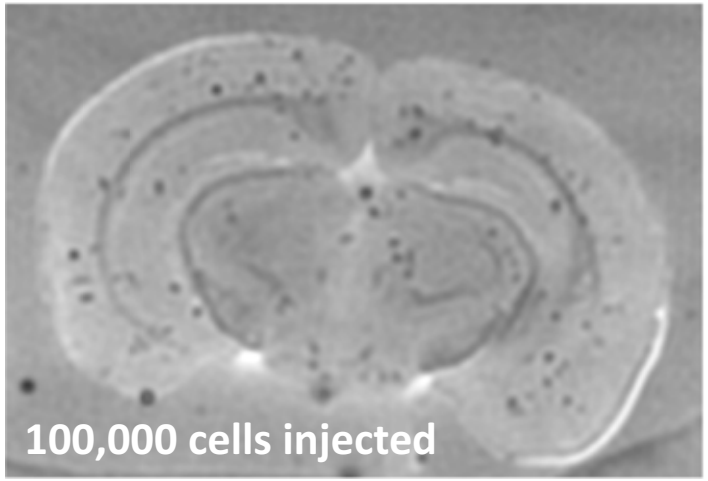
BLI





100,000 cells

MRI of ex vivo mouse brain 231BR cells loaded with Molday (3.5pg Fe/Cell)



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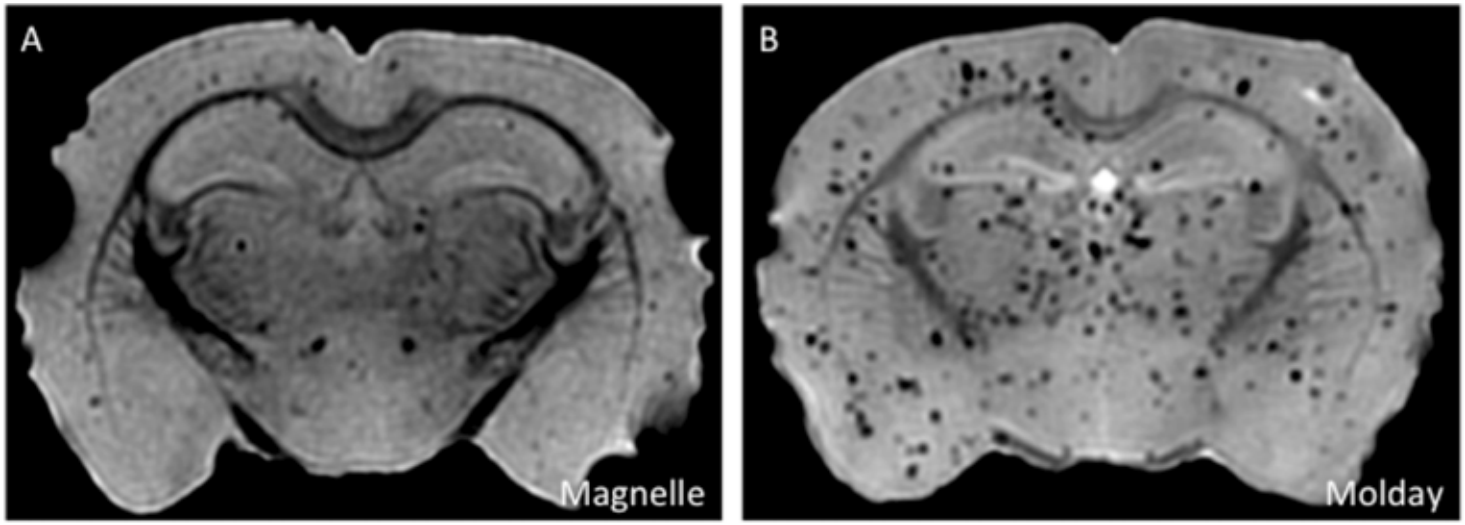
Single Cell detection in mouse brain



MRI image of single metastatic cells in mouse brain. A) magnelle labeled 231BR-luc cells and B) molday labeled 231BR-luc cells both with matched iron loading (~10pg of Fe per cell). Mice were given an intracardiac injection of $1.5x10^5$ labeled 231BR-luc metastatic cells prior to perfusion fixation with 4% paraformaldehyde. Brains were harvested fixed overnight and then placed into a tube containing Fomblin prior to the MRI scan. A bSSFP MRI sequence was used to visualize single cells as signal voids

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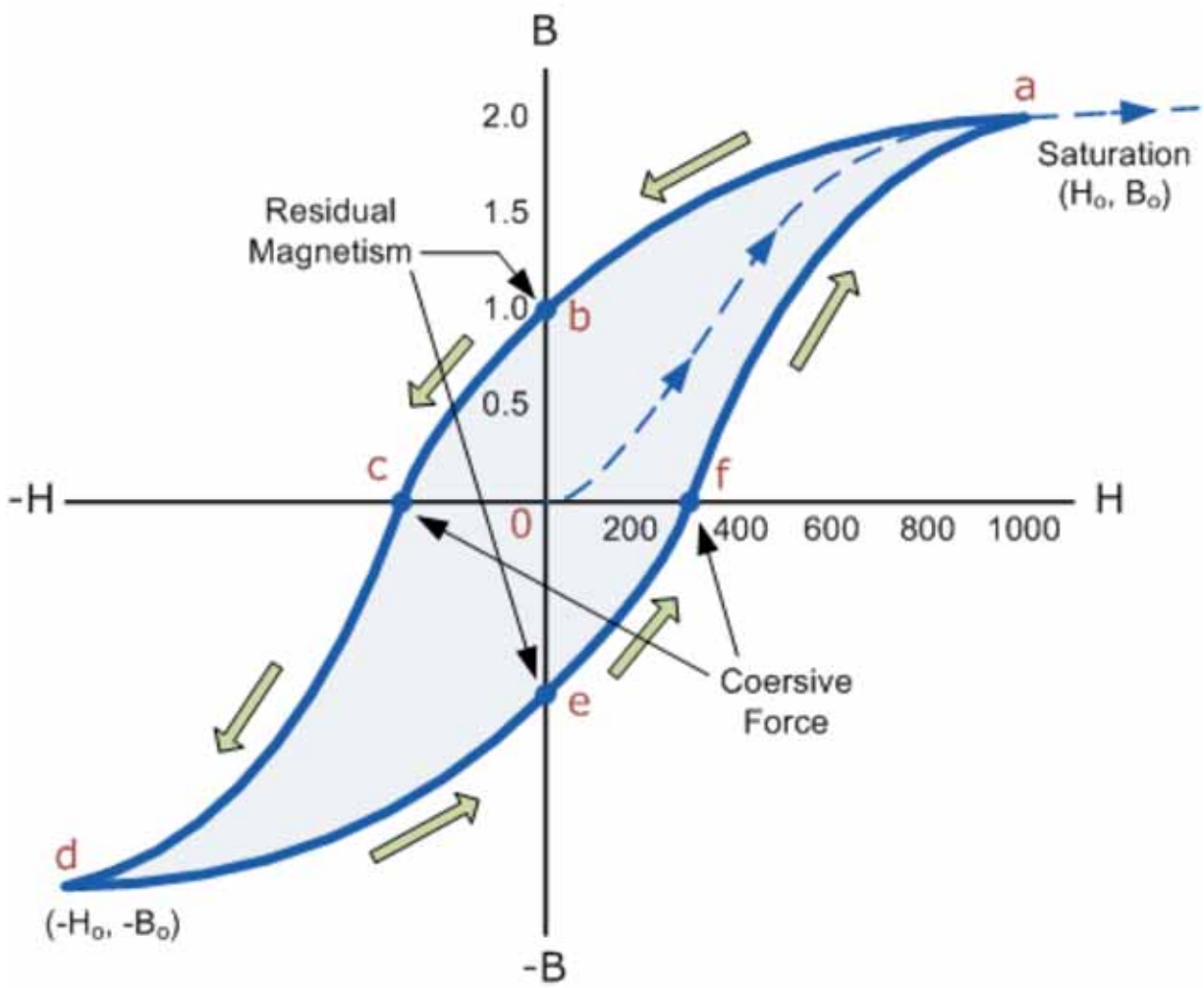
Magnetic Hysteresis Curve



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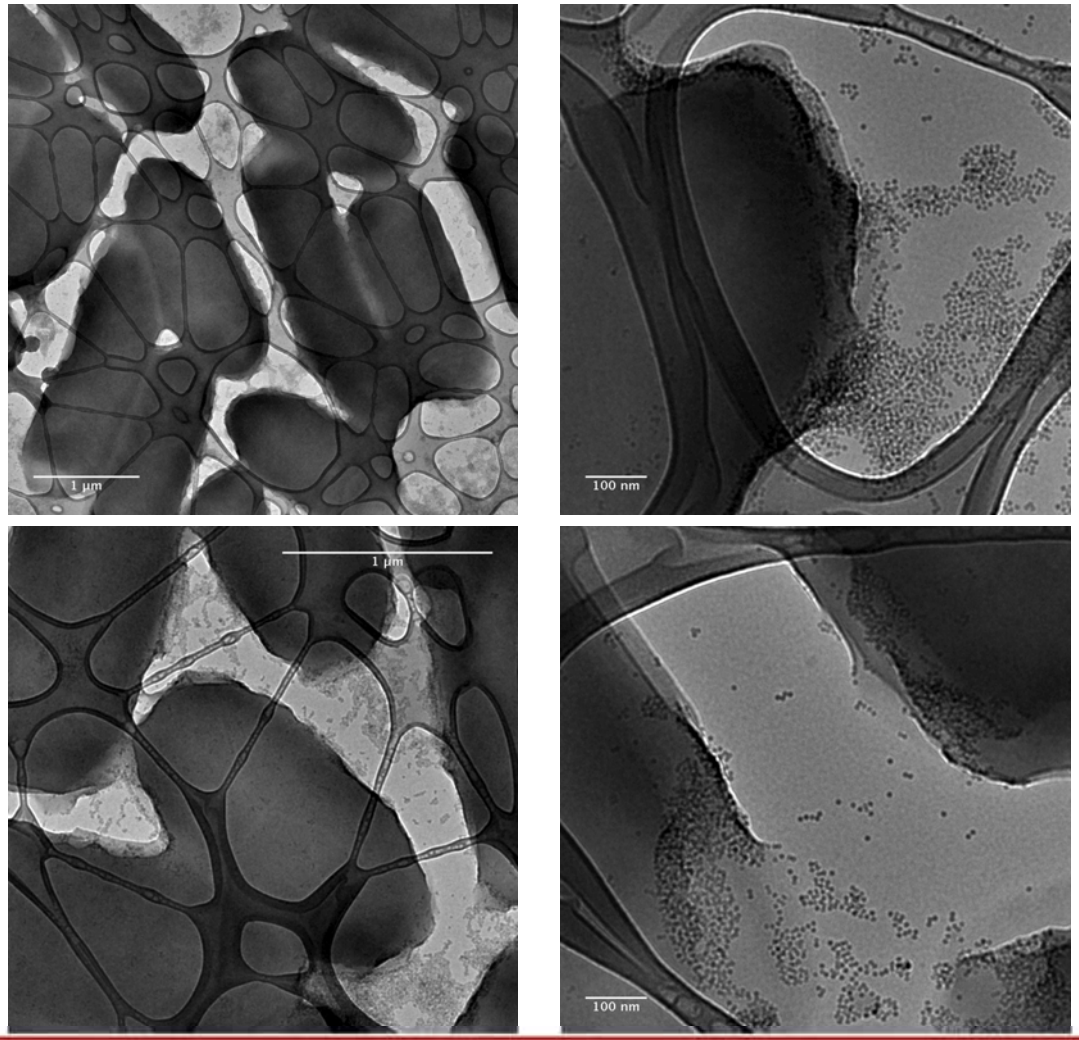
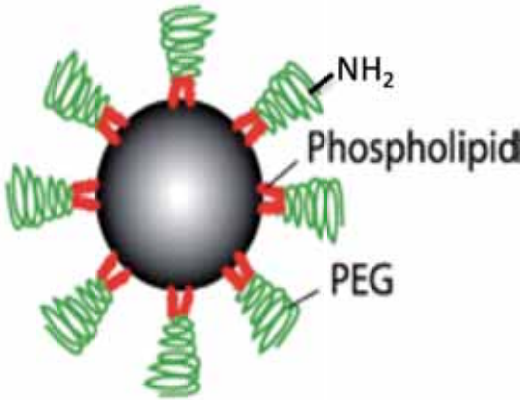
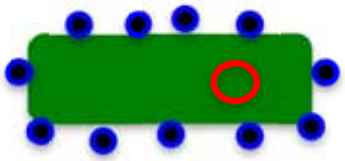
E.coli coated with Lipid/IONPs

TEM images of *E.coli* dried on C grids







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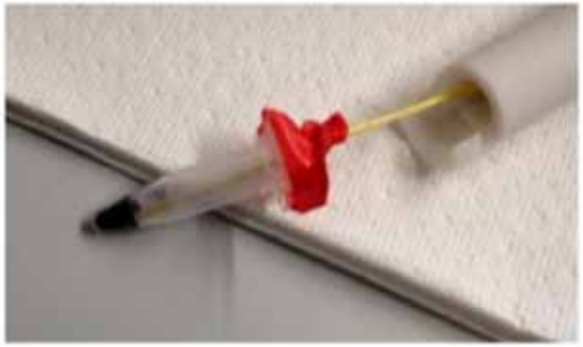
Experimental Setup



- Excitation LC tank.
  - Center f: 205 kHz.
  - Field: ~18 mT RMS.
  - Water cooling.
- Optical T sensors.
- Senior Scientific (SS) 25 nm particles.

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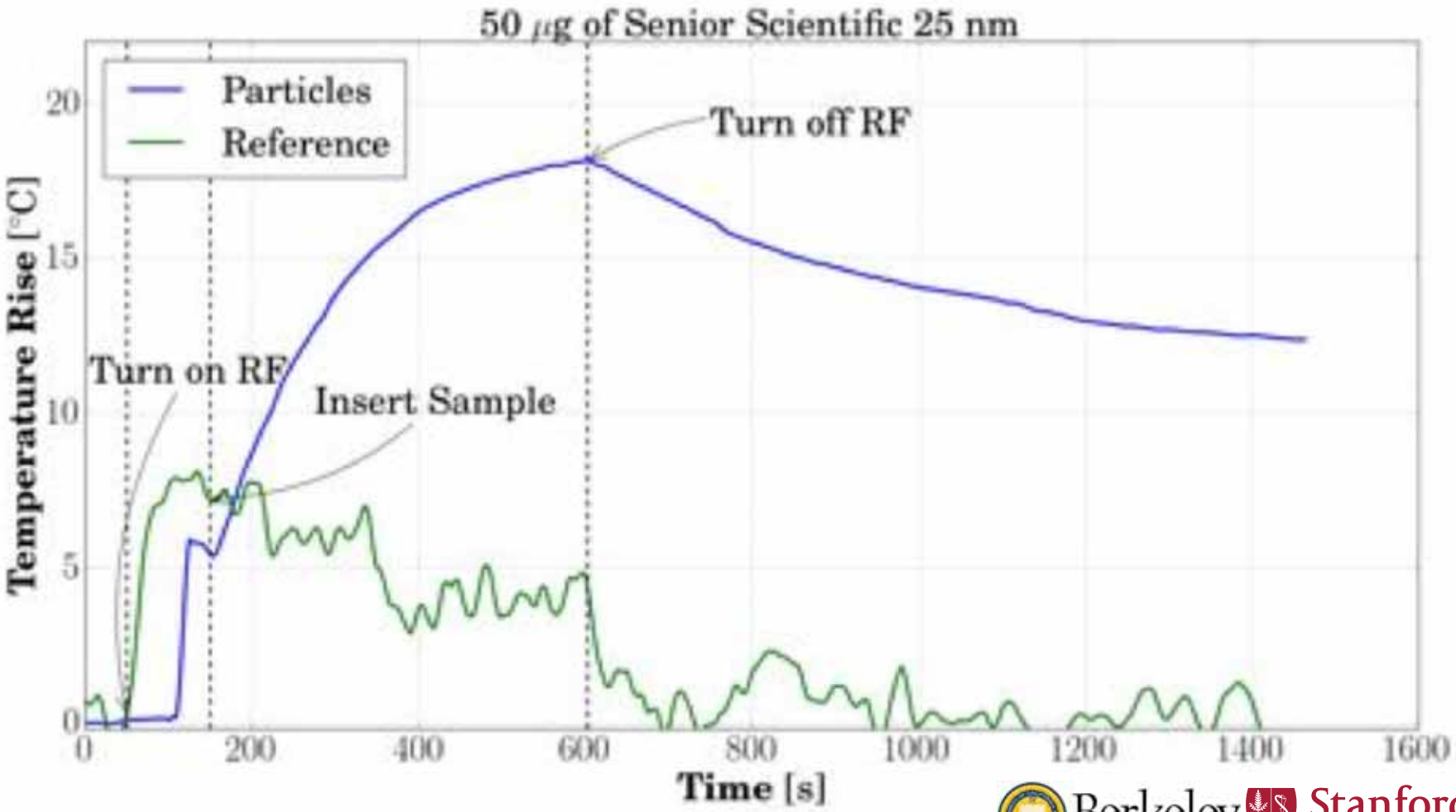
Results: SS 1 to 5 Dilution



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Results

- Demonstrated 4.8 C per minute average heating rate with SS particles.
  - 250 μL of 5 mg/mL particles (250 μg).
- Demonstrated 1.6 C per minute average heating rate with SS particles
  - 250 μL of 1 mg/mL particles (50 μg).

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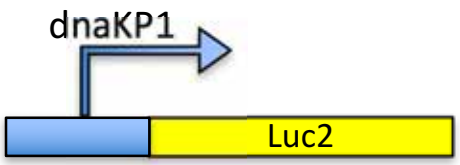
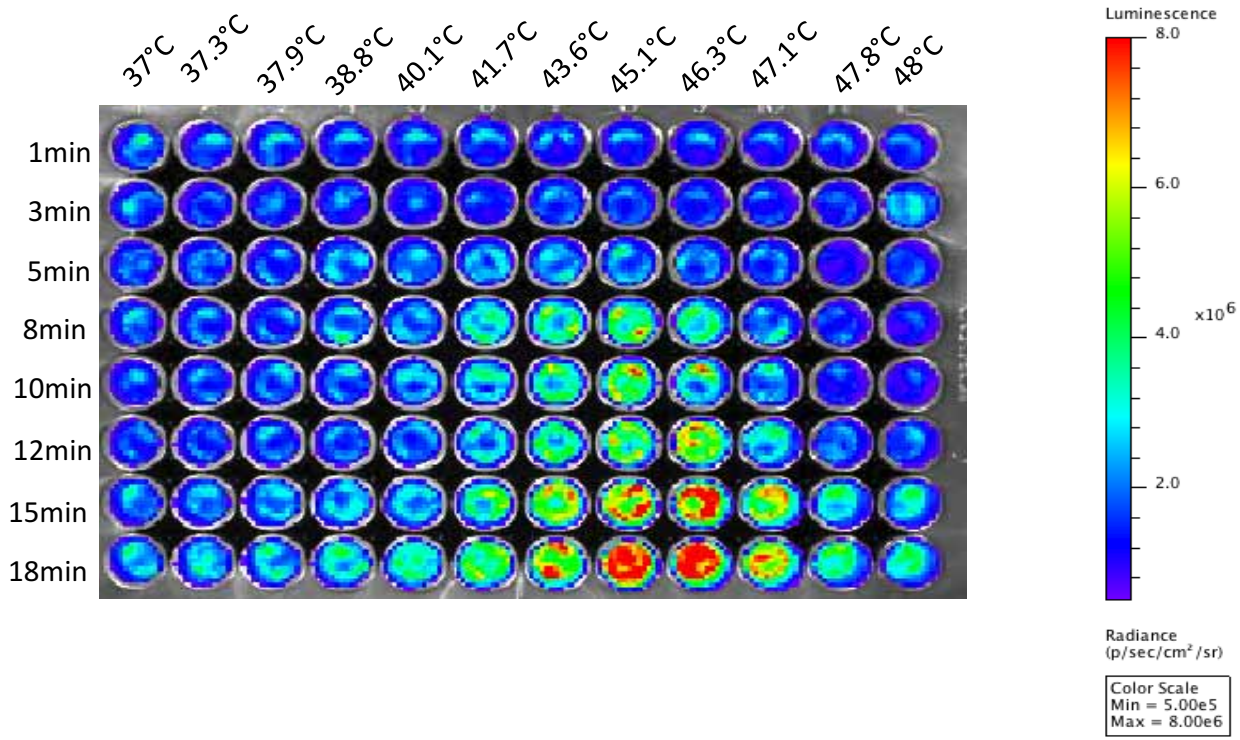
Optimized conditions for thermally induced gene expression using E.coli the dnak promoter





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MagneTherm Heating Results

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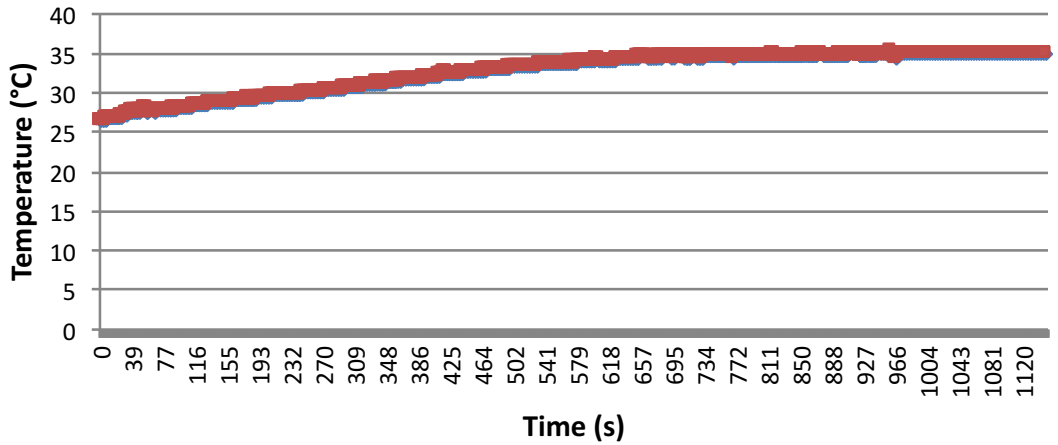
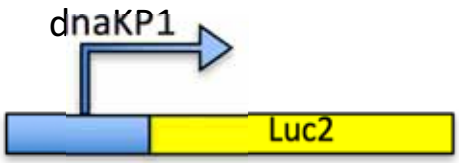
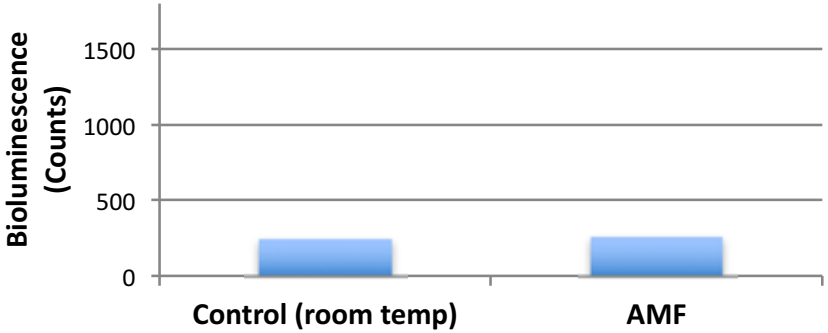
AMF Coil Control



MangeTherm HFS Settings	
Seq	2
Freq (kHz)	103.6
VDC	60
Amps	12.7
Vpp	2800
Field (mT)	50
Chiller (°C)	25





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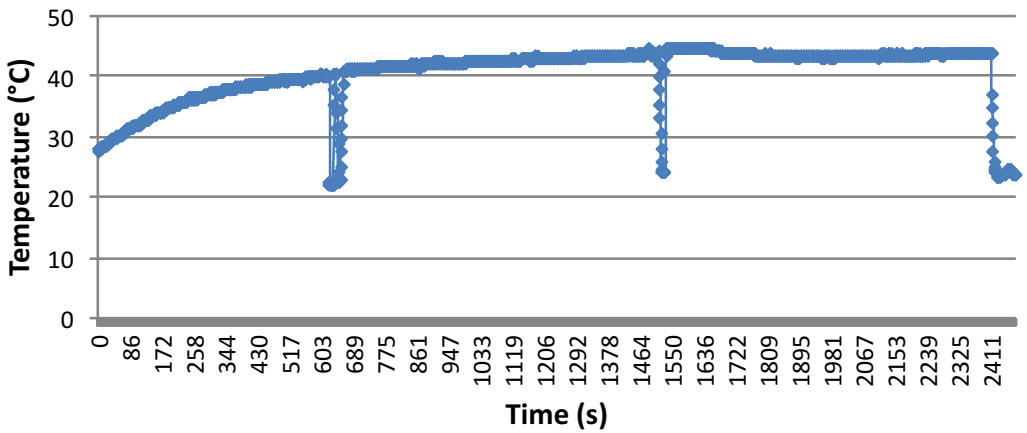
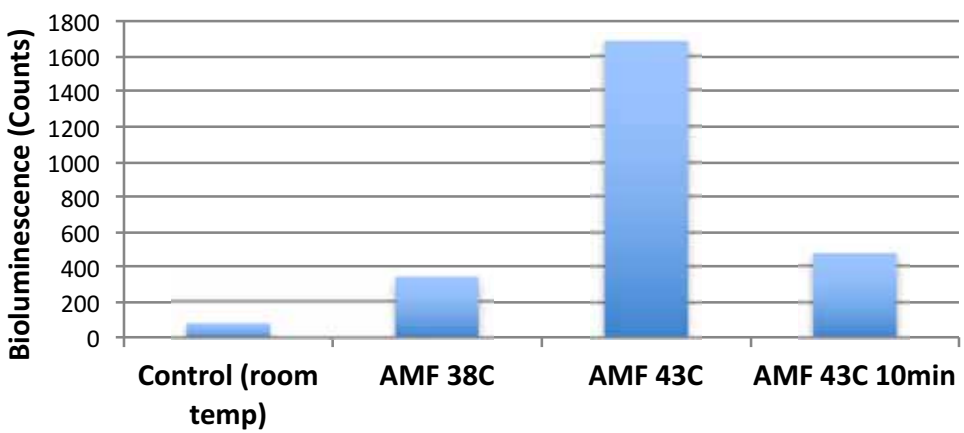
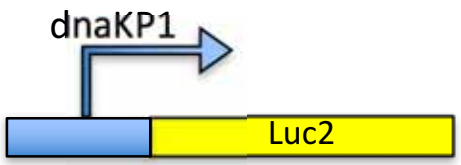
AMF Induced Gene Expression





> 20 fold induction



1 mg/ml Fe SPIOS (25 nm)

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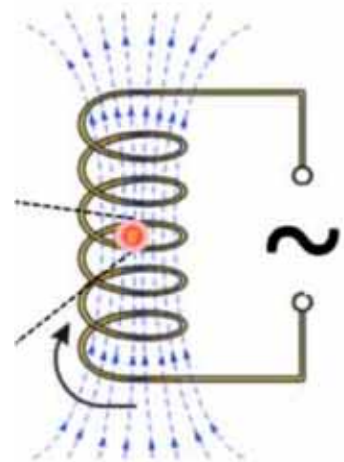
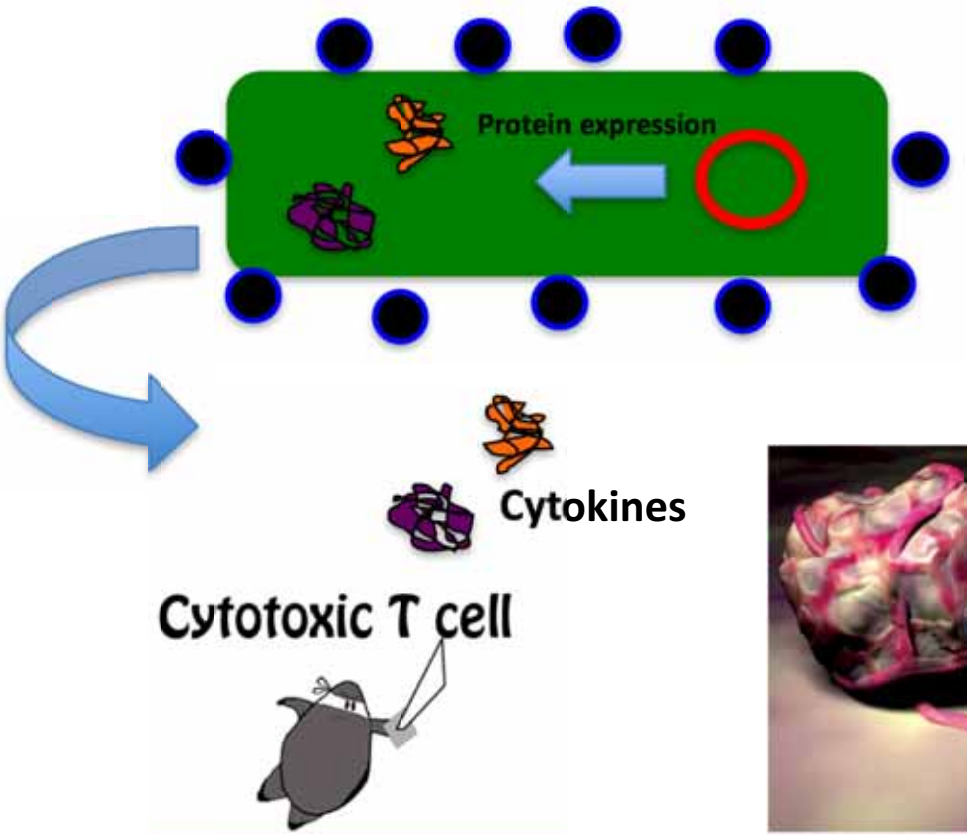
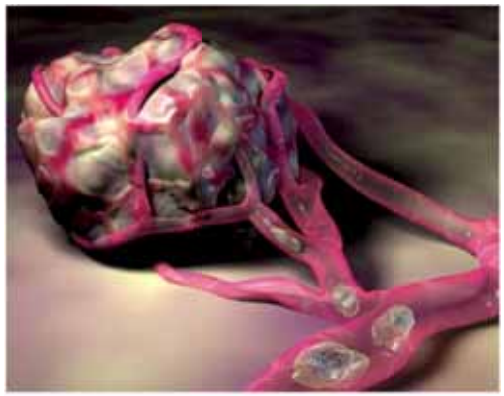
Overall Future Goal

AMF Field Generator







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