Engineering and Purification of Surrogate Markers of Biological Pharmaceuticals to Enable Visualization and Quantification of Pharmacological Assessments of Drug Delivery

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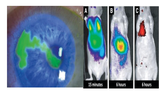
LSUHealthNewOrleans HEALTH SCIENCES CENTER Stanley S. Scott Cancer Center

A Dillard University - LSUHSC Collaboration
Minority Health & Health Disparities RESEARCH CENTER

BACKGROUND



Therapeutic Need

Carriers for efficient therapeutic delivery of already developed biological pharmaceuticals

2010: Top 30
Biological pharmaceuticals
$84.6 billion

2011: Top 10
Biological pharmaceuticals
$90.6 billion

Trade Name	Description	2011 Revenues
Humira	TNF Mab, rDNA, human	7.932 billion
Enbrel	TNF Receptor-IgG Fc, rDNA	7870 billion
Remicade	TNF Mab, rDNA	7157 billion
Avastin	VEGF Mab, rDNA	6596 billion
Rituxan	CD20 Mab, rDNA	6596 billion
Herceptin	HER2 receptor Mab, rDNA	5770 billion
Lantus	Insulin glargine, rDNA	5190 billion
Neulasta	G-CSF, rDNA, PEG-	3950 billion
Epogen/Procrit	EPO, rDNA	3730 billion
Lucentis	VEGF Mab Fab, rDNA	3723 billion

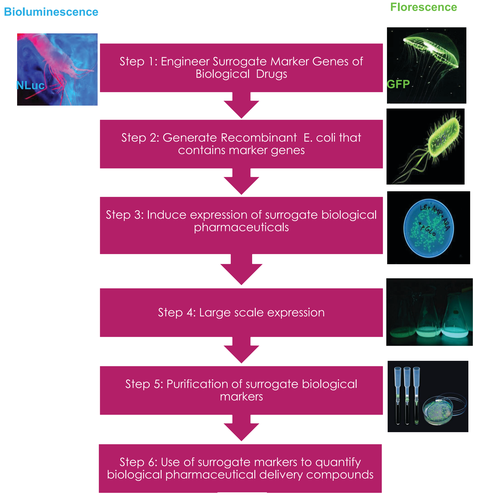
PHARMACOLOGICAL NEEDS, PROBLEM, & HYPOTHESIS

•**NEED:** There is an intense need for developing carrier compounds of biological drugs that alter their immune recognition, drug availability, activity, delivery, retention, and pharmacological properties.

•**PROBLEM:** Accurate assessment of pharmacological properties of specific carrier compounds for biological drugs is a complex process. Complications in modifying biological drugs for visualization and detection slows carrier drug development and assessment.

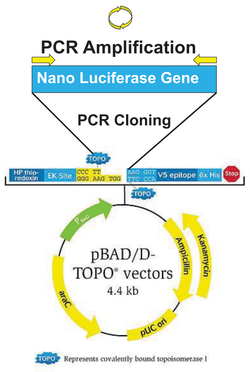
•**HYPOTHESIS:** Bioluminescent and fluorescent proteins can be utilized as surrogate markers of biological pharmaceuticals to assist in development of carrier compounds.

EXPERIMENTAL PLAN



RESULTS: Engineering Recombinant Surrogate Markers of Biological Drugs

Nano Luciferase



Green Fluorescent Protein

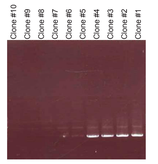
Generation of Recombinant E.coli That Contains Marker Genes

Transformed E. coli

Isolate Individual Bacterial Colonies

Diagnostics for Positive Clones

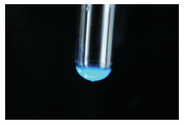
PCR Screen for Positive Clones



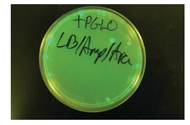
Restriction Digestion Confirmation

Induction of Surrogate Biological Pharmaceuticals Expression

Nano Luciferase

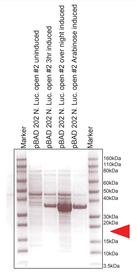


Green Fluorescent Protein



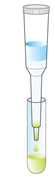
Large Scale Protein Expression

Scaled up to 1 Liter cultures



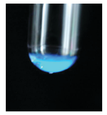
SDS- PAGE of Nano Luciferase Expression

Purification of Surrogate Biological Markers

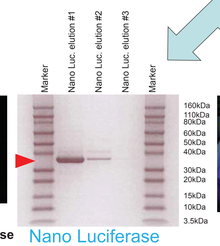


Hydrophobic (GFP) or AFFINITY (Nano-Luciferase) Interaction Chromatography Column

PROTEIN (GFP) Bound to Column

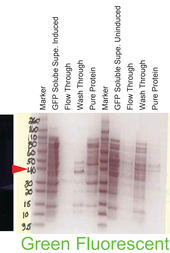


Purified Nano Luciferase



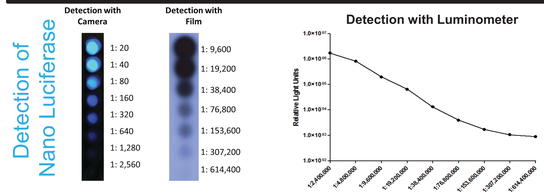
Nano Luciferase

Purified GFP



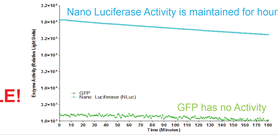
Green Fluorescent Protein

Characterization of Nano Luciferase Enzymatic Activity

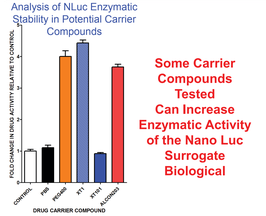


Analysis of Nano Luciferase Enzymatic Activity Every 1 Minute Over 3hr Time Period:

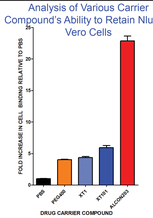
Nano Luciferase Activity is STABLE!



Utilization of Nano Luciferase to Assess Efficacy of Biological Carrier Compounds



Some Carrier Compounds Tested Can Increase Enzymatic Activity of the Nano Luc Surrogate Biological



Although all Carrier Compounds Enhanced Biological Drug Binding to Cells, the ALCON203 Formulation Was Significantly More Efficient in Cellular Retention of Biological Drugs

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