Recombinant Protein Applications:

Choosing the best cytokine, growth factor or chemokine for your experimental system

Matthew Riolo, Ph.D.

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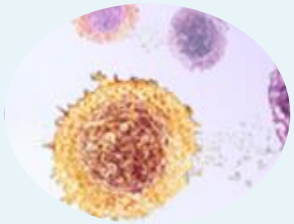
GenScript: Products

Protein Analysis

Antibodies

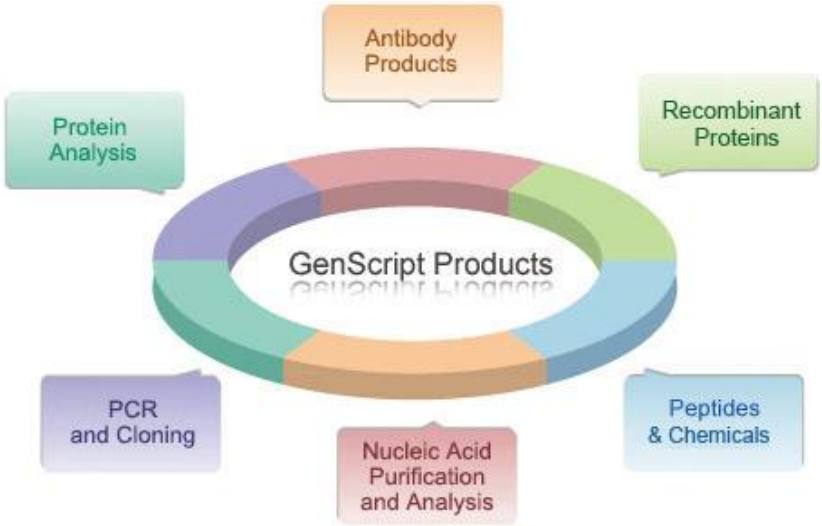


Cytokines

PCR reagents

ELISA Kits

Peptides



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Webinar Overview

1. Cytokine characterization
2. Key regulatory signaling pathways
3. T_H1, T_H2, and T_H17 immune response
4. Cytokines used in differentiation
5. Best lab practices

Cytokine Nomenclature

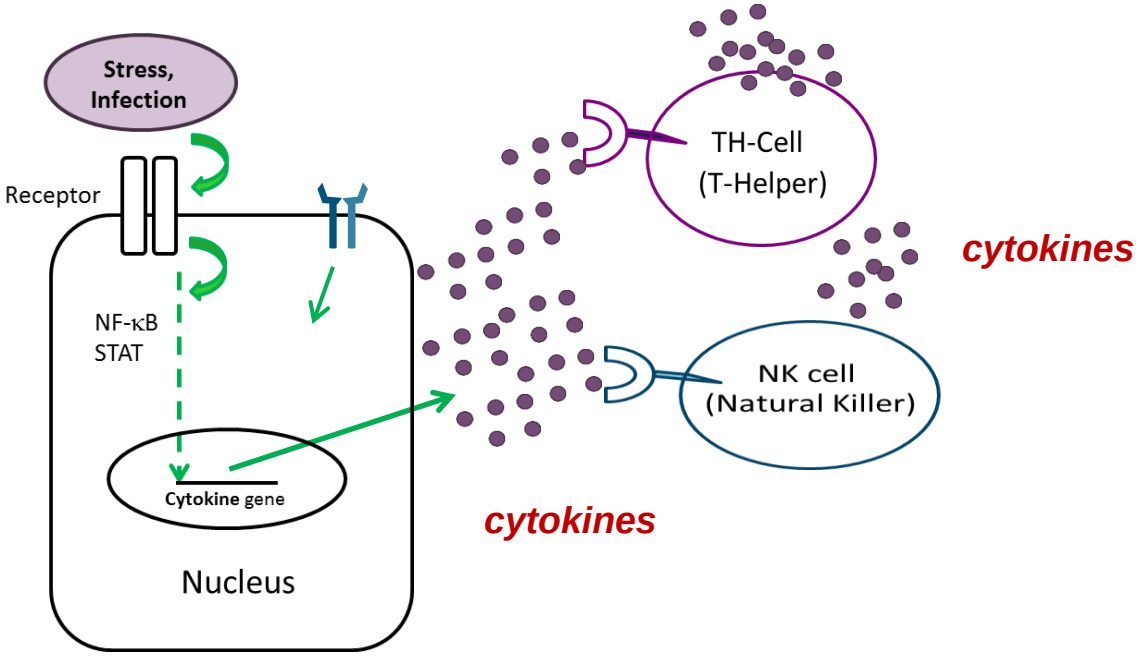
Interleukins:	Growth Factors:	Interferons:	Chemokines:
• IL-2 • IL-6 • IL-8 • IL-10 • IL-12 • IL-17 • IL-33	• GM-CSF • EGF • FGF • VEGF	• IFN-γ • IFN-α • IFN-β	• CXCL1 • CXCL12 • CXCL13 • CCL2 • CCL5 • CCL20

Cytokine Categorization

Pro-inflammatory Cytokines	Anti-inflammatory Cytokines	Differentiation Cytokines
IL-1α, IL-1β, TNF-α, IL-2,		
IL-6, IL-8, IL-9, IL-12, L-15	IL-6, IL-10, IL-11	IL-3, IL-4
IL-16, IL-17, IL-18, IL-21	IL-13, IL-19, IL-20, IL-22	IL-5, IL-7, IL-21
IL-22, IL-25 , IL-31, IL-33		

Cytokine Secretion

- Proteins
- Secreted by immune cells
- Regulate other immune cells

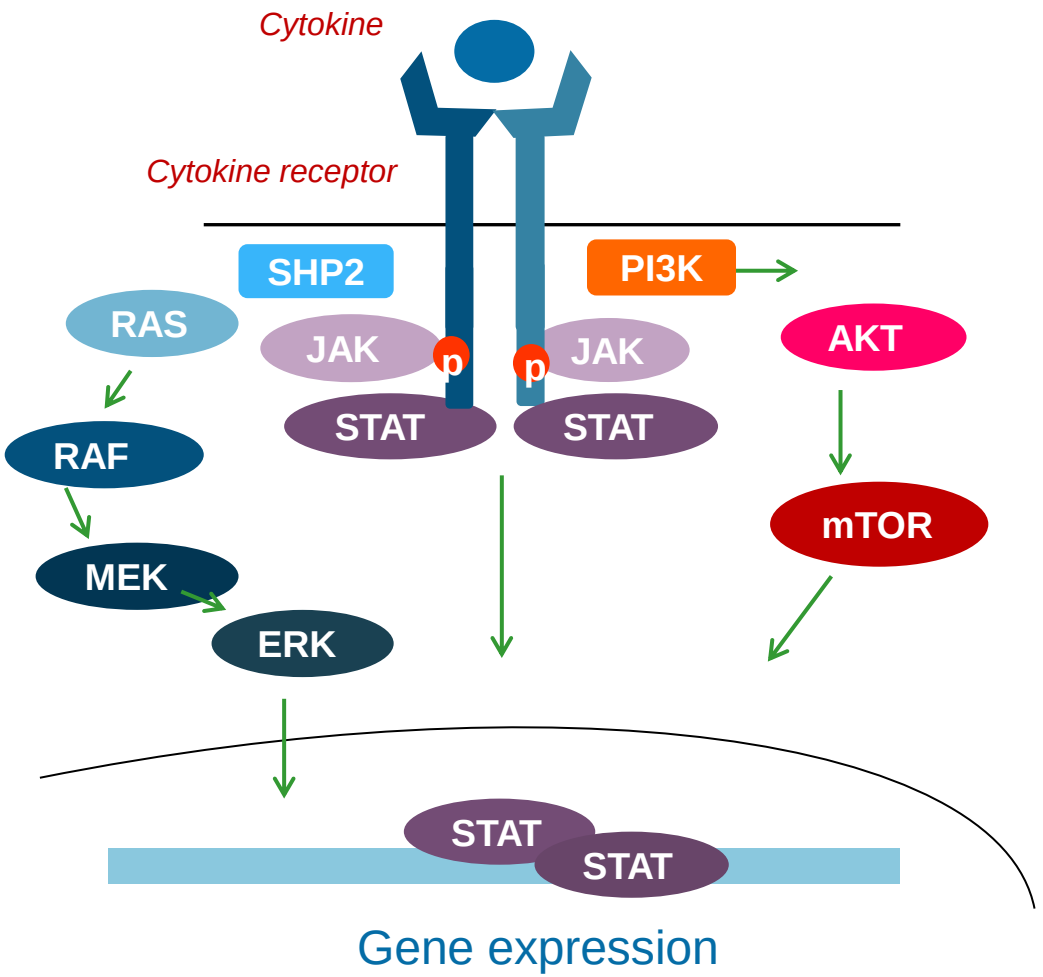


Cytokine Stimulated Intracellular Pathways

Cytokine Receptor

Intracellular Signal Transduction

Physiological Endpoint

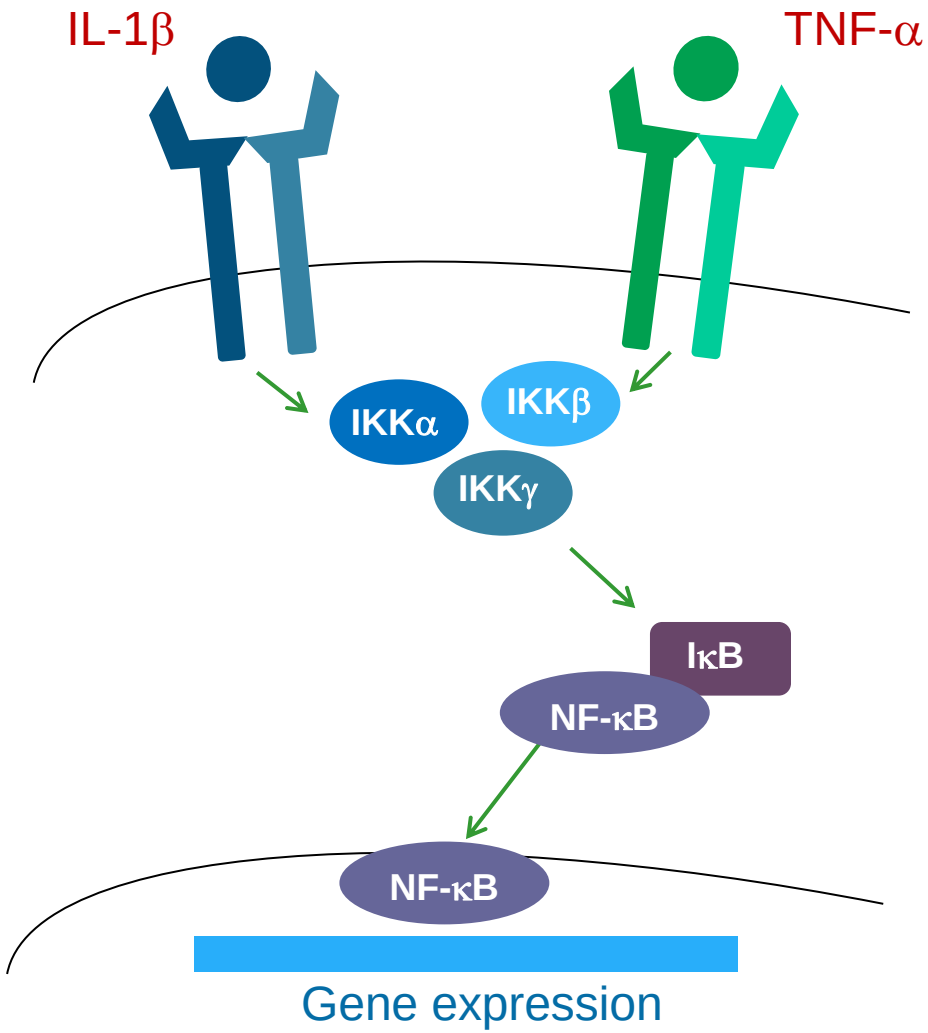


IL-1β and TNFα Can Activate the NF-κB Pathway

Cell types
Monocytes
Endothelial cells
Breast cancer cell lines
Prostate cancer cell lines

Pathway Activation
NF-κB

Application
Dose: 50ng/ml
Time: 30 min



IL-10 Can Inhibit TNF-α and TLR Mediated Activation of NF-κB

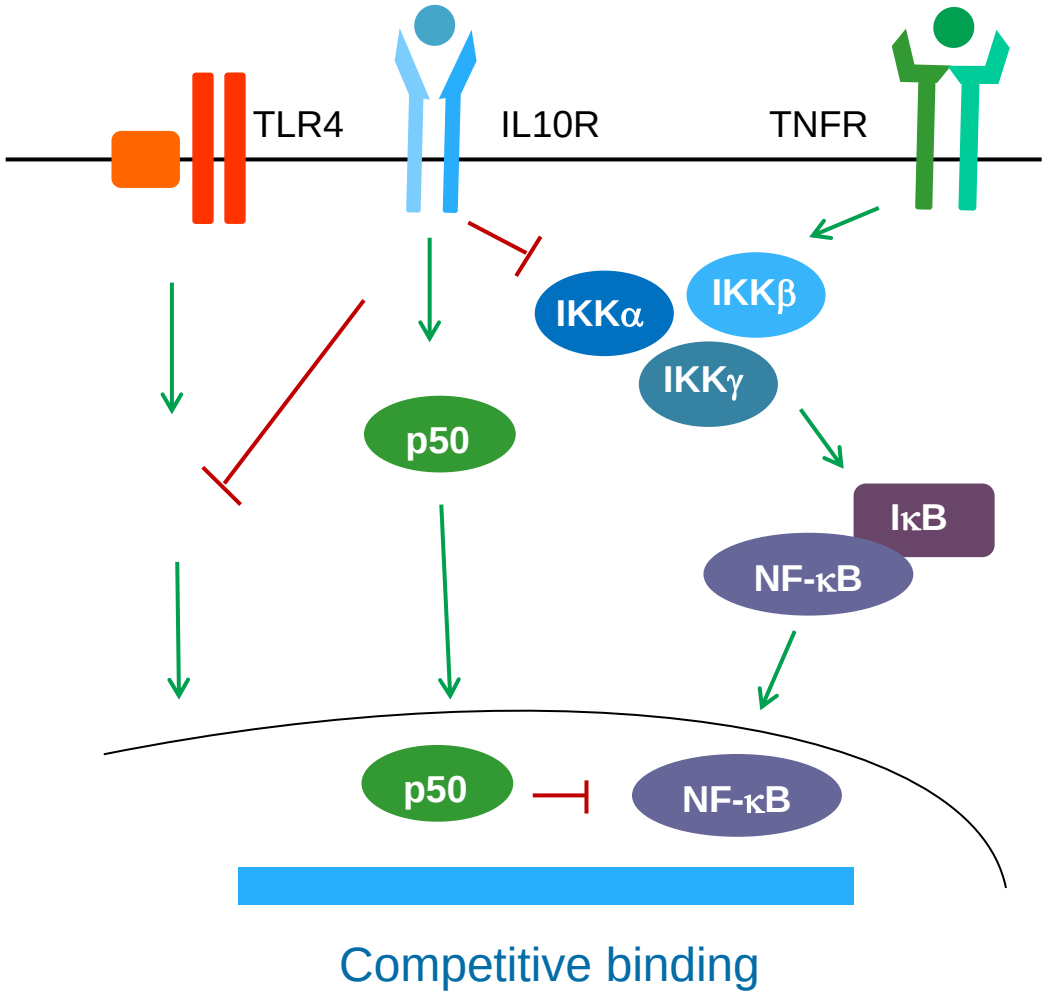
Interleukin 10

Produced by monocytes

Inhibits LPS induced production of pro-inflammatory cytokines

Inhibits NF-κB Nuclear translocation

Inhibits TNF-α activation of NF-κB pathway



T_H1 Immune Response

- CD4+ T Helper Cells
- Pro-inflammatory cytokines
- Intracellular parasitic infections
- Autoimmune response

Cytokines secreted by T_H1 T cells
IL-2
TNF-α
IFN-γ

T_H2 Immune Response

- Counteract T_H1 response
- IL4, IL5, IL-10, IL-13
- Produced by Macrophages
- Innate immune cells

T_H17 Immune Response

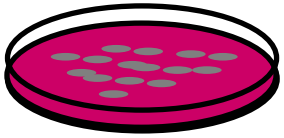
- Secreted by T helper lymphocytes (T_H17 cells)
- IL-17A, IL-17F, IL-22
- Promote the expression of pro-inflammatory cytokines & chemokines
- Autoimmune disease

Macrophages in T_H2 Response

In-vitro

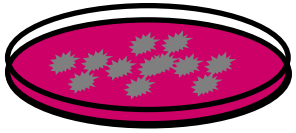
Bone marrow containing hematopoietic stem cells (HSCs) is flushed from mouse femur and collected

HSCs are cultured in the presence of M-CSF for 7 days and differentiated into macrophages



Hematopoietic stem cells

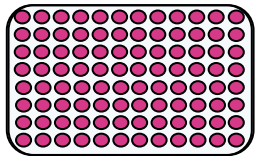
M-CSF



Macrophages

Treated with IL-33

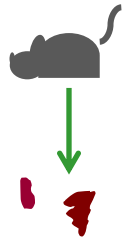
Macrophages treated with IL-33 for 6, 12, 24 ,48 and 72hrs: Supernatants are collected then subject to ELISA for IL-13



IL-13

In-vivo

Mice are treated i.p. (1µg) with IL-33 daily for 3 days



Spleen and small intestine are harvested and analyzed by qPCR for mRNA expression of T_H2 cytokines

Significant increase in mRNA levels IL-13, IL-9, CCL2, CCL17 & CCL24

Macrophage depletion decreased IL-13

Adoptive transfer significantly reduced nematode infection

Yang Z, Grinchuk V, Urban JF Jr, Bohl J, Sun R, Notari L, Yan S, Ramalingam T, Keegan AD, Wynn TA, Shea-Donohue T, Zhao A. Macrophages as IL-25/IL-33-Responsive Cells Play an Important Role in the Induction of Type 2 Immunity. PLoS One. 2013;8(3) e5944

Interferons

Type I	IFN-α, IFN-β IFN-τ, IFN-ω	IFNAR1 IFNAR2	Innate Immune cells	Dendritic Cell Activation / CD8 T cell stimulation
Type II	IFN-γ	IFNGR1 IFNGR2	CD4+, CD8+, NK cells	Macrophages / JAK-STAT pathway

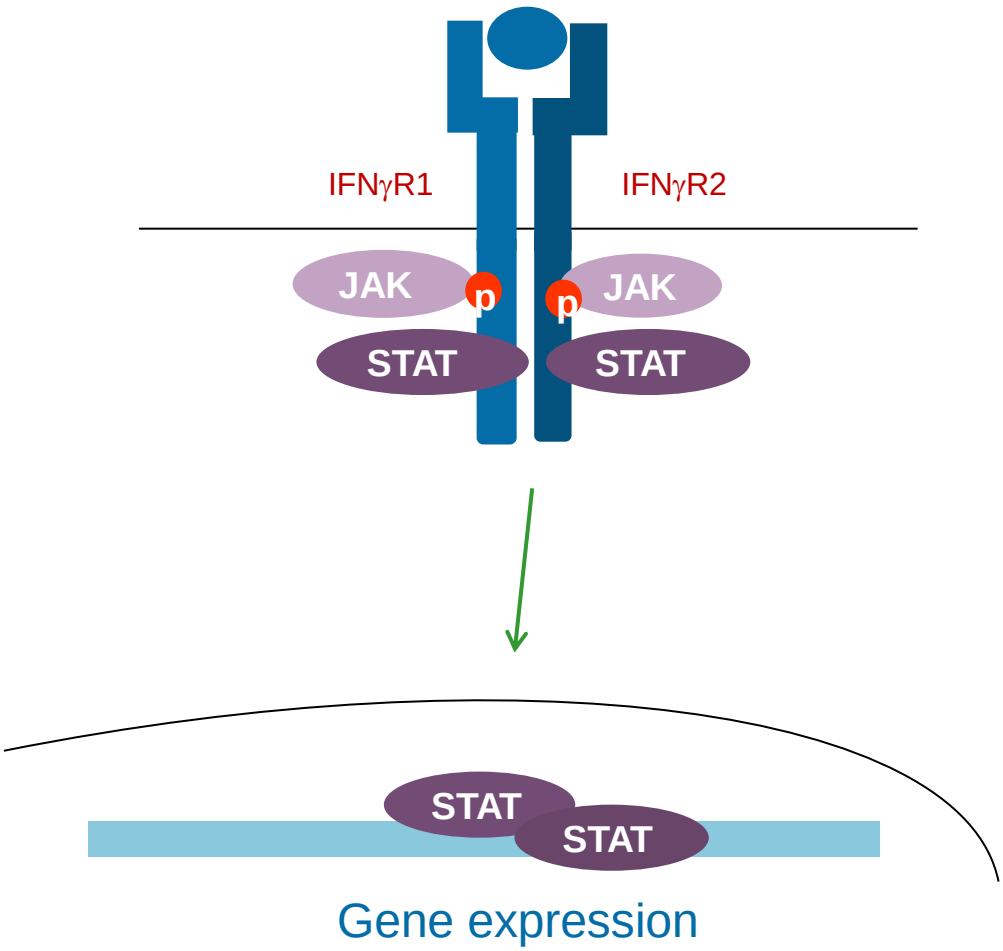
Interferon-γ Signaling

Produced by: NK cells, T cells

T-cells (low expression)

B-cells (intermediate expression)

Macrophages
Dendritic Cells
Neutrophils



Cytokines Involved in T_H17 Regulation and NK Cell Development

IL-2

Activate Tregs

Inhibits T_H17 regulation

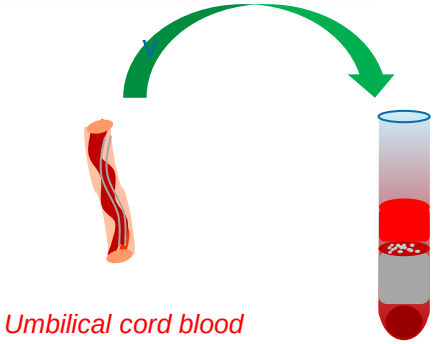
Inhibits $T_{FH}17$ differentiation

IL-15

NK Cell development

CD8 T-cell survival

Case Study: NK Cell Differentiation



Umbilical cord blood is collected

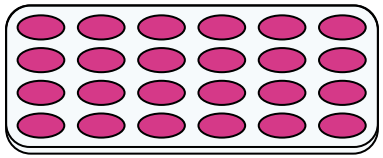
CD34+ isolation

CD34+ cells are isolated

Cells are added into the well of a multi-well plate and cultured under 1 of 4 different conditions

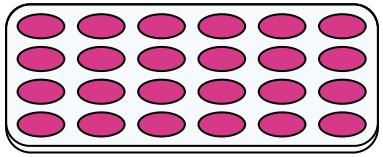
Conditions
1. Cytokines Alone
2. Cytokines + Feeder Cells
3. Cytokines + Hydrocortisone
4. Cytokines + Feeder Cells + HDC

\+ human AB serum
Ethanolamine
Ascorbic acid
Sodium selenite
βME
Pen/strep



IL-3, IL-7, IL-15, SCF, FLT-3L

Week 1



IL-7, IL-15, SCF, FLT-3L

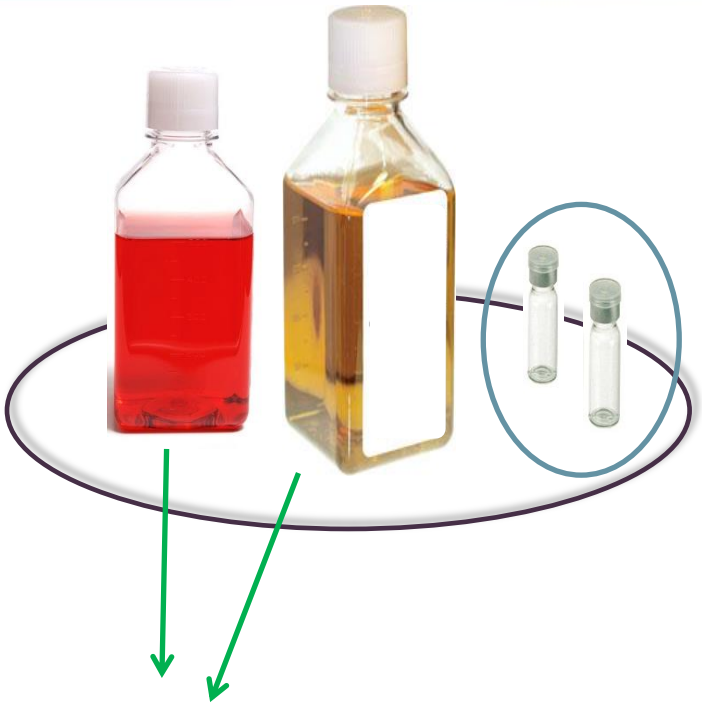
Week 2, 3, 4

Supplemented DMEM media used to culture and differentiate CD34+ cells into NK cells

Cell viability and NK cell differentiation determined after 2, 3 & 4 weeks: Differentiation is highest under condition 4

Flow chart adapted from : Grzywacz B, Kataria N, Kataria N, Blazar BR, Miller JS, Verneris MR. Natural killer-cell differentiation by myeloid progenitors. Blood. 2011; 117(13):3548-58

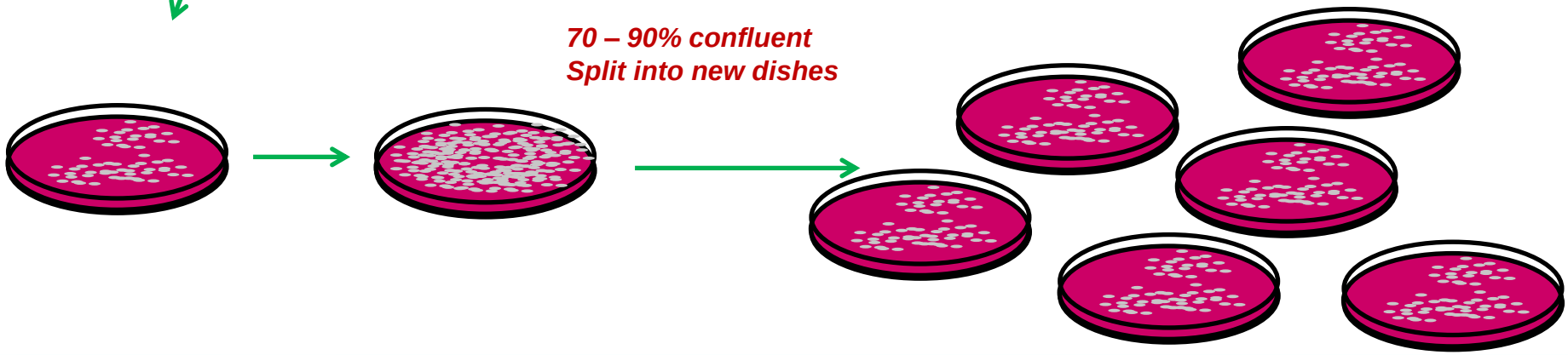
Cell Culture: Routine Expansion



Cancer Cells
EGF
IGF

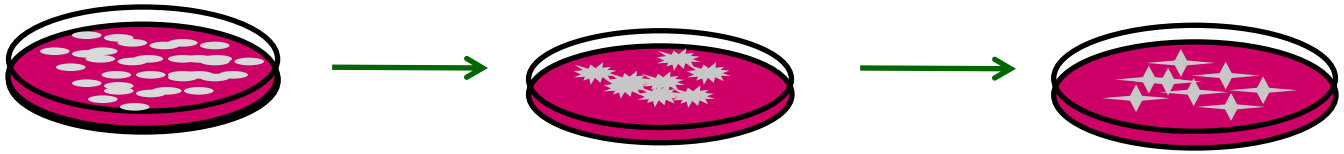
Immune Cells
GM-CSF
FLT-3
M-CSF
G-CSF

70 – 90% confluent
Split into new dishes

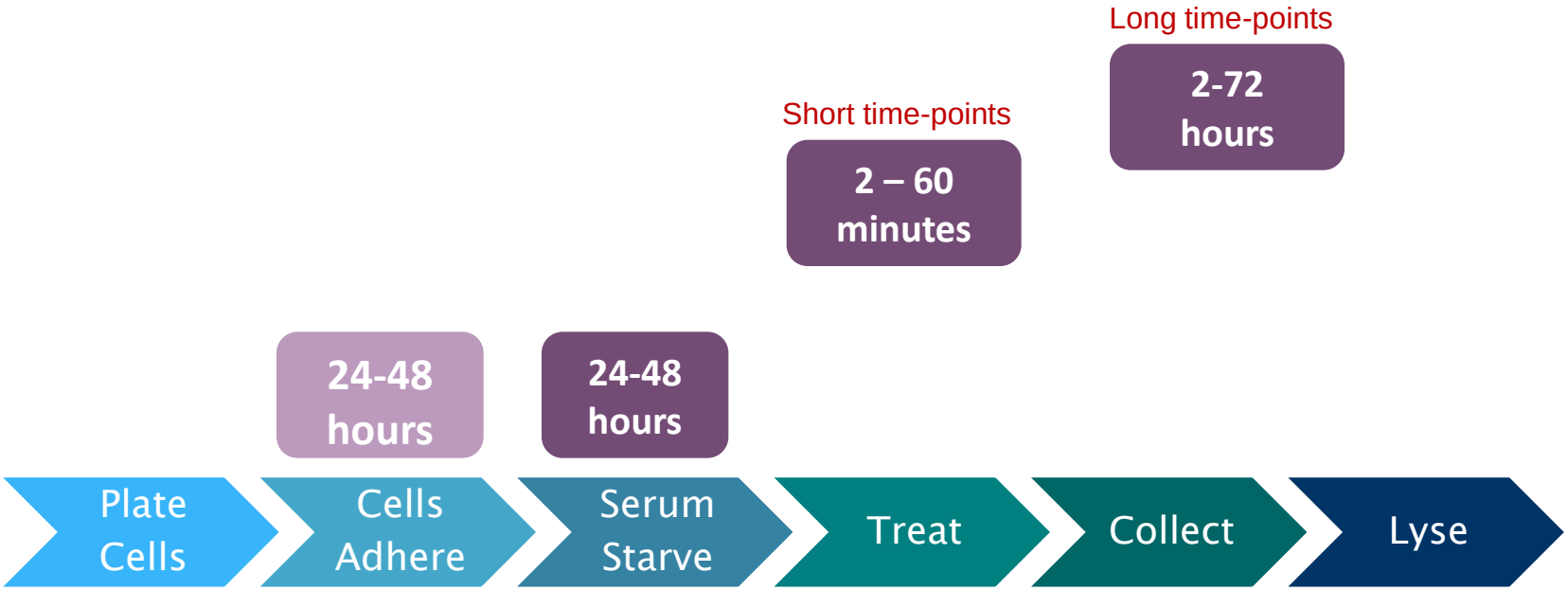


Cell Culture: Expansion and Differentiation

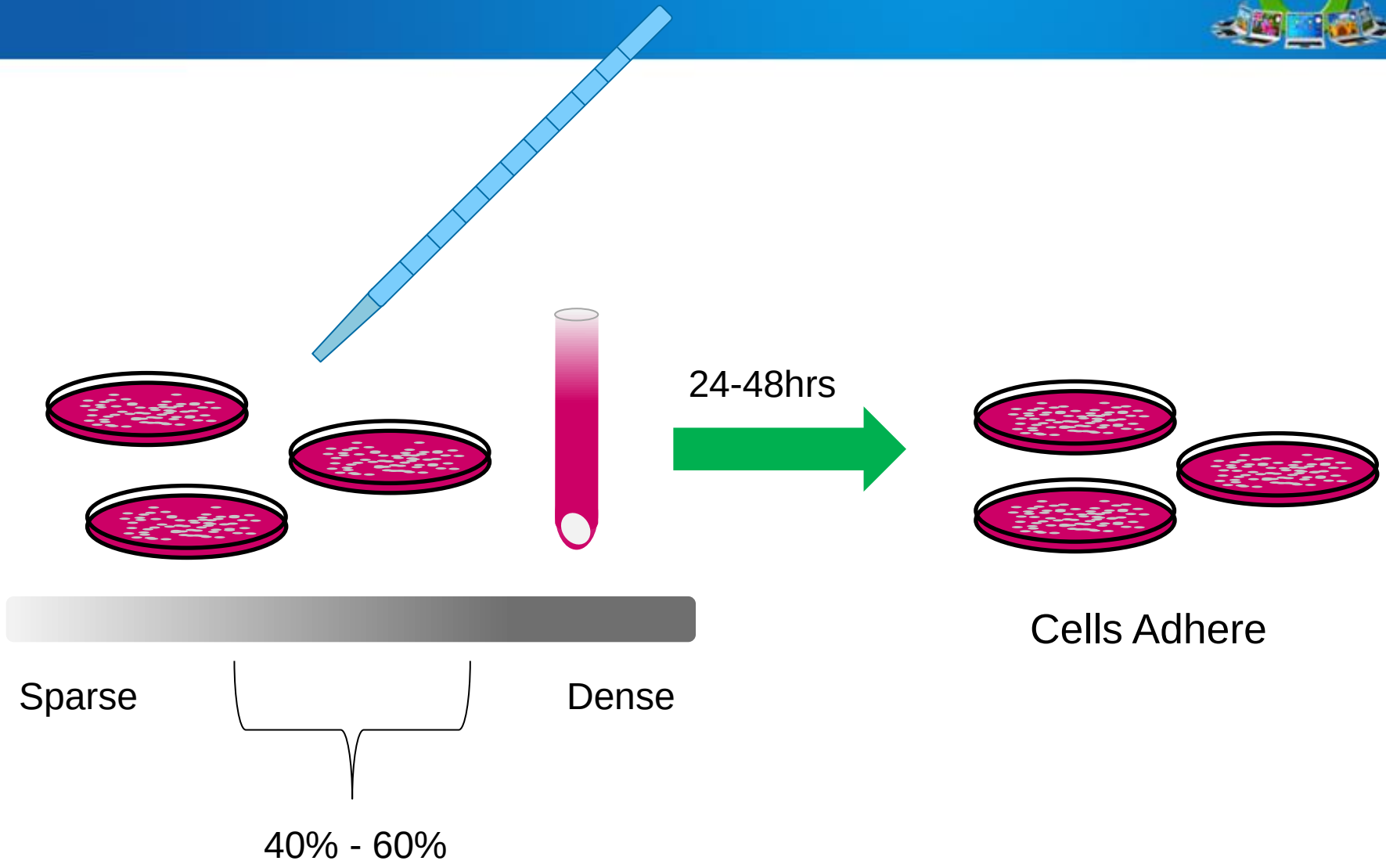
	7 days	5-7 days	2 days
	Expansion	Differentiation	Maturation
	SCF	GM-CSF	TNF-α
	TPO	IL-4	
	FLT-3 ligand		



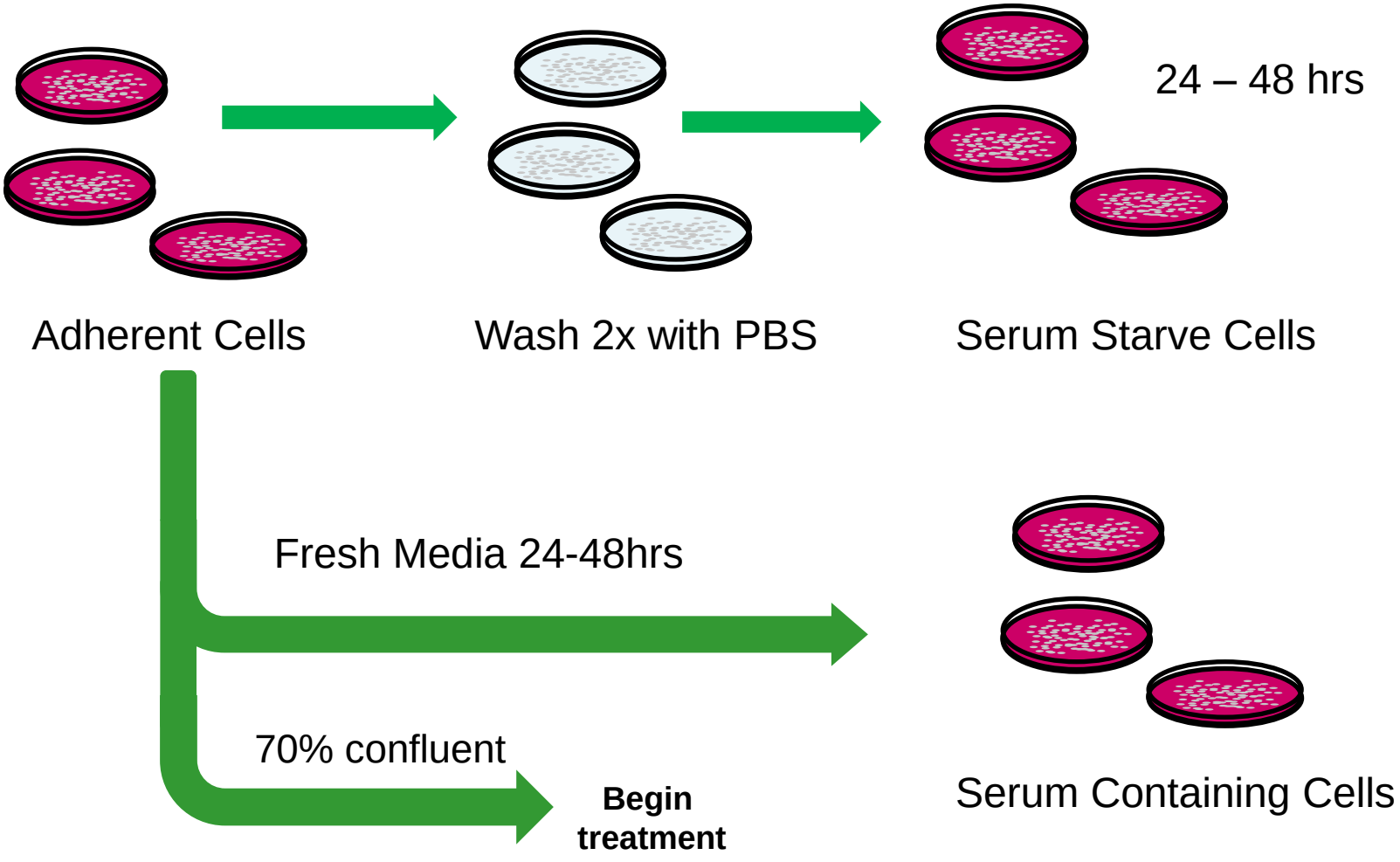
In-vitro Work Flow



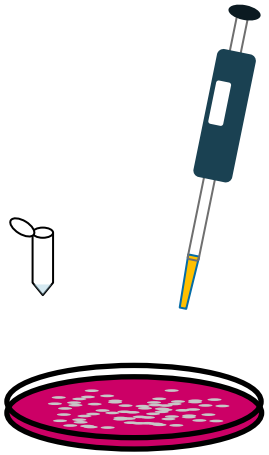
Preparing Cells for *in-vitro* Treatment



Serum Starve Cells *in-vitro* Prior to Treatment

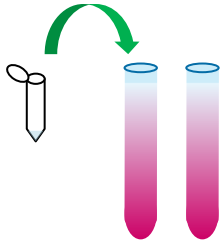


Cytokine *in-vitro* Treatment Techniques

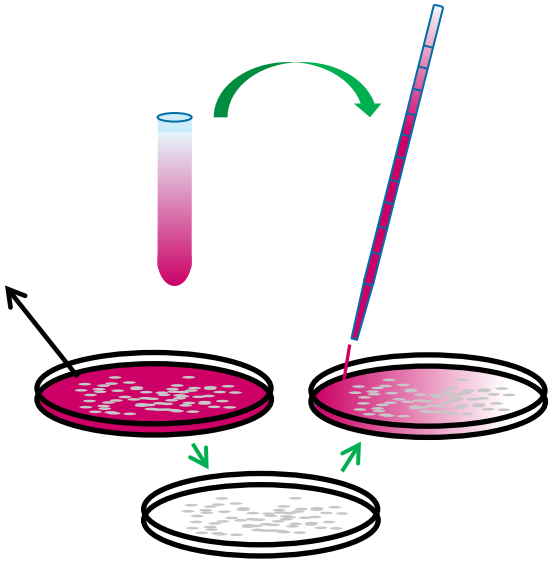


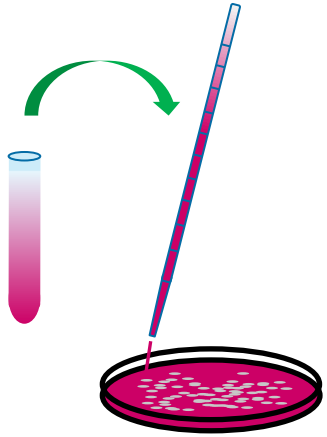
Potential for uneven treatment

Dilute in media



Assimilate 1hr





Factors that Can Affect Experimental Results

Variable	Issue	Solution
Endotoxin Level	Increase immune response False-positive Pyrogenic effect; off target activation	Use recombinant proteins with extremely low endotoxin levels
Purity	Affect immune response	Use recombinant proteins with a purity > 95%
Bioactivity	May affect the concentration needed for a desired result	Consider bioactivity when calculating concentration needed
Lot-to-lot consistency	Even small differences can affect results	Check data sheets Order in bulk

GenScript: Cytokines

Catalog #	Cytokine	Price		
Z01001 Z02918	TNF-α	Human Mouse	50μg -$95 20μg -$85	1mg - $625 1mg - $1200
Z02978 Z02988	IL-β	Human Mouse	50μg -$195 50μg -$249	1mg - $1750 1mg - $1680
Z03121 Z03161	IL-10	Human Mouse	10μg -$85 10μg -$85	1mg - $2100 1mg - $2100
Z02991 Z02765	IL-3	Human Mouse	50μg -$139 10μg -$175	1mg - $1200 1mg - $3275
Z03198 Z02772	IL-33	Human Mouse	50μg -$275 10μg -$159	1mg - $2100 1mg - $3600
Z03017 Z03178	IGF	Human Mouse	50μg -$75 50μg -$85	1mg - $125 1mg - $625
Z00333 Z02972	EGF	Human Mouse	50μg -$75 50μg -$25	1mg - $150 1mg - $125
Z02695 Z02979	GM-CSF	Human Mouse	50μg -$275 50μg -$275	1mg - $2100 1mg - $2100
Z02915 Z02916	IFN-γ	Human Mouse	100μg -$85 100μg -$85	1mg - $425 1mg - $425

Matthew.Riolo@GenScript.com

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Until the end of 2014

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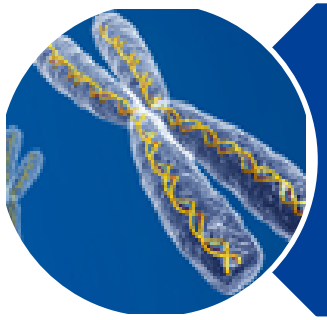
Biologics drug discovery: Steps to achieving an antibody drug candidate

October 22, 2014 8:00am

Presented by Frank Fan Ph.D.



Codon optimization: Why & how to design DNA sequences for optimal soluble protein expression

October 29, 2014 8:00am and 2:00pm

Presented by Rachel Speer Ph.D.