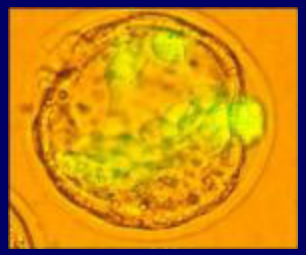
Validation of Gene Expression Signatures Employed in Directed Stem Cell Differentiation with Small Molecule Perturbagens

Charisma N. Garcia
Cancer Genomics
Summer Undergraduate Research Program

BROAD INSTITUTE



What is a stem cell?

- Potential to differentiate into numerous cell types
- Capacity to self-replicate indefinitely
- When in culture, can remain in an undifferentiated state for many generations

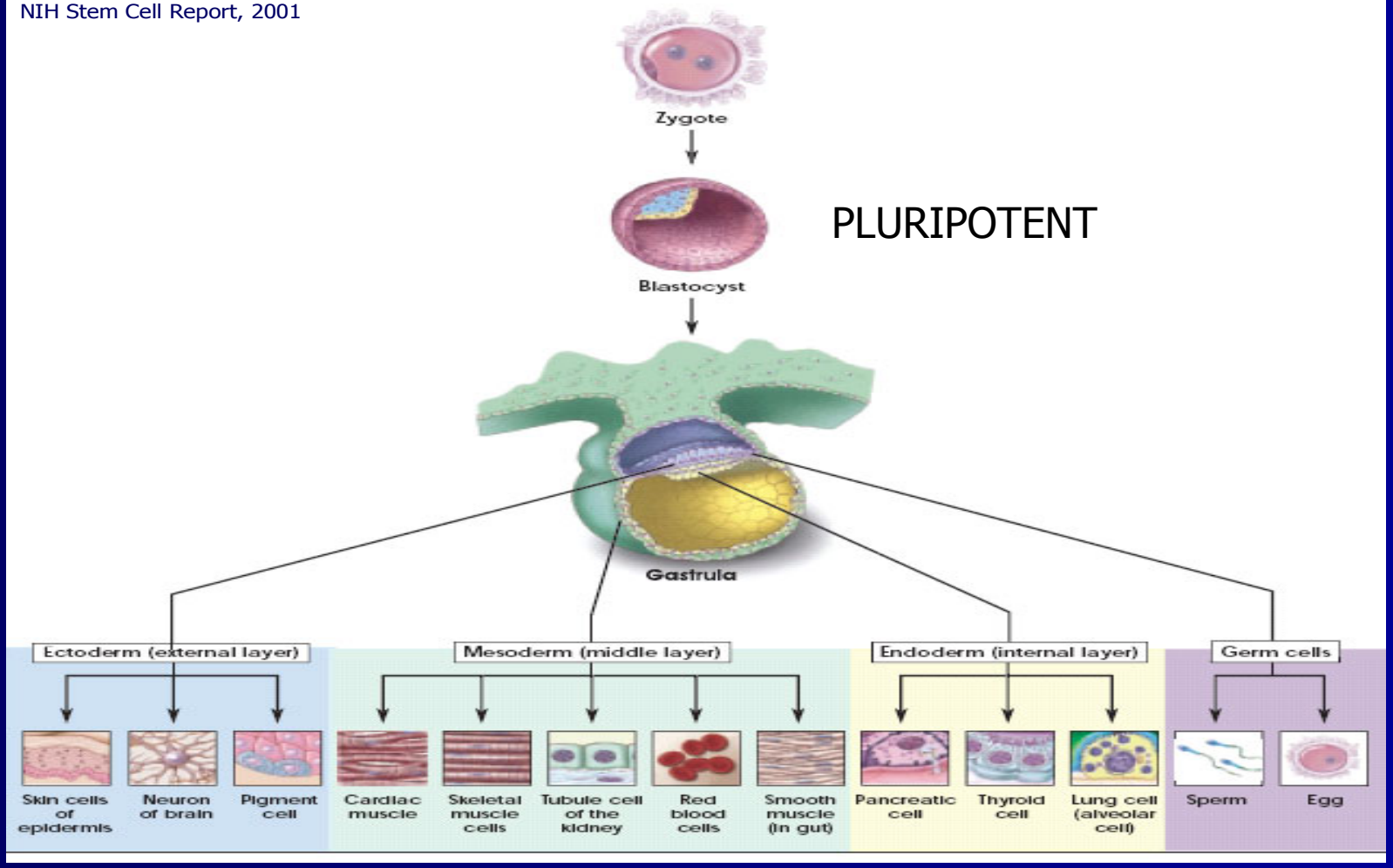
Embryonic stem cells


NIH stem cell report, 2001

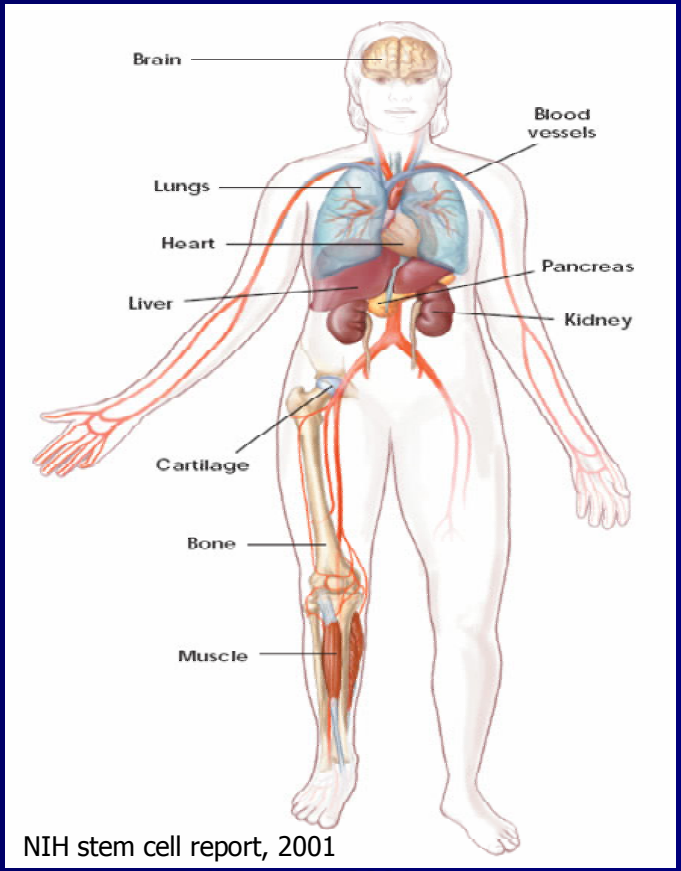
- Source: Inner cell mass of the pre-implantation blastocyst
  - Blastocyst: 4-5 day embryo

Embryonic stem cells

NIH Stem Cell Report, 2001



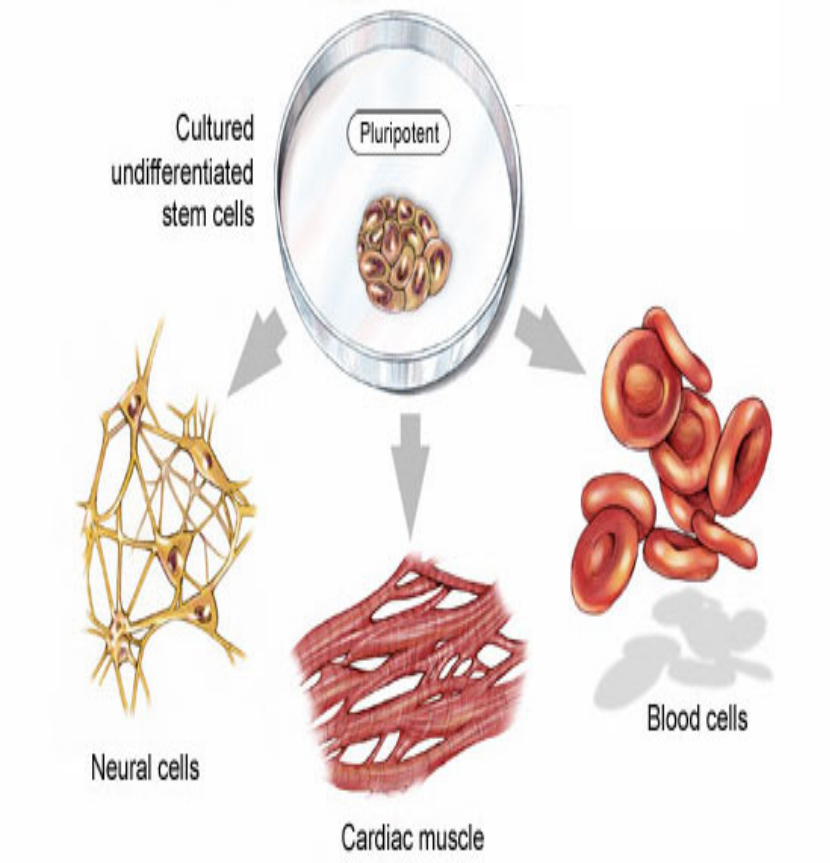
Potential stem cell applications



NIH stem cell report, 2001

- Monitoring developmental biology
- Genetic engineering
- Pharmaceutical testing
- Toxicology
- Therapeutic transplants
  - Chronic heart disease
  - End-stage kidney disease
  - Liver failure
  - Cancer
  - Parkinson's disease
  - Spinal cord injury
  - Multiple sclerosis
  - Alzheimer's disease
  - Amyotrophic lateral sclerosis
  - Diabetes
  - Skin grafts
  - Purkinje cell degeneration
  - Duchenne's muscular dystrophy
  - Osteogenesis imperfecta

Directed differentiation



http://www.stemcellresearchfoundation.org/index.htm

Using laboratory techniques:

Unspecialized cell

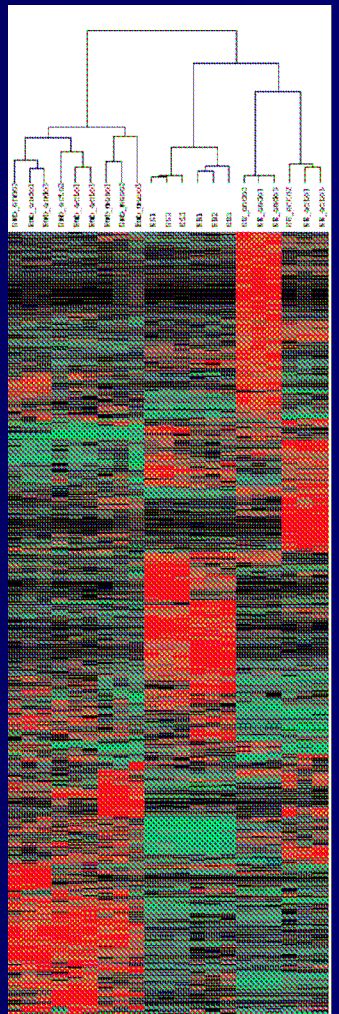
Small molecule perturbagen

Specialized cell

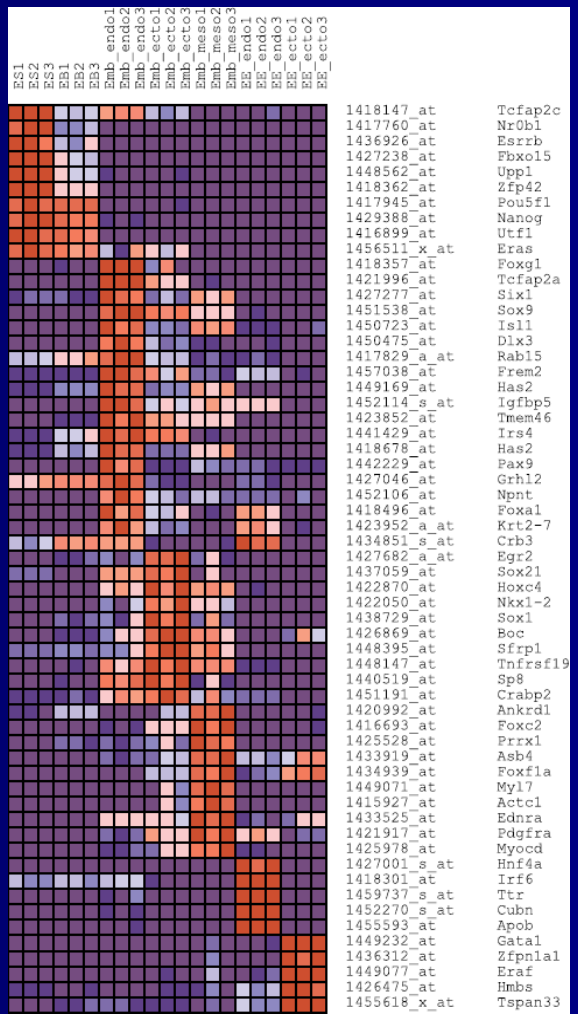
[determined by gene expression signatures, i.e. expression of marker genes]

Microarray Gene Expression Profiling of Embryonic Stem Cells and Dissected Embryonic Tissues for Marker Selection

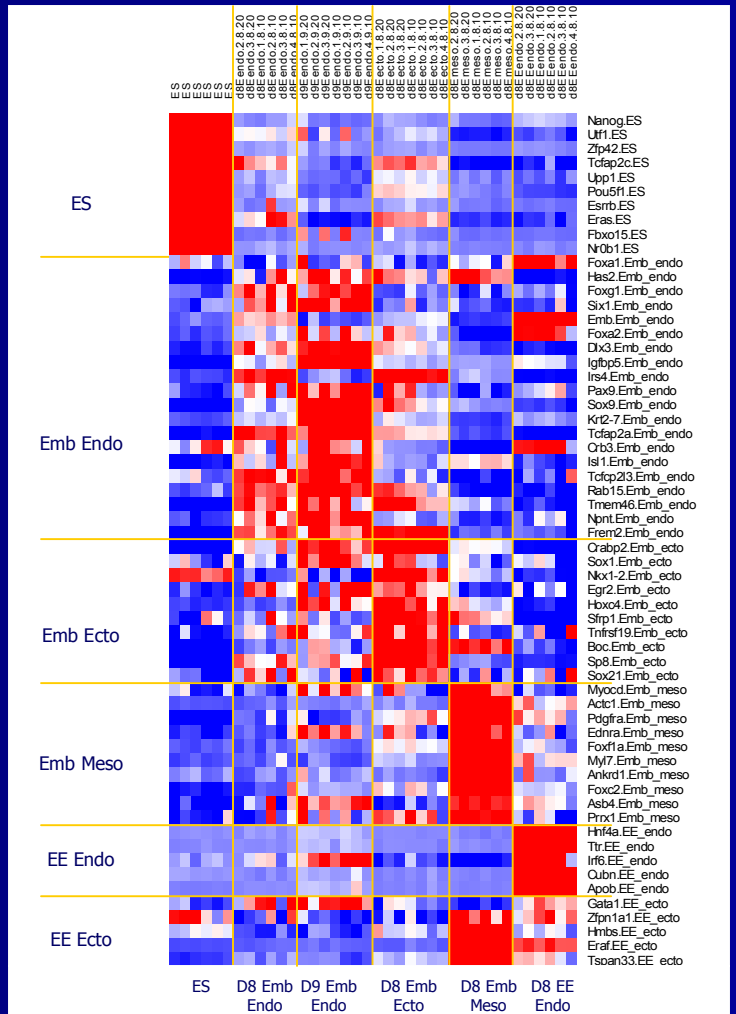
Microarrays



Microarrays



GE-HTS



Gene Expression Signatures

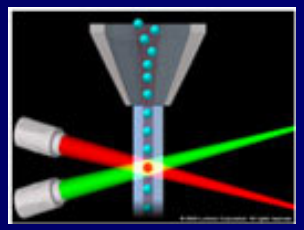
Embryonic Stem cell	Embryonic Endoderm	Embryonic Ectoderm	Embryonic Mesoderm	Extra-embryonic Endoderm	Extra-embryonic Ectoderm	Housekeeping Genes
Tcfap2c	Foxg1	Egr2	Ankrd1	Hnf4a	Tspan33	Gapdh
Nr0b1	Tcfap2a	Sox21	Foxc2	Ttr	Hmbs	Actb
Esrrb	Six1	Hoxc4	Prrx1	Irf6	Eraf	Pgk1
Fbxo15	Sox9	Nkx1-2	Asb4	Cubn	Gata1	Ldh1
Uppl	Is11	Sox 1	Myl7	Apob	Zfpn1a1	
Zfp42	Dlx3	Boc	Actc1			
Pou5f1	Rab15	Sfrp1	Ednra			
Nanog	Frem2	Tnfrsf19	Pdgfra			
Utf1	Has2	Sp8	Myocd			
Eras	Igfbp5	Crabp2	Fox1a			
	Tmem46					
	Irs4					
	Pax9					
	Npnt					
	Foxa1					
	Krt2-7					
	Emb					
	Tcfcp2l3					
	Foxa2					

Project Overview

GOAL: Validate gene signatures in tissue culture

1. Start and maintain a mouse embryonic stem cell (mES) line

2. Treat the mES with the chosen compounds, at various dilutions, for 4 days



3. Determine if differentiation occurred by comparing gene expression signatures

Treatments to be tested for differentiation

Treatment	Hypothesized Target Cell	Germ Layer
All-trans-Retinoic Acid	Neurons	Ectoderm
5-Azacytidine	Cardiomyocytes	Mesoderm
Fluoxetine Hydrochloride	Neurons	Ectoderm
Mitomycin C	Dopamine Neurons	Ectoderm
Reversine	Myocytes	Mesoderm
Scriptaid	Induces hemoglobin	Mesoderm

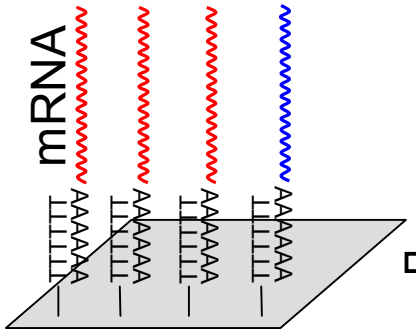
DILUTIONS:
6.4 μM
3.2 μM
1.6 μM
0.8 μM
0.4 μM
0.2 μM
0.1 μM
0.05 μM

96 Well Layout

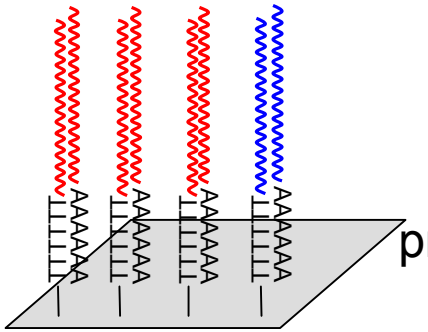
	1	2	3	4	5	6	7	8	9	10	11	12
A	DMSO	LIF	ATRA 0.05 µM	5AZA 0.05 µM	*	FLUOX 0.05 µM	MITOC 0.05 µM	*	REVERS 0.05 µM	SCRIPT 0.05 µM	DMSO	LIF
B	DMSO	LIF	ATRA 0.1 µM	5AZA 0.1 µM	*	FLUOX 0.1 µM	MITOC 0.1 µM	*	REVERS 0.1 µM	SCRIPT 0.1 µM	DMSO	LIF
C	DMSO	LIF	ATRA 0.2 µM	5AZA 0.2 µM	*	FLUOX 0.2 µM	MITOC 0.2 µM	*	REVERS 0.2 µM	SCRIPT 0.2 µM	DMSO	LIF
D	DMSO	LIF	ATRA 0.4 µM	5AZA 0.4 µM	*	FLUOX 0.4 µM	MITOC 0.4 µM	*	REVERS 0.4 µM	SCRIPT 0.4 µM	DMSO	LIF
E	DMSO	LIF	ATRA 0.8 µM	5AZA 0.8 µM	*	FLUOX 0.8 µM	MITOC 0.8 µM	*	REVERS 0.8 µM	SCRIPT 0.8 µM	DMSO	LIF
F	DMSO	LIF	ATRA 1.6 µM	5AZA 1.6 µM	*	FLUOX 1.6 µM	MITOC 1.6 µM	*	REVERS 1.6 µM	SCRIPT 1.6 µM	DMSO	LIF
G	DMSO	LIF	ATRA 3.2 µM	5AZA 3.2 µM	*	FLUOX 3.2 µM	MITOC 3.2 µM	*	REVERS 3.2 µM	SCRIPT 3.2 µM	DMSO	LIF
H	DMSO	LIF	ATRA 6.4 µM	5AZA 6.4 µM	*	FLUOX 6.4 µM	MITOC 6.4 µM	*	REVERS 6.4 µM	SCRIPT 6.4 µM	DMSO	LIF

Ligation-mediated amplification (LMA) & Gene expression-based highthroughput (GE-HTS) experimental overview

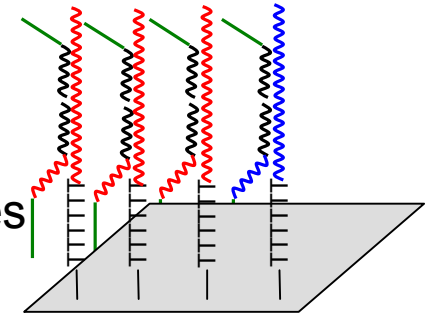
Treated cells



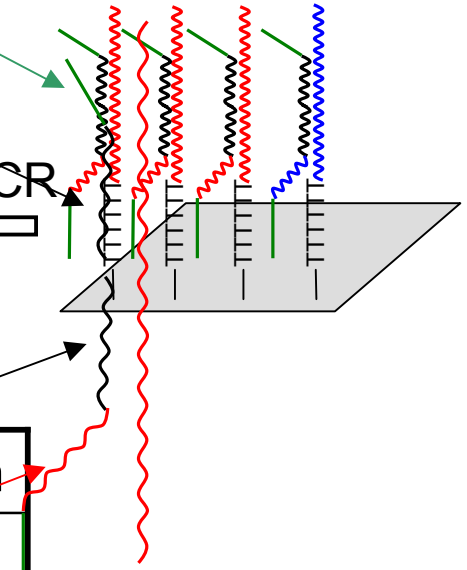
RT



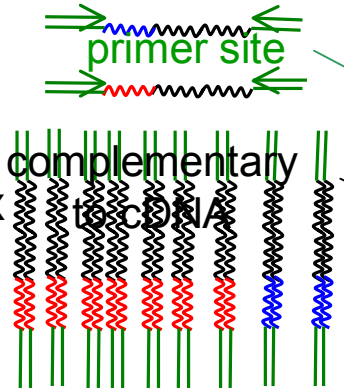
probes



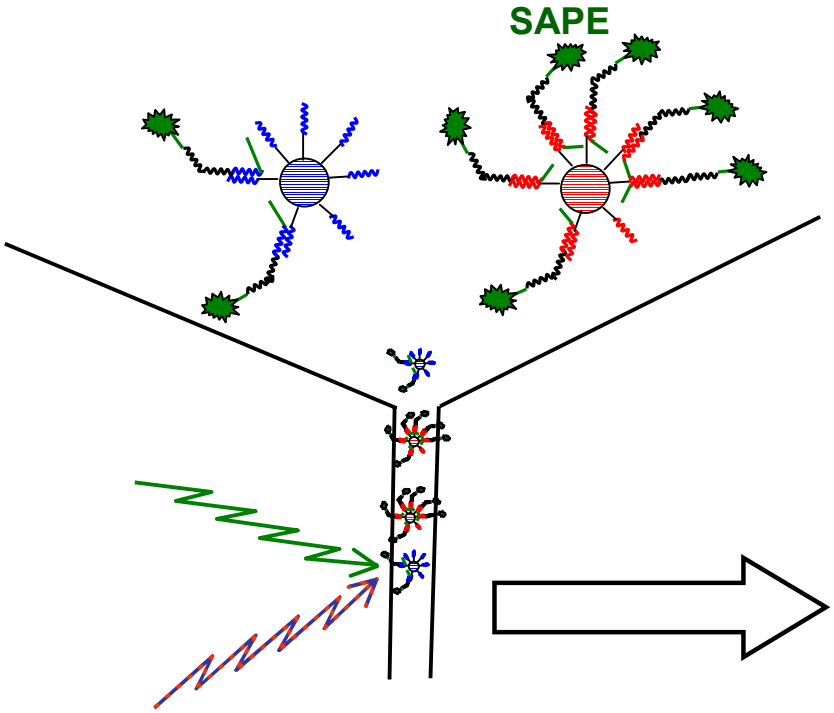
ligation



PCR



Luminex



Marker	Expression
1 probe specific tag	3
2 primer site	1

Courtesy of Cristian Jitianu

Acknowledgements:

- Todd Golub
- Cristian Jitianu

- Angela Brunache
- Bruce Birren