Molecular classification of breast cancer using Quantigene Plex 2.0 assay

Principal Investigator: Dr. Godfrey Grech

Presenter: Mr Shawn Baldacchino

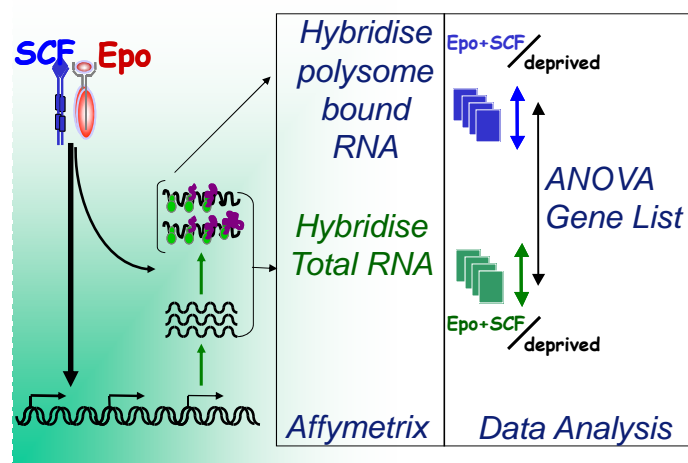# Molecular classification of breast cancer using Quantigene Plex 2.0 assay

- Background
- Introduction to current research objectives
- Project overview and Infrastructure
- Quantigene 2.0
- Criteria for Biomarker Selection
- Results – Classification of Breast cancer and biomarker discovery

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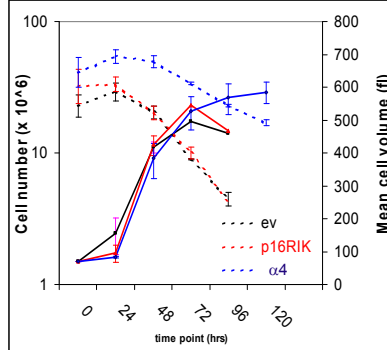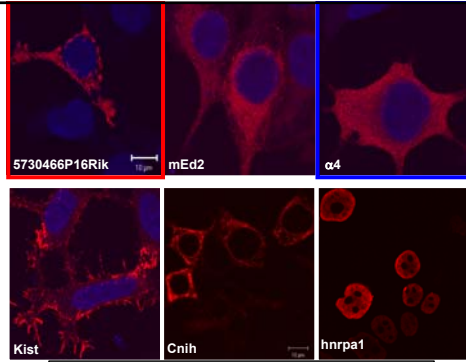

Background

Novel list of transcripts that are recruited to polysomes by Growth Factor Signaling

Erasmus MC
University Medical Center Rotterdam

Background
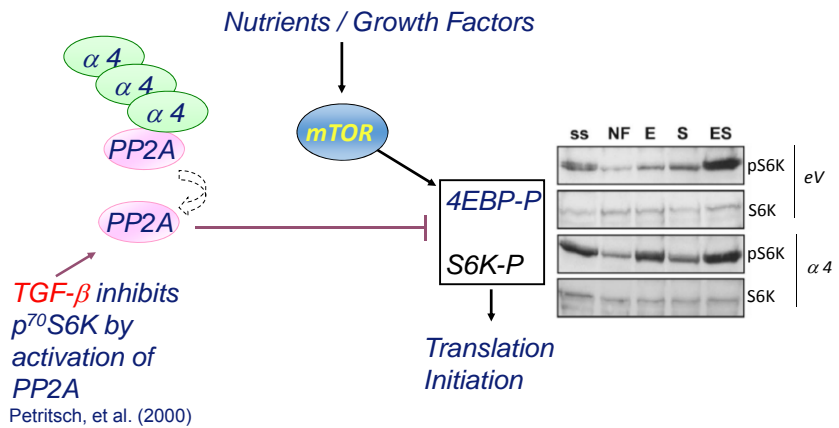
Constitutive expression of α4 blocks differentiation in the erythroid model.

Background

TGF-β rescues block of differentiation.

FTY720 activates pp2a resulting in the release of differentiation block by Stem Cell Factor.

Nutrients / Growth Factors

mTOR

4EBP-P

S6K-P

TGF-β inhibits p^{70}S6K by activation of PP2A
Petritsch, et al. (2000)

Translation Initiation

Grech, G., et al. (**2008**)
Blood Oct 1;112(7):2750-60.

Suppressed PP2A activity maintains mTOR signals resulting in enhanced proliferation and block of differentiation.

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PP2A Mechanism – Breast Cancer
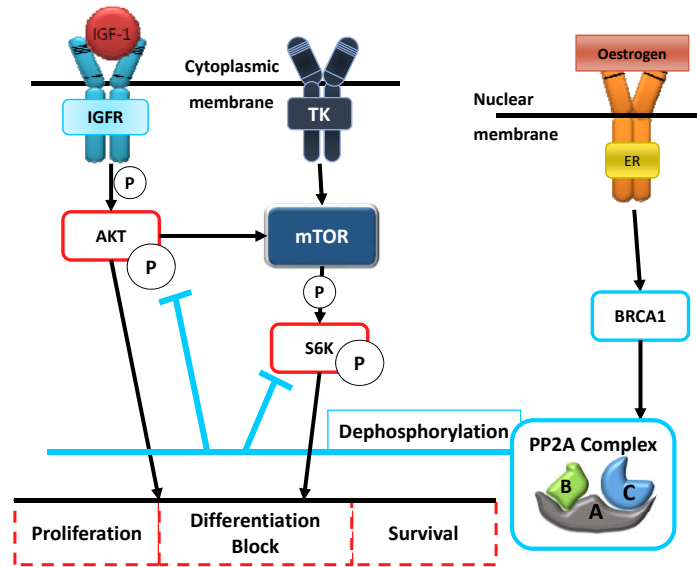
PP2A is a versatile complex.

Binding subunits regulate target specificity and activity.
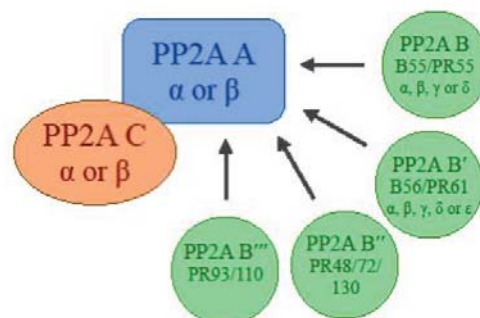
PP2A Mechanism – Breast Cancer

High p-AKT is a common event in Triple Negative breast cancer.

Activation of the PI3K/AKT can be blocked by the PP2A activator,FTY720 (Borg et al., 2014)

Nigel Borg, Shawn Baldacchino, Christian Saliba, Sharon Falzon, James DeGaetano, Christian Scerri, Godfrey Grech: Phospho-Akt expression is high in a subset of Triple Negative breast Cancer Patients. Xjenza. 03/2014
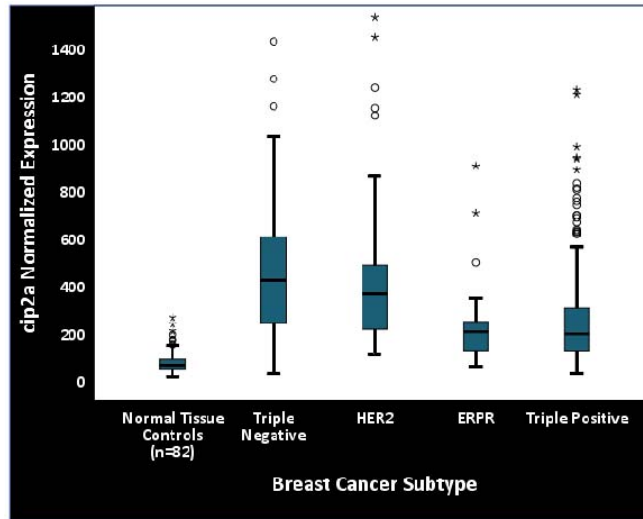

PP2A Mechanism – Breast Cancer

PP2A inhibitory subunits, a potential list of cancer biomarkers.
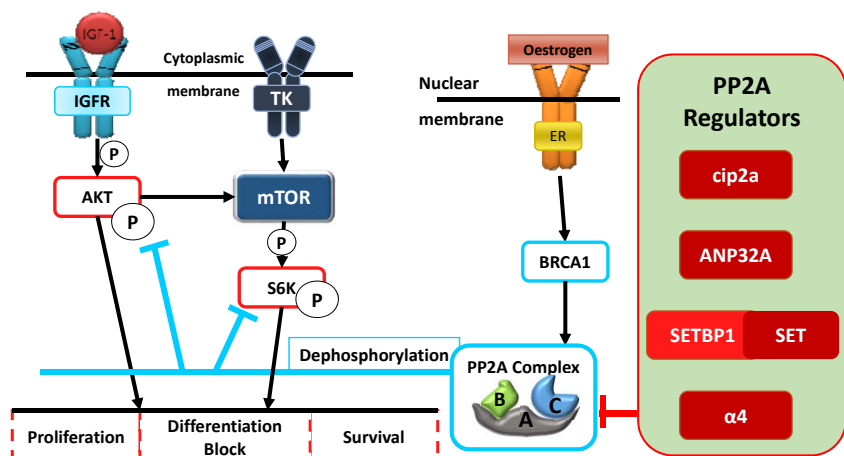
PP2A deregulation is a common event

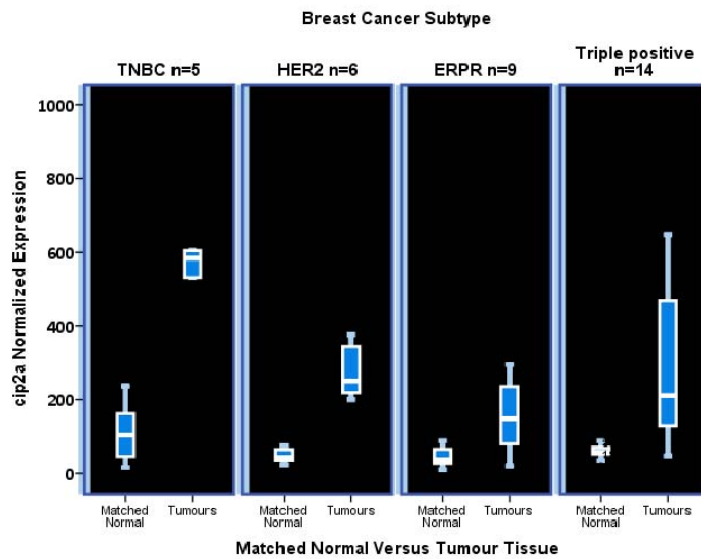CIP2A expression is upregulated in TNBC and Her2-enriched samples.

PP2A deregulation is a common event

High expression of Cip2a in tumour tissue when compared to matched normal.

PP2A deregulation is a common event

PP2A Deregulation Criteria	Triple Negative (N=84)	ERPR (N=308)	HER2 (N=26)	Triple Positive (N=59)	Total (N=477)
PPP2CA HOMDEL EXP <-2	**17%**	2%	4%	0%	4%
PPP2CB HOMDEL EXP <-2	24%	16%	31%	19%	19%
PPP2R2A HOMDEL EXP <-2	24%	15%	46%	19%	18%
PPP2R2B HOMDEL EXP <-2	1%	0%	0%	0%	0%
CIP2A AMP EXP>2	**18%**	5%	12%	10%	8%
SETBP1 AMP EXP>2	2%	7%	8%	2%	6%
SET AMP EXP>2	**15%**	5%	**31%**	2%	8%
α4 AMP EXP>2	2%	6%	4%	2%	5%
Total PP2A deregulation	**67%**	38%	**74%**	36%	45%

cBioPortal
for Cancer Genomics

The Cancer Genome Atlas

Understanding genomics to improve cancer care

Baldacchino S, Saliba C, Petroni V, Fenech AG, Borg N, Grech G: Deregulation of the phosphatase, PP2A is a common event in breast cancer, predicting sensitivity to FTY720. The EPMA Journal 2014 Jan 25; 5(1):3


Aims and Relevance of Study

Generate a predictive gene set to classify patients with low PP2A activity

Define actionable biomarkers providing knowledge on the application of PP2A activators in specific therapeutic groups.

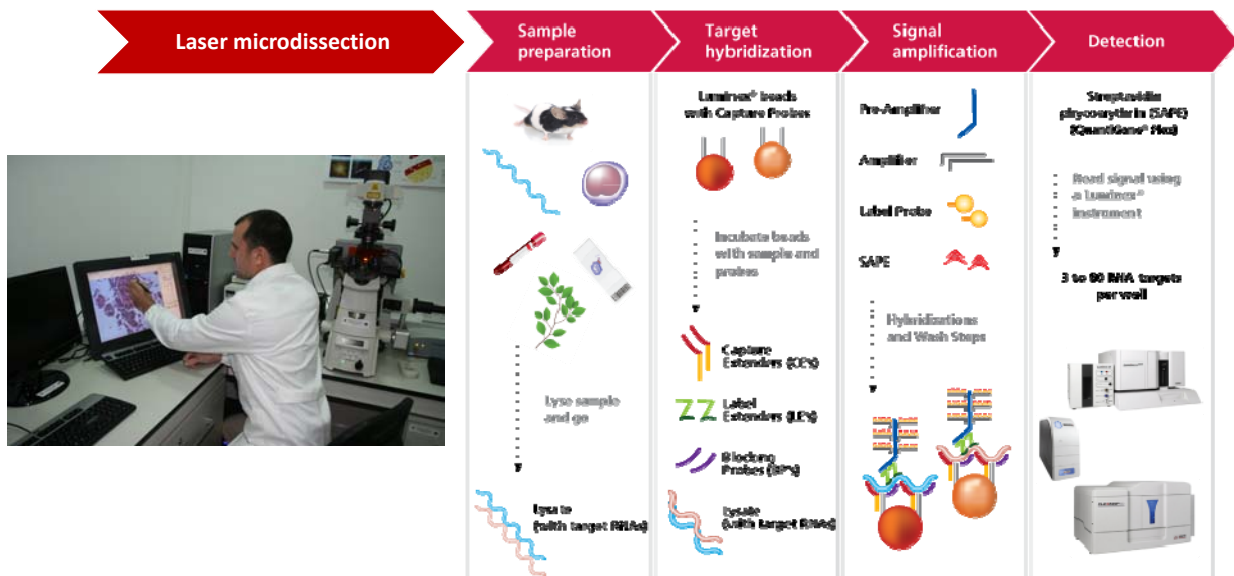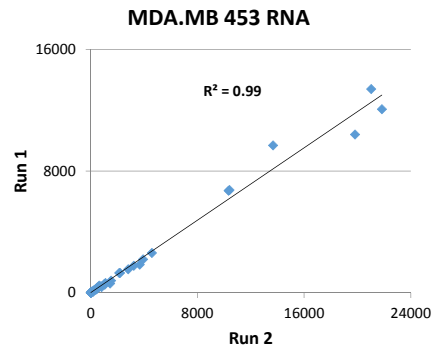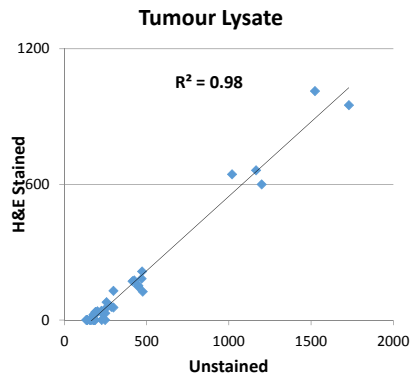# Molecular classification of breast cancer using Quantigene Plex 2.0 assay

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Project Outline
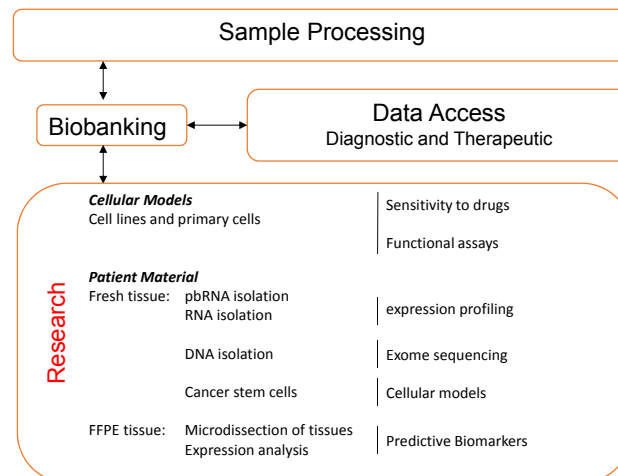
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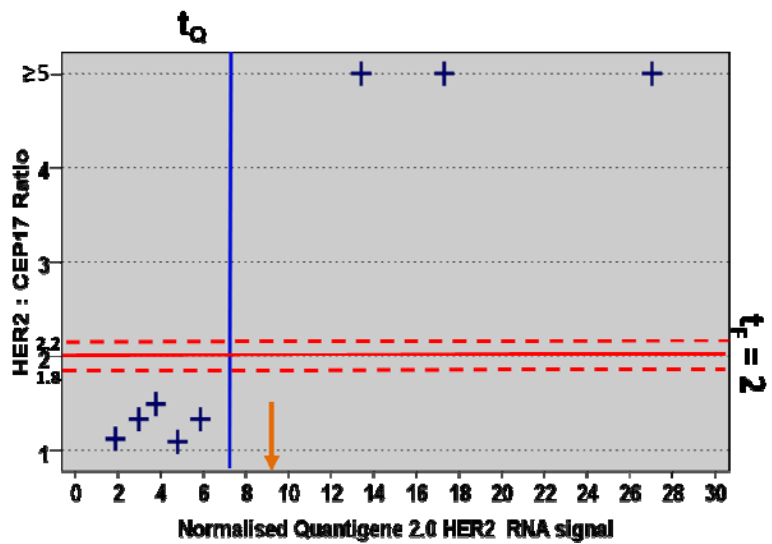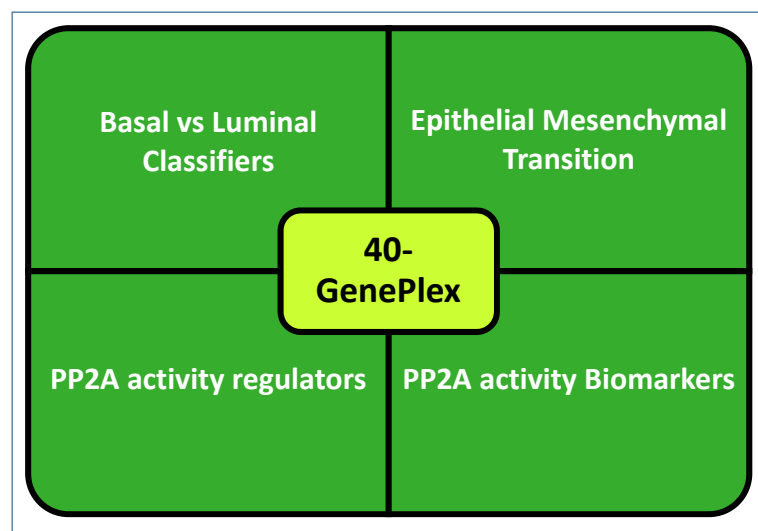
Quantigene 2.0 - Affymetrix Technology

Quality Control

No significant difference between replicates
And no significant effect when stained or unstained

MDA.MB 453 RNA

Tumour Lysate

Quality Control

Quantigene 2.0 expression
data for HER2 classified HER2
positive patient by FISH or
IHC correctly – to change

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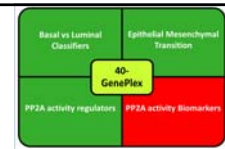- Results – Classification of Breast cancer and biomarker discovery

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Biomarker selection

A panel of genes was selected to identify:
- Molecular classification
- Epithelial or Mesenchymal phenotype (EMT)
- PP2A deregulation
- Potential biomarkers for PP2A activity

| Basal vs Luminal Classifiers	Epithelial Mesenchymal Transition
40-GenePlex	
PP2A activity regulators	PP2A activity Biomarkers

PP2A Activity Biomarker Selection

1. List of Genes of Interest

2. Use criteria to group patients into suppressed or normal PP2A activity

3. Selection of five (5) biomarker genes associated with low PP2A activity

PP2A Activity Biomarker Selection
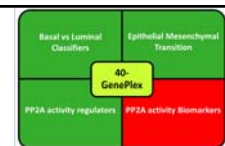
1. **List of Genes of Interest**

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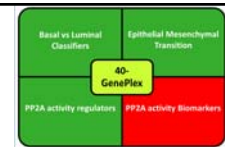## PP2A Activity Biomarker Selection
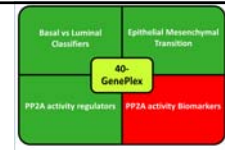
Used publicly available gene sets from genome-wide expression patterns that:

1. predict clinical outcome or survival in breast cancer
2. define Molecular and Novel tumour subclasses
3. persist in distant tumour metastasis
4. are differentially expressed in Breast Cancer
5. cip2a expression signature in breast cancer
6. proteins interacting with PP2A complex

1) (Van 't Veer et al., 2001)
   (Sotiriou et al., 2003)
   (Cobleigh et al., 2005)
   (Paik et al., 2007)
   (Lyng et al., 2013)

2) (Hedenfalk et al., 2001)
   (Sotiriou et al., 2003)
   (Ma et al., 2003)
   (Sotiriou et al., 2006)

3) (Wang et al., 2005)

4) (Zhang et al., 2013)
   (Ma et al., 2003)

5) (Niemela et al., 2012)
   Ingenuity, IPA
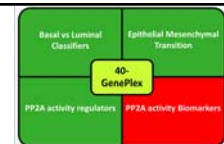

PP2A Activity Biomarker Selection

1. List of Genes of Interest

2. **Use criteria to group patients into suppressed or normal PP2A activity**

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PP2A Activity Biomarker Selection
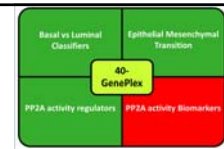
Criteria defining PP2A Deregulation	
PPP2CA	
PPP2CB	HOMOZYGOUS DELETION OR
PPP2R2A	EXPRESSION < -2 RNASeq (z-score)
PPP2R2B	
CIP2A	
SETBP1	AMPLIFICATION OR
SET	EXPRESSION > 2 RNASeq (z-score)
α4	

PP2A Activity Biomarker Selection

1. List of Genes of Interest

2. Use criteria to group patients into suppressed or normal PP2A activity

3. **Selection of five (5) biomarker genes associated with low PP2A activity**

   Prioritisation based on:

   a. Ingenuity Pathway Analysis (IPA)
   b. Function and implications in tumours (Literature)
   c. Possible relation with the PP2A pathway

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Quantigene 2.0 Results – Dataset and Data Analysis

Dataset
Annotated cell lines
(Quantigene results)
[N=10]

Patient cases (FFPE)
(Quantigene results)
[N=44]

TCGA annotated dataset
(RNASeq data)
[N=835 Tumours and N=82
Normal]

Statistical Analysis
z-score weighting
Principal Component Analysis (PCA)

Algorithm Training
Decision Tree
Random Forests
Rule Model
Support Vector Machine (SVM)
Neural Networks

rapidminer

Quantigene 2.0 - Data Processing
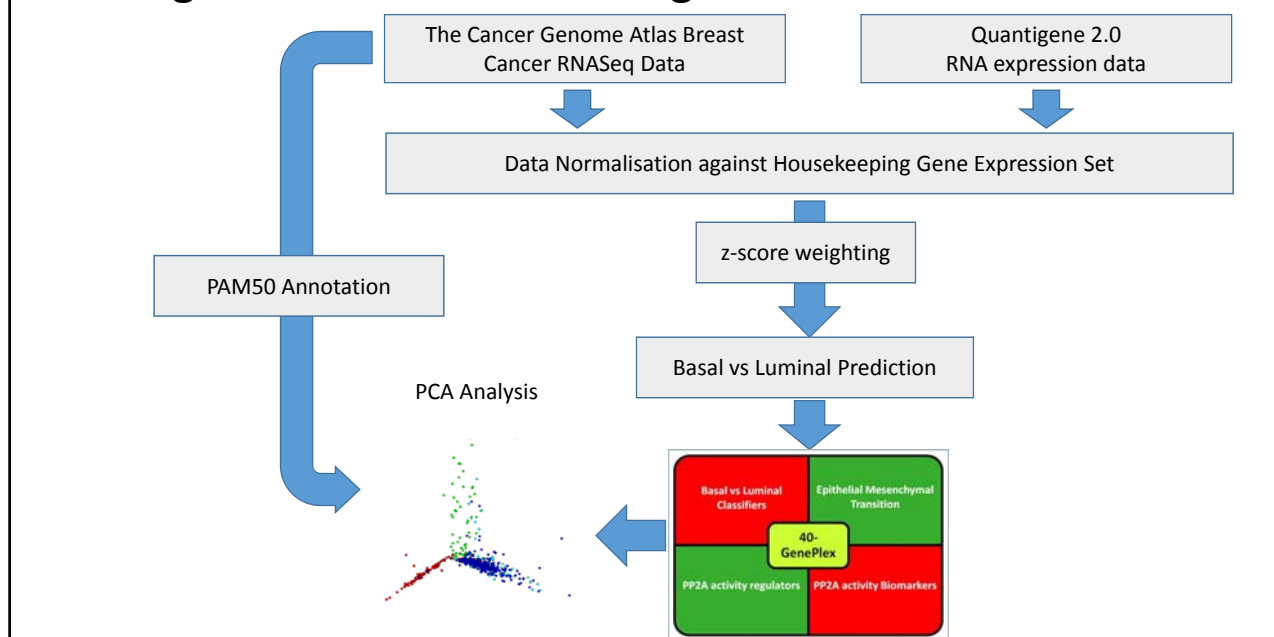
Quantigene 2.0 Results – Dataset and Data Analysis

Analysis:
Principal Component Analysis
(PCA) using the
Basal vs Luminal Classifier gene
set and ERBB2.

Sample:
TCGA patients annotated with
PAM50 annotation
N = 520

98.2% overlap between
molecular classification of
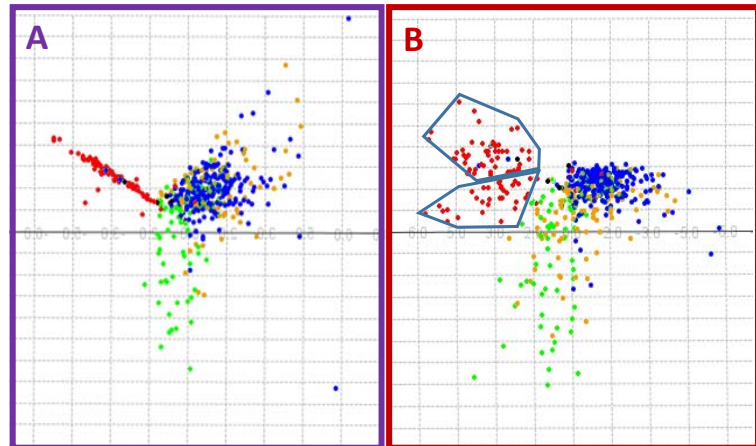Quantigene 2.0 and PAM50.
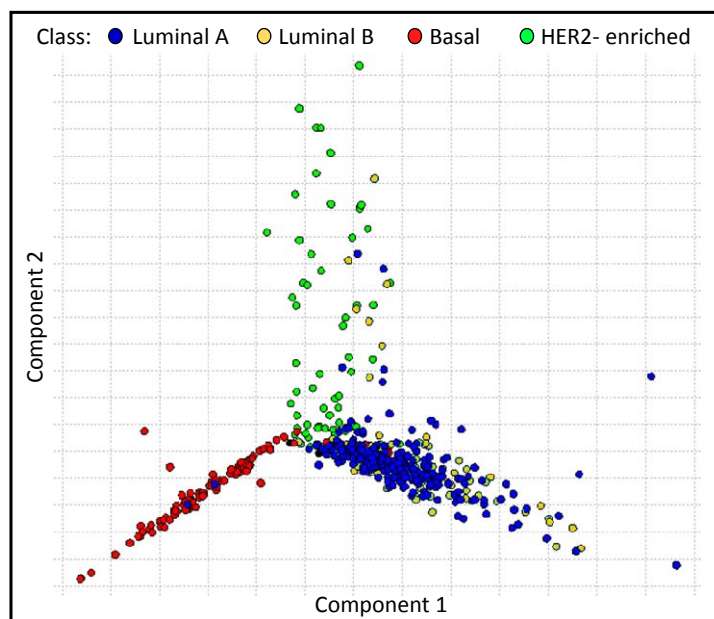
Our Vision - Classifiers

PCA Analysis:

A: Basal vs Luminal Classifiers
ERBB2

B: Basal vs Luminal Classifiers
ERBB2
PP2A activity Biomarkers

Sample:
TCGA patients annotated with
PAM50 annotation
N = 520

Class: ● Luminal A ○ Luminal B ● Basal ● HER2- enriched
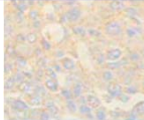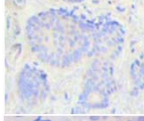

Quantigene 2.0 - Data Processing

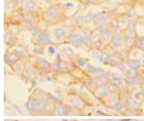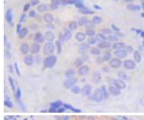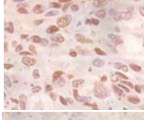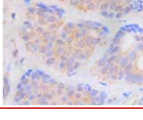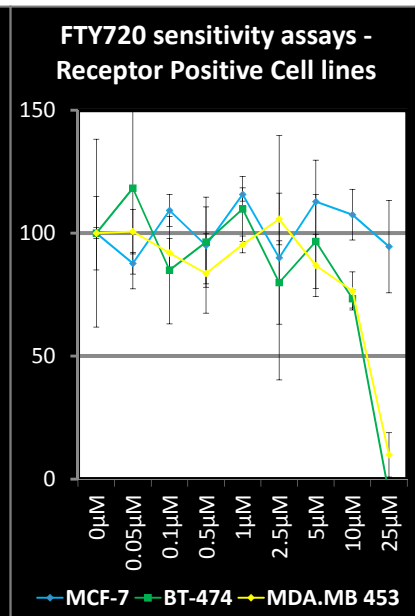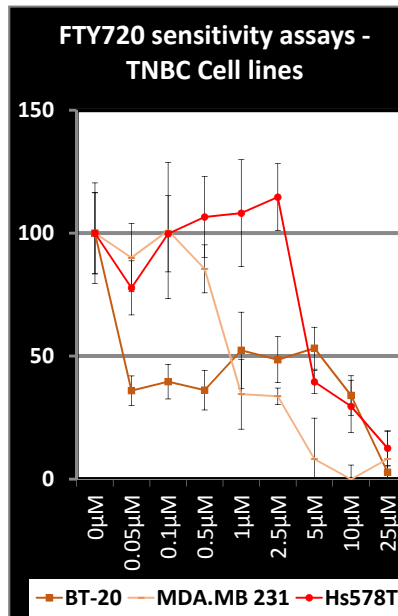# Biomarker Validation

IHC Validation:
PP2A activity using pS6K

IHC Annotation:
PP2A complex regulators
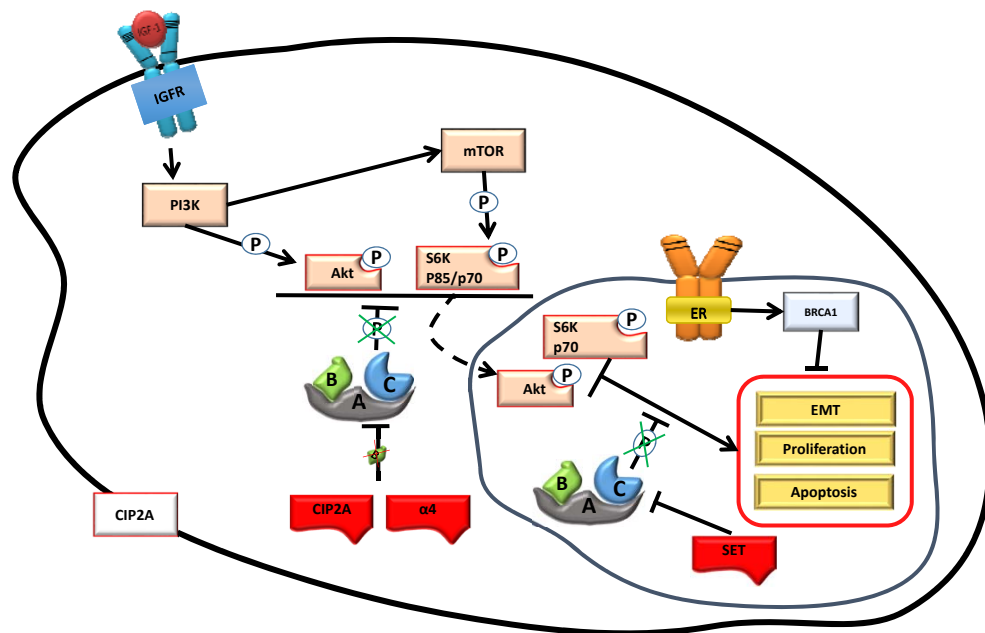
Sample:
FFPE material
N = 40

Concordance	ER Positive	TNBC
Z-score Algorithm	99.4% Luminal	95% Basal
p-S6K		
cip2a		
SET		
α4		

Potential Biomarkers to define a new Therapeutic Subtype

Out of 11 Breast cancer cell lines, 3 TNBC cell lines are sensitive to FTY720.
(Baldacchino et al., 2014)

FTY720 sensitivity assays - TNBC Cell lines
BT-20   MDA.MB 231   Hs578T

FTY720 sensitivity assays - Receptor Positive Cell lines
MCF-7   BT-474   MDA.MB 453

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Ongoing Studies

Conclusions

1. Quantigene results classified datasets into Luminal and Basal subtypes with high accuracy.

2. Quantigene results provided evidence for a novel Basal subtype.

3. The quantigene assay provides the technology to utilise FFPE material for gene expression studies

4. The novel subtype based on PP2A activity biomarkers, is potentially sensitive to the PP2A activator, FTY720.

Acknowledgments

PostDoc Position: Dr Christian Saliba
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MSc Students: Dr Keith Sacco; Mr Robert Gauci