



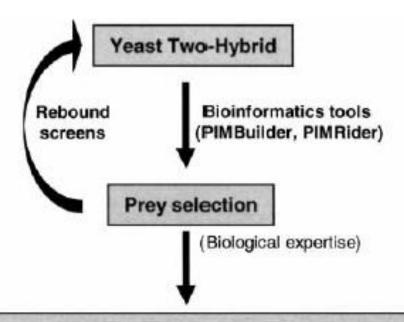
Functional Proteomics Mapping of a Human Signaling Pathway

Assignment in Biotechnology, ITMB
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Supervisors:

Zoidakis Ieronymos, Investigator, BRFAA

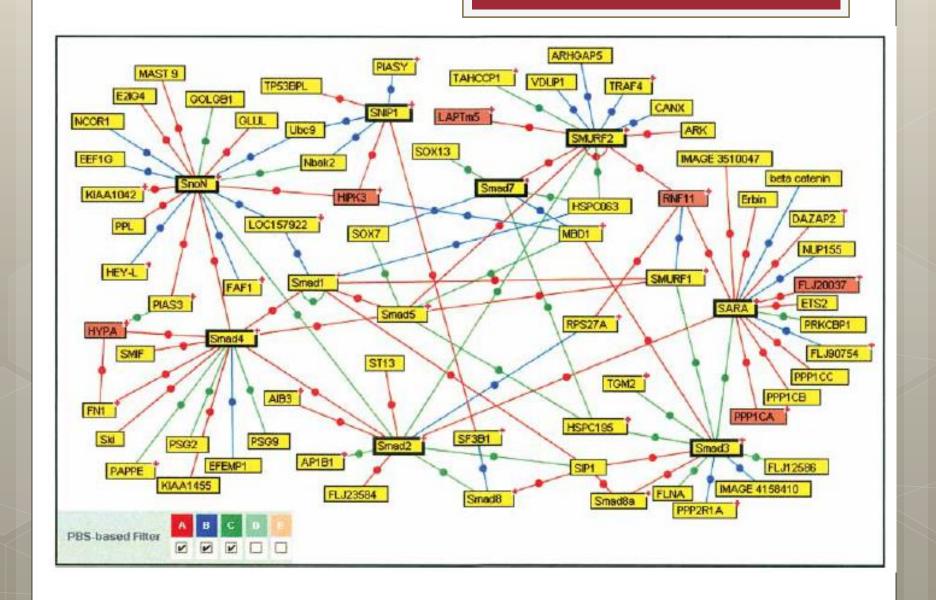
Vlachou Antonia, Investigator (Professor Level), BRFAA



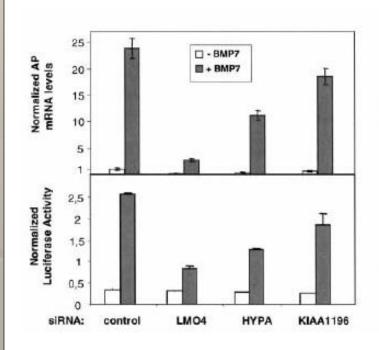
Functional validation in mammalian cells

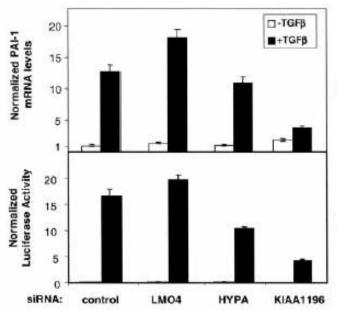
- TGFβ/BMP inducibility
- Protein overproduction/ Reporter assays
- siRNA/Reporter assays
- siRNA/Q-PCR assays

Protein name	Accession number	Bait fragments (amino acids)				
Primary screens						
Smad1	NM 005900	v1 (1-465); v2 (1-268); v3 (242-465)				
Smad2	NM 005901	v1 (1–467); v2 (1–271); v4 (403–467)				
Smad3	NM 005902	v1 (1–425); v2 (1–145); v3 (200–425); v4 (1–229)				
Smad4	NM 005359	v1 (1–552); v2 (1–318); v3 (251–552)				
Smad5	U59913	v1 (1–268)				
Smad7	NM 005904	v1 (251–426)				
Smad8	NM 005905	v1 (1-430); v2 (1-233); v3 (209-430)				
SARA	AF104304	v5 (665–1323)				
Smurf2	AF301463	v1 (1–335)				
SnoN	X15219	v1 (1-684); v2 (1-370); v3 (290-684)				
SNIP1	AK022615	v1 (1-396); v2 (1-198)				
Rebound screens						
LAPTm5	NM 006762	v1 (1-262); v2 (219-262)				
PTPN12	NM 002835	v1 (69-457); v2 (100-336)				
PPP1CA	NM 002708	v1 (11–330); v2 (1–324)				
HIPK3	NM 005734	v1 (667–1152); v2 (643–929)				
p621	NM 018179	v1 (1–709)				
PKD2	XM 028271	v1 (487–643)				
HYPA	AF049528	v1 (1–452)				
LMO4	NM 006769	v1 (1–165)				
RNF11	NM 014372	v1 (232–154); v2 (1–154)				
KIAA1196	XM 028968	v1 (486–774); v2 (644–833)				
FLJ20037	NM 017633	v1 (1–447); v2 (27–252)				
ZNF8	XM 034263	v1 (245–436)				



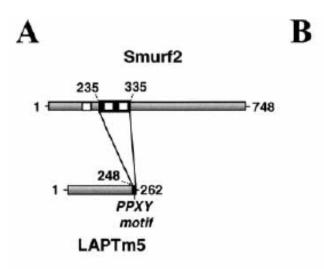
Selected proteins	Bait proteins	Rebound screens	mRNA Induction assay		Gene reporter activity					
					Protein overproduction		siRNA interference		Endogenous gene expression	
			TGF β	ВМР7	TGF β	ВМР7	TGF β	ВМР7	TGF β (PAI-1)	BMP7 (AP)
LAPTm5	Smurf2	+	A +++	_	▼++	_	A +	_	A ++	_
MAPK13	Smad4	nd	_	_	_	_	_	_	_	_
PTPN12	Smad5	_	_	_	_	_	_	_	_	_
PPP1CA	SARA	+	_	_	A++	_	▼+	_	_	_
HIPK3	SNIP1	_	_	_	nd	nd	_	_	_	_
	SnoN	_								
p621	Smad4	_	_	_	nd	nd	_	_	_	_
PKD2	Smad1	_	_	_	_	_	_	_	_	_
	Smad8	_								
MAN1	Smad1	nd	_	_	_	_	_	▼+	_	▼++
	Smad8	nd								
HYPA	Smad4	_	_	_	_	_	▼+	▼+	_	▼+
LMO4	Smad8	_	_	_	_	_	_	▼+++	_	▼+++
RNF11	Smurf2	+	_	_	_	_	nd	nd	▼+++	_
	SARA	_								
KIAA1196	Smad1	+	_	_	nd	nd	▼+++	_	▼++	_
FLJ20037	SARA	_	_	_	_	_	_	_	_	_
ZNF8	Smad1	+	_	_	_	_	_	▼++	_	▼++
	Smad5	_								
	Smad8	_								

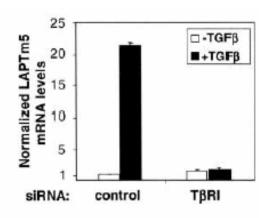


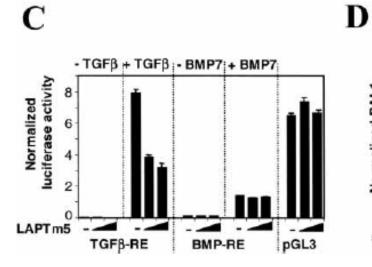


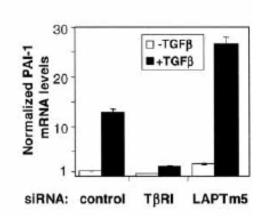
Q-PCR assays

Reporter assays









- ■What Questions is the paper trying to answer?
- Abstract
- ■What kind of experiments did they do, what can it show?
- What do they claim the figure shows?
- What is the specific evidence for the claim?
- ■What could be wrong with the claims, what controls address that?
- What inferences do they make in the Discussion, based on these results?

Thank you!

Begetis Nikolaos PIV0111