Results Summary

YChemH SCREEN
HBX129756 - NCGC00689148-01
vs Human Placenta_RP7

Tue, Aug 31, 2021 - 06:53 PM

Screen Parameters

Nature	cDNA
Chemical Probe	HBX129756 - NCGC00689148-01
Screen Name	PLA_RP7_hgx5814v1
Prey Library	Human Placenta_RP7
Vector(s)	pB409 (N-LexA-eDHFR-C)
Processed Clones	8 (pB409_A)
Analyzed Interactions	0 E0 (pB409_A)
Chemical Probe Concentration	5.0 µM (pB409_A)
Other Conditions	pH 4.5-5 standard (pB409_A)

All clones displayed in the list below are not dependent on the TMP-tagged compound HBX129756

Global PBS®

Global PBS (for Interactions represented in the Screen)		Nb	%
A	Very high confidence in the interaction	0	0.0%
B	High confidence in the interaction	0	0.0%
C	Good confidence in the interaction	0	0.0%
D	Moderate confidence in the interaction This category is the most difficult to interpret because it mixes two classes of interactions : - False-positive interactions - Interactions hardly detectable by the Y2H technique (low representation of the mRNA in the library, prey folding, prey toxicity in yeast)	2	28.6%
E	Interactions involving highly connected prey domains, warning of non-specific interaction. The threshold for high connectivity is 6. They can be classified in different categories: - Prey proteins that are known to be highly connected due to their biological function - Proteins with a prey interacting domain that contains a known protein interaction motif or a biochemically promiscuous motif	5	71.4%
F	Experimentally proven technical artifacts	0	0.0%
Non Appliable			
N/A	The PBS is a score that is automatically computed through algorithms and cannot be attributed for the following reasons : - All the fragments of the same reference CDS are antisense - The 5p sequence is missing - All the fragments of the same reference CDS are either all OOF1 or all OOF2 - All the fragments of the same reference CDS lie in the 5' or 3' UTR		

HYBRIGENICS CONFIDENTIAL - 1190087 - NIH-NCATS-PMorris

Prey Fragment Analysis

Symbols	Means
✠	The fragment contains the full length ORF
↖	Fragment is fully in 5' UTR
↘	Fragment is fully in 3' UTR
x	Fragment contains at least one In Frame STOP codon in 5' UTR
x	Fragment contains at least one In Frame STOP codon in CDS
x	Fragment contains the natural STOP codon
[NR]	Fragment was found to be non relevant (poor quality, high N density)
IF OOF1 OOF2	With regard to the theoretical frame of each corresponding ORF (GenBank),fragments are cloned in frame (IF) if they are in the same frame as Gal4AD.In general, polypeptides synthesized from OOF fragments are not considered of biological interest, unless found together with another frame. However, some of the proteins expressed from an OOF fragment can be translated in the correct frame,due to the existence of natural frame-shift events during translation in yeast. No stop codons are displayed for OOF fragments.
??	Unidentified frame when : - The clone sequence is antisense - The 5p sequence is missing
N	Antisense
Start...Stop	Position of the 5p and 3p prey fragment ends, relative to the position of the ATG start codon (A=0)

Clone Name	Type Seq	Gene Name (Best Match)	Start..Stop (nt)	Frame	Sense	%Id 5p	%Id 3p	PBS
pB409_A-7	5p/3p	Homo sapiens - HEYL	24..650	IF		99.8	98.9	E
pB409_A-8	5p/3p	Homo sapiens - MAGI1	1341..2076	IF		100.0	97.7	E
pB409_A-2	5p/3p	Homo sapiens - MAGI1	1350..1977	IF		99.8	100.0	E
pB409_A-4	5p/3p	Homo sapiens - PLAG1	369..1109	IF		99.4	100.0	E
pB409_A-9	5p/3p	Homo sapiens - PRDM15	2265..2976	IF		99.7	99.6	E
pB409_A-10	5p/3p	Homo sapiens - RUNX1	39..1115	IF		100.0	98.3	D
pB409_A-6	5p/3p	Homo sapiens - TAF1	3087..4141	IF		99.8	99.1	D
pB409_A-11	5p/3p	Homo sapiens - ZNF624	948..1649	IF		99.8	99.8	E

HYBRIGENICS CONFIDENTIAL - 1190087 - NIH-NCATS-PMorris

Results Summary

YChemH SCREEN
HBX129755 - NCGC00687962-01
vs Mouse Adult Brain_RP2

Tue, Aug 31, 2021 - 07:19 PM

Screen Parameters

Nature	cDNA
Chemical Probe	HBX129755 - NCGC00687962-01
Screen Name	AMB_RP2_hgx5813v1
Prey Library	Mouse Adult Brain_RP2
Vector(s)	pB409 (N-LexA-eDHFR-C)
Processed Clones	14 (pB409_B)
Analyzed Interactions	0 E0 (pB409_B)
Chemical Probe Concentration	20.0 µM (pB409_B)
Other Conditions	ph4.5-5 (pB409_B)

All clones displayed in the list below are not dependent on the TMP-tagged compound HBX129755

Global PBS®

Global PBS (for Interactions represented in the Screen)		Nb	%
A	Very high confidence in the interaction	0	0.0%
B	High confidence in the interaction	2	50.0%
C	Good confidence in the interaction	0	0.0%
D	Moderate confidence in the interaction This category is the most difficult to interpret because it mixes two classes of interactions : - False-positive interactions - Interactions hardly detectable by the Y2H technique (low representation of the mRNA in the library, prey folding, prey toxicity in yeast)	2	50.0%
E	Interactions involving highly connected prey domains, warning of non-specific interaction. The threshold for high connectivity is 6. They can be classified in different categories: - Prey proteins that are known to be highly connected due to their biological function - Proteins with a prey interacting domain that contains a known protein interaction motif or a biochemically promiscuous motif	0	0.0%
F	Experimentally proven technical artifacts	0	0.0%
Non Appliable			
N/A	The PBS is a score that is automatically computed through algorithms and cannot be attributed for the following reasons : - All the fragments of the same reference CDS are antisense - The 5p sequence is missing - All the fragments of the same reference CDS are either all OOF1 or all OOF2 - All the fragments of the same reference CDS lie in the 5' or 3' UTR		

HYBRIGENICS CONFIDENTIAL - 1193464 - NIH-NCATS-PMorris

Prey Fragment Analysis

Symbols	Means
✠	The fragment contains the full length ORF
↖	Fragment is fully in 5' UTR
↗	Fragment is fully in 3' UTR
x	Fragment contains at least one In Frame STOP codon in 5' UTR
x	Fragment contains at least one In Frame STOP codon in CDS
x	Fragment contains the natural STOP codon
[NR]	Fragment was found to be non relevant (poor quality, high N density)
IF OOF1 OOF2	With regard to the theoretical frame of each corresponding ORF (GenBank),fragments are cloned in frame (IF) if they are in the same frame as Gal4AD.In general, polypeptides synthesized from OOF fragments are not considered of biological interest, unless found together with another frame. However, some of the proteins expressed from an OOF fragment can be translated in the correct frame,due to the existence of natural frame-shift events during translation in yeast. No stop codons are displayed for OOF fragments.
??	Unidentified frame when : - The clone sequence is antisense - The 5p sequence is missing
N	Antisense
Start...Stop	Position of the 5p and 3p prey fragment ends, relative to the position of the ATG start codon (A=0)

Clone Name	Type Seq	Gene Name (Best Match)	Start..Stop (nt)	Frame	Sense	%Id 5p	%Id 3p	PBS
pB409_B-10	5p/3p	Mus musculus - Armcx1	240..782	IF		99.8	99.1	D
pB409_B-8	5p/3p	Mus musculus - Magi1	613..1295	OOF1		99.5	99.4	N/A
pB409_B-7	5p/3p	Mus musculus - Magi1	613..1295	OOF1		99.2	99.3	N/A
pB409_B-4	5p/3p	Mus musculus - Magi1	613..1295	OOF1		99.5	99.5	N/A
pB409_B-13	5p/3p	Mus musculus - Magi1	613..1295	OOF1		99.5	99.0	N/A
pB409_B-11	5p/3p	Mus musculus - Prdm15	1599..2723	IF		99.8	91.0	B
pB409_B-14	5p/3p	Mus musculus - Prdm15	1599..2723	IF		99.8	96.0	B
pB409_B-15	5p/3p	Mus musculus - Prdm15	2421..3236	IF		100.0	100.0	B
pB409_B-2	5p/3p	Mus musculus - Zfp521	150..774	IF		99.8	98.0	D
pB409_B-5	5p/3p	Mus musculus - Zfp87	891..1538	IF		99.3	99.5	B
pB409_B-12	5p/3p	Mus musculus - Zfp87	891..1538	IF		99.3	98.5	B
pB409_B-9	5p/3p	Mus musculus - Zfp87	891..1538	IF		99.1	100.0	B
pB409_B-6	5p/3p	Mus musculus - Zfp87	891..1538	IF		98.5	99.0	B
pB409_B-3	5p/3p	Mus musculus - Zfp87	936..1552	IF		99.0	99.1	B

HYBRIGENICS CONFIDENTIAL - 1193464 - NIH-NCATS-PMorris

Results Summary

YChemH SCREEN
HBX129755 - NCGC00687962-01
vs Human Placenta_RP7

Tue, Aug 31, 2021 - 06:29 PM

Screen Parameters

Nature	cDNA
Chemical Probe	HBX129755 - NCGC00687962-01
Screen Name	PLA_RP7_hgx5813v1
Prey Library	Human Placenta_RP7
Vector(s)	pB409 (N-LexA-eDHFR-C)
Processed Clones	24 (pB409_A)
Analyzed Interactions	0 E0 (pB409_A)
Chemical Probe Concentration	20.0 µM (pB409_A)
Other Conditions	PH 4.5-5 (pB409_A)

All clones displayed in the list below are not dependent on the TMP-tagged compound HBX129755

Global PBS®

Global PBS (for Interactions represented in the Screen)		Nb	%
A	Very high confidence in the interaction	0	0.0%
B	High confidence in the interaction	0	0.0%
C	Good confidence in the interaction	0	0.0%
D	Moderate confidence in the interaction This category is the most difficult to interpret because it mixes two classes of interactions : - False-positive interactions - Interactions hardly detectable by the Y2H technique (low representation of the mRNA in the library, prey folding, prey toxicity in yeast)	1	12.5%
E	Interactions involving highly connected prey domains, warning of non-specific interaction. The threshold for high connectivity is 6. They can be classified in different categories: - Prey proteins that are known to be highly connected due to their biological function - Proteins with a prey interacting domain that contains a known protein interaction motif or a biochemically promiscuous motif	7	87.5%
F	Experimentally proven technical artifacts	0	0.0%
Non Appliable			
N/A	The PBS is a score that is automatically computed through algorithms and cannot be attributed for the following reasons : - All the fragments of the same reference CDS are antisense - The 5p sequence is missing - All the fragments of the same reference CDS are either all OOF1 or all OOF2 - All the fragments of the same reference CDS lie in the 5' or 3' UTR		

HYBRIGENICS CONFIDENTIAL - 1184617 - NIH-NCATS-PMorris

Prey Fragment Analysis

Symbols	Means
✻	The fragment contains the full length ORF
↖	Fragment is fully in 5' UTR
↘	Fragment is fully in 3' UTR
x	Fragment contains at least one In Frame STOP codon in 5' UTR
x	Fragment contains at least one In Frame STOP codon in CDS
x	Fragment contains the natural STOP codon
[NR]	Fragment was found to be non relevant (poor quality, high N density)
IF OOF1 OOF2	With regard to the theoretical frame of each corresponding ORF (GenBank),fragments are cloned in frame (IF) if they are in the same frame as Gal4AD.In general, polypeptides synthesized from OOF fragments are not considered of biological interest, unless found together with another frame. However, some of the proteins expressed from an OOF fragment can be translated in the correct frame,due to the existence of natural frame-shift events during translation in yeast. No stop codons are displayed for OOF fragments.
??	Unidentified frame when : - The clone sequence is antisense - The 5p sequence is missing
N	Antisense
Start...Stop	Position of the 5p and 3p prey fragment ends, relative to the position of the ATG start codon (A=0)

Clone Name	Type Seq	Gene Name (Best Match)	Start..Stop (nt)	Frame	Sense	%Id 5p	%Id 3p	PBS
pB409_A-16	5p/3p	Homo sapiens - ARHGAP21	2157..2995	IF		100.0	99.8	E
pB409_A-24	5p/3p	Homo sapiens - ARHGAP21	2265..2929	IF		100.0	100.0	E
pB409_A-15	5p/3p	Homo sapiens - HEYL	15..646	IF		99.6	99.8	E
pB409_A-26	5p/3p	Homo sapiens - HEYL	15..646	IF		99.8	99.8	E
pB409_A-11	5p/3p	Homo sapiens - MAGI1	1194..2287	IF		100.0	100.0	E
pB409_A-7	5p/3p	Homo sapiens - MAGI1	1194..2287	IF		99.8	100.0	E
pB409_A-14	5p/3p	Homo sapiens - MAGI1	1347..2294	IF		100.0	100.0	E
pB409_A-4	5p/3p	Homo sapiens - MAGI1	1350..1977	IF		100.0	100.0	E
pB409_A-8	5p/3p	Homo sapiens - PLAG1	282..1477	IF		100.0	99.8	E
pB409_A-17	5p/3p	Homo sapiens - PLAG1	510..1243	IF		100.0	100.0	E
pB409_A-12	5p/3p	Homo sapiens - PLAG1	510..1243	IF		100.0	100.0	E
pB409_A-3	5p/3p	Homo sapiens - PLAG1	510..1243	IF		100.0	100.0	E
pB409_A-13	5p/3p	Homo sapiens - PRDM15	2217..2932	IF		99.8	99.4	E
pB409_A-22	5p/3p	Homo sapiens - PRDM15	2217..2932	IF		99.8	99.4	E
pB409_A-20	5p/3p	Homo sapiens - PRDM15	2229..3368	IF		99.7	99.8	E
pB409_A-18	5p/3p	Homo sapiens - PRDM15	2229..3368	IF		99.6	99.8	E
pB409_A-21	5p/3p	Homo sapiens - PRDM15	2229..3368	IF		99.7	99.8	E
pB409_A-10	5p/3p	Homo sapiens - PRDM15	2256..2902	IF		99.8	99.8	E
pB409_A-9	5p/3p	Homo sapiens - PRDM15	2265..2976	IF		99.7	99.6	E
pB409_A-6	5p/3p	Homo sapiens - PRDM15	2265..2976	IF		99.7	99.6	E
pB409_A-25	5p/3p	Homo sapiens - PRDM15	2265..2976	IF		99.7	99.8	E
pB409_A-5	5p/3p	Homo sapiens - ZNF521	-544..373	IF		100.0	100.0	E
pB409_A-19	5p/3p	Homo sapiens - ZNF624	948..1649	IF		99.8	100.0	E
pB409_A-23	5p/3p	Homo sapiens - GenMatch	-1	IF		100.0	86.1	D

HYBRIGENICS CONFIDENTIAL - 1184617 - NIH-NCATS-PMorris

Results Summary

YChemH SCREEN
HBX129755 - NCGC00687962-01
vs Mouse Adult Brain_RP2

Tue, Mar 2, 2021 - 09:34 AM

Screen Parameters

Nature	cDNA
Chemical Probe	HBX129755 - NCGC00687962-01
Screen Name	AMB_RP2_hgx5813v1
Prey Library	Mouse Adult Brain_RP2
Vector(s)	pB409 (N-LexA-eDHFR-C)
Processed Clones	21 (pB409_A)
Analyzed Interactions	103 millions (pB409_A)
Chemical Probe Concentration	5.0 µM (pB409_A)
Other Conditions	pH 4,5-5 (pB409_A)

Global PBS®

List below = all HIS+ colonies isolated from the screen:
- none of them dependent to the presence of the tagged compound HBX129755
- clone pB409_A-4 potentially dependent of the presence of HBX129755. Needs confirmation

Global PBS (for Interactions represented in the Screen)		Nb	%
A	Very high confidence in the interaction	0	0.0%
B	High confidence in the interaction	0	0.0%
C	Good confidence in the interaction	0	0.0%
D	Moderate confidence in the interaction This category is the most difficult to interpret because it mixes two classes of interactions : - False-positive interactions - Interactions hardly detectable by the Y2H technique (low representation of the mRNA in the library, prey folding, prey toxicity in yeast)	0	0.0%
E	Interactions involving highly connected prey domains, warning of non-specific interaction. The threshold for high connectivity is 6. They can be classified in different categories: - Prey proteins that are known to be highly connected due to their biological function - Proteins with a prey interacting domain that contains a known protein interaction motif or a biochemically promiscuous motif	0	0.0%
F	Experimentally proven technical artifacts	0	0.0%
Non Appliable			
N/A	The PBS is a score that is automatically computed through algorithms and cannot be attributed for the following reasons : - All the fragments of the same reference CDS are antisense - The 5p sequence is missing - All the fragments of the same reference CDS are either all OOF1 or all OOF2 - All the fragments of the same reference CDS lie in the 5' or 3' UTR		

HYBRIGENICS CONFIDENTIAL - 1182168 - NIH-NCATS-PMorris

Prey Fragment Analysis

Symbols	Means
✠	The fragment contains the full length ORF
↖	Fragment is fully in 5' UTR
↘	Fragment is fully in 3' UTR
x	Fragment contains at least one In Frame STOP codon in 5' UTR
x	Fragment contains at least one In Frame STOP codon in CDS
x	Fragment contains the natural STOP codon
[NR]	Fragment was found to be non relevant (poor quality, high N density)
IF OOF1 OOF2	With regard to the theoretical frame of each corresponding ORF (GenBank),fragments are cloned in frame (IF) if they are in the same frame as Gal4AD.In general, polypeptides synthesized from OOF fragments are not considered of biological interest, unless found together with another frame. However, some of the proteins expressed from an OOF fragment can be translated in the correct frame,due to the existence of natural frame-shift events during translation in yeast. No stop codons are displayed for OOF fragments.
??	Unidentified frame when : - The clone sequence is antisense - The 5p sequence is missing
N	Antisense
Start...Stop	Position of the 5p and 3p prey fragment ends, relative to the position of the ATG start codon (A=0)

Clone Name	Type Seq	Gene Name (Best Match)	Start..Stop (nt)		Frame	Sense	%Id 5p	%Id 3p	PBS
pB409_A-5	5p/3p	Mus musculus - Magi1	366..1118		IF		100.0	95.2	N/A
pB409_A-11	5p/3p	Mus musculus - Magi1	613..1295		OOF1		99.5	99.3	N/A
pB409_A-19	5p/3p	Mus musculus - Magi1	613..1295		OOF1		99.6	99.6	N/A
pB409_A-20	5p/3p	Mus musculus - Prdm15	1599..2723		IF		99.8	100.0	N/A
pB409_A-15	5p/3p	Mus musculus - Prdm15	1599..2723		IF		99.8	100.0	N/A
pB409_A-8	5p/3p	Mus musculus - Prdm15	1599..2723		IF		99.8	100.0	N/A
pB409_A-18	5p/3p	Mus musculus - Prdm15	2211..2860		IF		100.0	100.0	N/A
pB409_A-3	5p/3p	Mus musculus - Prdm15	2211..2860		IF		99.8	96.7	N/A
pB409_A-10	5p/3p	Mus musculus - Prdm15	2211..2860		IF		100.0	100.0	N/A
pB409_A-6	5p/3p	Mus musculus - Prdm15	2211..2860		IF		100.0	99.1	N/A
pB409_A-13	5p/3p	Mus musculus - Zbtb16	1311..2104	x	IF		99.5	98.3	N/A
pB409_A-1	5p/3p	Mus musculus - Zfp521	147..677		IF		100.0	100.0	N/A
pB409_A-17	5p/3p	Mus musculus - Zfp87	891..1538		IF		99.3	99.5	N/A
pB409_A-21	5p/3p	Mus musculus - Zfp87	891..1538		IF		99.3	99.5	N/A
pB409_A-9	5p/3p	Mus musculus - Zfp87	891..1538		IF		96.0	97.0	N/A
pB409_A-12	5p/3p	Mus musculus - Zfp87	891..1538		IF		99.3	99.5	N/A
pB409_A-14	5p/3p	Mus musculus - Zfp87	891..1538		IF		99.3	99.5	N/A
pB409_A-2	5p/3p	Mus musculus - Zfp87	891..1538		IF		99.3	97.8	N/A
pB409_A-16	5p/3p	Mus musculus - Zfp87	891..1538		IF		99.3	99.5	N/A
pB409_A-7	5p/3p	Mus musculus - Zfp87	936..1552		IF		99.5	99.5	N/A
pB409_A-4	5p/3p	Mus musculus - Zfp87	993..2046	x	IF		99.0	98.8	N/A

HYBRIGENICS CONFIDENTIAL - 1182168 - NIH-NCATS-PMorris

CONFIDENTIAL

Evry, August 31st, 2021

From:

Marie-Edith GOURDEL,
Ph.D.
Director Chemistry

HYBRIGENICS SERVICES SAS

To:

NIH / NCATS

Patrick MORRIS
Division of Preclinical Innovation
NCATS
9800 Medical Center Drive
Rockville, MD, 20850

Copy:

YChemH studies
-
HBX129755 and HBX129756

Final Report (v2)

1 INTRODUCTION

Two TMP-tagged chemical probes were synthesized to perform a YChemH target deconvolution study and identify the protein target(s) of ketamine metabolites. The compounds were sent to Hybrigenics and submitted to permeability in yeast and cell viability assessment first. Results from the Perm/Tox assays were delivered in report v1. The 2 tagged compounds were screened against several different cDNA libraries. The work performed and the results are described in this final report (report v2), including the perm/tox results.

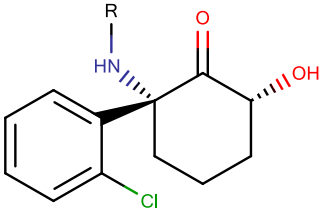


Figure 1 : Ketamine Metabolites (R = H, HydroxyNorketamine, R = CH3, Hydroxyketamine)

2 CHEMICAL PROBES

Chemical structures and IDs, together with physicochemical parameters calculations are described in the figures below.

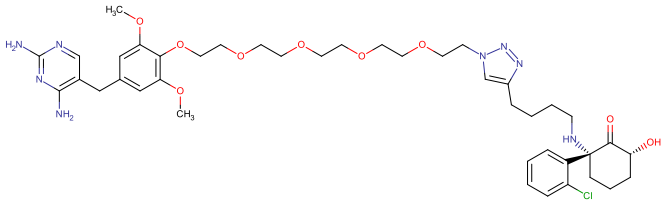
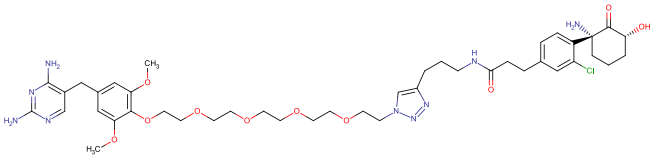
NCGC00687962-01 (bar-code 3EA5)	HBX129755	
NCGC00689148-01 (bar-code 5PEW) TFA salt	HBX129756	

Figure 2 : Chemical structures of the TMP chemical probes

HGX ID #	Alias Name	NIH/NCATS	MW probe	Amount delivered (mg)
HBX129755-1	TMP-PEG4-TR-C4-KET1	NCGC00687962-01	841,4	120
HBX129756-1	TMP-PEG4-TR-NCO-KET2	NCGC00689148-01	1012,48	36,8

Figure 3 : Compounds reference numbers

HGX ID #	LogP	LogD pH 2	LogD pH 7.4	LogD pH 9	TPSA (pH7.4)	HBA	HBD
HBX129755	4,2	-0,5	3,6	4,2	222,5	16	4
HBX129756	2,8	-1,7	2,3	2,8	265,6	17	5

Figure 4 : calculations of physico-chemical properties for the chemical probes (Chemaxon tools)

3 PERMEABILITY EVALUATION

3.1 Principle:

Permeability in yeast for a tagged-compound X of interest is assessed using a competition assay. Figure 5 describes its principle. This involved a positive control system consisting of the 3 following hybrids:

- An anchor binding protein (or hook): dihydrofolate reductase DHFR,

- A prey protein : Glucocorticoid receptor, GR
- A hybrid ligand: Trimethoprim-tagged Dexamethasone (TMP-But-PEG3-DEX, 2µM). TMP is a ligand of DHFR, and DEX a ligand of Gr. DEX is the bait small molecule.

Within this positive control system and on selective medium, yeast growth occurs as DEX-GR interaction takes place if HIS3 is used as a reporter gene (1).

When adding both TMP-tagged-DEX and TMP-tagged-Compound X molecules at the same time (3), yeast growth decrease or suppression means that TMP-tagged Compound X competes with TMP-tagged-DEX, and therefore enters yeast cells. The tagged-compound X to test is used at different concentrations (e.g. 5, 10, 20 µM).

In parallel, we also use TMP as a reference compound to compete with the positive control compound TMP-tagged-DEX (2).

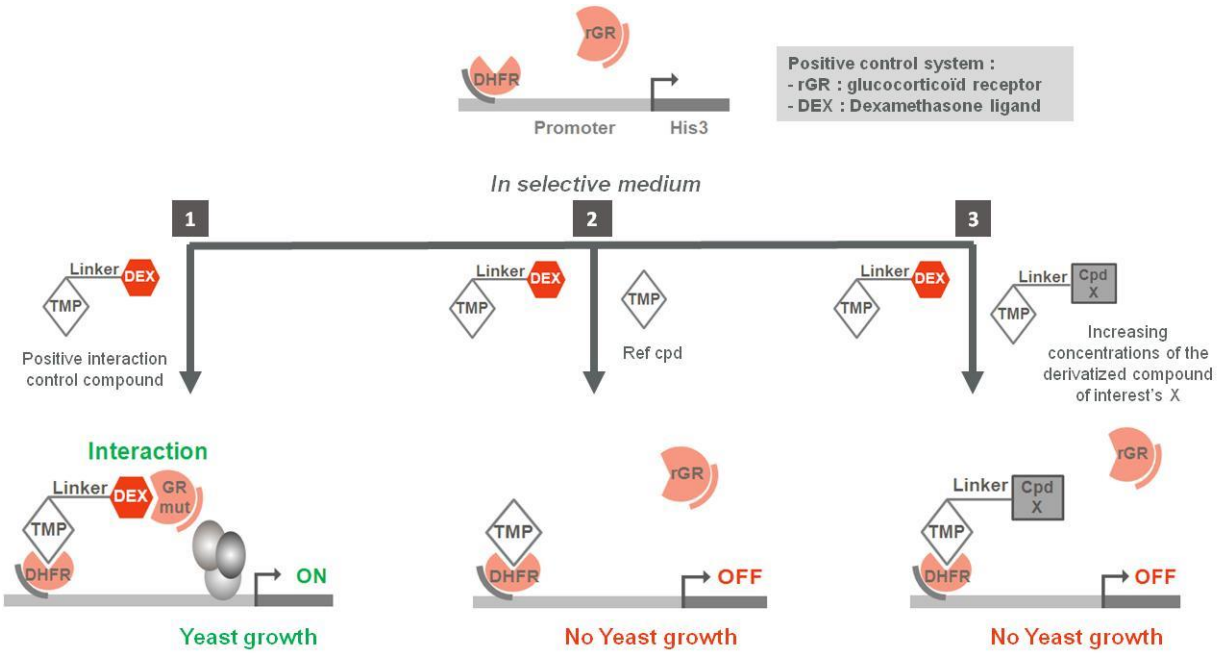


Figure 5 : permeability assessment principle

The same principle is used to check the non-toxicity of the tagged compound on yeast. Cell viability is assessed by working in non-selective medium (interaction non-dependent). Yeast growth is observed whatever the chemical compound used. We compare the normal growth of the yeast without any tested compound (DMSO only) with the growth when a chemical compound is added at different concentrations.

Two kinds of yeast strains are used:

- Strain#1: HGX permeable strains deleted from 5 genes involved in drug transport (haploid strain)
- Strain#2: HGX hemizygote strain obtained after mating between strain#1 and a wild type strain. This strain is close to the conditions used in Hybrigenics' mating procedure. (diploid strain)

3.2 Experimental procedure

The permeability was assessed through two experiments carried out at two different pH conditions: standard conditions at pH 4,5-5 and the other experiment at pH 6,5.

Experiment #1:

Solution preparation: Each TMP-tagged probe 10 mM DMSO stock solution was diluted with 100% DMSO to provide 1, 2, 4 and 8 mM solutions of the chemical probe to be evaluated. An EtOH solution containing FK506 (10 µM) and the positive interaction control compound TMP-But-PEG3-DEX (2 µM) were added to each previous DMSO solutions. Dilutions were then carried out with the yeast Drop Out medium (-L-W-H, pH 4.5-5) to give respectively 0.25, 0.5, 1 and 2 mM concentration clear solutions (Probe solutions #1). The solutions were then spread on the plates to get the expected final concentrations of the compound (5, 10, 20 and 40 µM) and 0.5% DMSO.

Growth assay on -His plates: One DO-W-L-H solid medium (agar) plate was used per chemical compound and concentration. Each probe solution #1 was spread on a distinct agar plate. After these solutions were adsorbed, OD_{600} 1, 1/10, 1/100 and 1/1000 solutions of the yeast cultures (strains #1 and #2) were spotted on all agar plates. Plates were incubated at 30°C for 3 days.

Experiment #2:

Solution preparation: Same conditions were used as for experiment #1. The difference was that the pH of the yeast Drop out medium (-L-W-H) was adjusted to 6.5 with NaOH 30%. All probe solutions #2 were clear solutions.

Growth assay on -His plates: same conditions as for experiment #1

No solubility issues were observed.

3.3 Results for HBX129755 TMP probe

In Figures 6 to 7 are displayed the pictures from the toxicity/permeability assay using a competition assay. This assay explores yeast growth under 3 different conditions:

1) DMSO only **1** ,
2) Reference compound (Trimethoprim, TMP) **2**: to check if YChemH competition assay works properly
3) Compound to evaluate **3**,

All petri dishes contain:

- The positive interaction control compound (e.g. TMP-But-PEG3-DEX, 2 µM).
- FK506, an inhibitor of the Pdr5 transporter, was also added to enhance the permeability of the yeast.

3.3.1 HBX129755 - Exp #1: yeast culture medium at pH 4,5-5

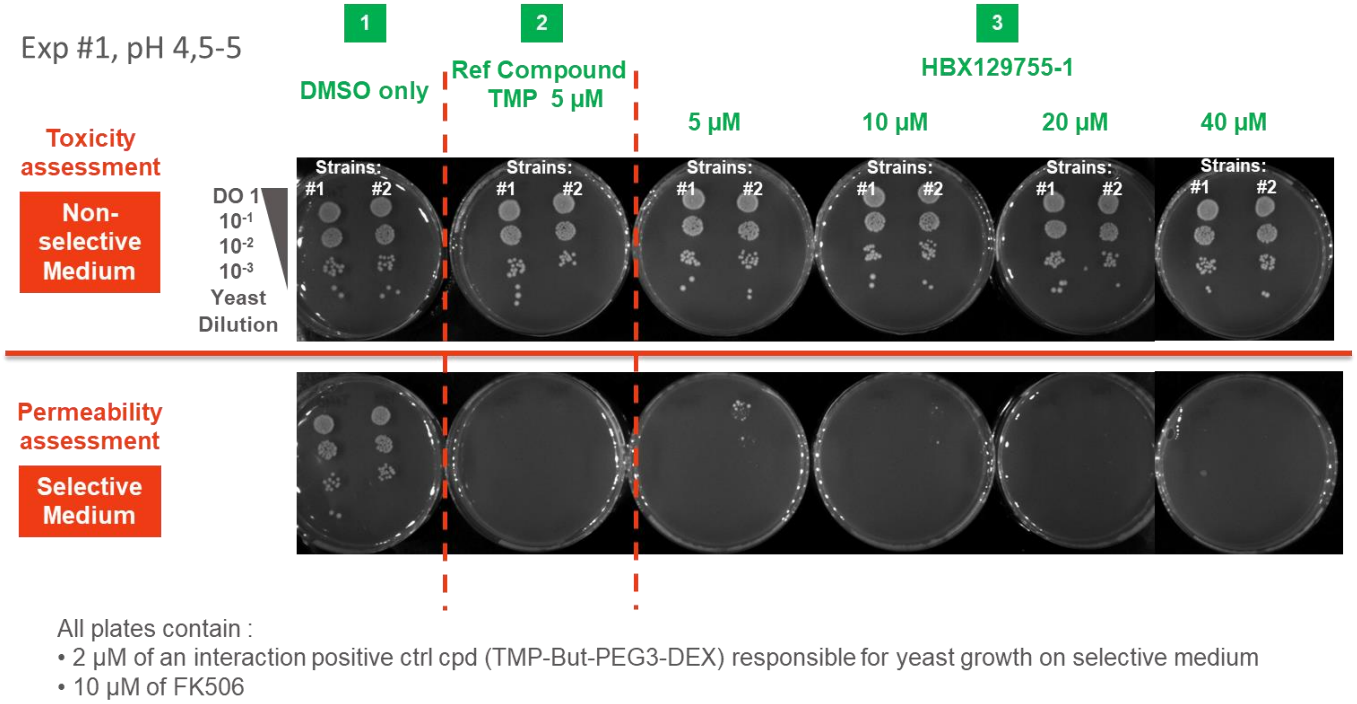


Figure 6: Permeability and toxicity assay (HBX129755) at 5, 10, 20 and 40 µM) – Exp#1 pH 4,5-5

Toxicity evaluation (non-selective medium):

In non-selective medium, no difference in yeast growth is observed between condition 1 and condition 3 when HBX129755 is used whatever its concentration. No toxicity was observed on the yeast for HBX129755.

Permeability evaluation (selective medium):

- On condition 1 with DMSO only, yeast growth occurs as the positive interaction control compound (TMP-But-PEG3-DEX) is present and interaction takes place.
- TMP (reference compound, 5 µM, condition 2) suppresses yeast growth (strains #1 and #2), confirming that TMP competes with the positive control, and therefore enters yeast cells. It validates that the competition assay works well.
- For HBX129755 (condition 3):
  - Strain #1: No yeast growth is observed.
  - Strain #2: Remaining yeast growth happens at the highest inoculum at a 5 µM concentration of HBX129755 only.
  - As HBX129755 suppresses yeast growth. This means that it competes with the positive control compound and therefore enters yeast cells. As this is observed at 5 µM, HBX129755 has a very good permeability in yeast cells
  - According to the results, we would perform the YChemH screen at a 5 µM concentration of HBX129755.

3.3.2 HBX129755 - Exp #2: yeast culture medium at pH 6,5

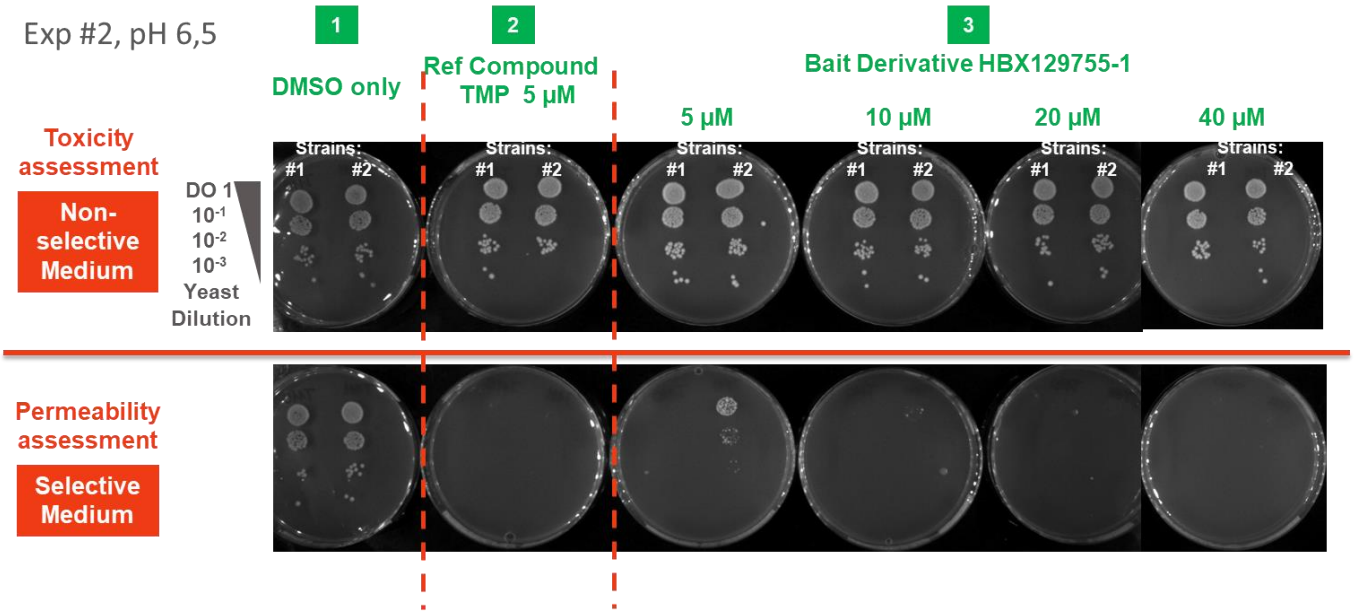


Figure 7: Permeability and toxicity assay (HBX129755 at 5, 10, 20 and 40 µM) – Exp#2 pH 6,5

For the experiment at pH 6,5:

- No toxicity is observed
- Permeability:
  - One can see the same behavior when the experiment is performed at pH 6,5 instead of 4,5-5.
  - Strain #1: very good permeability as no yeast growth happened already from 5 µM
  - Strain #2: remaining yeast growth at 5 µM.

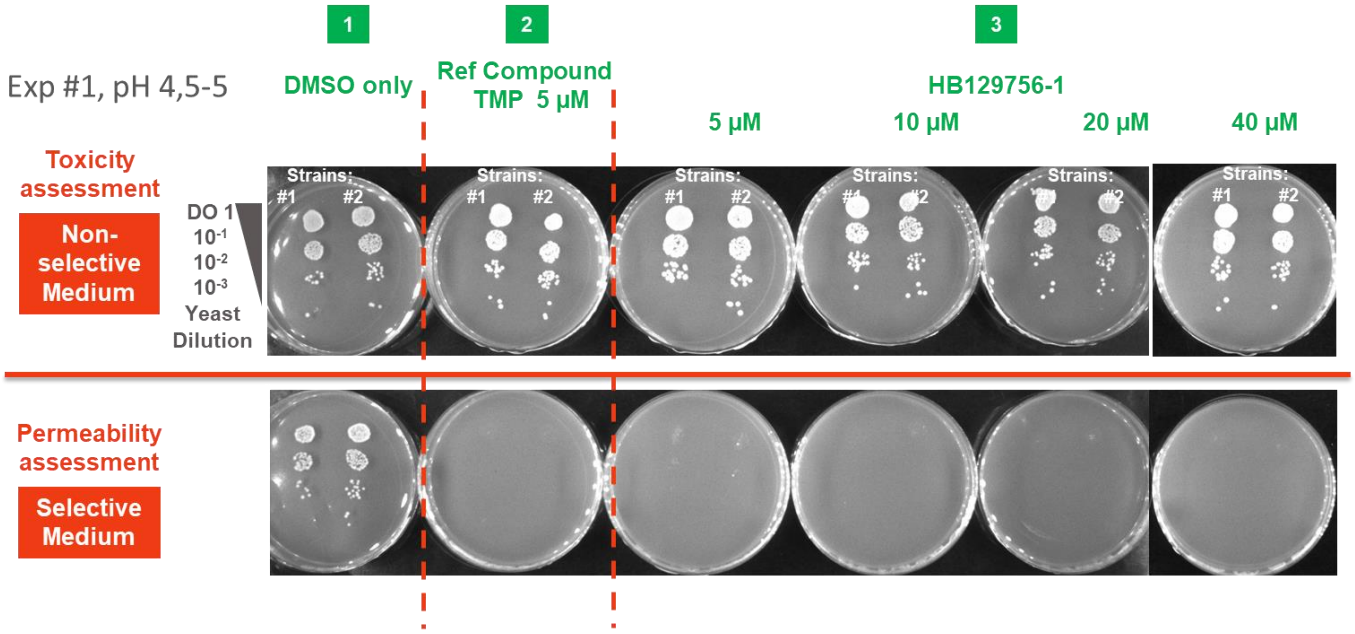
3.3.3 Comparison of both experiments

HBX129755 tagged probe showed very good permeability in yeast cells whatever the pH. The screen will be carried out at the standard pH (4,5-5).

3.4 3.3 Results for HBX129756 TMP probe

Same conditions were used to carry out the experiments for HBX129756. Pictures are displayed for the experiments at standard pH (4,5-5), and at pH 6,5. Then the permeability profiles are compared to see if there is any benefit in running the YChemH screen at a given pH.

3.4.1 HBX129756 - Exp #1: yeast culture medium at pH 4,5-5



All plates contain :
- 2 µM of an interaction positive ctrl cpd (TMP-But-PEG3-DEX) responsible for yeast growth on selective medium
- 10 µM of FK506

Figure 8: Permeability and toxicity assay HBX129756 at 5, 10, 20 and 40 µM – Exp#1 pH 4,5-5

Toxicity evaluation (non-selective medium):

In non-selective medium, no difference in yeast growth is observed between condition 1 and condition 3 when HBX129756 is used whatever its concentration. HBX129756 is not toxic for the yeast cells..

Permeability evaluation (selective medium):

- Control plates showed yeast growth or suppression as expected:
  - condition 1 , DMSO only: yeast growth in the presence only of the positive interaction control compound (TMP-But-PEG3-DEX)
  - condition 2, competition with Trimethoprim observed
- For HBX129756 (condition 3):
  - The compound suppressed yeast growth whatever the yeast strain (#1 or #2) and whatever the concentration used. This means that HBX129756 competes with the positive interaction control, and therefore is highly permeable in yeast cells.
  - According to the results, we would perform the YChemH screen at 5 µM concentration.

3.4.2 HBX129756 - Exp #2: yeast culture medium at pH 6,5

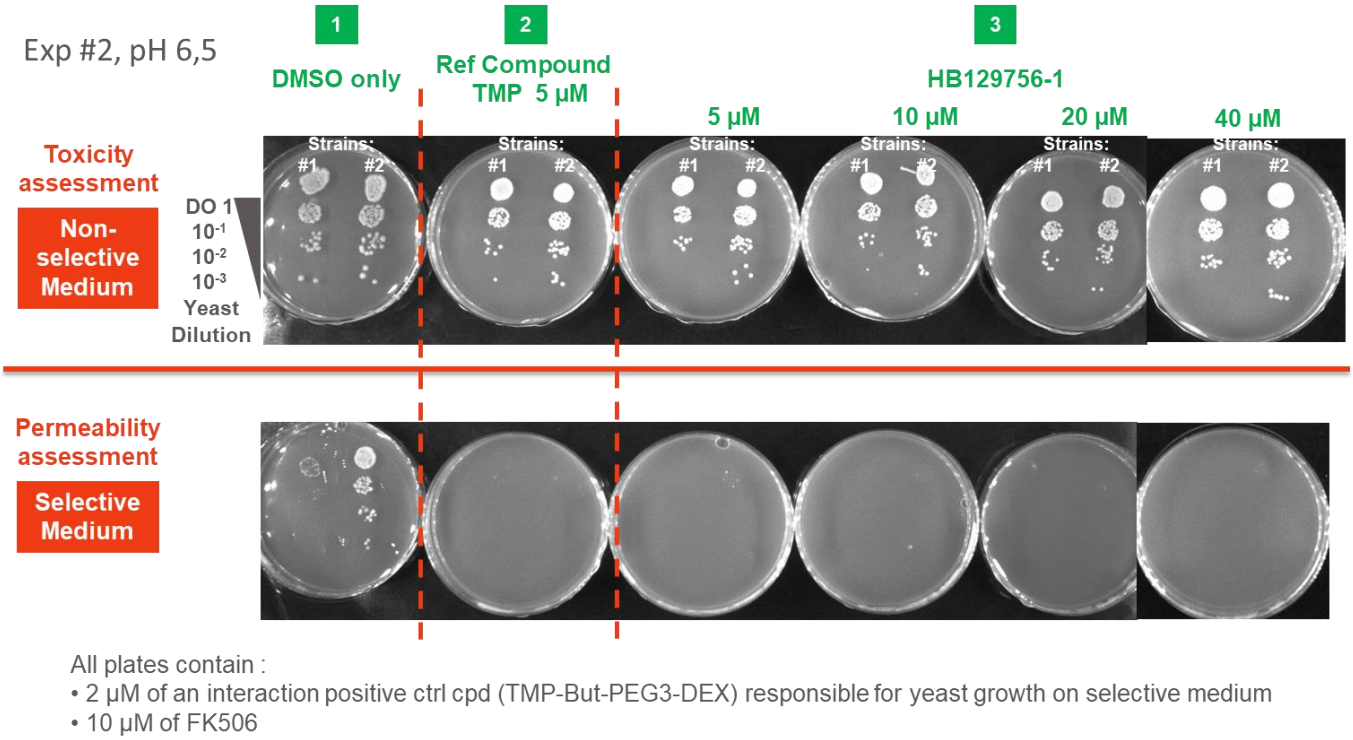


Figure 9: Permeability and toxicity assay HBX129756 at 5, 10, 20 and 40 µM – Exp#2 pH 6,5

No toxicity is observed for HBX129756.

Permeability: HBX129756 tagged compound showed the same behavior at pH 6,5 and can suppress yeast growth already at 5 µM.

3.4.3 Comparison of both experiments

Yeast growth decrease/supression is comparable between the two experiments at pH 5,5-5 and 6,5. Standard pH conditions will be used for the YChemH screen.

3.5 CONCLUSION

HBX129755 and HBX129756 TMP-tagged probes were evaluated for permeability in yeast cells and cell viability.

No toxicity was observed for both compounds whatever the conditions (concentration and pH).

Both compounds are highly permeable in yeast. They can be moved to the YChemH screening step. Both YChemH screens will be carried out at standard pH and at a 5 µM concentration of each TMP-tagged probe.

4 YChemH screens

4.1 YChemH Screens ID

HBX129755 and HBX129756 were screened against several cDNA libraries. The screens were carried out according to the optimized cell-to-cell mating protocol developed for Hybrigenics ULTImate Y2H™. Figure 10 summarizes compounds and screens ID, together with the concentration of the compound used for each screen. Seven YChemH screens were performed.

TMP-Tagged probe	Compound Ref #	YChemH Project	YChemH screen Project ID	Probe concentration
TMP-PEG4-TR-C4-KET1 NCGC00687962-01	HBX129755	hgx5813	AMB_RP2_hgx5813v1_pB409_A	5 µM
			AMB_RP2_hgx5813v1_pB409_B	20 µM
			PLA_RP7_hgx5813v1_pB409_A	20 µM
			HLUC_RP1_hgx5813v1_pB409_A	1 µM
			HBR_RP3_hgx5813v1_pB409_A	5 µM
TMP-PEG4-TR-NCO-KET2 NCGC00689148-01	HBX129756	hgx5814	AMB_RP2_hgx5814v1_pB409_A	5 µM
			PLA_RP7_hgx5814v1_pB409_A	5 µM

Figure 10: YChemH screens ID

cDNA Libraries used in the project:

- AMB: Mouse Adult Brain
- PLA: Human placenta
- HLUC: Human Lung Cancer cells
- HBR: Human Adult Brain

Hybrigenics' cell-to-cell mating protocol (figure 11) involves a "HGX permeable" strain (type mata), containing the anchor plasmid (Dihydrofolate reductase, DHFR)", and a wild type strain (type matα), containing the prey plasmids. In addition to the experimental process, we have shown that the use of FK506, an inhibitor of the Pdr5 transporter, improves the permeability of the compounds into the wild type yeast cells. A 10 µM concentration of FK506 has been used in all the YChemH screens described in this report.

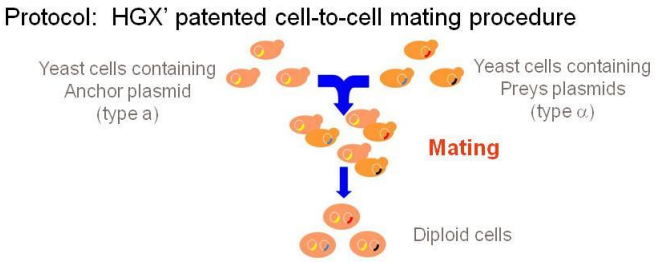


Figure 11: Hybrigenics' ULTImate YChemH screen protocol

4.2 Dependency assays

Clones obtained from YChemH screens are picked and evaluated in a dependency assay using spot assays in a 96-well plate format. Yeast growth for each clone is monitored under 3 conditions:

1) 1 : on non-selective medium (DO-2), we check normal growth for each selected clone without the tagged compound being evaluated (DMSO only)
2) 2 : growth on selective medium (DO-3) without the tagged compound being evaluated (DMSO only)
3) 3 : growth on selective medium (DO-3) with the tagged compound being evaluated.

Dependent clones are those which grow only under condition 3 but not under condition 2.

4.2.1 HBX129755

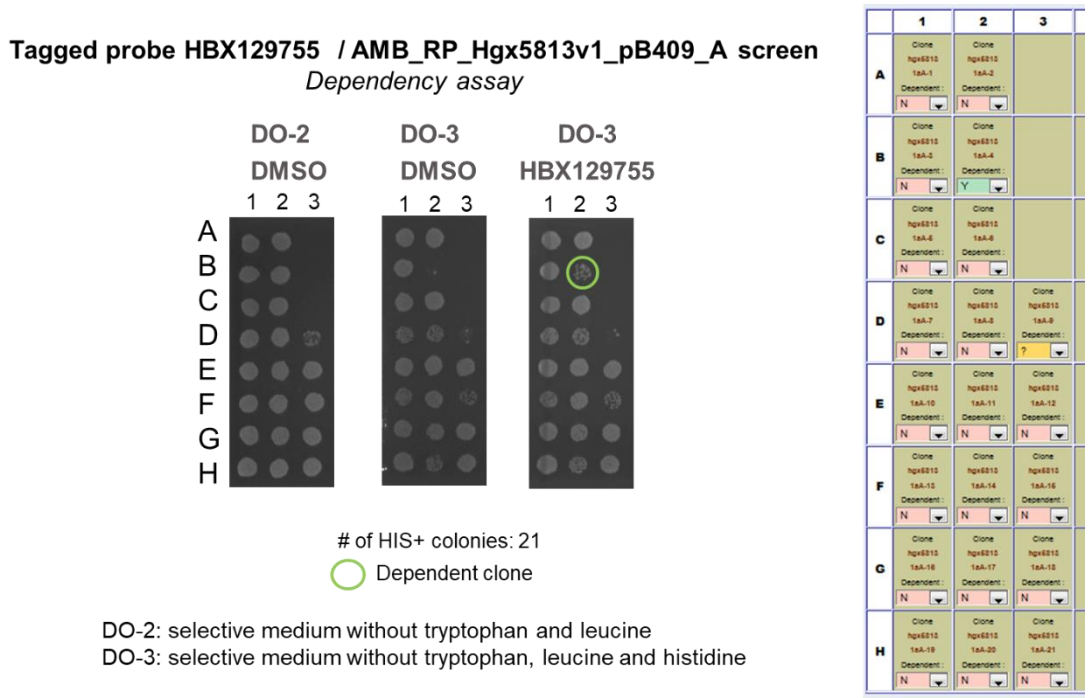
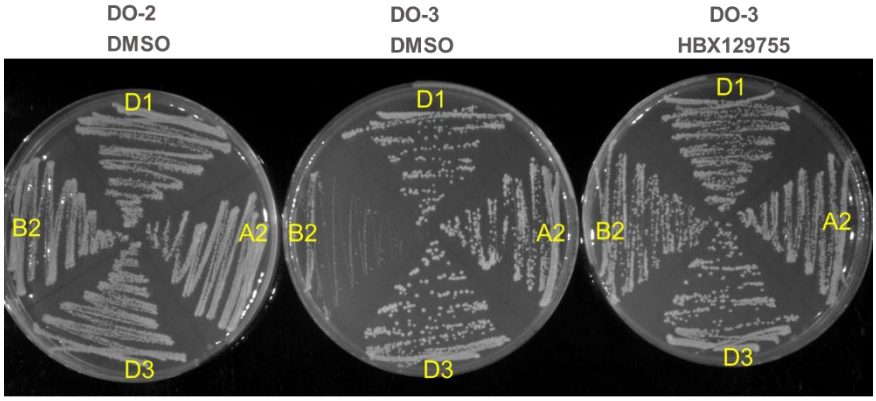


Figure 12: AMB_RP2_hgx5813v1_pB409_A dependency assay

The dependency assay showed only one clone (AMB_RP2_hgx5813v1_pB409_A-4) to be dependent on the presence of the tagged probe HBX129755. There were some ambiguities regarding the dependency of this clone, as the sequencing results indicated 9 clones corresponding to the same gene Zfp87 (list below)

Clone Name	Type Seq	Gene Name (Best Match)	Start..Stop (nt)	Frame	Sense	%Id 5p	%Id 3p	PBS
pB409_A-17	5p/3p	Mus musculus - Zfp87	891..1538	IF		99.3	99.5	N/A
pB409_A-21	5p/3p	Mus musculus - Zfp87	891..1538	IF		99.3	99.5	N/A
pB409_A-9	5p/3p	Mus musculus - Zfp87	891..1538	IF		96.0	97.0	N/A
pB409_A-12	5p/3p	Mus musculus - Zfp87	891..1538	IF		99.3	99.5	N/A
pB409_A-14	5p/3p	Mus musculus - Zfp87	891..1538	IF		99.3	99.5	N/A
pB409_A-2	5p/3p	Mus musculus - Zfp87	891..1538	IF		99.3	97.8	N/A
pB409_A-16	5p/3p	Mus musculus - Zfp87	891..1538	IF		99.3	99.5	N/A
pB409_A-7	5p/3p	Mus musculus - Zfp87	936..1552	IF		99.5	99.5	N/A
pB409_A-4	5p/3p	Mus musculus - Zfp87	993..2046	IF		99.0	98.8	N/A

So, we checked again the dependency with streaks for few clones of this gene. The picture below showed that there was a difference between clone (AMB_RP2_hgx5813v1_pB409_A-4) and the other ones that are clearly non-dependent.



Well	Clone Name	Gene Name (Best Match)	Start	Stop	Dependency assay	Dependency checking with streaks
D3	AMB_RP2_hgx5813v1_pB409_A-9	Mus musculus - Zfp87	891	1538	?	No
A2	AMB_RP2_hgx5813v1_pB409_A-2	Mus musculus - Zfp87	891	1538	No	No
D1	AMB_RP2_hgx5813v1_pB409_A-7	Mus musculus - Zfp87	936	1552	No	No
B2	AMB_RP2_hgx5813v1_pB409_A-4	Mus musculus - Zfp87	993	2046	Yes	+/- Yes

We then performed a 1by1 assay to investigate this in more details. It is described in part 4.3 in this report.

We then screen the same cDNA library at a higher concentration to see if we can fish additional interactions.

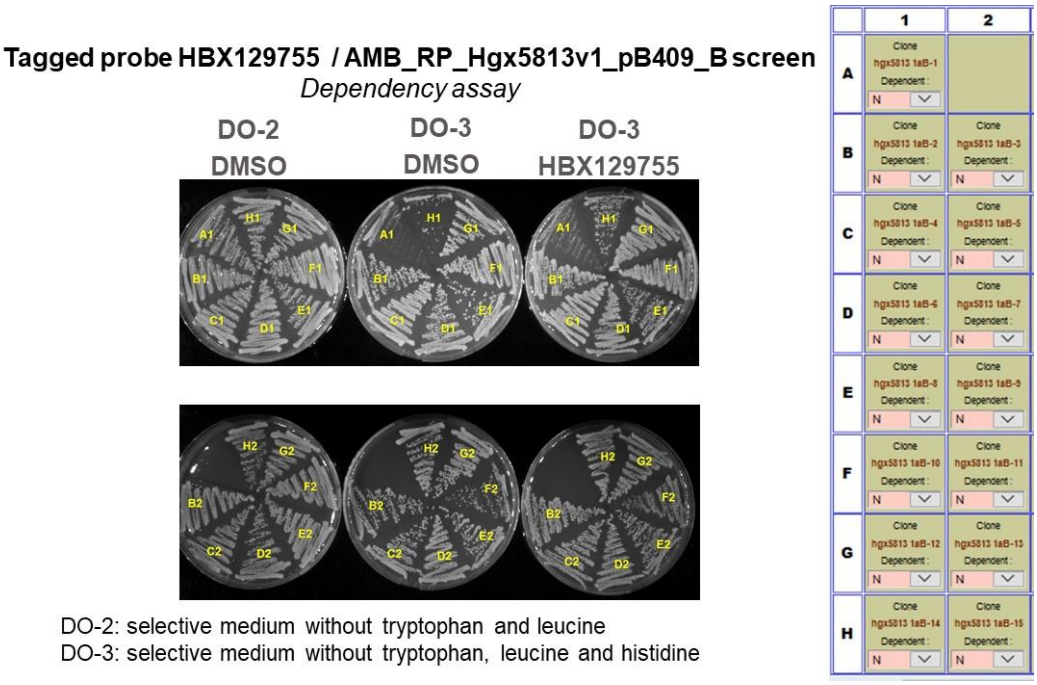


Figure 13: AMB_RP2_hgx5813v1_pB409_B dependency assay

None of the positive clones were shown dependent on the presence of the tagged probe HBX129755.

It was then decided to screen one of ours two main generic libraries (Human Placenta PLA and Human Lung Cancer cells HLUC), as they have a broad transcripts representation.

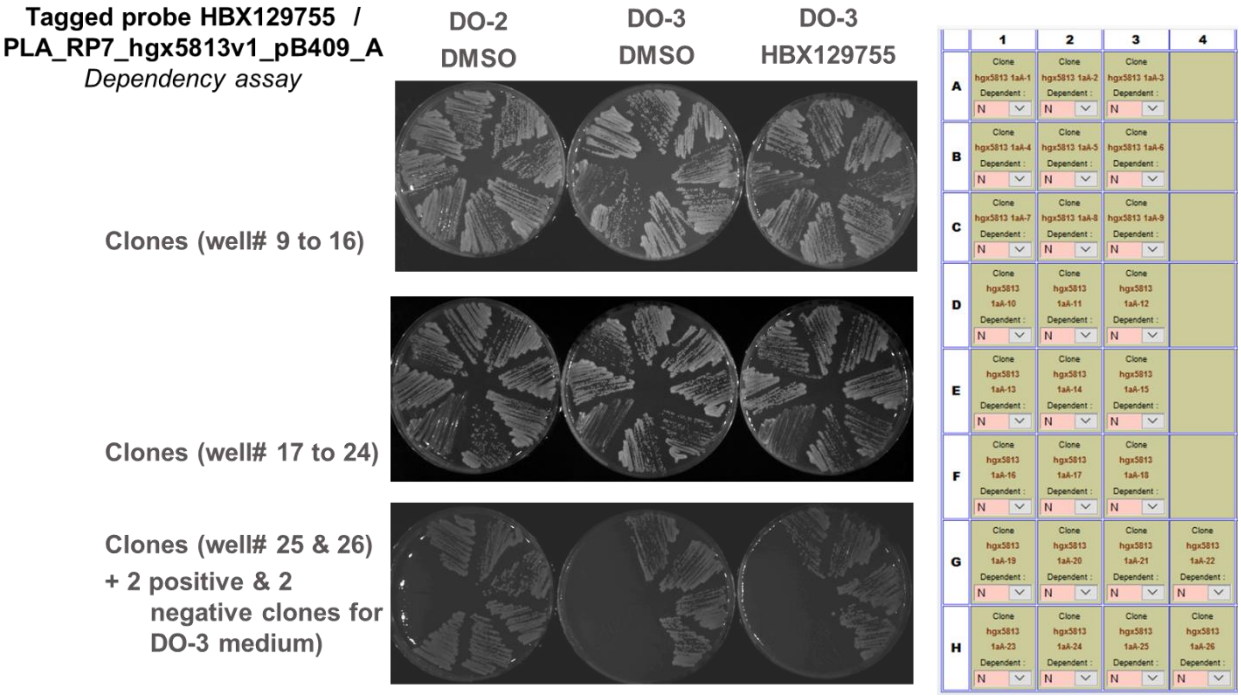


Figure 14: PLA_RP7_hgx5813v1_pB409_A dependency assay

The screen against the PLA library gave 26 positive clones but none of them were dependent on the tagged compound as shown above.

The list of the positive but non-dependent genes is displayed in separate documents (screen summary results). All genes are well-known false positive interactions that we regularly identified in our YChemH screens.

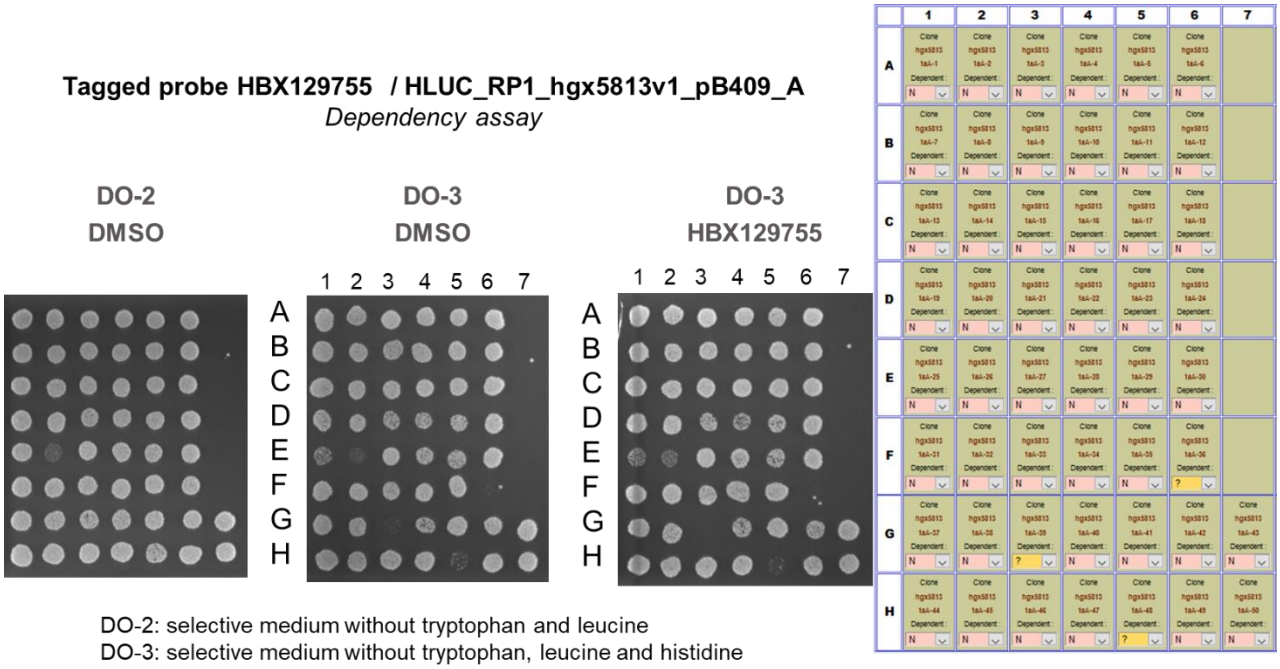


Figure 15: HLUC_RP1_hgx5813v1_pB409_A dependency assay

None of the clones from the screen were dependent on the presence of the tagged compound.

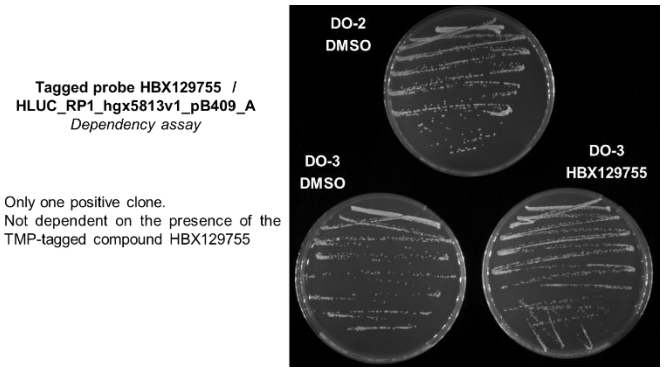


Figure 16: HBR_RP3_hgx5813v1_pB409_A dependency assay

4.2.2 HBX129756

We performed 2 YChemH screens with the tagged probe HBX129756. None of the colonies isolated were dependent on the presence of HBX129756.

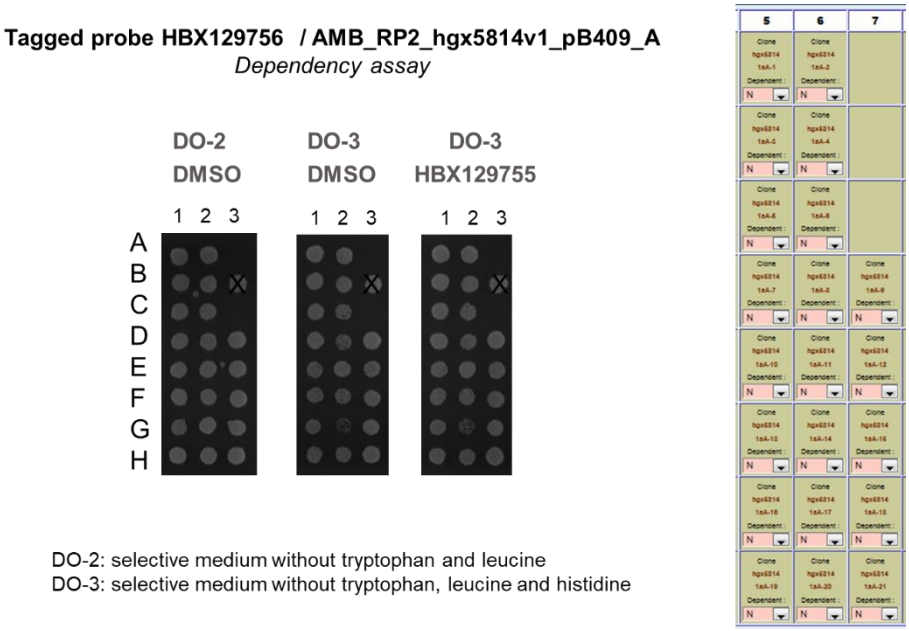


Figure 17: AMB_RP2_hgx5814v1_pB409_A dependency assay

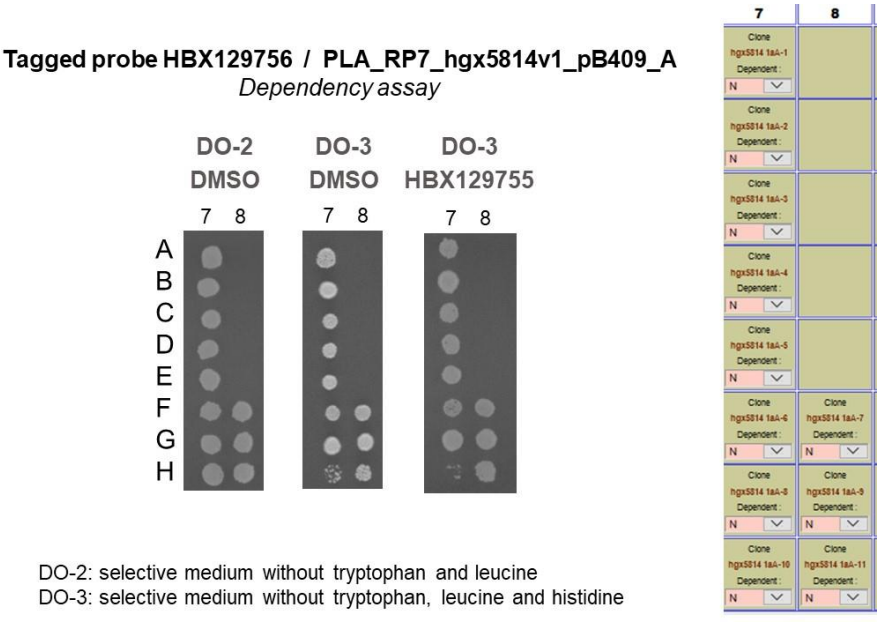


Figure 18: PLA_RP7_hgx5814v1_pB409_A dependency assay

4.3 1by1 YChemH assay

4.3.1 Principal

To check dependency and specificity for the identified protein preys, Hybrigenics' first validation assay consists in a 1-by-1 experiment whose principle is described in Figure 2. Each experiment involves one identified prey fragment and a tagged chemical probe (HBX129755). Interaction is confirmed if we observe yeast growth on selective medium. If yeast growth is observed with HBX129755, but not with any negative controls (HBX24786 Trimethoprim TMP, or HBX129634 TMP-PEG5-OH, DMSO alone), the interaction is considered as specific of the tested compound.

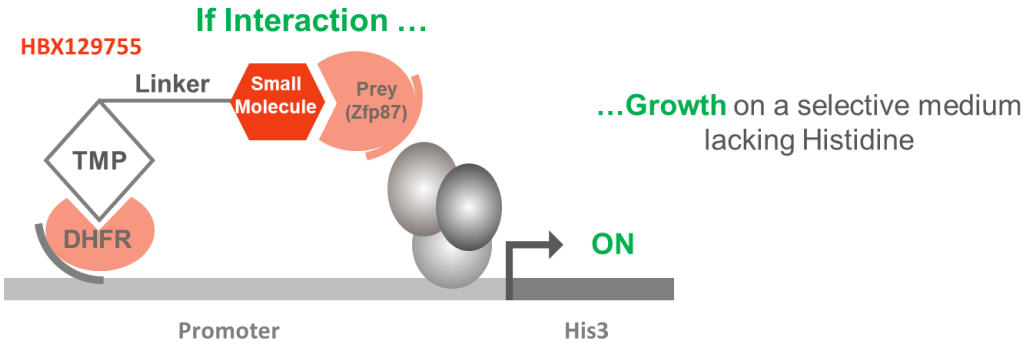


Figure 19 : *1-by-1 experiment for one Zfp87 prey fragment and HBX129755 TMP chemical probe*

4.3.2 Selected prey fragments

Two experimental fragments were selected for the 1-by-1 assay as indicated below, and one negative fragment.

Interaction #	HGX reference	Protein prey name	Dependency assay
1	Empty prey vector		/
2	AMB_RP2_hgx5813v1_pB409_A-4	zpf87	+/- yes
3	AMB_RP2_hgx5813v1_pB409_B-12	zpf87	no

Figure 20 : experimental fragment selected for the 1-by-1 assay

The plasmid from the experimental fragments were extracted from yeast cells, amplified in *E. coli* to obtain purified plasmid DNA, which was sequenced again to validate the sequence and subsequently re-transformed into fresh YHGX13 yeast cells.

A mini-mating was carried out between YHGX13 strains (*MAT*α) transformed with the prey plasmids and YPT6AT yeast cells (*MAT*a) transformed with the DHFR hook (Dihydrofolate reductase) to produce a diploid yeast culture.

4.3.3 Growth assay on +/-His plates (Drops method)

For each interaction, serial dilutions at OD_{600} 1, 1/10, 1/100 and 1/1000 of the diploid yeast culture expressing both hook and prey constructs were spotted on a solid selective medium without tryptophan, leucine and histidine and supplemented with the chemical compounds (as indicated below) and FK506. Interactions were tested in duplicate. One plate was used per chemical compound and concentration (DMSO, 1, 5, 10 and 20 µM of HBX129755, 10 µM of HBX24786 Trimethoprim (TMP) and 10 µM of HBX129634 (TMP-PEG5-OH)). Plates were incubated at 30°C for 3 days.

As shown in Figure 5, the experiment was designed as follows:

- 1 : we check normal growth of the clones on non-selective medium without any tagged compound (yeast growth independent of the interaction)
- 2 : DMSO only. This is a first negative control plate under selective medium. We expect no yeast growth. If yeast growth is observed, the interaction is not specific to the tested compound
- 3 : Selective medium with a chemical compound:
    - 1, 5, 10, and 20 µM of HBX129755
    - negative controls 10 µM of TMP and 10 µM of TMP-PEG5-OH

4.3.4 Results

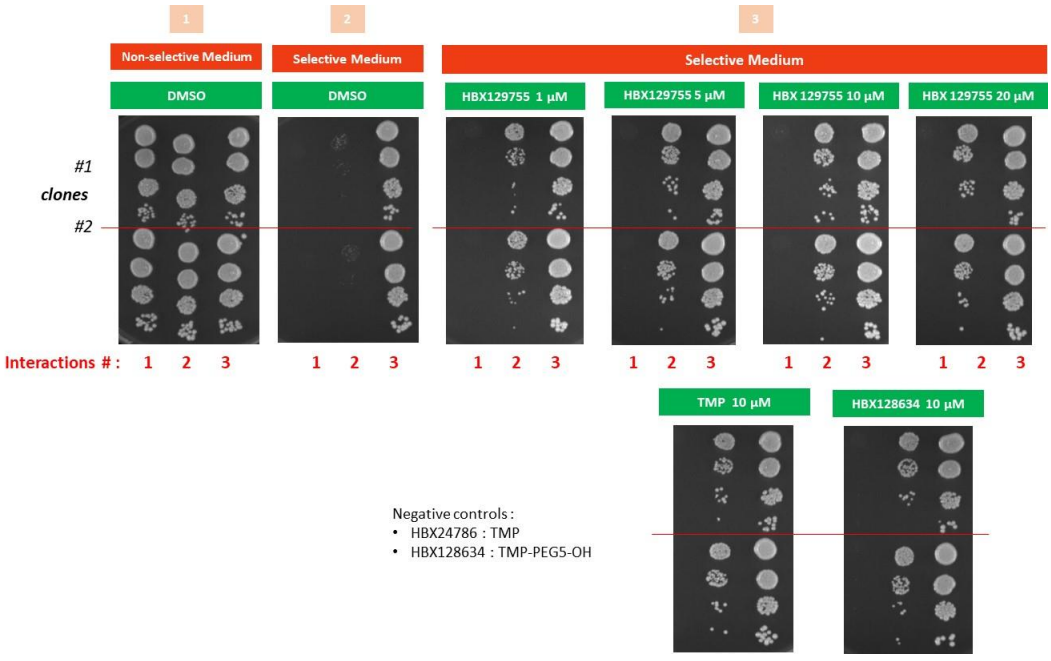


Figure 21 : Pictures from 1-by-1 experiment with HBX129755 chemical probe

Interaction #1: empty prey vector (negative control). No yeast growth

Interaction #3: clone AMB_RP2_hgx5813v1_pB409_B-12. This clone from the second screen wasn't dependent on the presence of the TMP-tagged probe HBX129755. The pictures shows that yeast growth happened with HBX129755, but also with all 3 other negative controls: DMSO, TMP and TMP-PEG5-OH. So, it's not specific of HBX129755

Interaction #2: clone AMB_RP2_hgx5813v1_pB409_A-4 that was shown dependent on the dependency assay. The binding is confirmed with HBX129755. There was no yeast growth with the DMSO plate, which was promising. But Yeast growth is observed with TMP and TMP-PEG5-OH negative controls. So, the interaction is not specific of HBX129755.

4.4 YChemH Screens Results Summary

Attached you will find the results summary for each screen as pdf documents. They only content non-dependent clones.

However, this type of documents is typically delivered and contain only the clones and corresponding prey proteins that are dependent of the tagged compound used in a YChemH screen.

For your project and for you to see what kind of results we got, we decided to keep the non-dependent clones. These clones are not relevant. They are usually false positives that we fish regularly with the technology. As an example, for the screens performed against PLA and HLUC libraries, two libraries that we often screen, confidence score (PBS, Predicted biological scores), most of the interactions received a E score, meaning that they are flagged as false positive as they come often in such screens. And we usually have a good correlation between the non-dependent colonies and the flagged false positives.

5 CONCLUSION

We performed a YChemH target ID study with two tagged chemical probes HBX129755 and HBX129756 that were shown to be permeable in yeast and not toxic for the yeast.

We started to screen the Mouse Adult Brain library. For both compounds, a low to medium range of HIS+ colonies were obtained and none of them were dependent on the tagged compounds.

We then screen some of our most generic libraries, the human placenta and the human lung cancer cells libraries, without any more success.

We carried out a total of 7 YChemH screens, and despite our efforts, these screens couldn't deliver any relevant prey protein for the ketamine metabolites.

Results Summary

YChemH SCREEN
HBX129755 - NCGC00687962-01
vs Human Lung Cancer_RP1

Tue, Aug 31, 2021 - 06:06 PM

Screen Parameters

Nature	cDNA
Chemical Probe	HBX129755 - NCGC00687962-01
Screen Name	HLUC_RP1_hgx5813v1
Prey Library	Human Lung Cancer_RP1
Vector(s)	pB409 (N-LexA-eDHFR-C)
Processed Clones	47 (pB409_A)
Analyzed Interactions	0 E0 (pB409_A)
Chemical Probe Concentration	1.0 µM (pB409_A)
Other Conditions	pH 4.5_5.5 (pB409_A)

All clones displayed in the list below are not dependent on the TMP-tagged compound HBX129755

Global PBS®

Global PBS (for Interactions represented in the Screen)		Nb	%
A	Very high confidence in the interaction	0	0.0%
B	High confidence in the interaction	0	0.0%
C	Good confidence in the interaction	0	0.0%
D	Moderate confidence in the interaction This category is the most difficult to interpret because it mixes two classes of interactions : - False-positive interactions - Interactions hardly detectable by the Y2H technique (low representation of the mRNA in the library, prey folding, prey toxicity in yeast)	4	50.0%
E	Interactions involving highly connected prey domains, warning of non-specific interaction. The threshold for high connectivity is 6. They can be classified in different categories: - Prey proteins that are known to be highly connected due to their biological function - Proteins with a prey interacting domain that contains a known protein interaction motif or a biochemically promiscuous motif	4	50.0%
F	Experimentally proven technical artifacts	0	0.0%
Non Appliable			
N/A	The PBS is a score that is automatically computed through algorithms and cannot be attributed for the following reasons : - All the fragments of the same reference CDS are antisense - The 5p sequence is missing - All the fragments of the same reference CDS are either all OOF1 or all OOF2 - All the fragments of the same reference CDS lie in the 5' or 3' UTR		

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Prey Fragment Analysis

Symbols	Means
✠	The fragment contains the full length ORF
↶	Fragment is fully in 5' UTR
↷	Fragment is fully in 3' UTR
x	Fragment contains at least one In Frame STOP codon in 5' UTR
x	Fragment contains at least one In Frame STOP codon in CDS
x	Fragment contains the natural STOP codon
[NR]	Fragment was found to be non relevant (poor quality, high N density)
IF OOF1 OOF2	With regard to the theoretical frame of each corresponding ORF (GenBank),fragments are cloned in frame (IF) if they are in the same frame as Gal4AD.In general, polypeptides synthesized from OOF fragments are not considered of biological interest, unless found together with another frame. However, some of the proteins expressed from an OOF fragment can be translated in the correct frame,due to the existence of natural frame-shift events during translation in yeast. No stop codons are displayed for OOF fragments.
??	Unidentified frame when : - The clone sequence is antisense - The 5p sequence is missing
N	Antisense
Start...Stop	Position of the 5p and 3p prey fragment ends, relative to the position of the ATG start codon (A=0)

Clone Name	Type Seq	Gene Name (Best Match)	Start..Stop (nt)	Frame	Sense	%Id 5p	%Id 3p	PBS
pB409_A-12	5p/3p	Homo sapiens - ARHGAP21	1998..3334	IF		100.0	99.7	E
pB409_A-50	5p/3p	Homo sapiens - ARHGAP21	2157..2993	IF		100.0	100.0	E
pB409_A-10	5p/3p	Homo sapiens - ARHGAP21	2157..3056	IF		99.8	100.0	E
pB409_A-34	5p/3p	Homo sapiens - ARHGAP21	2214..3551	IF		100.0	100.0	E
pB409_A-44	5p/3p	Homo sapiens - ARHGAP21	2274..3163	IF		100.0	100.0	E
pB409_A-37	5p/3p	Homo sapiens - ARHGAP21	2274..3163	IF		100.0	100.0	E
pB409_A-4	5p/3p	Homo sapiens - ARHGAP21	2274..3163	IF		100.0	100.0	E
pB409_A-49	5p/3p	Homo sapiens - ARHGAP21	2394..3050	IF		100.0	100.0	E
pB409_A-22	5p/3p	Homo sapiens - ARHGAP21	2397..2927	IF		100.0	100.0	E
pB409_A-40	5p/3p	Homo sapiens - CEL	145..31	??	N	99.0	100.0	N/A
pB409_A-25	5p/3p	Homo sapiens - CEL	145..31	??	N	100.0	100.0	N/A
pB409_A-7	5p/3p	Homo sapiens - CEL	145..31	??	N	100.0	100.0	N/A
pB409_A-17	3p	Homo sapiens - CEL	259..146	??	N		100.0	N/A
pB409_A-48	5p/3p	Homo sapiens - CENPA	-115..425 ✠ x	IF		98.5	98.9	D
pB409_A-35	5p/3p	Homo sapiens - DNM1L	-579..-89 ↶	OOF1		100.0	100.0	N/A
pB409_A-31	5p/3p	Homo sapiens - DNM1L	-549..-90 ↶	OOF1		98.0	98.8	N/A
pB409_A-27	5p/3p	Homo sapiens - DNM1L	-549..-90 ↶	OOF1		100.0	100.0	N/A
pB409_A-13	5p/3p	Homo sapiens - DNM1L	-549..-90 ↶	OOF1		100.0	99.8	N/A
pB409_A-45	5p/3p	Homo sapiens - ENO1	1242..653	??	N	100.0	99.8	N/A
pB409_A-38	5p/3p	Homo sapiens - HNRNPA2B1 varB1	834..1414 x	IF		96.8	85.0	D
pB409_A-28	5p/3p	Homo sapiens - PRDM15	1851..3398	IF		99.7	99.5	E
pB409_A-24	5p/3p	Homo sapiens - PRDM15	2124..3385	IF		100.0	99.5	E
pB409_A-20	5p/3p	Homo sapiens - PRDM15	2124..3385	IF		100.0	99.6	E
pB409_A-47	5p/3p	Homo sapiens - PRDM15	2124..3385	IF		100.0	99.8	E
pB409_A-9	5p/3p	Homo sapiens - PRDM15	2124..3385	IF		100.0	99.8	E
pB409_A-41	5p/3p	Homo sapiens - PRDM15	2127..3535	IF		99.8	99.8	E

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Clone Name	Type Seq	Gene Name (Best Match)	Start..Stop (nt)	Frame	Sense	%Id 5p	%Id 3p	PBS
pB409_A-32	5p/3p	Homo sapiens - PRDM15	2127..3535	IF		99.8	99.8	E
pB409_A-18	5p/3p	Homo sapiens - PRDM15	2127..3535	IF		90.8	92.0	E
pB409_A-15	5p/3p	Homo sapiens - PRDM15	2127..3535	IF		99.7	99.6	E
pB409_A-14	5p/3p	Homo sapiens - PRDM15	2127..3535	IF		99.8	99.5	E
pB409_A-11	5p/3p	Homo sapiens - PRDM15	2127..3535	IF		99.8	99.8	E
pB409_A-6	5p/3p	Homo sapiens - PRDM15	2127..3535	IF		98.0	97.0	E
pB409_A-1	5p/3p	Homo sapiens - PRDM15	2127..3535	IF		99.8	100.0	E
pB409_A-43	5p/3p	Homo sapiens - PRDM15	2304..3370	IF		99.5	99.8	E
pB409_A-30	5p/3p	Homo sapiens - PRDM15	2304..3370	IF		99.7	99.6	E
pB409_A-16	5p/3p	Homo sapiens - PRDM15	2304..3370	IF		99.5	99.8	E
pB409_A-8	5p/3p	Homo sapiens - SAFB	1155..2392	IF		99.8	99.8	E
pB409_A-23	5p/3p	Homo sapiens - SAFB	1218..2519	IF		99.8	97.9	E
pB409_A-29	5p/3p	Homo sapiens - SAFB	1218..2519	IF		100.0	98.5	E
pB409_A-39	3p	Homo sapiens - STAT3	..2077	??			99.6	D
pB409_A-36	5p/3p	Homo sapiens - STAT3	96..2077	IF		99.7	100.0	D
pB409_A-3	5p/3p	Homo sapiens - TXNDC5	1419..993	??	N	99.8	99.8	N/A
pB409_A-42	5p/3p	Homo sapiens - ZNF585B	42..799	IF		100.0	99.6	E
pB409_A-5	5p/3p	Homo sapiens - ZNF585B	42..799	IF		99.8	99.3	E
pB409_A-26	5p/3p	Homo sapiens - ZSCAN25	1889..-3	??	N	100.0	99.8	N/A
pB409_A-21	5p/3p	Homo sapiens - GenMatch	-1..608 X	IF		100.0	99.8	N/A
pB409_A-2	5p/3p	Homo sapiens - GenMatch	-1..190 X	IF		100.0	70.3	D

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