

# Quantitative measurement of multiple signal transduction pathway activities in cell and tissue culture, including cancer, fibroblasts, and immune cell types: a new way forward to standardization of cell culture experiments



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## Summary

**Problem:** phenotypic differences between genotypically identical cell lines interfere with experimental reproducibility across labs and effective drug development. Reference: Ben-David U, *et al.* Nature. 2018 Aug;560(7718):325-330.

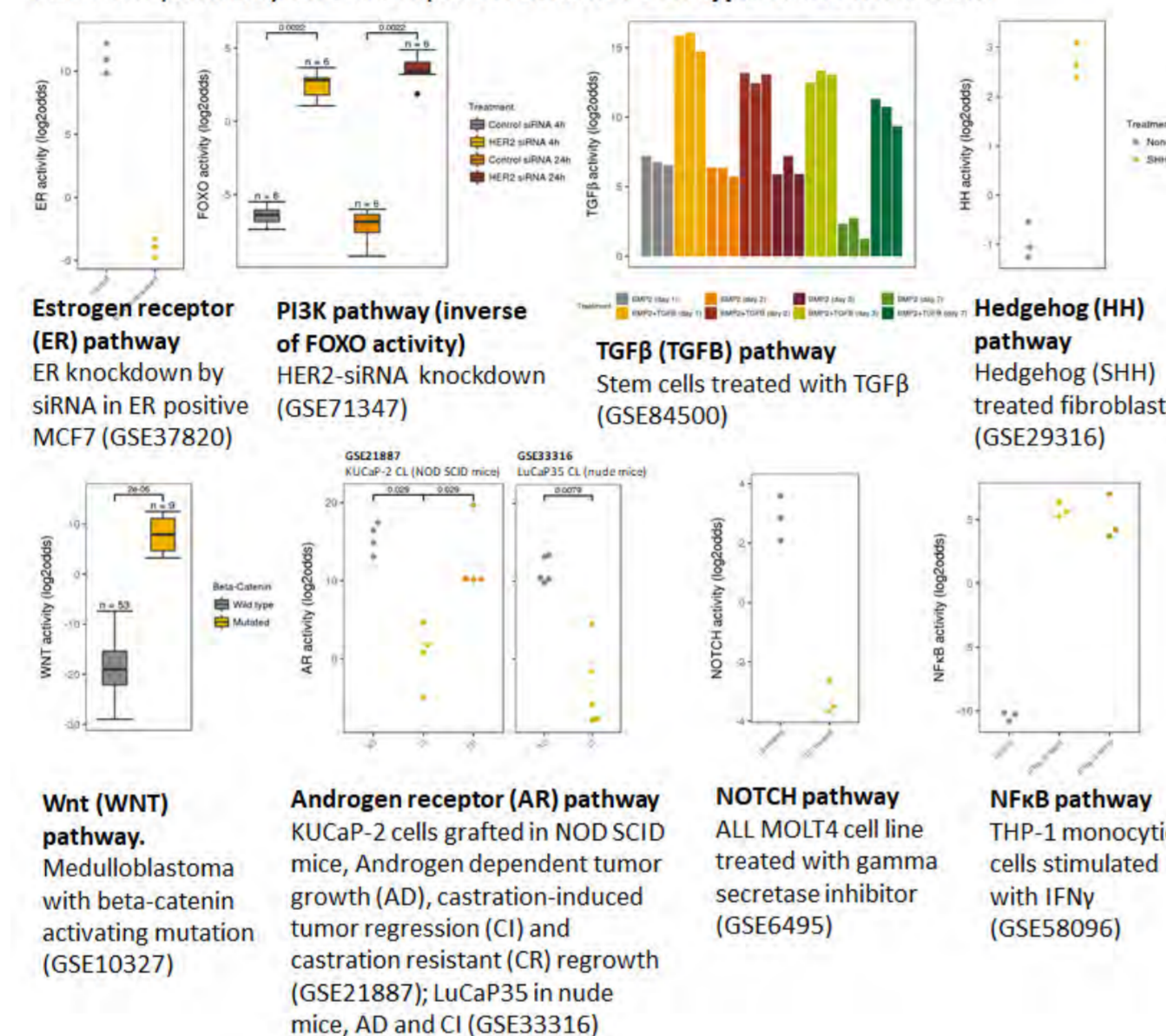
**Solution:** a novel *biologically validated* method for quantitative measurement of activity of the estrogen receptor (ER), androgen receptor (AR), FOXO-PI3K, Wnt, TGFβ, Hedgehog, Notch, NFκB, MAPK-AP1, JAK-STAT1/2 and STAT3 signal transduction pathways in *any cell or tissue type*. The method is based on knowledge-based Bayesian models which interpret measurements of target mRNAs of pathway-associated transcription factors to provide quantitative pathway activity scores on individual samples.

## Assays for quantitative measurement of signal transduction pathway activity

Activity level of different signal transduction pathways is assessed by measuring mRNA expression of downstream target genes of pathway transcription factors using Affymetrix HG-U133Plus2.0 or RT-qPCR (for FFPE tissue). Knowledge-based Bayesian models translate mRNA expression levels into a quantitative pathway activity score as described in Cancer Res 2014;74(11):2936-45.

## Biological validation of pathway models on different cell types

Pathway models have been calibrated on one cell type. For each pathway one example on another cell type is shown below.



## Analysis of signaling pathway activity to quantitatively characterize cell lines and compare across different labs

MCF-7 breast cancer cell line

LNCaP prostate cancer cell line

MAPK-AP1 (AP1), AR, ER, PI3K-FOXO, Hedgehog (HH), NFκB, Notch, JAK-STAT1/2 type I interferon (IFN), JAK-STAT1/2 type II IFN, STAT3, TGFβ, Wnt pathway activities were measured per cell sample. Note: FOXO is the reverse of PI3K pathway activity (in the absence of oxidative stress, van Ooijen, Am.J.Pathol, 2018).

Culture conditions	Dataset	GEO sample annotation	Pathway Activity (log2)											
			AP1	AR	ER	FOXO	HH	NFκB	NOTCH	STAT1/2 (IFN I)	STAT3 (IFN II)	TGFβ	WNT	
Estradiol depleted	GSE19639	MCF-7, rep 1	-14.8	-17.8	-4.7	-3.9	-8.2	-10.2	0.5	-3.7	-4.8	3.5	-17.0	-16.7
		MCF-7, rep 2	-14.4	-17.4	-4.5	-4.4	-9.1	-10.4	1.4	-3.8	-4.8	3.2	-17.7	-17.5
		MCF-7, rep 3	-14.3	-17.9	-3.4	-4.2	-8.4	-9.4	1.2	-4.1	-4.6	4.0	-16.4	-17.2
	A3753	MCF7, control, replicate #1	-15.0	-16.9	-7.0	-0.9	-11.2	-21.0	2.8	-7.7	-8.0	-0.4	-17.4	-20.2
		MCF7, control, replicate #2	-14.8	-15.0	-5.8	0.6	-11.4	-20.3	3.0	-7.8	-7.6	-0.6	-16.7	-20.0
		MCF7, control, replicate #3	-14.3	-10.5	-5.5	1.6	-12.7	-20.5	4.6	-7.8	-8.6	-1.2	-16.0	-16.2
Standard culture	GSE79761	MCF7 Veh Rep1	-11.8	-13.3	-10.8	0.4	-12.8	-16.7	10.5	-7.5	-8.9	-2.7	-13.7	-15.8
		MCF7 Veh Rep2	-14.5	-17.3	-8.3	3.3	-14.6	-17.7	3.3	-8.2	-9.9	-3.0	-19.4	-8.5
		MCF7 Veh Rep3	-12.4	-12.5	-14.4	1.9	-13.7	-15.5	10.0	-8.3	-9.5	-2.6	-9.5	-17.2
	GSE95529	Gr. 1 = MCF7C WT Control 1	-12.2	-18.6	0.7	-5.2	-10.4	-15.6	1.4	-8.8	-10.1	4.5	-15.3	-13.5
		Gr. 1 = MCF7C WT Control 2	-11.5	-19.8	0.5	-5.4	-10.8	-16.1	1.7	-9.1	-9.8	4.9	-15.6	-14.4
		Gr. 1 = MCF7C WT Control 3	-11.4	-18.7	-0.6	-5.3	-10.1	-16.8	2.3	-9.0	-10.3	5.4	-14.8	-13.5
	GSE1232	Parental.replicate1	-10.7	-8.4	8.1	-6.3	-13.0	-16.1	12.7	-8.2	-9.0	3.5	-12.3	-14.8
		Parental.replicate2	-10.8	-8.7	8.6	-7.0	-12.8	-17.1	11.5	-8.1	-8.8	4.2	-12.8	-15.2
		Parental.replicate3	-13.5	-10.4	6.9	-5.7	-13.2	-16.6	9.8	-7.5	-8.6	2.1	-12.7	-18.2
	GSE43657	MCF7 cell line	-12.4	-14.7	-4.6	-4.5	-9.9	-14.0	12.2	-8.1	-8.2	0.5	-11.2	-16.9
		MCF7 sphere	-10.3	-14.6	-8.7	-5.1	-8.6	-11.0	17.3	-7.7	-8.6	0.9	-16.6	-17.1
		MCF7_Mock	-11.8	-15.1	9.3	-2.5	-12.2	-18.3	12.9	-8.6	-9.5	3.8	-15.6	-17.3
Unknown	GSE8742	Rep. 1 for MCF7untreated	-13.5	-20.6	17.7	-14.0	-12.5	-15.2	11.4	-7.5	-8.0	4.4	-15.8	-17.9
		Rep. 2 for MCF7untreated	-13.4	-19.9	19.3	-13.4	-12.5	-15.9	12.3	-8.0	-8.4	4.5	-15.5	-17.5
		Rep. 3 for MCF7untreated	-13.3	-19.9	18.4	-13.6	-12.2	-15.4	12.2	-7.8	-8.1	4.4	-15.8	-17.8
	GSE10890	Rep. 1 for MCF7control	-14.1	-19.4	18.9	-11.3	-11.9	-15.5	12.2	-7.8	-8.6	4.4	-15.9	-17.6
		Rep. 2 for MCF7control	-13.6	-19.9	18.3	-12.6	-11.9	-15.1	12.4	-7.4	-8.5	4.3	-16.3	-17.1
		Rep. 3 for MCF7control	-13.8	-19.8	18.5	-11.6	-11.6	-14.8	12.6	-7.7	-8.5	4.4	-16.7	-17.8
EMTAB37	MCF7 - Replicate 1	-11.2	-16.2	4.8	-11.4	-12.1	-18.5	11.2	-7.2	-7.7	3.1	-14.8	-17.9	
	MCF7 - Replicate 2	-12.2	-15.2	1.2	-8.6	-11.5	-15.7	4.3	-7.9	-9.3	-0.3	-17.6	-17.2	
	MCF7 - Replicate 3	-11.5	-15.6	4.4	-10.2	-12.4	-18.4	9.8	-8.1	-8.9	1.2	-15.9	-17.9	

MCF-7 breast cancer cell line, reported culture conditions:

- GSE19639 estrogen depletion. estrogen-depleted (LTED), 10% dextran-coated charcoal-treated fetal bovine serum (FBS) for 24 hrs. J Clin Invest 2010 Jul;120(7):2406-13;
- A3753 estrogen depletion. 48 hr estradiol deprivation, 10% dextran-coated charcoal-treated FBS. Unpublished.
- GSE79761 estrogen depletion. DMEM supplemented with 10% FBS, 1% penicillin/streptomycin. For estradiol stimulation: phenol red-free DMEM containing 2.5% charcoal-stripped serum for 4 days total (with a media change after 48h). Mol Cancer Res 2016 Aug;14(8):707-19;
- GSE95529 MEM. Breast Cancer Res Treat 2016 Jun;157(2):253-265;
- GSE51232 standard culture;
- GSE43657 DMEM supplemented with 10% FBS; spheroid culture in serum-free DMEM-F12 supplemented with 2% B-27, 20 ng/mL epidermal growth factor, 5 µg/mL insulin, and 0.4% BSA. Biomed Res Int 2013;2013:510905.
- GSE8742 no information on cell culture.
- GSE10890 no information on cell culture
- EMTAB37 no information on cell culture

Culture conditions	Dataset	GEO sample annotation	Pathway Activity (log2)											
			AP1	AR	ER	FOXO	HH	NFκB	NOTCH	STAT1/2 (IFN I)	STAT3 (IFN II)	TGFβ	WNT	
Mixed standard and androgen deprived	GSE8702	LNCaP_Control_Time0	-15.4	8.0	-17.5	0.8	-12.1	-20.4	-3.3	-8.2	-10.3	3.8	-18.1	-13.5
		LNCaP_Control_3week	-14.4	14.7	-20.4	2.8	-11.7	-19.7	-0.6	-8.2	-10.1	1.3	-14.7	-13.6
		LNCaP_AndrogenDeprived_3week_1	-12.0	-16.2	-20.8	4.3	-14.4	-21.9	-1.1	-9.7	-9.5	0.1	-16.4	-15.0
		LNCaP_AndrogenDeprived_3week_2	-11.7	-16.4	-22.4	2.9	-14.9	-19.8	-0.8	-9.2	-9.7	0.3	-18.8	-11.7
		LNCaP_Control_1month	-14.3	14.0	-20.5	2.1	-12.2	-18.2	-0.5	-8.4	-9.5	0.8	-15.1	-12.7
		LNCaP_AndrogenDeprived_1month_1	-12.0	-13.8	-23.0	4.4	-13.8	-21.5	-0.6	-8.6	-9.0	-0.3	-16.7	-16.4
	GSE4589	LNCaP_Control_1month_2	-11.4	-15.8	-23.0	4.5	-12.7	-20.6	-0.9	-8.8	-9.4	-0.1	-17.9	-16.9
		LNCaP_Control_5month	-15.3	1.7	-17.8	1.8	-12.0	-17.7	-1.1	-8.0	-10.0	1.8	-17.6	-14.6
		LNCaP_AndrogenDeprived_5month_1	-14.6	-12.9	-19.8	1.2	-13.0	-15.0	-1.0	-10.3	-10.4	-1.2	-14.1	-8.7
		LNCaP_AndrogenDeprived_5month_2	-15.0	-13.2	-18.5	1.3	-12.5	-15.1	-0.9	-10.3	-11.0	-1.1	-13.5	-8.2
		LNCaP_Control_12month	-14.9	-11.8	-18.8	2.3	-11.1	-18.0	2.6	-8.6	-10.6	5.1	-17.5	-14.3
		LNCaP_AndrogenDeprived_12month_1	-15.7	-20.3	-20.8	-3.8	-10.6	-17.2	0.2	-9.7	-11.8	1.2	-17.1	-7.5
Androgen deprived	GSE39354	LNCaP_AndrogenDeprived_12month_2	-15.9	-20.6	-20.4	-3.2	-10.3	-17.5	0.9	-9.4	-11.1	0.6	-17.7	-7.5
		LNCaP_AndrogenDeprived_11month_1	-15.5	-20.5	-20.9	-1.8	-9.1	-14.7	1.4	-9.2	-11.7	1.8	-16.1	-10.8
		LNCaP_AndrogenDeprived_11month_2	-16.0	-20.8	-21.1	-1.2	-10.1	-14.6	1.1	-9.2	-11.6	2.3	-17.7	-10.6
	GSE40794	LNCaP_Control_CGF	-15.2	14.9	-15.4	1.2	-12.1	-18.3	0.2	-8.4	-10.0	3.0	-21.3	-17.2
		LNCaP + vehicle rep 1	-11.0	-14.9	-20.7	-1.0	-13.3	-16.1	0.5	-8.4	-9.5	-2.6	-16.3	-18.0
		LNCaP + vehicle rep 2	-11.1	-15.4	-20.2	-1.8	-13.3	-15.5	0.3	-8.4	-9.2	-2.9	-16.6	-17.8
Standard culture	GSE56352	LNCaP-shControl-ETOH-A	-10.5	-4.1	-19.6	4.4	-9.9	-13.8	0.1	-8.7	-10.7	1.7	-18.2	-14.2
		LNCaP-shControl-ETOH-B	-11.7	-4.8	-20.1	4.7	-10.3	-18.0	-0.3	-9.0	-11.0	1.7	-17.5	-14.4
		LNCaP-shControl-ETOH-C	-11.2	-5.5	-20.0	4.1	-9.7	-17.7	0.0	-8.8	-10.5	1.7	-18.4	-14.5
	GSE36133	LNCaP-GFP-ETOH-1	-15.3	-12.2	-19.0	4.5	-10.7	-18.1	-0.4	-9.2	-10.5	-0.4	-19.8	-13.1
		LNCaP-GFP-ETOH-2	-14.0	-11.2	-15.1	2.2	-11.6	-19.5	0.4	-9.3	-11.0	0.5	-20.5	-14.1
		LNCaP-GFP-ETOH-3	-14.9	-6.8	-16.0	3.9	-12.3	-20.2	0.6	-9.2	-10.9	2.5	-19.9	-14.1
Standard culture	GSE56352	LNCA P DMSO vehicle at 24 hrs, Biological rep1	-7.5	19.8	-12.3	-8.8	-11.1	-11.6	3.3	-8.2	-10.2	4.5	-15.1	-11.4
		LNCA P DMSO vehicle at 24 hrs, Biological rep2	-7.5	22.5	-12.4	-8.3	-12.2	-12.5	3.5	-8.4	-10.5	4.6	-15.1	-10.4
		LNcaP	-14.1	25.2	-14.3	1.8	-6.8	-16.8	0.0	-8.3	-10.4	7.1	-16.3	-14.6
GSE36133	LNcaP clone FGC	-13.6	27.0	-14.2	9.1	-9.5	-20.2	-3.3	-9.8	-11.5	2.1	-16.4	-16.3	

LNcaP prostate cancer cell line, reported culture conditions:

- GSE8702 Androgen proficient and androgen deprivation. RPMI plus 10% FBS, 1% P/S; for deprivation culture in charcoal/dextran filtered FBS. Prostate 2008 May 15;68(7):698-714..
- GSE34589. Androgen deprivation. RPMI 1640 with 10% charcoal stripped FBS 10% FBS, 100 units/ml penicillin, 100 g/ml streptomycin, 2 mM L-glutamine mixture; sodium pyruvate (1 mM) for 48 hrs. J Biol Chem 2013 Apr 19;288(16):11047-65.
- GSE39354 Androgen deprivation. Standard culture and hormone-depletion for 48 hrs (not specified). Genes Dev 2013 Mar 15;27(6):683-98.
- GSE40794 androgen proficient (5% Fetal Bovine Serum (FBS) in IMEM) or androgen depleted (5% Charcoal Dextran Treated (CDT)) media for 48 or 72 hours respectively. J Clin Invest 2013 Jan;123(1):493-508.
- GSE56352 RPMI-1640 medium containing 10% FBS, 2 mM GlutaMAX, 1 mM sodium pyruvate. No androgen deprivation Oncotarget 2013 Dec;4(12):2419-29.
- GSE57083 Standard culture. No publication
- GSE36133 Standard culture. Nature 2012 Mar 28;483(7391):603-7

## Analysis of signaling pathway activity to quantitatively compare Peripheral Blood Mononuclear Cells (PBMC) across labs

PBMCs obtained from peripheral blood of healthy individuals. MAPK-AP1 (AP1), AR, ER, PI3K-FOXO, Hedgehog (HH), NFκB, Notch, JAK-STAT1/2 type I interferon (IFN), JAK-STAT1/2 type II IFN, STAT3, TGFβ, Wnt pathway activities were measured per cell sample. Note: FOXO is the reverse of PI3K pathway activity (in the absence of oxidative stress, van Ooijen, Am.J.Pathol, 2018).

Cell type	Dataset	GEO sample annotation	Pathway Activity (log2)												
			AP1	AR	ER	FOXO	HH	NFκB	NOTCH	STAT1/2 (IFN I)	STAT3 (IFN II)	TGFβ	WNT		
PBMCs, donor	GSE11103	PBMCs, donor	-9.3	-10.6	-10.9	4.4	-12.0	0.9	-6.0	-8.1	-7.4	-7.5	-13.7	-20.6	
		PBMCs, donor	-6.8	-10.8	-14.6	4.2	-9.5	2.4	-4.6	-6.5	-5.6	-8.3	-14.1	-24.5	
		PBMCs, donor	-7.7	-10.9	-11.0	9.0	-13.0	1.6	-3.4	-7.1	-7.1	-5.7	-11.8	-23.1	
	GSE11281	PBMCs, donor	-7.8	-11.0	-11.5	6.8	-13.0	0.2	-3.7	-6.8	-6.2	-6.4	-11.4	-19.7	
		PBMCs untreated donor1	-3.8	-8.8	-13.7	5.9	-13.4	26.8	-0.4	2.9	0.8	19.0	4.6	-26.5	
		PBMCs untreated donor2	-5.2	-8.5	-14.2	7.4	-15.0	26.2	1.8	5.1	4.9	15.9	8.0	-19.0	
	PBMC controls	GSE17114	PBMCs untreated donor3	-5.4	-8.7	-14.0	8.3	-13.4	25.8	1.6	2.3	0.1	15.0	5.0	-20.9
			peripheral blood mononuclear cells	-6.8	-10.4	-11.1	10.7	-11.5	6.5	-1.5	-8.7	-8.2	-6.7	-9.4	-19.8
			peripheral blood mononuclear cells	-5.3	-11.0	-11.7	4.0	-9.6	4.0	-2.1	-7.3	-7.1	-4.4	-13.1	-15.8
		GSE27562	peripheral blood mononuclear cells	-9.5	-11.1	-11.6	5.0	-12.0	2.8	-2.2	-8.4	-7.8	-7.5	-10.4	-18.9
			peripheral blood mononuclear cells	-8.3	-11.5	-12.5	11.7	-12.3	15.4	-1.7	-7.9	-7.7	-2.5	-10.3	-19.7
			peripheral blood mononuclear cells	-8.4	-10.8	-11.3	16.3	-10.5	15.7	-3.2	-7.5	-7.2	3.0	-7.8	-21.1
GSE43210		peripheral blood mononuclear cells	-9.3	-10.8	-9.2	11.8	-9.0	6.4	0.9	-8.1	-8.5	-4.9	-9.7	-20.4	
		peripheral blood mononuclear cells	-9.9	-11.0	-13.1	7.1	-9.9	4.2	-4.1	-7.8	-7.8	3.2	-13.3	-20.8	
		peripheral blood mononuclear cells	-8.7	-12.0	-12.1	14.5	-14.1	9.5	-1.5	-7.7	-7.3	-4.2	-9.0	-20.1	
GSE43210		peripheral blood mononuclear cells	-6												