

NormaCurve: a SuperCurve-based method that simultaneously quantifies and normalizes Reverse Phase Protein Array data

S. Troncale, A. Barbet, L. Coulibaly, E. Henry, **B. He**, E. Barillot, T. Dubois, P. Hupé, L. de Koning.

Institut Curie, Paris, France

Contact: normacurve@curie.fr

NORMALIZED DATA

Normalized data correspond to relative protein levels estimated by NormaCurve for each sample. Dilution factors no longer appear, since NormaCurve uses all dilution factors to estimate one single value per sample. Technical replicates no longer appear either, since NormaCurve calculates the mean of all replicates. Extraction buffer and blanks are not taken into account and eliminated by NormaCurve.

For details concerning experimental procedures and normalization by NormaCurve, please refer to the publication.

The following arrays are available:

00152292	Chk2 labeling
00152293	negative control (CTRL) array
00152294	Chk2 labeling
00152295	negative control (CTRL) array
00152296	Chk2 labeling
00152297	negative control (CTRL) array
00152298	Chk2 labeling
00152299	negative control (CTRL) array
00152300	Chk2 labeling
00152391	negative control (CTRL) array
00152287	Sypro Ruby total protein stain
00152288	Sypro Ruby total protein stain
00152289	Sypro Ruby total protein stain
00152290	Sypro Ruby total protein stain
00152291	Sypro Ruby total protein stain

For several extracts, starting concentrations of 0.8, 0.9, 1, 1.1 and 1.2 mg/ml were used, complemented or not with respectively 0.033, 0.038, 0.042, 0.046 and 0.05 ng/ml purified Chk2. The aim of these varying starting concentrations is to introduce a variability in the spotted amount of total protein, in order to test the ability of our models to correct for this.

Dissection of sample names:

BSA	Bovine serum albumin
3T3, MCF10A, BT20, jurkat, T47D	cell line used
+SVF	grown in presence of fetal calf serum

-SVF	grown in absence of fetal calf serum (serum starved)
_Chk2	purified Chk2 has been added
_siRNA_Chk2-10_48h	short interfering RNA against Chk2 was transfected and cells were harvested 48h later. ¹
_siRNA_CTL_48h	control (nonsense) short interfering RNA was transfected and cells were harvested 48h later. ¹
_0.8 or 0.9 or 1 or 1.1 or 1.2	starting concentration (of dilution 1) in mg/ml

¹ Please note that siRNA against Chk2 was found to be ineffective (no diminished Chk2 protein levels, neither in Western Blot nor in RPPA). These samples were therefore not taken into account in our publication.