Approach for Customer Antibody Validation
(antibody validation for RPPA analysis)

Big Data Publicly Available

CCLE mRNA data (Affymetrix): 1934 cell lines

https://portals.broadinstitute.org/ccle/browseData

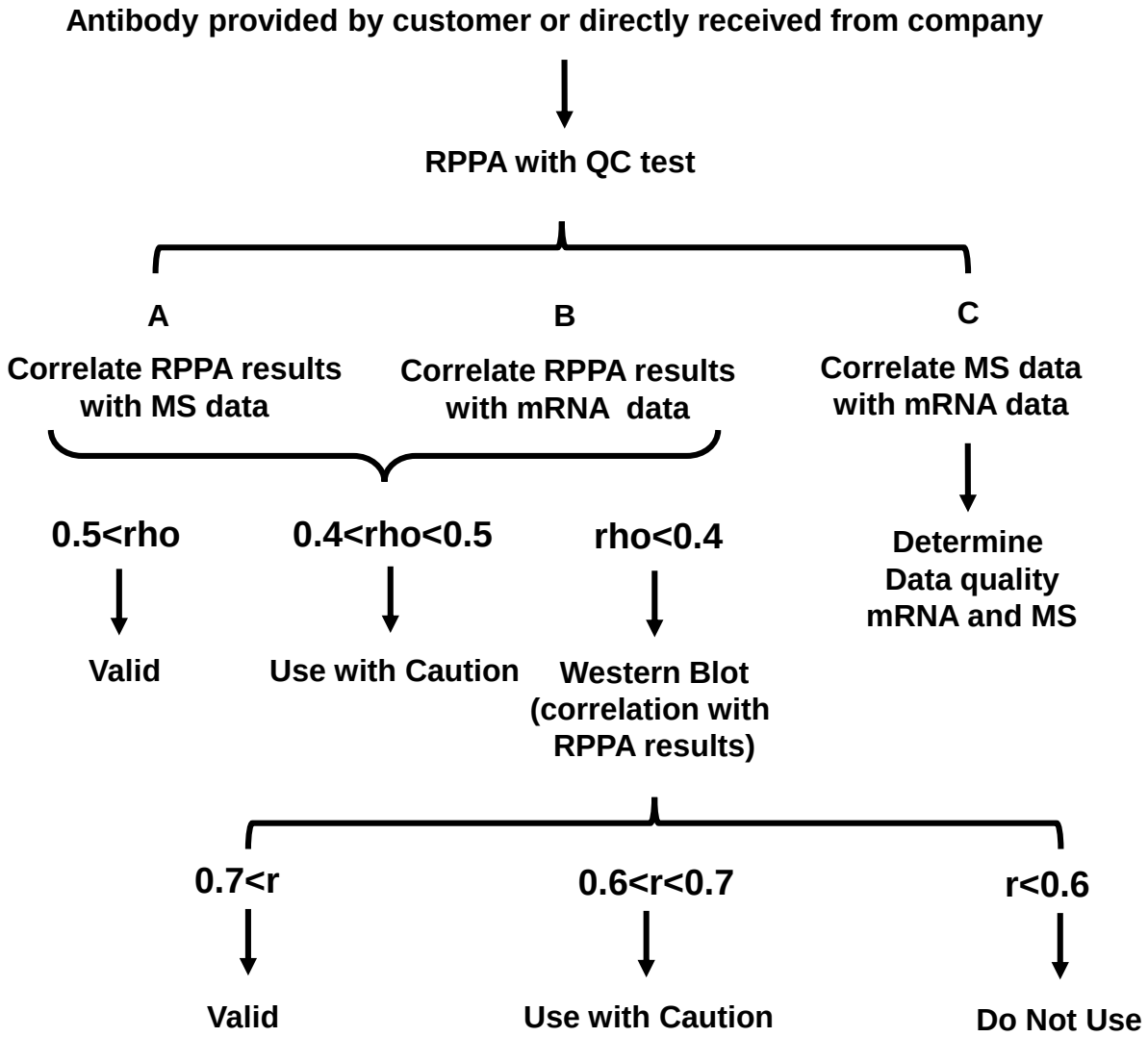
The data file has expression data for 54,675 probes corresponding to 20,067 unique genes.

Barretina, J., et al. 2012. The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. *Nature* 483: 603-607.

NCI60 MS data: 59 cell lines

http://129.187.44.58:7070/NCI60/

Gholami, A. M., et al. 2013. Global proteome analysis of the NCI-60 cell line panel. *Cell Rep* 4(3): 609-620.



Validation Process

1) Perform RPPA on cell line superslide with the antibody provided by customer or received directly from company.
2) Analyze RPPA results with QC test.
3) Correlate RPPA results with mRNA and/or MS data (Spearman Correlation).
4) If rho >0.5, the antibody is “Valid” for RPPA analysis.
5) If rho <0.5 but >0.4, the antibody status is “Use with Caution” for RPPA analysis.
6) If rho <0.4, the antibody status is “Do Not Use” for RPPA analysis. We will perform a western blot and review results for final status.
7) For antibodies targeting modified proteins (phosphorylation or cleavage, etc.), we emphasize on western blot results. Correlate RPPA results with western blot results.
    - If r >0.7, the antibody is “Valid”.
    - If r <0.7 but >0.6, antibody is “Use with Caution”.
    - If r <0.6, antibody is “Do Not Use”.

Example: RSK1 CST #8408 Lab ID #2425 Status: Valid

WB: 1:1000 RPPA: 1:500 GxR

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