CRISPR Gene Editing and Metabolic Phenotype Expression in a Haploid Cell Line

Summary

Clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-Cas systems are revolutionizing the field of genome editing. With the capability to achieve highly flexible and specific targeting, CRISPR-Cas systems can be modified and redirected to become powerful tools for site specific mutagenesis in mammalian cells.

Biolog's OmniLog® Phenotype MicroArray™ system can now be used in conjunction with CRISPR-Cas systems to enable genotype-phenotype studies with mammalian cell models, as has already been done for 14 years with microbial cells (1). One can directly analyze for phenotypic changes resulting from CRISPR gene editing.

Results

HAP1 is an adherent human cell line that is near-haploid. For this experiment, the CRISPR/Cas9 system was used to engineer a specific gene knockout of mannose-6-phosphate isomerase (MPI), an enzyme catalyzing the interconversion of mannose-6-phosphate and fructose-6-phosphate.†

Since the engineered cell lines are single cell clones bearing frameshift mutations, they are "complete" knockouts (2).

The resulting cell line, HAP1_MPI_00124-16, was compared to its isogenic precursor (HAP1) with Biolog PM-M plates containing diverse carbon sources and using standard assay protocols and data analysis (3). In Figure 1, the mutant cell line (HAP1_MPI_00124-16-- color-coded green) is directly compared to the control cell line (HAP1-- color-coded red). Where they overlap in substrate utilization is color-coded yellow. It is immediately apparent that for the mutant cell line, the ability to utilize mannose as an alternative energy source is significantly reduced compared to the HAP1 cells (Figure 1, well C-5).

To further confirm the genotype-phenotype relationship between HAP1 cells and the MPI knockout strain, Biolog IF-NB media (Biolog IF-M1 with no buffering capacity) was used with trypsinized cells from both cell lines in PM-M1 plates as described in the figure legend (Figure 2) to assess cell metabolism induced "acidification" of the medium using Phenol Red as a pH indicator. While both cell lines clearly utilize glucose (wells B-4, 5, 6) with resultant acidification of the medium, the HAP1_MPI_00124-16 mutant cells fail to acidify well C-5 containing mannose.

† These haploid cell lines were created and generously provided by Haplogen Genomics GmbH.

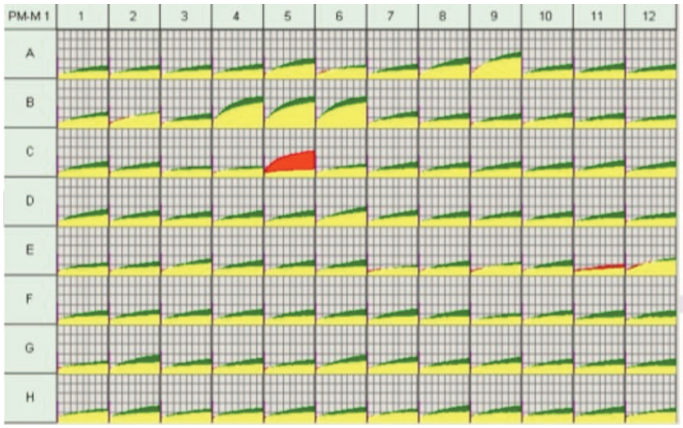

Figure 1: Comparison of HAP1 and HAP1_MPI_00124-16 (MPI knockout) on Biolog Redox Dye MB reduction. *Both cell lines were dispensed into PM-M1 MicroPlates at 20,000 cells per well in 50 uL IF-M1, supplemented with 0.3 mM Gln, 5% FCS and Pen/Strep. Plates were incubated at 37 in 5% CO_2 for 20 hrs before adding 10 µL of Dye MB to each well and placing in a 37° C OmniLog. Dye reduction was measured over 10 hrs and is the average of 4 runs.*

Conclusions

The CRISPR-Cas technology is a powerful tool for genomic editing. Biolog's Phenotype MicroArray system is a multiplexed, label free technology that enables efficient testing of hundreds of phenotypes to assess which ones change as a consequence of the engineered mutation. The OmniLog Phenotype MicroArray system is an ideal companion technology to CRISPR gene editing technology. It is a fast, reliable, sensitive and cost effective method for measuring metabolic and other phenotypic changes correlated with gene alterations in genotype-phenotype studies.

In the examples shown with the HAP1 and HAP1-derived cell lines, we evaluated different carbon energy substrate pathways to obtain a metabolic fingerprint of isogenic cell lines. Metabolic phenotypes are relevant in testing any gene that affects cellular metabolism in important areas of research such as cancer, diabetes, obesity, nutrition, toxicology, etc. However, in addition to carbon metabolism, the Phenotype MicroArray technology can assay for phenotypic changes in nitrogen metabolism, mineral metabolism, effects of hormones, and sensitivity of cells to diverse anti-cancer agents, a total of nearly 1,400 phenotypes.

[1]Bochner B.R. (2003) New Technologies to Assess Genotype-Phenotype Relationships. Nat Rev Genet Apr;4 (4):309-14

[2]Haplogen Genomics GmbH, Campus Vienna Biocenter 5, 1030 Vienna, Austria

[3]Bochner B.R., Siri M., Huang R.H., Noble S., Lei X.-H., Clemons, P.A., Wagner, B.K., (2011) Assay of the Multiple Energy-Producing Pathways of Mammalian Cells. PLoS ONE 6(3):e18147. doi:10.1371/journal.pone.0018147

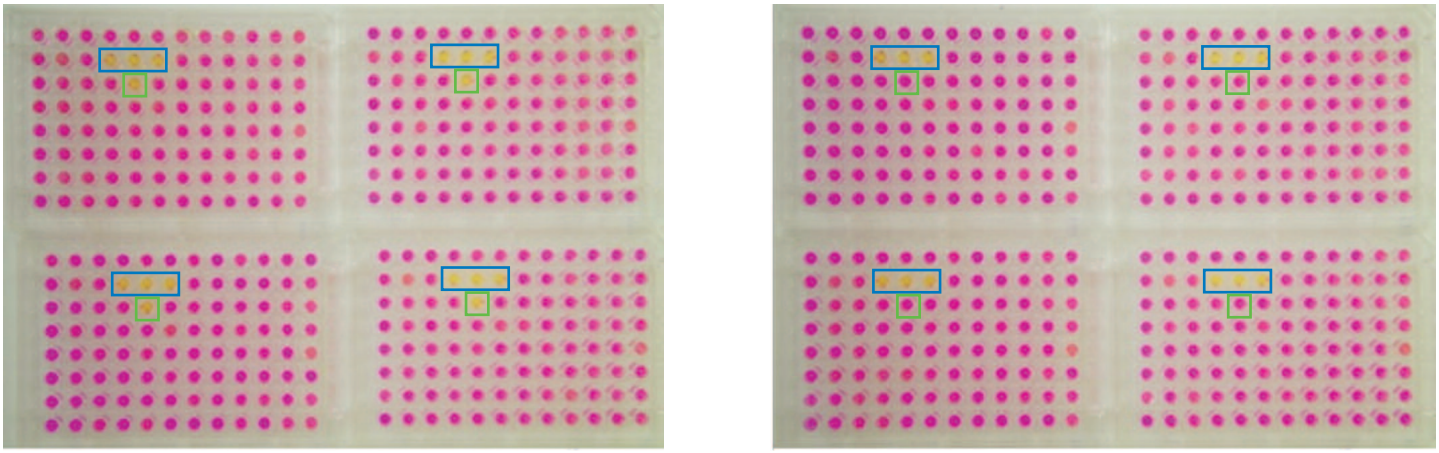

Figure 2: Comparison of HAP1 (parental haploid) and HAP1_124-16 (mannose phosphate isomerase mutant) cell-mediated acidification.
Cells were suspended in 76% IF-NB (IF-M1 with no buffering capacity)/24% IF-M1 +0.3 mM Gln+ Pen/Strep +150 µM Phenol Red and dispensed in PM-M1 MicroPlates at 80,000 cells per well. Plates were incubated in a 37° C OmniLog and photos were taken at 24 hr. Wells outlined in blue are wells containing glucose, wells outlined in green contain mannose.