sufficiently rich snapshot of disease physiology. In such cases, complementary technologies, which measure mRNA expression, protein levels, genetic mutation, copy number variation, gene silencing or regulatory RNA expression, could be considered. Alternatively, the best technology may vary by tumor type. High-throughput sequencing, in particular, offers advantages over microarrays in that coverage of the genome is less biased and the dynamic range is larger[6]. With luck, the results of MAQC-II will be useful for shepherding other high-throughput technologies toward the clinic as well.

COMPETING FINANCIAL INTERESTS
The author declares no competing financial interests.

1. DeRisi, J.L., Iyer, V.R. & Brown, P.P. *Science* **278**, 680–686 (1997).
2. Dumur, C.I. *et al. J. Mol. Diagn.* **10**, 67–77 (2008).
3. Buyse, M. *et al. J. Natl. Cancer Inst.* **98**, 1183–1192 (2006).
4. The MicroArray Quality Control (MAQC) consortium. *Nat. Biotechnol.* **28**, 827–838 (2010).
5. Luo, J. *et al. Pharmacogenomics J.* **10**, 278–291 (2010).
6. Schuster, S.C. *Nat. Methods* **5**, 16–18 (2008).

Shaking up genome engineering

KA Tipton & John Dueber

A new method generates genome-scale modified bacteria with unprecedented ease.

Systematic approaches to mutate and characterize the function of every gene in a microbe have been hampered by the need to manually create thousands of separate strains through tedious genetic manipulation. In this issue, Warner *et al.*[1] describe an approach to create and characterize rationally modified versions of almost every gene in *Escherichia coli*. Using this strategy, the authors quickly zero in on genes that influence industrially relevant traits, such as tolerance to toxins in a biofuel feedstock. The method enables single genome modifications to be probed rapidly and comprehensively and correlated to a phenotype, yielding information that lays a foundation for gene mapping and for engineering strains with desired phenotypes.

Until now, systematic phenotyping of mutants in yeasts[2,3] and *E. coli*[4] has been accomplished by Herculean manual efforts to create thousands of mutant strains, each with a different single-gene knockout. Although the resulting strain collections have proven valuable, it remains a challenge to create, on a genome scale, new collections of mutants for targeted applications or to control gene expression levels using a strong promoter, an inducible promoter or a low-efficiency ribosome binding site.

In contrast, the method of Warner *et al.*[1]—trackable multiplex recombineering (TRMR), pronounced 'tremor' (**Fig. 1**)—offers a fast and cheap approach for creating collections of mutants. Impressively, the authors were able to construct libraries containing up- and downregulated versions of 96% of the genes in the *E. coli* genome in one week at a materials cost of ~$1 per targeted gene.

The first step in TRMR is to obtain thousands of 189-base-pair oligonucleotides that target and uniquely identify every *E. coli* gene. Each of these oligos consists of a barcode tag unique to a gene and regions of homology that flank the targeted gene in the genome. Warner *et al.*[1] purchased the oligos, which were made on a programmable microarray. Next, using a clever cloning strategy, they appended the oligos to DNA elements that modulate gene expression. Attaching the targeting oligos to the strong $P_{LtetO-1}$ promoter created a DNA cassette that was expected to upregulate the targeted gene after incorporation into the genome. Conversely, attaching the targeting oligo to a weak ribosome binding site produced a DNA cassette that downregulated the targeted gene. An antibiotic resistance gene allowed selection for the genetic modifications. As a result of the DNA synthesis and manipulation steps, Warner *et al.*[1] created two libraries of linear DNA fragments, each with 4,077 DNA cassettes pooled together in a single tube.

These libraries of DNA oligonucleotides were used to modify the *E. coli* genome by means of recombineering, a homologous recombination–based method in *E. coli* expressing λ phage recombination factors (λgam, bet and exo)[5]. Growth on antibiotic medium selects for successful recombinants, and the sites of recombination are determined by homology of the targeting oligos to genomic regions flanking each gene.

The resulting collections of modified *E. coli* strains were then challenged by growth in environmental conditions of interest. Warner *et al.*[1] measured the relative fitness of each

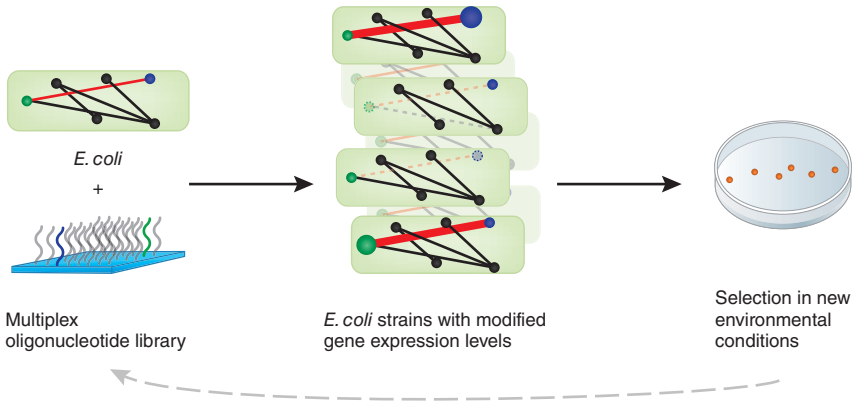

Figure 1 TRMR enables genome-scale selection of rational modifications to the expression of single genes. A multiplex library of oligonucleotides is synthesized to encode a unique barcode tag and regions of homology flanking individual target genes in the *E. coli* genome (left). A series of cloning steps generates linear DNA fragments that contain sequences necessary for up- or downregulating the expression of each target gene. *E. coli* are transformed with this library of linear fragments to create a collection of genetically modified strains (middle, green cells containing a modified genetic network). The modifications alter the functional linkages between genes. (Lines in the networks represent linkages, with thickness being the strength of the link. Circles represent genes, with translucency and a dashed outline representing attenuated expression). The *E. coli* strain collection is grown on medium containing an environmental challenge of interest (right). The identities and relative abundances of individual survivors are determined by sequencing colonies using universal primer sequences. Alternatively, survivors are determined in bulk by microarray analysis of the barcode tags. Importantly, the basic TRMR strategy is amenable to rapid iteration such that the most promising gene modifications are used to seed subsequent cycles of mutation and selection (dotted arrow).

KA Tipton and John Dueber are at the University of California Berkeley, Berkeley, California, USA.
e-mail: jdueber@berkeley.edu

modified strain by isolating genomic DNA, amplifying the barcode tags using PCR and hybridizing the amplified DNA to a microarray that contains probes complementary to each tag. A signal on the microarray identifies strains that grew. To demonstrate the approach, the authors selected for growth in media containing salicin, D-fucose, valine or methylglyoxyl. These compounds inhibit cell growth by different mechanisms. Salicin is a carbon source that normally cannot be metabolized. D-fucose is an analogue of arabinose that inhibits the ability of *E. coli* to metabolize this sugar. Valine acts as a feedback inhibitor of growth-limiting leucine and isoleucine biosynthesis. Methylglyoxal presents an oxidative stress if present in elevated concentrations. These conditions demonstrated the effectiveness of TRMR in identifying gene-trait relationships and in identifying genes that were not expected to be involved in resistance to the given cellular stress, thus supporting the power of a genome-scale, unbiased approach.

In a particularly challenging and exciting application of TRMR, Warner *et al.*[1] grew their libraries of strains in lignocellulosic hydrolysate derived from corn stover. Hydrolysates represent a complex potpourri of molecules toxic to *E. coli*. It has been difficult to predict a priori which genes would best confer resistance to growth inhibitors in the hydrolysates[6]. This problem is thus well suited to test the authors' methods. Among the modified genes that conferred improved growth were genes with expected functions as well as several with seemingly disparate cellular functions, including primary metabolism, RNA metabolism, sugar transporters, secondary metabolism, vitamin processes and antioxidant activities. In one notable result, the authors identified the antioxidant *ahpC*, a gene not previously linked to growth on hydrolysates, which, when upregulated, considerably improved both growth rate and final biomass levels.

TRMR has many potential uses. Warner *et al.*[1] note that it could easily be applied iteratively, with strains selected after one round of TRMR used as the starting strains for a second round, thereby accumulating beneficial genome alterations (**Fig. 1**, dotted arrow). Such iterative processing can take advantage of the same pool of oligos already synthesized. Parallel microarray analysis of the barcode tags present in the selected survivors should produce additional layers of information about genetic contributors to fitness. For instance, the ability to track combinations of alterations in a stepwise fashion as they accumulate has the potential to provide snapshots of genetic interaction data that, if taken at a high enough frequency, may uncover network connections in conditions particularly relevant to industrial and biotechnological settings.

TRMR is also valuable because it identifies genes and network connections that could form the basis for further strain optimization. For instance, a particularly powerful combination of technologies would be to first use TRMR to identify relevant genes and then apply the recently developed multiplex automated genome engineering (MAGE) method[7], which finely tunes the expression levels of a limited number of genes. In microbial engineering applications, such as the creation of a strain of *E. coli* that can metabolize lignocellulose sugars, TRMR should complement existing technologies, including directed evolution, genome-scale metabolic modeling and synthetic biology approaches for redox balancing, flux improvement and limiting the production of undesirable and toxic metabolic products.

In addition to TRMR, other approaches based on genome-wide modifications are increasingly providing scientists with the ability to generate large, information-rich data sets from which new genetic information may be extracted[2–4,8,9]. TRMR heralds an approach to genetic analyses in which phenotypes are rapidly mapped to genetic modifications across the genome, simultaneously producing improved strains for immediate practical use as well as data sets enabling future rational creation of sophisticated strains.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

1. Warner, J. *et al. Nat. Biotechnol.* **28**, 856–862 (2010).
2. Giaever, G. *et al. Nature* **418**, 387–391 (2002).
3. Kim, D.U. *et al. Nat. Biotechnol.* **28**, 617–623 (2010).
4. Baba, T. *et al. Mol. Syst. Biol.* **2**, 2006.0008 (2006).
5. Datta, S., Costantino, N. & Court, D.L. *Gene* **379**, 109–115 (2006).
6. Mohagheghi, A. & Schell, D.J. *Biotechnol. Bioeng.* **105**, 992–996 (2010).
7. Wang, H.H. *et al. Nature* **460**, 894–898 (2009).
8. Tong, A.H. *et al. Science* **294**, 2364–2368 (2001).
9. Mnaimneh, S. *et al. Cell* **118**, 31–44 (2004).

The expanding family of dendritic cell subsets

Hideki Ueno, A Karolina Palucka & Jacques Banchereau

The recent identification of human CD141$^+$ dendritic cells as a counterpart of mouse CD8$^+$ dendritic cells may be useful in developing vaccines and immunotherapies.

Dendritic cells (DCs) are central players in the control of immunity and tolerance, and investigation of their properties is expected to illuminate many diseases of the immune system and lead to innovative therapies. Four recent reports[1–4] in *The Journal of Experimental Medicine* mark new progress in our understanding of the biology of a particular human DC subset identified by co-expression of CD141 (thrombomodulin, BDCA-3) and the C-type lectin CLEC9A (DNGR-1). Collectively, the papers show that CD141$^+$ DCs are the human counterpart of mouse CD8$^+$ DCs. As mouse CD8$^+$ DCs are important for the induction of cytotoxic T-lymphocyte responses through their exceptional capacity to present exogenous antigens in an HLA class I pathway (so-called cross-presentation)[5], this discovery could have significant clinical impact if human CD141$^+$ DCs have a similar role.

DCs were discovered in 1973 by Ralph Steinman as a novel cell type in the mouse spleen and are now recognized as a group of related cell populations that efficiently present antigens. Both mice and humans have two major types of DC: myeloid DCs (mDCs, also called conventional or classical DCs), and plasmacytoid DCs (pDCs). pDCs are considered the front line in anti-viral immunity as they rapidly produce abundant type I interferon in response to viral infection. In their resting state, pDCs may be important in tolerance, including oral tolerance[6,7]. pDCs are

Hideki Ueno, A. Karolina Palucka and Jacques Banchereau are at the Baylor Institute for Immunology Research and INSERM U899, Dallas, Texas, USA; A. Karolina Palucka is at the Sammons Cancer Center, Baylor University Medical Center, Dallas, Texas, USA; and A. Karolina Palucka and Jacques Banchereau are in the Department of Gene and Cell Medicine and Department of Medicine, Immunology Institute, Mount Sinai School of Medicine, New York, New York, USA.
e-mail: jacquesb@baylorhealth.edu