tein detection sensitivity for SERS into the femtomolar range, which is similar to detection limits reported for fluorescence-based assay formats[2–4], and the specialized planar waveguide variation (**Fig. 1b**)[5]. Moreover, by fabricating the SWNTs with different carbon isotopes, Chen *et al.* produced sensitive Raman labels of different colors that allow for multiplexed detection (**Fig. 1a**).

This method joins an exciting offering of detection techniques stemming from nanotechnology. Carbon nanotube and nanowire sensing systems (**Fig. 1c**), based on conductivity changes in response to variation in their surface electric field, provide sensitivities ranging from femtomolar (nanowire sensor arrays[6]) to attomolar (nanoparticle bio-bar codes[7]). The most widely available and best-characterized nanotechnology-based detection technique is SPR, which measures changes in the refractive index induced by the binding of molecules at the surface of metal (**Fig. 1d**). As a label-free method, SPR can monitor binding events in real time, thereby allowing measurement of binding kinetics and affinity. SPR was adapted to protein microarrays through a modification called SPR imaging[8]. SPR imaging fixes on a single incidence angle and records the reflected light for the whole array surface as a function of time. It can simultaneously monitor >1,000 interactions in real time with a detection limit close to 1 attomole[8].

A promising new cousin of SPR is the nanohole array, a real-time sensing technique based on changes in the intensity of extraordinary optical transmission[9]. The ability of nanohole arrays to detect light directly without a prism (for example, with high-density sensors) and their enhanced resolution may simplify the instrumentation and dramatically increase array feature density (**Fig. 1e**). Ellipsometry is another optical reflection technique based on light polarization changes that is reported to enable label-free detection of biomolecular interactions for nearly 3,000 features on protein microarrays, although the sensitivity is not as good as that of SPR imaging[10]. A recently reported label-free interferometric technique, spectral reflectance imaging biosensor (**Fig. 1f**), monitors the optical phase difference resulting from the accumulation of biological material on solid support with picomolar sensitivity, real-time kinetics, multiplexing and protein microarray compatibility[11].

Many factors determine the success of detection techniques, including high sensitivity, resolution and detection limit; simplicity of operation; avoidance of interference from tags; real-time monitoring; broad applicability; multiplexing and high-throughput capability; and biomedical relevance. No single technology has achieved the ideal, but, as shown here by Chen *et al.*[1], the strategy of creatively combining elements from existing tools will bring us closer. The capabilities of most of the new detection techniques have been shown using very strong antibody-antigen interactions, and it will be important to conduct tests with real-life, weak protein interactions. Nevertheless, biomedical researchers will benefit from having a surfeit of methodological options available.

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The many ways to make an iPS cell

William E Lowry & Kathrin Plath

Several new approaches for generating induced pluripotent stem cells reduce the risk of insertional mutagenesis.

Despite their immense promise, induced pluripotent stem (iPS) cells face several major hurdles on the road to clinical application. One immediate challenge is that current reprogramming methods involve expression of putative oncogenes by retroviral vectors, which may themselves cause cancer by integrating into the genome in a way that disrupts endogenous gene expression. Now, three new studies[1–3] suggest that the problem of genomic integration can be surmounted through alternative methods of transgene delivery and the use of small molecules. Writing in *Science,* Stadtfeld *et al.*[1] describe the production of mouse iPS cells with adenoviral vectors, and Okita *et al.*[2] achieve reprogramming of mouse cells using plasmid transfection. A report in this issue by Huangfu *et al.*[3] shows that one of the reprogramming factors can be replaced by a small molecule, enabling the generation of human iPS cells with only two factors.

Reprogramming of a somatic cell to an embryonic-like state by overexpression of a set of genes that are highly expressed in embryonic stem (ES) cells is commonly referred to as the 'Yamanaka method', after its founder, Shinya Yamanaka[4,5]. There are now variations on the original cocktail of genes that induce pluripotency[6], but the only method for delivering these factors described to date relies on integrating retroviruses, including lentiviruses (**Fig. 1a**). Introduction of the reprogramming factors activates a panoply of endogenous genes normally expressed only in ES cells and represses the differentiated cells' expression program, resulting in ES cell–like morphology and functionality[7]. Although the mechanism underlying this dramatic transformation remains murky, it is clear that the method can be applied to multiple somatic cell types[8].

The three new papers describe different strategies for avoiding genomic integration by retroviral vectors. Adenoviruses do not integrate into the host genome as part of their life cycle. Indeed, Stadtfeld *et al.*[1] did not detect integration of the adenoviral vector in their iPS cell clones, as measured by both PCR and Southern blotting (**Fig. 1b**). The authors succeeded in producing iPS cells from mouse hepatocytes through repeated adenoviral infection, but the efficiency of reprogramming was much lower than that described for retroviral delivery (**Table 1**). A possible explanation for the low efficiency is that expression of exogenous genes by the replication-deficient adenoviral vectors used in this study is maintained for only 3–8 days[1]. In the case of mouse fibroblasts, iPS cell generation has been shown to require transgene expression for at least 8 days[9], suggesting that it could be difficult to maintain sufficiently high levels of the reprogramming factors with the adenoviral method.

Stadtfeld *et al.*[1] found that mouse fibroblasts, the cell type most commonly used in

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reprogramming experiments, could be reprogrammed only by expressing three factors (Sox2, c-Myc anf Klf4) adenovirally in fibroblasts that carry a single copy Oct4 transgene. The vectors they used are based on human adenovirus serotype 5, which infects cultured human epithelial cells through an interaction with the coxsackievirus and adenovirus receptor (CAR). The virus does not bind efficiently to mouse CAR and therefore does not infect mouse cells effectively. *In vivo*, the virus infects hepatocytes through binding to blood factors—a recently discovered pathway that works equally well for mouse and human hepatocytes[10]. These facts might explain why Stadtfeld *et al.*[1] could reprogram mouse hepatocytes more readily than fibroblasts and suggest that human cell types expressing CAR will be well suited to adenoviral reprogramming. It is also possible that mouse hepatocytes were reprogrammed more easily than fibroblasts because their epigenetic and transcriptional state is more similar to that of ES cells[8].

Okita *et al.*[2] avoided viral vectors altogether, delivering the reprogramming factors by simple transfection (**Fig. 1c**). Remarkably, by expressing Oct4, Klf4 and Sox2 from one polycistronic vector and c-Myc from a separate plasmid, they reprogrammed mouse embryonic fibroblasts by repeated transfection over 7 days. As with the adenoviral method, reprogramming efficiency was much lower than that of retroviral expression (**Table 1**). Of course, plasmid transfection can lead to random integration into the genome at a low frequency. However, Okita *et al.*[2] detected integration in only some of the clones; others were free of any ectopic DNA as determined by PCR and Southern blotting.

Previous studies had suggested that the specific sites of proviral integration are not likely to be crucial to reprogramming success because the many lines that have been generated thus far do not show significant overlap of integration sites[8]. The results of Stadtfeld *et al.*[1] and Okita *et al.*[2] establish that reprogramming does not require the genomic disruption caused by integrating viruses. Moreover, transient overexpression of the four factors, whether by adenoviral vectors or by plasmid transfection, should also circumvent the tumor formation, and possibly the perinatal death, observed in iPS cell–derived chimeric mice, which are both thought to be caused by ectopic expression of the four factors[8,11] (**Fig. 1**). However, reprogramming of human fibroblasts takes even longer than that of mouse cells, and the timing and efficiency of both new methods will probably have to be optimized for application to human cells.

Another strategy for avoiding insertional mutagenesis by retroviral vectors is to identify small molecules that can take the place of the reprogramming transgenes. Such compounds could also increase the overall efficiency of a transfection or adenoviral approach, allowing integration-free reprogramming of human cells. Huangfu *et al.*[3] exploited the common retroviral delivery method to determine whether small molecules can replace one or more of the reprogramming factors. They found that adding just two factors (Oct4 and Sox2) in combination with a histone deacetylase inhibitor, valproic acid (VPA), is sufficient to reprogram human fibroblasts to an embryonic state. VPA has now been shown to dramatically increase the efficiency of reprogramming in both human and mouse[12] cells, whether induced with two, three or four factors. These experiments highlight

Table 1 Efficiencies of reprogramming methods reported in refs 1–3

Strategy	Cell type	Efficiency[a]	Reference
Retroviral transduction			
Four factors (Oct4, Sox2, Klf4, c-Myc)	Mouse embryonic fibroblasts	0.1%	2
Three factors (Oct4, Sox2, Klf4)	Mouse embryonic fibroblasts	0.01%	2
Three factors (Oct4, Sox2, Klf4)	Human fibroblasts	0.001%	3
Three factors (Oct4, Sox2, Klf4) + VPA	Human fibroblasts	1%	3
Two factors (Oct4, Sox2)	Human fibroblasts	No iPS colonies obtained	3
Two factors (Oct4, Sox2) + VPA	Human fibroblasts	0.001%	3
Adenoviral transduction			
Four factors (Oct4, Sox2, Klf4, c-Myc)	Mouse postnatal fibroblasts	No iPS colonies obtained	1
	Mouse hepatocytes	0.0006%	1
Plasmid transfection			
One plasmid with Oct4-2A-Klf4-2A-Sox2[b] + one plasmid with c-Myc	Mouse embryonic fibroblasts	0.0015%	2

[a]Without correcting for transfection or transduction efficiency. [b]2A, foot-and-mouth disease virus self-cleaving peptide.

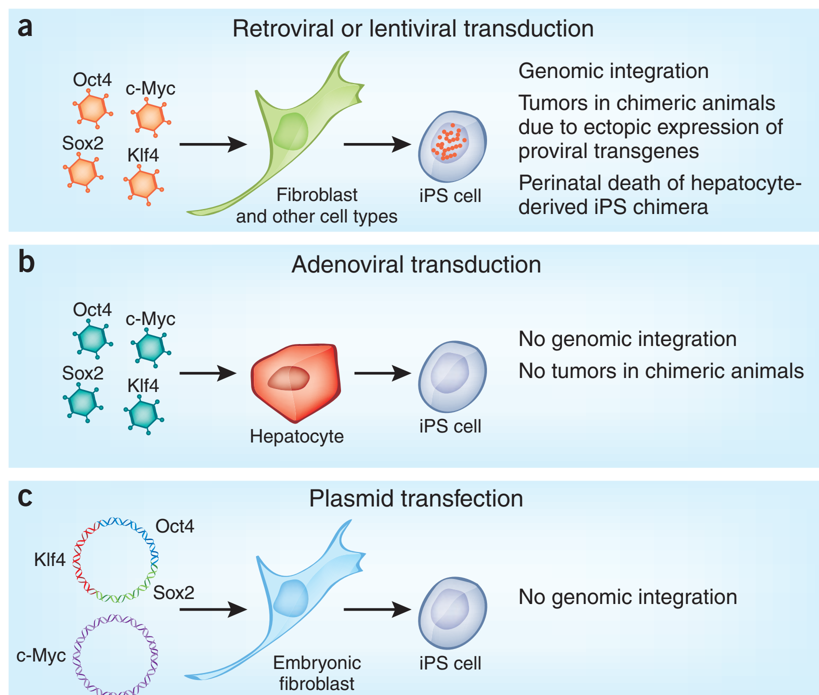

Figure 1 Three strategies to generate mouse iPS cells. (**a**–**c**) In contrast to the established retroviral reprogramming method (**a**), the new reprogramming strategies depicted in **b** and **c** rely on transient expression of the reprogramming factors without integration of ectopic DNA into the genome. Problems associated with retroviral insertion, such as tumors and perinatal death in iPS chimeric mice, may be overcome using these new methods.

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the still-undefined contribution of chromatin remodeling to the reprogramming process.

It will be interesting to see whether combining VPA with the adenoviral and plasmid methods increases reprogramming efficiency. Taken together, the findings of Stadtfeld *et al.*[1] and Okita *et al.*[2] argue that the mechanisms underlying reprogramming do not vary with the mode of transgene delivery and suggest that VPA should have the same effect in each instance. It is thought that eventually a combination of small molecules will be found that can reprogram somatic cells to an embryonic state without the use of transgenes. However, until we have a better understanding of the reprogramming process, we will not know whether any side effects induced by chemicals are less harmful than those associated with retroviral or transient expression of oncogenes. Transient expression of the four factors with the adenoviral or plasmid methods might even turn out to be the method of choice for generating Good Tissue Practice–grade human iPS cell lines for clinical use.

Having created mouse iPS cells without genomic integration, the nascent reprogramming field still has many interesting questions to explore. Can integration-free reprogramming be achieved in human cells? How do the reprogramming factors act? Are there significant genomic alterations or instabilities induced by or required for reprogramming? Are there specific epigenetic attributes that make certain cell types more or less amenable to reprogramming? How different are iPS cells functionally and molecularly from ES cells? Does the method of delivery of reprogramming factors or the source of starting material influence the potential of the resulting iPS lines? iPS cells should not be applied clinically without thorough answers to these questions.

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Fast forward genetics

Alistair C Darby & Neil Hall

High-throughput sequencing rapidly connects microbial phenotypes and genotypes to guide metabolic engineering.

Over the past decade, the rate of genome data generation has far outstripped our ability to ascertain gene function. Many strategies for high-throughput functional genomics have been proposed, ranging from elegant reverse-genetic techniques to *de novo* prediction using computer models. A recent paper by Smith *et al.*[1] in *Genome Research* exemplifies what is perhaps the simplest solution: high-throughput sequencing of entire genomes to identify the mutations that underpin particular phenotypes. The authors use this approach to genetically characterize the enhanced fermentative capacity of a *Pichia stipitis* mutant[1]. The study suggests that as next-generation sequencing becomes a routine assay to study genome-wide sequence variation, transcriptomics, epigenomics and DNA-protein interactions with a single technical platform[2] (**Fig. 1**), microbial researchers will gain unprecedented access to new leads for engineering industrially relevant traits.

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The ability of the yeast *P. stipitis* to ferment the xylose and cellobiose in lignocellulosic biomass to ethanol is of particular interest in the field of biofuel development. The strain studied by Smith *et al.*[1], which was generated in part by chemical mutagenesis and is capable of ethanol production rates 50% greater than its parent strain, is known to be disrupted in an isoform of cytochrome *c*. However, the full extent of its mutations, some of which might also contribute to its high-ethanol phenotype, was not clear. Identification of these mutations could open the way to improved approaches for engineering bioethanol production.

Using three next-generation sequencing platforms—Roche's 454 GS FLX, Illumina's Genome Analyzer and Applied Biosystems' SOLiD platform—Smith *et al.*[1] resequenced the entire 15.4-Mb *P. stipitis* genome and found that the improved strain bears 14 point mutations distributed over seven of the eight chromosomes. Mapping small changes across multiple chromosomes would be very time-consuming and error prone by traditional approaches. All three sequencing technologies identified the same mutations, but the accuracy of detection was good for all platforms only when the genome sequence coverage was greater than tenfold. This level of coverage is easier and cheaper to achieve using the short-read approaches, such as Illumina and SOLiD, with SOLiD showing the lowest error rate. A similar approach was also used to screen for mutations in the genomes of *Myxococcus xanthus*[3] and *Caenorhabditis elegans*[4].

The use of whole-genome sequencing to map mutations depends on the availability of a good wild-type reference sequence. When Smith *et al.*[1] confirmed by Sanger sequencing the single-nucleotide changes they had discovered, they found three false mutations resulting from sequencing errors in the reference genome[5]. Although the generation of a high-quality reference sequence for mapping mutations would have been prohibitively costly only a few years ago, this can now be achieved rapidly and cheaply using hybrid assemblies of different sequencing technologies. A high-quality bacterial genome sequence was recently generated using the 454 platform to generate the contiguous sequence and the Illumina platform to correct the 454's homopolymer errors[6]. Thus, next-generation sequencing technologies will advance functional analysis beyond model systems and permit a massive acceleration in the ability to assign biological roles to genes.

Functional genomics aims to determine not only what genes do, but also when they are expressed. The astonishing sensitivity of next-generation technology can also be used to measure gene expression by sequencing the transcriptome directly from cDNA. A recent study of *Schizosaccharomyces pombe*[7] demonstrated this sensitivity by detecting transcriptional activity from >90% of the genome, including low-level transcription, which would normally be undetectable using microarrays. The content and detection of genes on microarrays are limited by the fact that microarrays are designed according to a specific genomic annotation—any nonannotated or incorrectly annotated genes will be missed. However, as direct sequencing is independent of annotation, one can identify novel transcripts and