considerably. Presumably, assembly is more robust because longer tracts of DNA are left after the amplification primers are removed. Thus, fewer starting fragments are needed to assemble each product. The method of Kosuri *et al.*[4] is still subject to the error rate of input oligos, although errors are reduced by using new, more accurate microarrays and by performing an enzymatic error correction step.

The article from Matzas *et al.*[5] directly addresses the problem of errors in oligonucleotide sequences and the labor needed to deal with them. They use a 454 sequencing instrument to identify microarray oligos with the correct sequence and then recover these oligos from the sequencer. In essence, this provides cheap oligos preverified by cheap sequencing. 454-beads bearing correct sequences are located with microscope cameras and picked using a micropipette. The oligos are subsequently assembled into larger products. Because the DNA assembly step can be virtually error free (even during the construction of whole genomes[1,2]), presequenced and clonal oligos have the potential to produce near-perfect assemblies. Even though Matzas *et al.*[5] find an error in one of the eight ~220-bp constructs made from their microarray, this error rate is likely to decrease upon further optimization. Although the starting oligos, polymerase fidelity and accuracy of 454 sequencing all contribute errors, the experiments indicate that major gains are to be had by improving bead localization and retrieval. Automated bead extraction is under development, and the authors hope to achieve recovery rates of two or three beads per minute. The error rate of the current system is estimated to reach about 1 in 21,000 bp.

These papers demonstrate that the use of inexpensive microarray oligonucleotides to produce long synthetic DNA is becoming more practical. Assembly technology is also undergoing rapid development; for example, oligos produced by any method can be assembled into larger sequences by a fast and essentially labor-free method[3]. At the same time, projects involving intensive DNA synthesis are generating advances such as empirically determined codon-optimization rules[8], new insights into host metabolism[9] and the first microbe controlled by a synthetic genome[2].

It is reasonable to wonder about how to make effective use of megabases of user-defined sequence. But with low-cost oligos and a simple means of assembling them, we can safely predict that our ability to understand and manipulate biology will only continue to improve, perhaps leading eventually to advances such as microbes engineered to be attenuated vaccines or to synthesize biofuels.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

1. Gibson, D.G. *et al. Science* **319**, 1215–1220 (2008).
2. Gibson, D.G. *et al. Science* **329**, 52–56 (2010).
3. Gibson, D.G. *et al. Nat. Methods* **7**, 901–903 (2010).
4. Kosuri, S. *et al. Nat. Biotechnol.* **28**, 1295–1299 (2010).
5. Matzas, M. *et al. Nat. Biotechnol.* **28**, 1291–1294 (2010).
6. LeProust, E.M. *et al. Nucleic Acids Res.* **38**, 2522–2540 (2010).
7. Xu, Q. *et al. Proc. Natl. Acad. Sci. USA* **106**, 2289–2294 (2009).
8. Welch, M. *et al. PLoS ONE* **4**, e7002 (2009).
9. Warner, J. *et al. Nat. Biotechnol.* **28**, 856–862 (2010).

No refuge for insect pests

Kongming Wu

The sterile insect technique offers an alternative to the refuge strategy for managing resistance to *Bt* toxins.

Transgenic cotton and corn expressing insecticidal proteins from the bacterium *Bacillus thuringiensis* (*Bt*) have been cultivated on >200 million ha worldwide over the past 15 years[1], reducing the use of chemical insecticides and increasing farmers' profits[2,3]. But the environmental and economic advantages of these crops are threatened by the tendency of insect pests to develop resistance to *Bt* toxins. A report in this issue by Tabashnik *et al.*[4] demonstrates a new approach for suppressing the emergence of resistance. Field trials in Arizona over four growing seasons show that releasing sterile male pink bollworm moths, which can mate with resistant females, succeeded in almost completely eradicating a major cotton pest. The study establishes that sterile insect release is a viable alternative to the so-called refuge strategy for preventing the emergence of resistance and can enhance the sustainability of *Bt* crops.

Continuous monoculture of crop varieties producing *Bt* toxins provides strong selective pressure for *Bt*-resistant insect pests (**Fig. 1a**). Indeed, although *Bt* crops remain effective against most targeted insect populations, several pests have evolved resistance[5]. The most promising strategy for delaying the emergence of resistance involves planting *Bt* crops in close proximity to 'refuges', which contain plants that do not express *Bt* toxin. Such refuges maintain populations of insects susceptible to *Bt* toxin, which are likely to mate with the rare, resistant insects. If the mode of inheritance of resistance is recessive, *Bt* plants will kill the hybrid progenies produced by such mating and thereby delay the evolution of *Bt* resistance[6].

Certain governments limit the proportion of crop that each farmer can plant to *Bt* crops and require a minimum proportion of non-*Bt* crop to serve as a refuge. For example, the United States and Australia mandate this approach for *Bt* cotton that produces only one *Bt* toxin[5]. Resistance monitoring data from these countries have suggested that the refuge strategy can significantly delay the evolution of insect resistance to *Bt* crops[7]. It is also beneficial to non-*Bt* crops in the refuges, which are exposed to fewer pests owing to the control exerted by neighboring *Bt* crops[2]. Moreover, as nontransgenic seed is cheaper, there is an economic incentive for farmers to adopt the refuge strategy.

Despite the benefits of the refuge strategy, it has proved difficult to implement in developing countries such as China and India, primarily because of the challenges associated with training and monitoring millions of smallholder farmers. During 2009, China planted 3.7 million ha (~70% of total cotton production) and India planted 8.4 million ha (~90% of total cotton production) to *Bt* cotton. In certain cases, however, it has been put in place unintentionally. In China, the primary pest targeted by *Bt* cotton is *Helicoverpa armigera*, which also feeds on other crops, including corn, soybean, vegetables and peanuts. These nontransgenic host plants are frequently planted near *Bt* cotton and serve as refuges for *H. armigera*. Thus far, continuous resistance monitoring has not detected any resistance of *H. armigera* populations to *Bt* toxin[7].

In contrast to the situation with *H. armigera* in China, reliance on noncotton host plants as refuges is not an option for controlling pink bollworm (*Pectinophora gossypiella*), which feeds almost exclusively on cotton in some regions[4]. This pest has evolved resistance to *Bt* cotton producing one toxin in western India, where farmers did not follow regulations requiring them to plant non-*Bt* cotton refuges[8]. In Arizona, where pink bollworm is an alien species first detected about a century ago, the efficacy of *Bt* cotton has been sustained for

Kongming Wu is at the State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China.
e-mail: kmwu@ippcaas.cn

more than a decade. From 1996 to 2005, farmers who grew *Bt* cotton also planted non-*Bt* cotton in compliance with the refuge strategy, and the susceptibility of pink bollworm to *Bt* did not decrease[4]. A disadvantage of this approach, however, is that populations of the insect pest must be maintained.

Tabashnik *et al.*[4] report that superimposing the sterile insect technique (SIT) on *Bt* cotton plants offers a compelling alternative to the refuge strategy while helping to eradicate pink bollworm. The authors' program, which spanned ~100,000 ha in Arizona from 2006 to 2009, showed that susceptibility to *Bt* cotton did not decrease, pink bollworm population density declined dramatically and insecticide sprays against this pest were eliminated. The SIT, a method of pest control using area-wide inundative releases of sterile insects to reduce fertility of a field population of the same species, was first developed in the United States more than 50 years ago[9]. The repeated release of sterile insects into the environment in numbers 10- to 100-fold in excess of the size of the native population can eventually drive the native population to extinction if the majority of native female insects mate with sterile males. Although the SIT has been successful in some cases, such as the screwworm fly (*Cochliomyia hominivorax*) eradication program, its application has been limited[9]. One challenge has been the cost of generating a sufficiently high ratio of sterile to wild insects at the start of a SIT program (**Fig. 1b**). Using another control method to lower the pest's population density can make it easier to attain a suitable ratio, but intensive use of conventional insecticides raises concerns about harm to nontarget organisms, including people.

The report of Tabashnik *et al.*[4] reveals how the SIT and *Bt* transgenic technology can be used in a complementary manner (**Fig. 1c**). On the one hand, expression of the *Bt* toxin suppresses the size of the native insect population enough to jump-start the efficacy of the SIT while increasing its economic feasibility. On the other hand, when female moths that have evolved resistance to the *Bt* toxin mate with sterile insects, the failure to produce fertile progeny delays the establishment of resistance based on dominant inheritance. More importantly, the combination of the SIT and *Bt* crops allows farmers to reduce or eliminate planting of non-*Bt* refuges. Meanwhile, because mating with sterile insects does not produce fertile progeny, the approach could delay pest resistance to *Bt* crops that is based on either dominant or recessive inheritance (**Fig. 1c**).

The stunning success of the Arizona program can be attributed to several favorable factors, including long-term public investment in SIT against pink bollworm. Since 1967, sterile pink bollworm moths have been released over cotton fields in the San Joaquin Valley of California to prevent establishment of this pest by moth immigration from southern California[9]. The Arizona program also benefits from the extremely high efficacy of *Bt* cotton against pink bollworm. Results from Arizona show that *Bt* cotton producing either one or two *Bt* toxins kills virtually 100% of susceptible pink bollworm larvae[4]. Coordinated contributions from cotton farmers, the US Department of Agriculture and university scientists have also been critical.

Despite the impressive results reported by Tabashnik *et al.*[4] and the potential of this combinatorial strategy for many situations, several alternatives to conventional *Bt* crops also show considerable promise. For instance, new insect-resistant transgenic crops may decrease native insect populations more efficiently than current *Bt* crops. They include proteins modified to counteract resistance development or the expression of two or more distinct toxins targeting

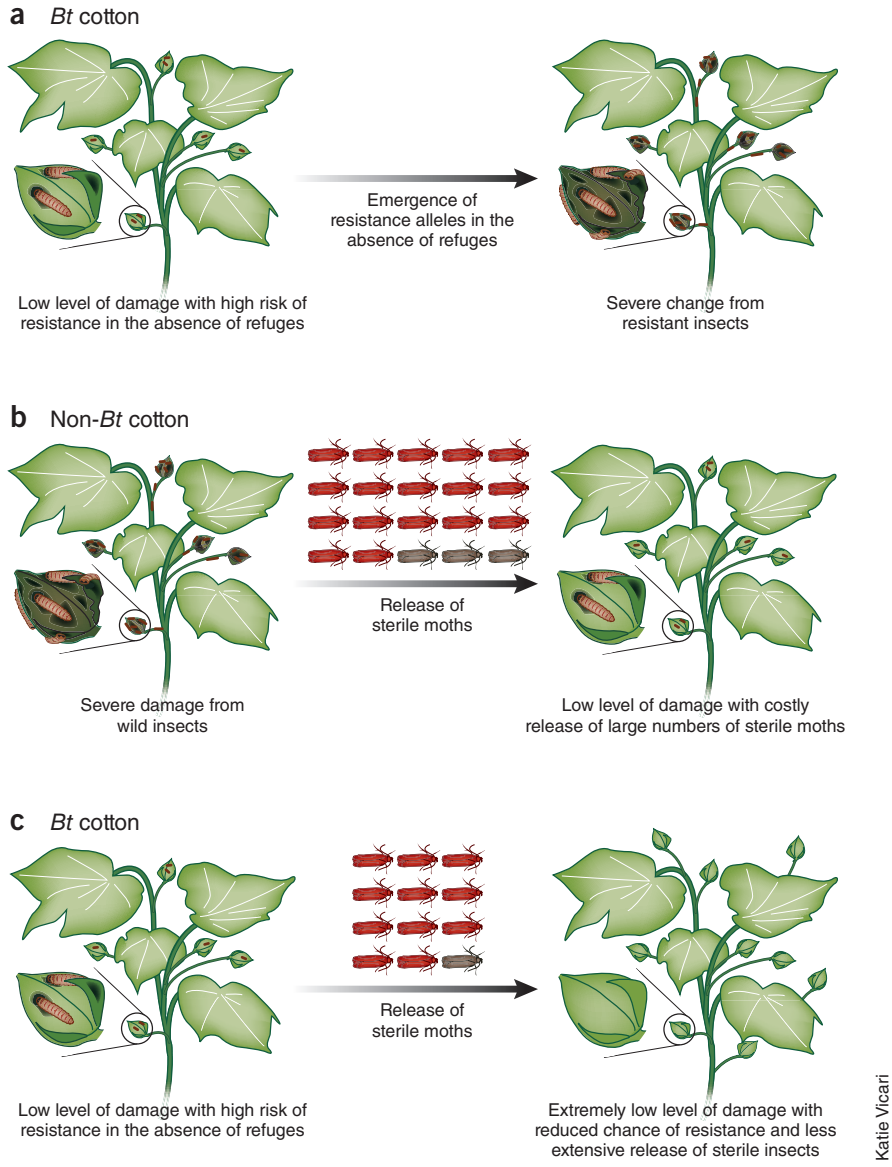

Figure 1 Use of the SIT together with transgenic cotton expressing the *Bt* transgene suppresses the growth of the pink bollworm population and facilitates management of resistance to *Bt* toxin. Pink bollworm feeds only on cotton bolls and does not damage other tissues. (**a**) Sustainable use of *Bt* cotton to control pink bollworm populations is threatened by the emergence of resistance. (**b**) Although costly, repeated release of sterile pink bollworm moths (red) in vast excess to the number of wild moths (brown) can suppress the growth of pink bollworm populations. (**c**) Combined use of *Bt* cotton and SIT ensures that the release of fewer sterile moths can suppress the growth of pink bollworm populations while preventing the emergence of resistance to *Bt* toxin.

the same pest species[10]. In another approach, the US Environmental Protection Agency has approved sales of mixtures of corn seeds with and without *Bt* toxins that kill corn rootworms. This ensures that farmers comply with the refuge strategy. However, its use is limited to the pest species whose larvae do not migrate between plants. Finally, there are also transgenic strategies to improve SIT efficiency by expressing dominant lethal genes, promoting refractoriness of the sterile insects to disease or facilitating strain marking, genetic sexing and molecular sterilization[11]. There thus seems considerable scope to further enhance the sustainability of *Bt* crops and develop new strategies for integrated pest management.

COMPETING FINANCIAL INTERESTS
The author declares no competing financial interests.

1. James, C. *Global Status of Commercialized Biotech/GM Crops: 2009* (ISAAA **41**, Ithaca, New York, USA, 2009).
2. Hutchison, W. *et al. Science* **330**, 222–225 (2010).
3. Wu, K. *et al. Science* **321**, 1676–1678 (2008).
4. Tabashnik, B. *et al. Nat. Biotechnol.* **28**, 1304–1307 (2010).
5. Tabashnik, B.E. *et al. J. Econ. Entomol.* **102**, 2011–2025 (2009).
6. Gould, F. *Annu. Rev. Entomol.* **43**, 701–726 (1998).
7. Liu, C. *et al. Sci. China Life Sci.* **53**, 934–941 (2010).
8. Bagla, P. *Science* **327**, 1439 (2010).
9. Klassen, W. & Curtis, C. in *Sterile Insect Technique* (eds. Dyck, V., Hendrichs, J. & Robinson, A.) 3–36 (Springer, The Netherlands, 2005).
10. Tabashnik, B. *Science* **330**, 189–190 (2010).
11. Alphey, N., Bonsall, M.B. & Alphey, L. *J. Econ. Entomol.* **102**, 717–732 (2009).

Nanoparticles in the lung

Wolfgang G Kreyling, Stephanie Hirn & Carsten Schleh

An imaging study begins to define the parameters that control the biodistribution of nanoparticles after pulmonary delivery.

Little is known about the fate of nanoparticles that enter the lungs, either deliberately through medical treatments[1] or incidentally through air pollution[2,3] and occupational exposure in the workplace. As inhaled nanoparticles can cross the air-blood barrier into the circulation and accumulate in secondary organs and tissues, a biokinetic analysis would be the first step in a dose-response study as part of a comprehensive risk assessment (**Fig. 1**). In this issue, Frangioni, Tsuda and colleagues[4] undertake such a biokinetic analysis in rats, using near-infrared imaging to follow the fate of intratracheally instilled nanoparticles that are varied systematically in size, surface modification and core composition. The authors study the parameters that control whether nanoparticles remain in the lung, are transported to regional lymph nodes and the bloodstream or are cleared from the body through the kidneys. Their findings should benefit several areas of research, including the design of nanoparticles for drug delivery and the evaluation of the toxicity of particulate air pollution.

Wolfgang G. Kreyling, Stephanie Hirn and Carsten Schleh are at the Comprehensive Pneumology Center, Institute of Lung Biology and Disease, and Focus Network Nanoparticles and Health, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg/Munich, Germany.
e-mail: kreyling@helmholtz-muenchen.de

The lungs represent a promising route for drug delivery because of their accessible, large surface area and because the air-blood barrier is rather thin[1]. After crossing the air-blood barrier, inhaled nanoparticles have been shown to reach detectable levels in the blood circulation and to enter specific cells and tissues[5] (**Fig. 1**). Translocation and accumulation in tissues seem to depend on physicochemical properties of the nanoparticle core and surface[5–7]. Thus far, however, few studies have described nanoparticle transport across the lungs quantitatively[5,6].

Frangioni, Tsuda and colleagues[4] find that noncationic nanoparticles smaller than ~34 nm in diameter that do not bind serum proteins reach the regional lymph nodes within 30 min. Nanoparticles larger than ~34 nm are consistently retained in the lungs. When the diameter falls below ~6 nm and the charge is zwitterionic, about half of the nanoparticles rapidly enter the bloodstream from the alveolar airspaces and are mostly cleared from the body by means of renal filtration. The authors also show that nanoparticle behavior depends strongly on the surface coating, which affects binding to proteins in body fluid.

Biodegradable nanoparticles are currently considered to be the best choice for targeted drug delivery, but biopersistent nanoparticles might also be used if they are cleared from the body quickly enough to minimize exposure and retention[8]. As demonstrated previously by the Frangioni laboratory, intravenously administered nanoparticles can be rapidly excreted through the kidneys if their physicochemical properties are carefully chosen[9,10]. Efficient renal clearance represents one elegant way of keeping the accumulation and, hence, the dose to secondary organs, low[9,10]. The present study[4] indicates how renal clearance could be achieved following deposition in the lungs.

The new work[4] also defines the requirements for targeting nanoparticles to regional lymph nodes, which may be useful for therapies directed to the immune system. However, further quantitative investigation is needed to differentiate between transport to the blood through lymphatic drainage and direct translocation across the air-blood barrier to the blood. Additional challenges will arise in extrapolating from the rather simple nanoparticles used in this study to highly functionalized nanosystems bearing targeting, therapeutic and diagnostic molecules.

Finally, the results of Frangioni, Tsuda and colleagues[4] may help in understanding the

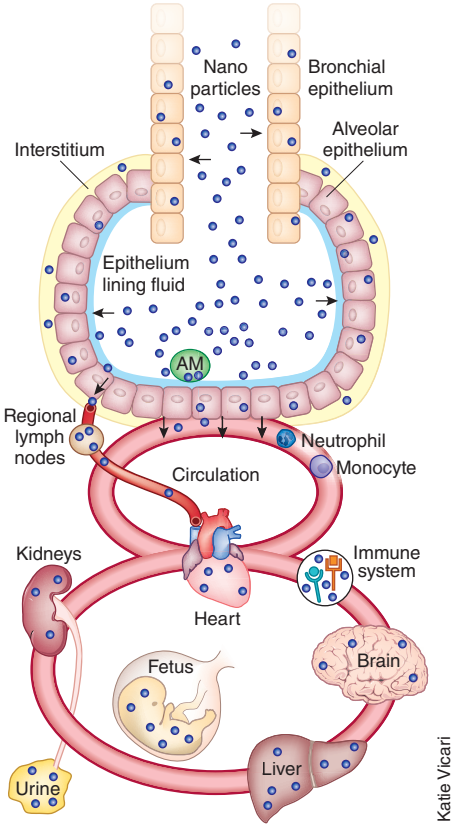

Figure 1 Schematic of nanoparticle translocation from the lung epithelium to regional lymph nodes and blood circulation. Once circulating in blood, nanoparticles may accumulate in various secondary organs of the body. The study by Frangioni, Tsuda and colleagues[4] highlights two important pathways of nanoparticle biodistribution: the pathway of lymphatic drainage toward regional lymph nodes and renal clearance from blood to urine. AM, alveolar macrophages.