

BRIEF COMMUNICATIONS

Dogs cloned from adult somatic cells

Two Afghan pups could help to unravel the genetics behind the assorted traits of other canine breeds.

Several mammals — including sheep, mice, cows, goats, pigs, rabbits, cats¹, a mule², a horse³ and a litter of three rats⁴ — have been cloned by transfer of a nucleus from a somatic cell into an egg cell (oocyte) that has had its nucleus removed. This technology has not so far been successful in dogs because of the difficulty of maturing canine oocytes *in vitro*. Here we describe the cloning of two Afghan hounds by nuclear transfer from adult skin cells into oocytes that had matured *in vivo*. Together with detailed sequence information generated by the canine-genome project^{5,6}, the ability to clone dogs by somatic-cell nuclear transfer should help to determine genetic and environmental contributions to the diverse biological and behavioural traits associated with the many different canine breeds^{7,8}.

Successful somatic-cell nuclear transfer (SCNT) depends on the quality, availability and maturation of the animal's unfertilized oocytes. Unlike other mammals, dogs ovulate at first meiotic prophase, and their oocytes mature for 2 to 3 days in the oviduct's distal regions. Previously, intra- and interspecific canine embryos have been constructed by canine SCNT into canine and bovine oocytes, respectively, but this did not result in viable offspring⁹.

We collected oocytes matured *in vivo* at metaphase II about 72 hours after ovulation by flushing the oviducts. (For details of methods, see supplementary information.) Donor fibroblasts were obtained from an ear-skin biopsy of a male Afghan hound and cultured for two to five passages (in which fully grown cells are transferred to a new culture dish). For SCNT, the chromosomes of the unfertilized canine oocytes were removed by micromanipulation, and a single donor cell was transferred into each enucleated oocyte. The couplets were fused and only successfully fused couplets (75%) were activated. The activated oocytes were then transferred into the oviducts or uterine horns of recipient dogs at times appropriate to the embryos' developmental stages. We collected an average of 12 oocytes from each female, and a total of 1,095 reconstructed canine embryos were transferred into 123 recipients.

Three pregnancies were confirmed by ultrasound scans at 22 days' gestation in recipients after transfer of constructs. Pregnancy was established only after embryo transfer of very-early-stage nuclear-transfer constructs (that is,



Figure 1 | Dog cloned by somatic-cell nuclear transfer. **a**, Snuppy, the first cloned dog, at 67 days after birth (right), with the three-year-old male Afghan hound (left) whose somatic skin cells were used to clone him. Snuppy is genetically identical to the donor Afghan hound. **b**, Snuppy (left) was implanted as an early embryo into a surrogate mother, the yellow Labrador retriever on the right, and raised by her.

less than 4 hours after oocyte activation). This transfer of early-stage embryos is a crucial factor in successful assisted reproductive technology for dogs. One fetus miscarried and two others were carried to term.

We named the first cloned dog Snuppy (for Seoul National University puppy); it is shown in Fig. 1a with the male Afghan fibroblast donor. Snuppy was delivered by caesarian section after 60 days (full term) from a yellow Labrador surrogate mother (Fig. 1b); his birth weight was 530 g. The second SCNT dog, NT-2, was carried by a mixed-breed surrogate, and was also delivered at 60 days, weighing 550 g (normal range for Afghans in a litter, 482–680 g). He experienced neonatal respiratory distress during the first week, seemed to recover, but died on day 22 as a result of aspiration pneumonia; no major anatomical anomalies were evident post mortem.

We tested whether the cloned dogs were genetically identical by microsatellite analysis of genomic DNA from the donor Afghan, the cloned dogs and the surrogates (see supplementary information). Analysis of eight canine-specific microsatellite loci confirmed that the cloned dogs were genetically identical to their donor dog. However, the efficiency of cloning is still very low (2 dogs from 123 recipients, or 1.6%) compared with the rates for cats¹ and horses³.

In addition to the benefits that cloning technology may generally provide (the preservation of rare species and therapeutic cloning

— once canine embryonic stem cells become available), this technology could become a useful research tool for studying the genetics of outcrossed populations.

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GREEN CHEMISTRY

Reversible nonpolar-to-polar solvent

Imagine a smart solvent that can be switched reversibly from a liquid with one set of properties to another that has very different properties, upon command. Here we create such a system, in which a non-ionic liquid (an alcohol and an amine base) converts to an ionic liquid (a salt in liquid form) upon exposure to an atmosphere of carbon dioxide, and then reverts back to its non-ionic form when exposed to nitrogen or argon gas. Such switchable solvents should facilitate organic syntheses and separations by eliminating the need to remove and replace solvents after each reaction step.

Chemical production processes often involve multiple reaction and separation steps, and the type of solvent that is optimum for a particular step may be different from the one needed in the next step. The solvent is therefore usually removed after each step and a new solvent added in preparation for the next, significantly adding to the economic cost and environmental impact of the process. This cumbersome procedure would be unnecessary if a solvent's properties could be adjusted for the following step while still in the reaction vessel, enabling the same solvent to be used for several consecutive reaction or separation steps. Moderate changes in temperature and pressure are incapable of triggering significant changes in the properties of conventional solvents. In contrast, supercritical fluids¹ and CO₂/organic solvent mixtures² can be modified by pressure changes, but unfortunately only above 40 bar.

The reaction we describe reversibly changes the nature and properties of a solvent but occurs under very mild conditions. We reasoned that switching a normal non-ionic liquid to an ionic liquid should induce a change in its properties: ionic liquids are often viscous and always polar, whereas non-ionic solvents are typically non-viscous and vary widely in polarity. We chose CO₂ as the 'switch' to elicit this change because it is a benign agent

and easily removed. (For methods, see supplementary information.)

We found that exposure of a 1:1 mixture of the two non-ionic liquids, namely DBU (1,8-diazabicyclo-[5.4.0]-undec-7-ene) and 1-hexanol, to gaseous CO₂ at one atmosphere and at room temperature causes conversion of the liquid mixture to an ionic liquid (Fig. 1a, b). This is readily converted back into a non-ionic liquid by bubbling N₂ or argon through

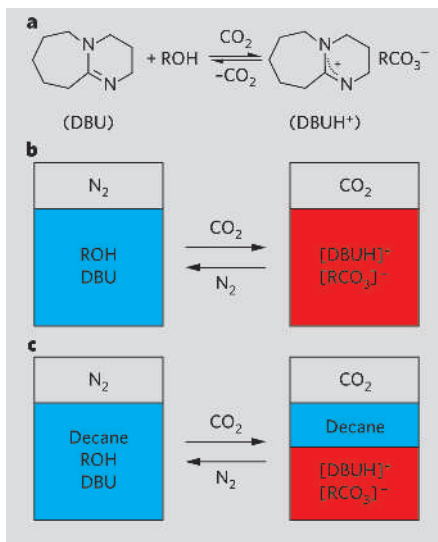


Figure 1 | The 'switching' of a switchable solvent.

a, Protonation of DBU (1,8-diazabicyclo-[5.4.0]-undec-7-ene) in the presence of an alcohol and carbon dioxide is reversed when CO₂ is removed. **b**, Polarity switching in the reaction shown in **a**, in which CO₂ causes a nonpolar liquid (shown in blue) mixture of hexanol and DBU to change over one hour into a polar, ionic liquid (shown in red); nitrogen gas reverses the process by stripping out CO₂ from the reaction. **c**, The different polarity of each liquid under the two conditions is illustrated by the miscibility of decane with the hexanol/DBU mixture under nitrogen, before exposure to CO₂; however, decane separates out once the mixture becomes polar in the presence of CO₂. Again, N₂ reverses the process.

the liquid at room temperature or, for a more rapid reaction, at 50 °C. These changes are demonstrated by chemical shifts in key protons, as revealed by ¹H-NMR spectroscopy, and by solvatochromic measurement of the polarity of the solvent before and after exposure to CO₂ (see supplementary information).

The reaction is exothermic and causes a marked increase in the viscosity of the liquid. The choice of alcohol is critical because the 1-hexylcarbonate salt (Fig. 1, right) is a viscous liquid at room temperature, whereas the bicarbonate^{3,4} and methylcarbonate (ref. 5, and A. D. Main, G. E. Fryxell and J. Linehan, unpublished results) salts are solids and so are not candidates for smart solvents.

Our non-ionic liquid is as nonpolar as chloroform, according to measurements using Nile Red as solvatochromic dye (see supplementary information), whereas the liquid under CO₂ is as polar as dimethylformamide or propanoic acid. The polarity changes in this switchable solvent system are demonstrated by testing the solubility of decane, a nonpolar compound, in each liquid: it is miscible with the liquid under N₂ but not with that under CO₂ (Fig. 1c). We conclude that N₂ and CO₂ at 1 bar can be used as triggers of miscibility and immiscibility, respectively.

We have built solvent switchability into molecules that are small enough to be liquid at room temperature. Further examples of switchable solvents, preferably ones less Lewis-basic than DBU, should eventually enable their application in the 'green' production of high-value chemicals such as pharmaceuticals.

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Corrigendum

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This communication contains an error in the methods section of the supplementary information. In the description of the fusion protocol on page 3, line 2, electrical pulses were delivered for 15 microseconds, and not for 15 seconds as published.