



## The Tet Technology: controlling gene expression in eukaryotes

TET Systems is marketing its proprietary Tet Technology, the most broadly and successfully applied system for controlled gene expression in eukaryotes. The Tet Technology allows the researcher to control the activity of a target gene specifically, quantitatively and reversibly *in vivo* and *in vitro*.

The Tet Technology (Fig. 1, 2) functions in cultured cells as well as in a wide spectrum of organisms, from animals and plants to various unicellular systems (Table 1). The potential to control individual gene activities specifically and in a temporally defined manner has allowed the dissection of gene functions and pathways *in vivo* and *in vitro* with unprecedented precision, yielding exciting new insights into such complex biological processes as development, disease and behavior.

The impact of the Tet Technology on basic and applied research as well as its commercial applications is reflected by more than 3,000 publications (see [http://www.tetsystems.com/main\\_references.htm](http://www.tetsystems.com/main_references.htm), or refs. 1–4 for reviews) and many applied-for and issued patents. Here we illustrate the power of the technology with examples of selected applications.

### Study of gene function in *Saccharomyces cerevisiae*

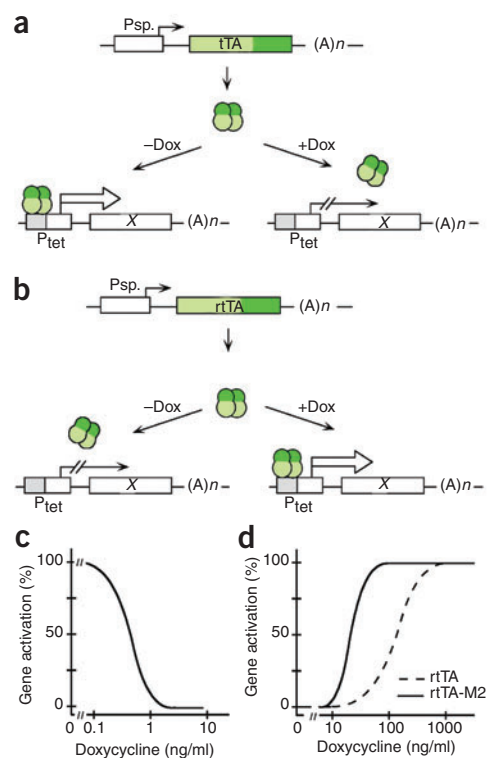
The systematic replacement of endogenous promoters by  $P_{tet}$  has allowed the production of libraries of yeast strains with titratable promoter alleles, the activity of which can now be controlled by doxycycline. Quantitative analyses of mutant phenotypes from these libraries have yielded a wealth of information on gene functions. Notably, the microarray analysis of libraries of conditional mutants permits the study of transcriptional patterns of gene clusters under specific conditions, thereby proving or disproving hypothetical gene functions<sup>5</sup>.

### Screening systems

Tet-based systems for high-throughput screening of compound libraries have proven most successful in *S. cerevisiae* and in defined animal cell lines. For such strategies, the target genes, whose products are frequently not tolerated when expressed constitutively, are kept under strict Tet control until required in the assay. An impressive example involves cell lines in which the hepatitis B viral genome was placed under Tet control<sup>6</sup> leading to the discovery of new and now widely marketed drugs for the treatment of hepatitis B and AIDS.

### Sabine Freundlieb

TET Systems Holding GmbH & Co. KG, Im Neuenheimer Feld 582, 69120 Heidelberg, Germany. Correspondence should be addressed to S.F. ([freundlieb@tet-systems.com](mailto:freundlieb@tet-systems.com)).



**Figure 1** | Principle of Tet regulation. (a–d)  $P_{tet}$  is composed of a minimal RNA polymerase II promoter fused downstream of operator sequences from the prokaryotic (Tn10) tetracycline-resistance operon.  $P_{tet}$  activity is thus dependent on the binding of tetracycline-controlled transcription activators tTA or rtTA. tTA is a fusion between the Tet repressor and eukaryotic transcriptional activation domains. tTA binds  $P_{tet}$  only in the absence of doxycycline (Dox). In contrast, rtTA contains mutations in the repressor moiety that reverse its phenotype so that it requires Dox to bind and activate  $P_{tet}$ . rtTA2-M2 (marketed by Clontech as ‘Tet-ON Advanced’) exhibits highly improved Dox sensitivity. Both tTA (a,c) and rtTA (b,d) are capable of tightly controlling  $P_{tet}$ -directed target gene expression and regulating gene activity over 4–6 orders of magnitude *in vivo* and *in vitro* depending on the presence or absence of Dox.  $P_{sp}$  designates tissue-specific promoters which, by restricting tTA or rtTA synthesis to cell types or tissues, can convey high specificity to Tet-controlled gene expression *in vivo* (Fig. 2).

**Table 1** | Systems in which Tet regulation has been applied

System	Examples
Mammals	Nonhuman primates, rats, mice
Amphibians and fish	<i>Xenopus laevis</i> , zebrafish
Insects	<i>Drosophila</i> , anopheles, medfly
Unicellular organisms	<i>S. cerevisiae</i> , <i>Dictyostelium</i> , <i>Toxoplasma gondii</i> , <i>Plasmodium falciparum</i> , <i>Candida</i> , <i>Aspergillus</i> , <i>Ustilago</i>
Cultured cells	Mammalian, amphibian, insect and plant
Viruses	Adenovirus, adeno-associated virus, retrovirus/lentivirus, herpesvirus, baculovirus

For specific publications please search the reference database on the TET Systems homepage (<http://www.tetsystems.com>).

### New mouse models for biomedical research

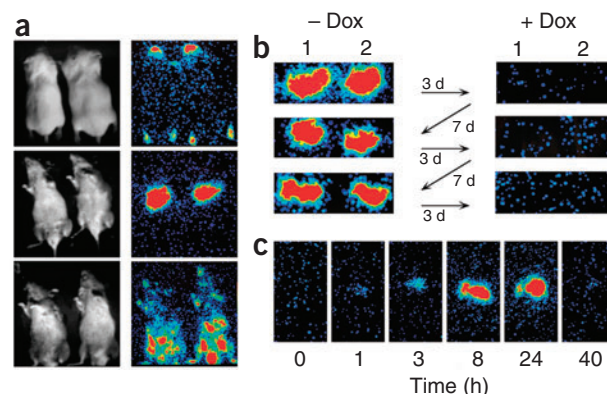
The feasibility of generating truly conditional mouse mutants via Tet Technology has led to the development of new animal models for human diseases. These allow the control of disease genes in a cell type-specific and temporally defined way, whereby the target gene may not only be turned on or off, but may also be adjusted to intermediate levels that mimic pathological states more closely than ‘all-or-none’ switches. Such mouse models can address early events during the onset of disease, disease progression, potential reversibility of pathologies and disease regression. Disease models of this quality are most valuable not only for the identification of genes and their products as pharmacological targets, but also for preclinical drug-efficacy studies.

Particularly intriguing mouse models have been generated by placing oncogenes such as *c-myc*, *ras12* and *ErbB2* under Tet control. These models have yielded new insights into tumor initiation, progression and reversibility or regression. Notably, this approach has also revealed unexpected dependencies of tumor cells on tumor-initiating oncogenes, a phenomenon named ‘oncogene addiction’<sup>3</sup>.

One important study, in which a ‘humanized’ Huntingtin gene was placed under Tet control, yielded results indicating that even the devastating neurodegenerative Huntington disease may be reversible once the disease-causing gene is inactivated<sup>3</sup>. The results of such sophisticated disease models are paving the way for new intervention strategies.

### Combining the Tet Technology with RNAi approaches

Knocking down gene activities via RNA interference (RNAi) has become a powerful technology for the study of gene functions, and genome-scale RNAi libraries have been generated that target virtually every human and mouse gene, a tremendous asset for drug discovery and development. Obviously, producing inhibitory RNAs in a temporally controlled and cell type-specific manner via Tet control will add another dimension to functional genomics studies. Indeed, several reports have demonstrated that inhibitory RNAs can be produced under Tet control by using modified doxycycline-dependent RNA polymerase III promoters<sup>7</sup> or by expressing microRNAs with a classical Tet Technology approach, based on RNA polymerase II transcription<sup>8</sup>. A successful application of Tet control of microRNA expression was described recently, in which tight control of p53 expression allowed investigators to monitor tumorigenesis and regression *in vivo*<sup>9</sup>.



**Figure 2** | Tissue-specific and reversible control of a target gene *in vivo* by Dox. **(a)** Mice expressing the luciferase gene in neurons (top), hepatocytes (middle), multiple tissues under tTA/P<sub>tet</sub> control (bottom). **(b)** Repeated induction and repression of luciferase in the liver of two mice by supply (+) and withdrawal (-) of Dox; d, days. **(c)** Kinetics of induction of luciferase in the liver by a single injection of Dox. Luciferase was under rtTA/P<sub>tet</sub> control.

### Access to Tet Technology

Based on its broad patent portfolio, TET Systems offers access to the Tet Technology to for-profit entities in the form of nonexclusive research licenses and individually tailored commercial licenses.

Academic institutions that acquire the Tet Technology by purchase of its components obtain automatically a cost-free license for use in internal academic research. This license excludes specifically the use of the technology in projects sponsored by for-profit institutions (unless the sponsor is a licensee) and the right to sell or otherwise transfer the Tet components to third parties.

Components of the Tet Technology as well as related products and services may be obtained from TET Systems licensed partners, which include Clontech, genOway, Jackson Laboratories, EMMA and IonGate.

- Baron, U. & Bujard, H. Tet repressor-based system for regulated gene expression in eukaryotic cells: principles and advances. *Methods Enzymol.* **327**, 401–421 (2000).
- Gossen, M. & Bujard, H. Studying gene function in eukaryotes by conditional gene inactivation. *Annu. Rev. Genet.* **36**, 153–173 (2002).
- Berger, S. & Bujard, H. Novel mouse models in biomedical research: the power of dissecting pathways by quantitative control of gene activities. in *Handbook of Experimental Pharmacology* (eds., Offermanns, S. & Hein, L.) 3–30 (Springer Verlag, Heidelberg, 2003).
- Jonkers, J. & Berns A. Conditional mouse models of sporadic cancer. *Nat. Rev. Cancer* **2**, 251–265 (2002).
- Mnaimneh, S. *et al.* Exploration of essential gene functions via titratable promoter alleles. *Cell* **118**, 31–44 (2004).
- Ladner S.K. *et al.* Inducible expression of human hepatitis B virus (HBV) in stably transfected hepatoblastoma cells: a novel system for screening potential inhibitors of HBV replication. *Antimicrob. Agents Chemother.* **41**, 1715–1720 (1997).
- Wiznerowicz, M. & Trono, D. Conditional suppression of cellular genes: lentivirus vector-mediated drug-inducible RNA interference. *J. Virol.* **77**, 8957–8961 (2003).
- Gossen, M. Conditional gene expression: Intelligent designs. *Gene Ther.* **13**, 1251–1252 (2006).
- Dickins, R.A. *et al.* Probing tumor phenotypes using stable and regulated synthetic microRNA precursors. *Nat. Genet.* **37**, 1289–1295 (2005).

This article was submitted to *Nature Methods* by a commercial organization and has not been peer reviewed. *Nature Methods* takes no responsibility for the accuracy or otherwise of the information provided.