KMOLBI Highlight 2006 No. 1:

Rapid knock-down-knock-in system for mammalian cells

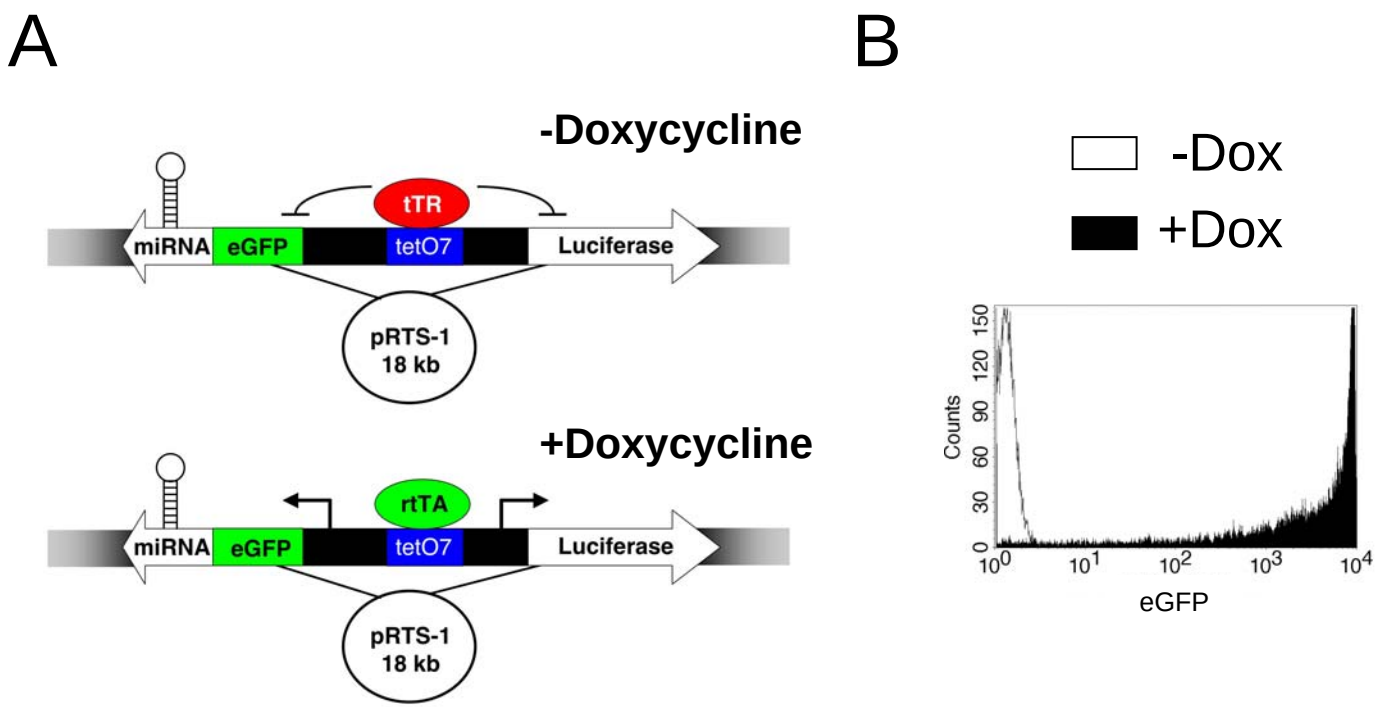
RNA interference (RNAi) is a powerful tool to analyze gene function in mammalian cells. However, the interpretation of RNAi knockdown phenotypes can be hampered by off-target effects and compound phenotypes, as many proteins combine multiple functions within one molecule and coordinate the assembly of multimolecular complexes. Replacing the endogenous protein after knock-down by ectopic wild-type or mutant forms can exclude off-target effects, preserve complexes and unravel specific roles of domains or modifications. Therefore we developed a rapid-knock-down-knock-in system for mammalian cells. Stable polyclonal cell lines where generated within two weeks by simultaneous selection of two episomal vectors. One mediated the reconstitution and the other the knock-down in a doxycycline-dependent manner to allow the analysis of essential genes. Knock-down was achieved either by synthetic siRNA or by an artificial miRNA-embedded siRNA targeting the untranslated region of the endogenous, but not the ectopic mRNA. To proof our system, we analysed mutants of Pes1 and WDR12, two factors essential for ribosome biogenesis and cell proliferation. Loss-off function phenotypes were rescued by the wild-type and specific mutant forms, but not by the remaining mutants. Thus, our system is suitable to exclude off-target effects and to functionally analyze mutants in cells depleted for the endogenous protein. This system allows for the first time the detailed and rapid genetic analysis of mammalian genes functions in cell culture.

Grimm, T., Hölzel., Rohrmoser, M., Harasim, T., Malamoussi, A., Gruber-Eber, A., Kremmer, E., and Eick, D. (2006) Dominant-negative Pes1 mutants inhibit ribosomal RNA processing and cell proliferation via incorporation into the PeBoW complex. *Nucleic Acids Research,* 34, 3030-3043

Hölzel, M., Rohrmoser, M., Grimm, T., Malamoussi, M., Harasim, T., Gruber-Eber, A., Kremmer, E., and Eick, D. (2006) The BRCT domain of mammalian Pes1 is crucial for nucleolar localization and rRNA processing. *Nucleic Acids Research,* in press

Hölzel, M., Rohrmoser, M., Orban, M., Hömig, C., Harasim, T., Malamoussi, A., Gruber-Eber, A., Heissmeyer, V., Bornkamm, G.W. and Eick, D. (2006) Rapid knock-down-knock-in system for mammalian cells. *Nucleic Acids Research,* in press

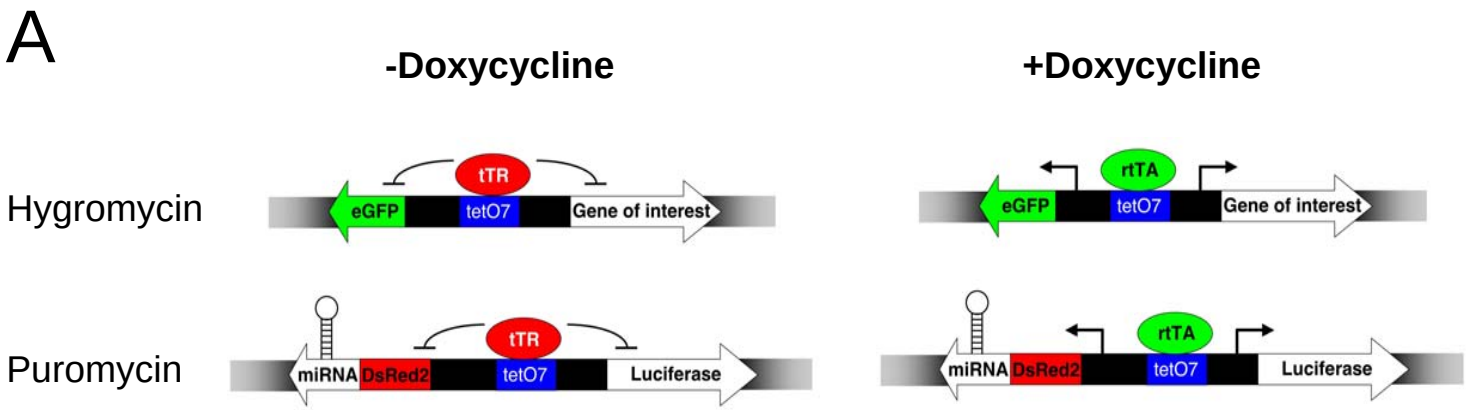
Rapid conditional knock-down-knock-in system for mammalian cells

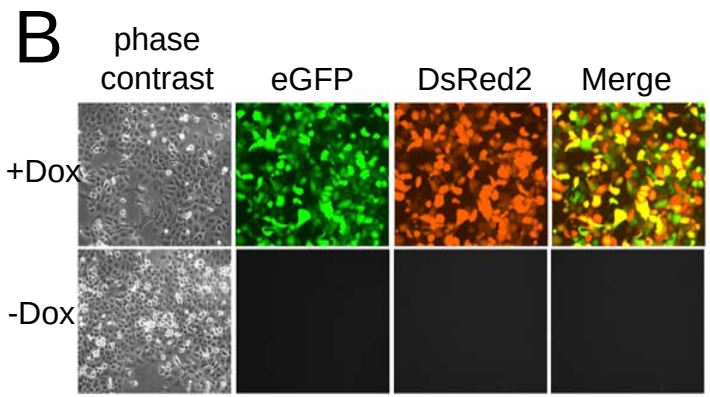


Conditional expression of miRNA-embedded siRNAs.

(A) Scheme of the pRTS construct harbouring a target gene specific siRNA sequence embedded in the modified murine miR-155 structure behind the eGFP open reading frame. Conditional gene activation is achieved in the presence of doxycycline by the tet-activator (rtTA, green), whereas active repression is mediated by the tet-repressor (rTR, red) in the absence of doxycycline. **(B)** Detection of eGFP positive cells by flowcytometry upon addition of doxycycline to the culture medium.

Rapid conditional knock-down-knock-in system for mammalian cells



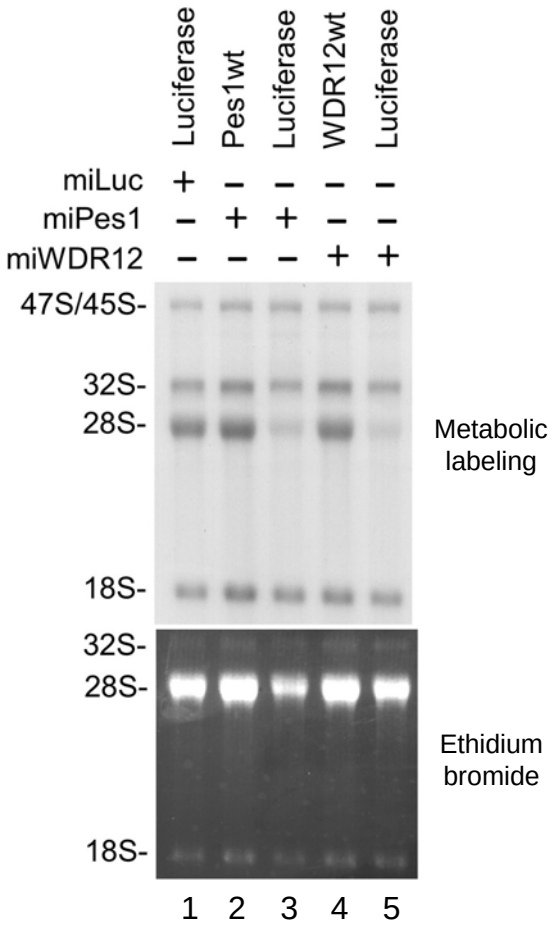


Development of a rapid knock-down-knock-in system for mammalian cells.

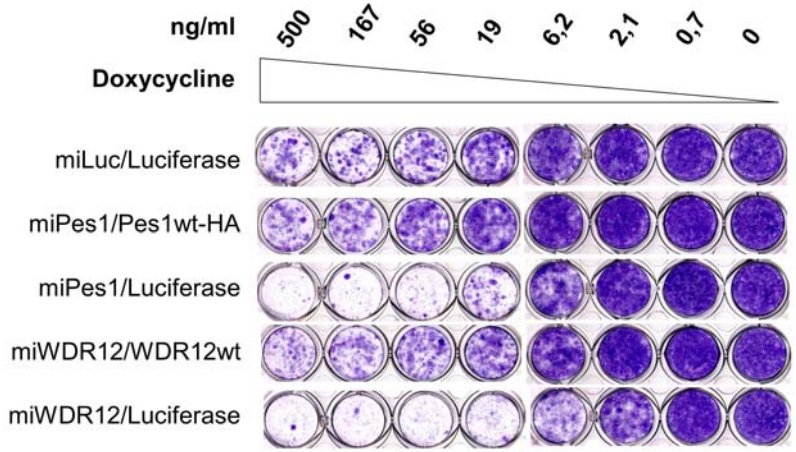
(A) Scheme of the knock-down-knock-in approach by using two pRTS plasmids.
(B) Verification of successful co-selection with hygromcyin and puromycin by monitoring co-expression of eGFP and DsRed2 in stably selected H1299 cells.

Knock-down-knock-in of Pes1 and WDR12 rescues processing of ribosomal RNA and cell proliferation

A



B



(A) Metabolically labelled total ribosomal RNA was separated on a 1% agarose-formaldehyde gel and visualized by autoradiography.
(B) Analysis of cell proliferation. The indicated cell lines were seeded in multiples at low density, fixed after 12 days with ice-cold methanol and stained with Giemsa. Representative wells are shown.