GENERATION OF CALUX® REPORTER CELL LINES CONTAINING MULTIPLE DIOXIN RESPONSIVE ELEMENTS

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Introduction

Dioxins are persistent contaminants that induce a variety of toxic effects. The DR CALUX® bioassay is used routinely to detect dioxin-like chemicals in various matrices. Upon exposure to dioxin-like compounds, the cells express luciferase in a dose-dependent manner. Here we describe the generation of a new reporter gene construct containing 20 repeats of the dioxin responsive element.

Aim

The current DR CALUX® assay makes use of a reporter gene construct containing four dioxin responsive elements (DREs)[1]. It has been suggested previously that the sensitivity and response can be improved by the integration of higher numbers of DREs into the plasmid[2,3].

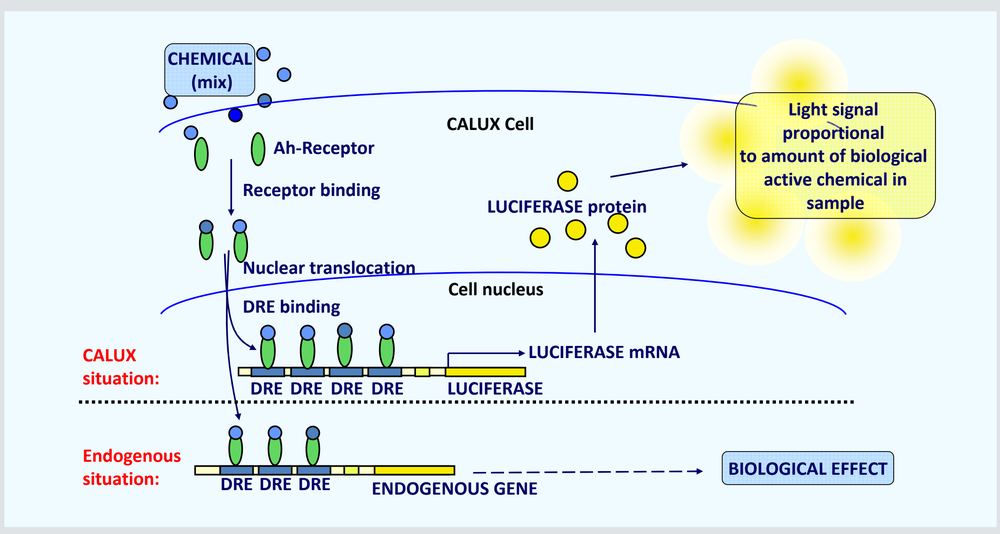

figure 1 - principle of the DR CALUX assay

Methods

Various DNA fragments with 10 or 20 DREs were cloned into different reporter gene expression vectors and compared to constructs with four DREs. The constructs were first tested transiently; based on those results (not shown), **pGudLuc1.5xCyp1a1** was selected for stable transfection.

Origin of DREs	No. of DREs	Plasmid (promoter)
5x mCyp1a1 promoter (-1313 to -820)	**20**	**pGudLuc1.1[1] (MMTV)**
5x mCyp1a1 promoter (-1313 to -820)	20	pGL2-tataluc[4,5] (minimal)
10x rCyp1a1 promoter (-985 to -979)	10	pGudLuc1.1 (MMTV)
10x rCyp1a1 promoter (-985 to -979)	10	pGL2-tataluc (minimal)
20x rCyp1a1 promoter (-985 to -979)	20	pGudLuc1.1 (MMTV)
20x rCyp1a1 promoter (-985 to -979)	20	pGL2-tataluc (minimal)

References

1. Garrison et al. (1996); *Fund. Appl. Tox.* 30(2): 194-203
2. He et al. (2008); *Organoha. Comp.* 70: 772-5
3. He et al. (2009); *Organoha. Comp.* 71: 2399-2403
4. Sonneveld et al. (2002); *Organoha. Comp.* 58: 369-72
5. Sonneveld et al. (2007); *Toxicol. Sci.* 99(2): 455-69

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Results: clone selection

Six clones were analysed in detail using 0.03 - 300 pM TCDD, and compared to the original DR CALUX® (figure 2). Clone #10 had the most favourable characteristics: low baseline RLUs, high fold induction and sensitivity, and was studied in more detail.

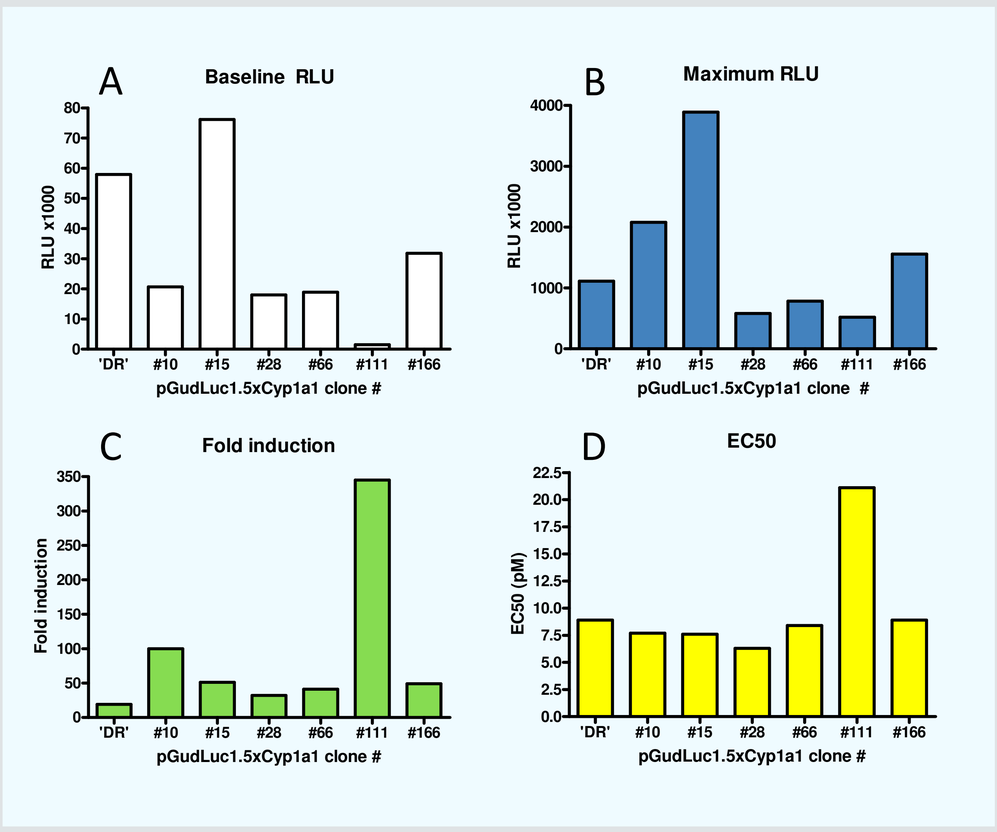

figure 2 – six stable clones of H4IIE pGudLuc1.5xCyp1a1 (20xDRE) were compared to the original DR CALUX (4xDRE): baseline- and maximum RLU values (A, B), fold induction (C) and EC50 values (D) were determined.

Results: sensitivity

At TCDD concentrations > 1 pM, the magnitude of response of clone #10 is significantly higher (figure 3).

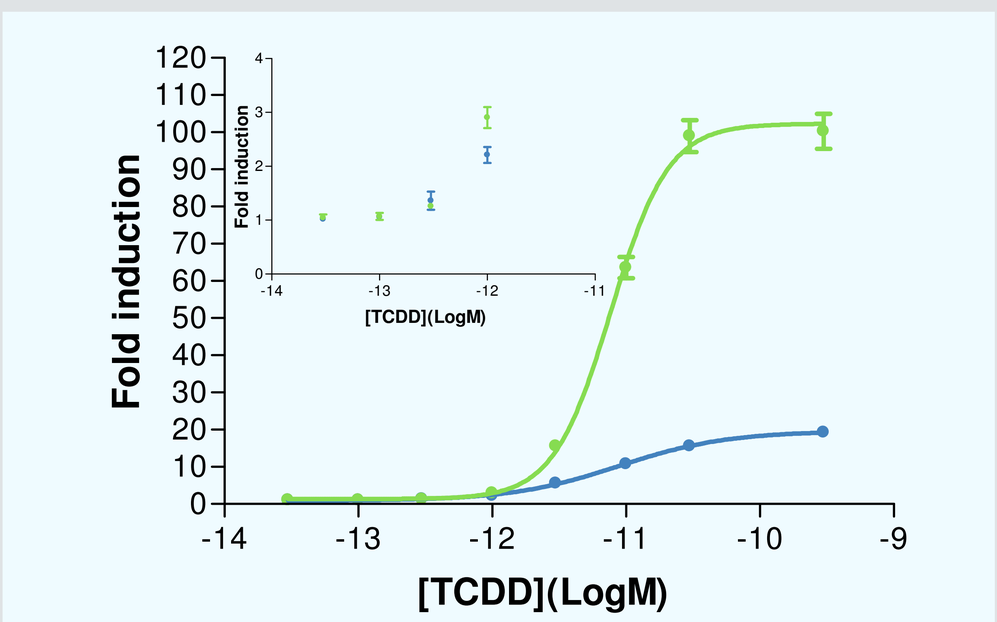

figure 3 - TCDD dose-response curves of the original DR CALUX® (blue) and the new 20xDRE-containing stable cell line H4IIE-pGudLuc1.5xCyp1a1#10 (green). Inset: zoom-in of 0.03 - 1 pM TCDD.

Conclusions

We constructed a 20xDRE CALUX line with similar sensitivity, but five times higher fold induction compared to the current DR CALUX®. Thus, in contrast to suggestions by others[2,3], increment of the number of DREs does not improve the sensitivity of the bioassay, but rather improves the response maximum. At TCDD concentrations > 1 pM the magnitude of response of the new cell line is significantly higher; this may facilitate accurate quantification in samples containing relatively low levels of dioxin-like compounds.