116th Annual Meeting of the Anatomische Gesellschaft Joint Meeting with the Anatomical Society 2022 Sept. 20-23

DFG Deutsche Forschungsgemeinschaft German Research Foundation

A novel approach for cell type-specific and systematic analysis of transcription factors *in vivo*

Christoph Wiegreffe[1,✉], Simon Ehricke[1], and Stefan Britsch[1]

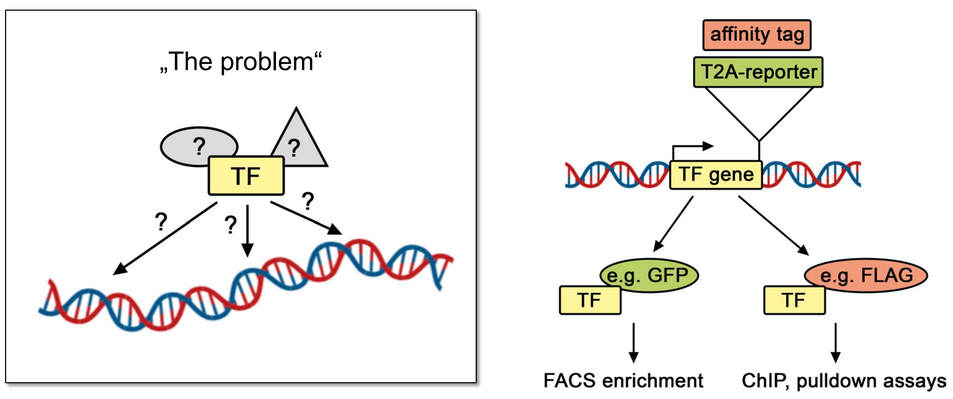
[1]Institute of Molecular and Cellular Anatomy, Ulm University, 89081 Ulm, Germany, ✉ christoph.wiegreffe@uni-ulm.de

ulm university universität ulm

Abstract

Transcription factors (TFs) regulate gene expression by direct DNA binding together with cofactors and in chromatin remodeling complexes. Their function is thus regulated in a spatiotemporally and cell type-specific manner. To analyze the functions of TFs in a cell type-specific context, genome-wide DNA binding as well as identification of the interacting proteins is required. We use an *in vivo* approach (*i*-GONAD) in mice to genetically modify TFs by adding reporter and affinity tags that can be exploited for enrichment of target cells, chromatin immunoprecipitation, and pull-down assays. Using this approach, we show functional and cell-type specific modification of Bcl11 TFs in newborn mice. iGONAD is a highly efficient procedure to modify TF coding genes by integration of small insertions, such as reporter and affinity tags. The novel Bcl11 strains described here, can be used to better understand Bcl11 function in neurodevelopment and disease.

I. Genome editing strategy

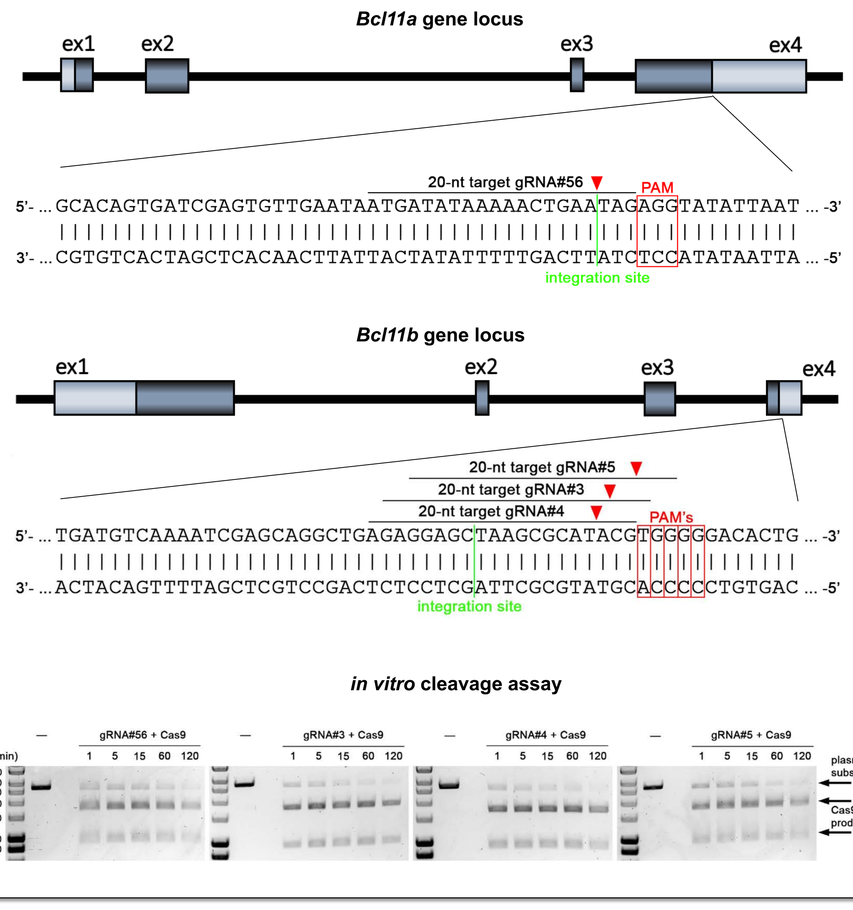


II. Selection and validation of gRNAs

in silico analysis of gRNAs targeting Bcl11 TF genes

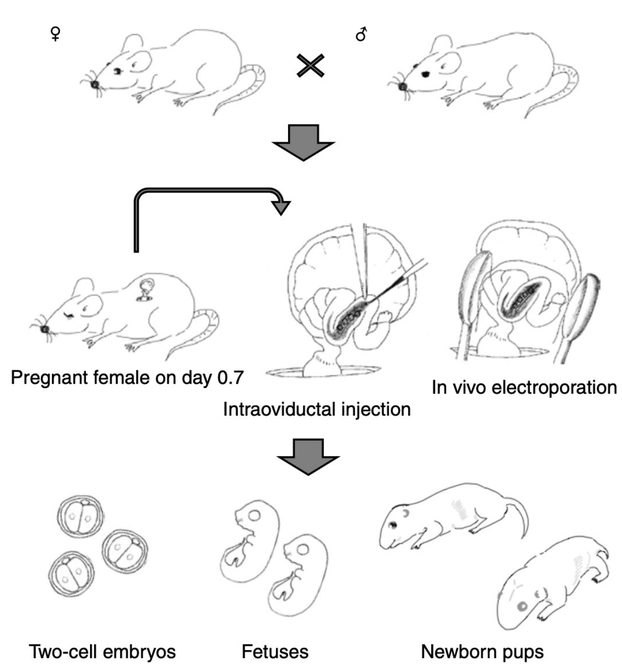
parameter	***Bcl11a*** gRNA#56	***Bcl11b*** gRNA#3	***Bcl11b*** gRNA#4	***Bcl11b*** gRNA#5
specificity score Zhang et al. (2013)	91.34%	99.45%	99.76%	99.84%
activity score Doench et al. (2016)	0.641	0.676	0.556	0.675
distance to insertion site	0	10	9	12

in vitro analysis of gRNA cutting efficiency



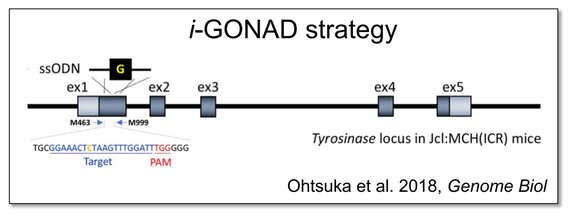
Wiegreffe, C., Ehricke, S., Britsch, S., *unpublished*

III. *i*-GONAD procedure

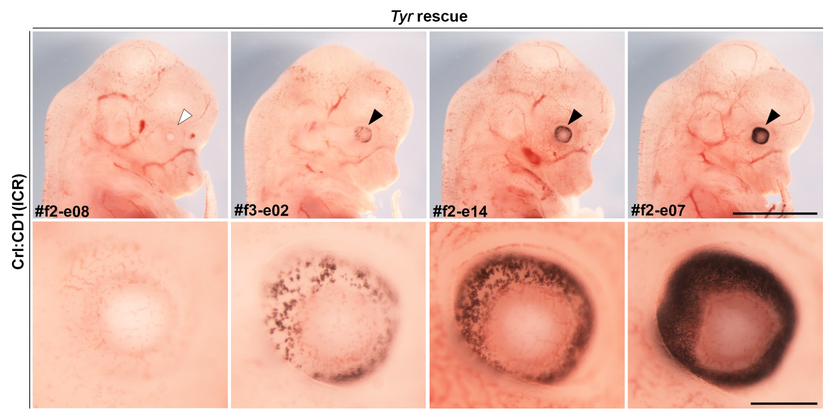


Gurumurthy et al., 2019 *Nat Protoc*

IV. Tyrosinase rescue experiment

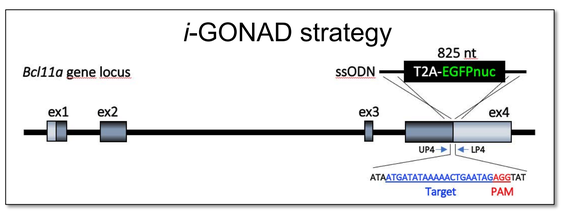


#	no. of embryos	eye pigmentation (E14)			success rate (%)
		albino	partial	complete	
1	14	11	1	2	27
2	5	3	0	2	40
3	7	3	2	2	57
total	26	17	3	6	**35**

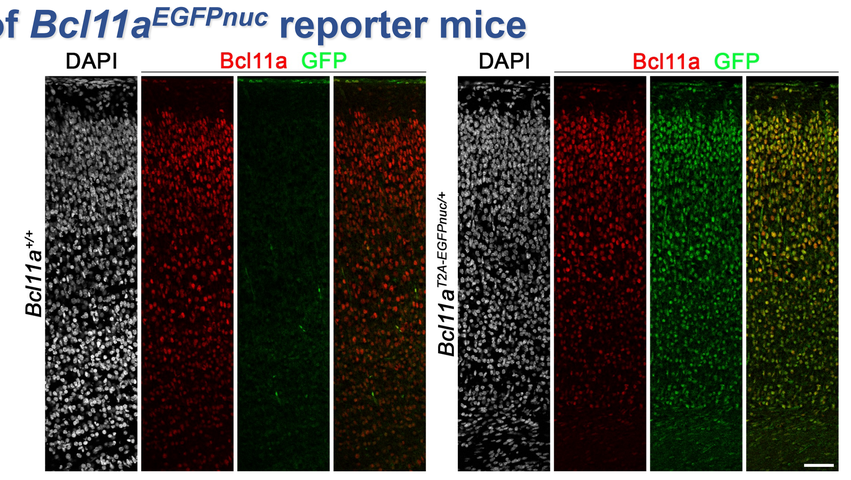


Wiegreffe, C., Ehricke, S., Britsch, S., *unpublished*

V. Generation of *Bcl11a*^{EGFPnuc} reporter mice

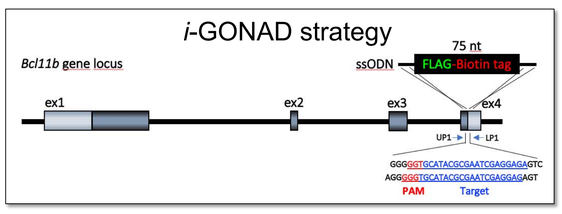


#	no. of embryos	genotype				success rate (%)
		wildtype	hetero-zygous	homo-zygous	other	
1	13	8	3	0	2	23
2	15	7	1	1	6	13
total	28	15	4	1	8	**18**



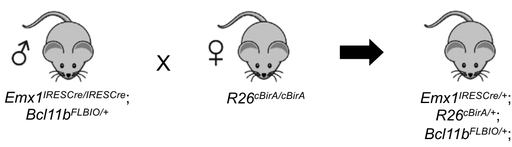
Wiegreffe, C., Ehricke, S., Britsch, S., *unpublished*

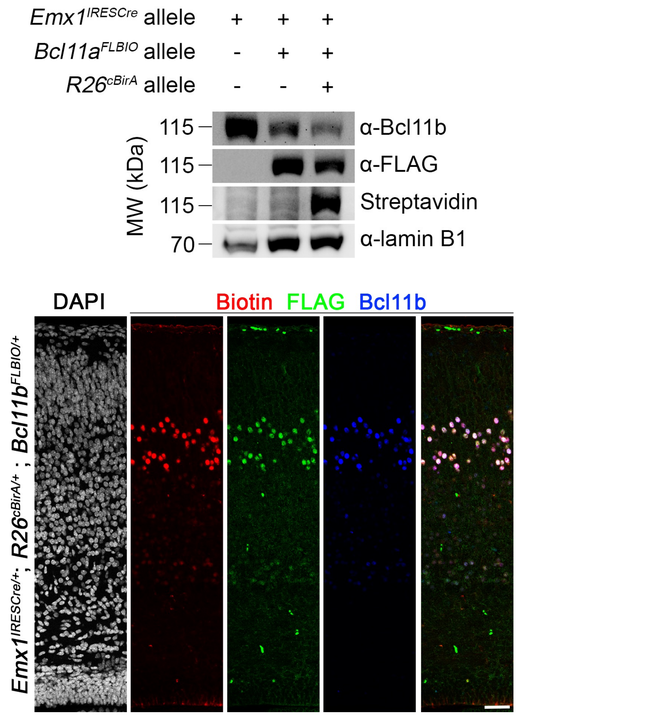
VI. Generation of *Bcl11b*^{FLBIO} affinity tag mice



#	no. of embryos	genotype				success rate (%)
		wildtype	hetero-zygous	homo-zygous	other	
1	11	8	3	0	0	27
2	12	5	2	1	4	25
3	14	7	4	1	3	36
4	12	5	6	1	0	58
5	12	6	5	0	1	42
6	10	1	6	1	3	70
total	71	32	26	4	9	**42**

Cre-specific biotinylation of Bcl11b *in vivo*





Wiegreffe, C., Ehricke, S., Britsch, S., *unpublished*

VII. Conclusions and perspective

- *i*-GONAD is a highly efficient method to generate novel mouse models.
- Careful selection and *in vitro* validation of candidate gRNAs is highly recommended.
- The DNA fragment size to be inserted limits the efficiency of *i*-GONAD (up to 1 kb is feasible).
- Novel Bcl11 mouse models will help identifying DNA binding sites as well as proteins interacting with Bcl11 TFs during regulation of gene expression in neurodevelopment.

VIII. References

Gurumurthy, C.B., Sato, M., Nakamura, A., Inui, M., Kawano, N., Islam, M.A., Ogiwara, S., Takabayashi, S., Matsuyama, M., Nakagawa, S., et al. (2019). Creation of CRISPR-based germline-genome-engineered mice without ex vivo handling of zygotes by i-GONAD. Nat Protoc 14, 2452-2482. 10.1038/s41596-019-0187-x.

Ohtsuka, M., Sato, M., Miura, H., Takabayashi, S., Matsuyama, M., Koyano, T., Arifin, N., Nakamura, S., Wada, K., and Gurumurthy, C.B. (2018). i-GONAD: a robust method for in situ germline genome engineering using CRISPR nucleases. Genome Biol 19, 25. 10.1186/s13059-018-1400-x.

Doench, J.G., Fusi, N., Sullender, M., Hegde, M., Vaimberg, E.W., Donovan, K.F., Smith, I., Tothova, Z., Wilen, C., Orchard, R., et al. (2016). Optimized sgRNA design to maximize activity and minimize off-target effects of CRISPR-Cas9. Nat Biotechnol 34, 184-191. 10.1038/nbt.3437.

Hsu, P.D., Scott, D.A., Weinstein, J.A., Ran, F.A., Konermann, S., Agarwala, V., Li, Y., Fine, E.J., Wu, X., Shalem, O., et al. (2013). DNA targeting specificity of RNA-guided Cas9 nucleases. Nat Biotechnol 31, 827-832. 10.1038/nbt.2647.

IX. Acknowledgements/Funding

Work was supported by grants from the Deutsche Forschungsgemeinschaft to S.B. (SFB 497/A9, BR 2215) and the Ulm University (Bausteinprogramm 3.2) to C.W.