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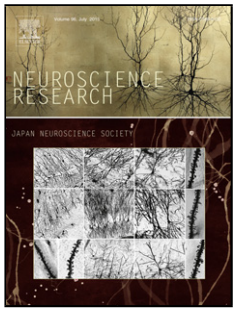

Title: Generation of *Pax6-IRES-EGFP* knock-in mouse via the cloning-free CRISPR/Cas9 system to reliably visualize neurodevelopmental dynamics

Authors: Yukiko U. Inoue, Yuki Morimoto, Mikio Hoshino, Takayoshi Inoue

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Generation of *Pax6-IRES-EGFP* knock-in mouse via the cloning-free CRISPR/Cas9 system to reliably visualize neurodevelopmental dynamics

Yukiko U. Inoue*, Yuki Morimoto, Mikio Hoshino, and Takayoshi Inoue

[1]Department of Biochemistry and Cellular Biology, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Ogawahigashi, 4-1-1, Kodaira, Tokyo 187-8502, Japan

*Corresponding Author, E-mail address; yinn3@ncnp.go.jp

Highlights

- We generate *Pax6-IRES-EGFP* knock-in mouse by the cloning-free CRISPR/Cas9 system.
- EGFP expressions in the knock-in mice recapitulate the endogenous Pax6 expressions.
- At embryonic stages, Pax6-expressing regions are detectable without PCR-genotyping.
- Visualization of Pax6 expression dynamics by this line offers useful research tools.

[Abstract]

Pax6 encodes a transcription factor that plays pivotal roles in eye development, early brain patterning, neocortical arealization, and so forth. Visualization of Pax6 expression dynamics in these events could offer numerous advantages to neurodevelopmental studies. While CRISPR/Cas9 system has dramatically accelerated one-step generation of knock-out mouse, establishment of gene-cassette knock-in mouse via zygote injection has been considered insufficient due to its low efficiency. Recently, an improved CRISPR/Cas9 system for effective gene-cassette knock-in has been reported, where the native form of guide RNAs (crRNA and tracrRNA) assembled with recombinant Cas9 protein are directly delivered into