

Resource Summary Report

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mCerulean-hCOL1A1

RRID:Addgene_140112

Type: Plasmid

Proper Citation

RRID:Addgene_140112

Plasmid Information

URL: <http://www.addgene.org/140112>

Proper Citation: RRID:Addgene_140112

Insert Name: Type I procollagen ?1

Organism: Homo sapiens

Bacterial Resistance: Ampicillin

Vector Backbone Description: Vector Backbone:pcDNA3.1; Vector Types:Mammalian Expression; Bacterial Resistance:Ampicillin

Comments: These human type I procollagen plasmids were designed and validated for human osteoblasts and fibroblasts similar to their previously published/shared murine counterparts e.g. eGFP-Pro?1 (Plasmid #119843) https://www.addgene.org/Sergey_Leikin/. Their expression in other cells might disrupt assembly, folding and trafficking of type I procollagen, resulting in artifacts. Even in human osteoblasts and fibroblasts, overexpression of exogenous pro?2 might cause intracellular trafficking and degradation of some transfected chains as monomers without integration into normal heterotrimeric (pro?1)2pro?2 procollagen molecules,. Overexpression of exogenous pro?1 might result in excessive formation of homotrimeric (pro?1)3 molecules, some of which might contain fluorescent tags on two or even all three pro?1 chains, potentially causing severe disruptions in cellular function. In our experience with the murine plasmids expressed in primary osteoblasts or the MC3T3 osteoblastic cell line, the best evidence of normal behavior of the transfected chains is the appearance of fluorescent extracellular collagen fibers ~ 12-24 h after transfection. These were harder to observe in human Saos2 osteoblast cell line transfected with the human type I procollagen plasmids (likely due to slower fibrillogenesis and more complete cleavage of the fluorescently-tagged N-propeptide prior to collagen fibril formation). We strongly recommend following these guidelines when using the plasmids 1) Only human cells that have high expression of endogenous type I procollagen and that express fewer transfected than endogenous chains are suitable for studies of physiologically-relevant

processes with these constructs. 2) At least 100 μ M ascorbic acid at and after transfection is required to ensure proper procollagen folding (for some cells, preincubation with ascorbic acid before transfection might be needed). 3) Stable transfection causes excessive accumulation of tagged chains in the ER over time, disruption of ER homeostasis, and disruptions and changes in cellular function. Therefore, low to moderate transient transfection is recommended for studies of physiologically relevant processes. Optimization of transfection efficiency is needed for all cell types. 4) Experiments beyond 24h after transfection are not recommended, to avoid the excessive accumulation of tagged chains in the ER and cell malfunction caused by increased procollagen synthesis. 5) Excessive expression of transfected chains might cause rapid accumulation of large procollagen aggregates in the ER, resulting in cellular malfunction and death. Contact information: Shakib Omari (shakib.omari@nih.gov), Sergey Leikin (leikins@mail.nih.gov).

Plasmid Name: mCerulean-hCOL1A1

Relevant Mutation: COL1A1 exons 2-3 replaced by fluorescent tag

Record Creation Time: 20220422T221810+0000

Record Last Update: 20260829T075636+0000

Ratings and Alerts

No rating or validation information has been found for mCerulean-hCOL1A1.

No alerts have been found for mCerulean-hCOL1A1.

Data and Source Information

Source: [Addgene](#)

Usage and Citation Metrics

We have not found any literature mentions for this resource.