iPSC Minicircle DNA User Manual

I. Introduction and Background

The minicircle DNA (mc-DNA) is an independent mammalian expression cassette void of any bacterial DNA element required for plasmid production. mc-DNA is generated by an intramolecular recombination catalyzed by the ΦC31 integrase, which is expressed by a special host *E.coli* bacterial strain together with the I-SceI endonuclease. The parental plasmid for the mc-DNA production contains multiple engineered I-SceI restriction sites that result in the destruction of the parental plasmid but not the mc-DNA (Jia, et al., 2010). Therefore, mc-DNA differs from the regular plasmid vectors by lacking the bacterial origin of replication or antibiotic resistance markers. Because of this, the expression cassette has been shown to have an extended life span with higher and longer expression levels of over 3 weeks in different cells studied (Chen, et al., 2003). In the end, the non-integrating mc-DNA gets degraded and loses the expression of target genes. Thus it is ideal for re-programming iPSCs due to its higher efficiency than iPSC proteins and its non-integrating nature as compared to lentiviral vectors.

abm is the first company to offer endotoxin-free iPSC mc-DNAs containing human or mouse Yamanaka or Thomson sets driven by the EF1a promoter. This mc-DNA vector is a truly xeno-free method for effectively generating iPSCs. The vector is deprived of the bacterial backbone sequence and utilizes the mammalian EF1a promoter rather than the viral CMV promoter. As the CMV promoter is the target for methylation and methlylation of the CMV promoter is often the reason leading to gene silencing, mc-DNA with the CMV promoter will significantly compromise efficacy of iPSC induction. This mc-DNA requires only a simple transfection and transgene expression can be easily monitored with GFP.

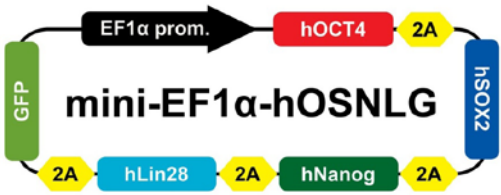

Figure 1. iPSC minicircle DNA map (Cat.# G389)

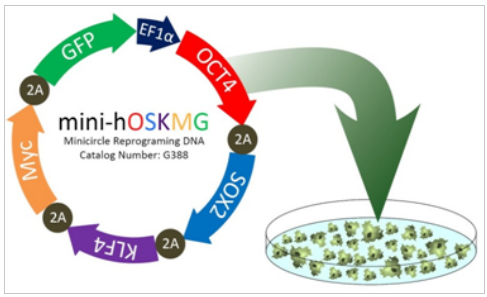

Figure 2. Transfection of mc-DNA to induce iPSC

II. Transfection of Minicircle DNA for reprogramming

iPSC induction with mc-DNA requires transfection reagents with high transduction efficiency and low toxicity. Unfortunately, transduction efficiency in most primary cells is very low and a minimum of 20% efficiency is required for successful iPSC generation. The human fibroblast was employed as the demonstrate cell line in the following protocol with Lentifectin™ (abm Cat. No. G074). Specialized optimization may be needed in different conditions. In general, reprogramming requires approximately 4ug per transfection per well in a 6-well plate three times.

1. Seed 10^5 human dermal fibroblast (HDF) cells in a 6-well plate (~70% confluent) one day before transfection, including one dish used for GFP control.
2. In a 1ml Eppendorf Tube A, dilute 4ug of mc-DNA in 100ul of medium without serum. In another tube B, dilute 10-20ul of Lentifectin™ in 100ul of medium without serum. Mix gently and incubate the two tubes for 5 min at room temperature.
3. Combine the medium in tube A and B, mix gently and incubate for another 20 minutes at room temperature.
4. Add 800ul serum-free medium to the complexes followed by gentle mixing.
5. Remove the medium from the cells, wash with PBS once and add the ~1ml complexes from the previous step to the 6-well plate without dislodging the cells. Incubate the cells for 5 hours at 37 °C.
6. After 5 hours, add 100ul FCS to the culture dish. Incubate overnight.
7. The following day, replace the medium with 2ml fresh iPSC medium.
8. Repeat mc-DNA transfection for at least 3 times to sustain activity of the mc-DNA for 7-14 days in the cells for the reprogramming process.
9. Colonies with morphologies similar to hESC colonies are clearly visible by day 18 after the initial transfection.
10. At day 26–28 after transfection, GFP-negative mc-iPSC colonies can be individually picked for further expansion and analysis.

Reference:

1. Jia FJ, Wilson KD, Sun N, Gupta DM, Huang M, Li ZJ, Panetta NJ, Chen ZY, Robbins RC, Kay MA, Longaker MT, Wu JC. (2010). A nonviral minicircle vector for deriving human iPS cells. Nature Methods 7(3):197-9.
2. Chen ZY, He CY, Ehrhardt A, Kay MA. (2003). Minicircle DNA vectors devoid of bacterial DNA result in persistent and high-level transgene expression in vivo. Molecular therapy 8(3):495-500.

Note: The mc-DNA technology is licensed from Standford University and commercial customers need to inquire about license agreement before purchasing. The product is only