# RNA Modifications in Direct Cardiac Reprogramming

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## Abstract

Heart disease is the leading cause of death within the United States, accounting for about 1 in 4 deaths annually.[1] An adult mammalian heart has extremely limited regenerative capabilities, so when cardiomyocytes are lost due to myocardial infarction, they are replaced with non-contractile fibrotic scar tissue. This in turn can lead to chronic heart failure and death. One promising approach for cardiac regeneration is direct reprogramming, which converts fibroblasts into induced cardiomyocytes (iCMs) through overexpression of cardiac transcription factors.[2,3] A better understanding of the molecular mechanisms underlying this process is necessary for future clinical applications. While the transcriptional and epigenetic regulation of direct reprogramming is well- studied, its post-transcriptional regulation, especially regarding mRNA modification changes, and the underlying regulatory mechanisms of direct reprogramming are largely unknown. This project seeks to better understand how mRNA modification changes affect direct cardiac reprogramming. Preliminary data found that knocking out Gene A, a m$^6$A reader, led to increased direct reprogramming yield.

## Background

- Heart disease accounts for about 1 in 4 deaths annually in the United States
- One form of post-transcriptional regulation is mRNA modification
- The most abundant mRNA modification is *N*6-methyladenosine (m$^6$A)
- Writer proteins create m$^6$A modifications, erasers remove them, and readers recognize them
- Recent studies have shown that m$^6$A modifications play a role in stem cell reprogramming[4,5,6]
- shRNA (small hairpin RNAs) are sequences of RNA that create a hairpin structure when they hybridize, knocking down gene expression

## Methods

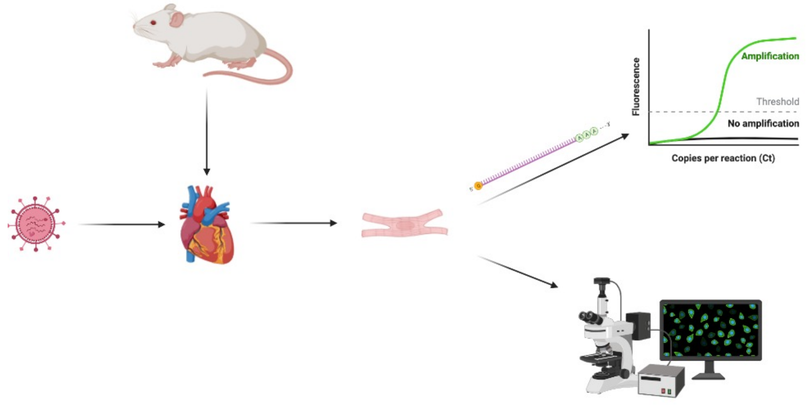


**Figure 1.** PlatE cells were used to package retroviruses for mouse cardiac cell infection. Immunofluorescence was used to stain cell nuclei and markers for cardiomyocytes. Images were taken and cells were counted. Cells were lysated using TRIzol and their RNA were extracted. This RNA was then reverse-transcribed into cDNA and analyzed using qRT-PCR to quantify the relative expression levels of mRNA.

## The Knockdown Efficiency of m$^6$A Regulators

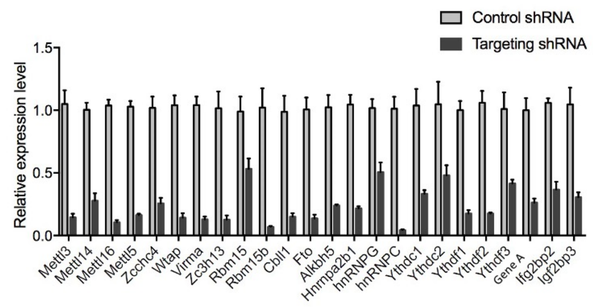


**Figure 2.** The effectiveness of shRNA designed for 24 m$^6$A regulators was tested through qRT-PCR analysis.

## Loss of Function Screen of m$^6$A Readers

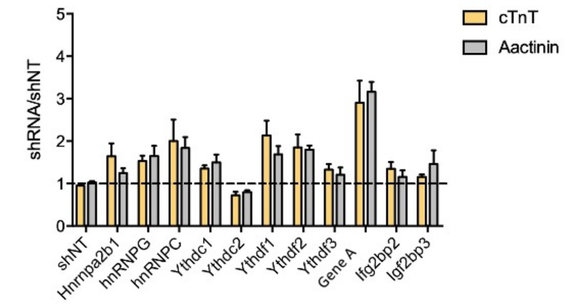


**Figure 3.** Induced cardiomyocytes were reprogrammed with the addition of certain shRNAs to knock down select genes. The level of cardiac marker proteins, cTnT and Aactinin were measured.

## Expression of Cardiomyocyte Markers and Fibroblast Genes

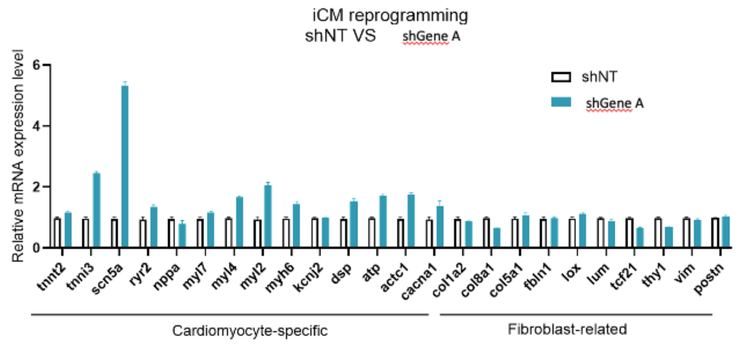


**Figure 4.** A qRT-PCR analysis was performed on induced cardiomyocytes reprogrammed in the presence of non-targeting shRNA or shRNA for gene A. Primers for cardiomyocyte-specific and fibroblast-related genes were selected to investigate improvements in efficiency.

## Immunocytochemistry for Reprogramming Efficiency

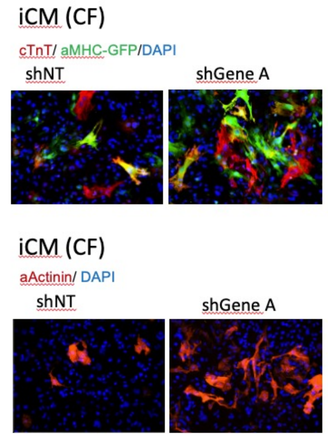


**Figure 5.** Induced cardiomyocytes were reprogrammed with and without knockdown RNA for Gene A. Immunocytochemistry was performed to visualize nuclei and cardiac marker proteins.

## References

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