THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF BIOCHEMISTRY & MOLECULAR BIOLOGY

# INVESTIGATING THE MECHANISM OF NEUROD1 IN ASTROCYTE REPROGRAMMING BY CHROMATIN IMMUNOPRECIPITATION

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# ABSTRACT

Many symptoms of neurological disorders, such as Alzheimer's, Parkinson's, and Huntington's, are based on loss of different types of neurons. When the brain gets damaged by a disease or an injury, astrocytes in the surrounding area become reactive, which means they start dividing and fill up the gap making up for the lost tissue. This is an effective immediate response for fixing the damage, however, in the long term, it causes further inhibition in the brain since many astrocytes secrete inhibitory GABA signals. For the extensive neuron loss, cell reprogramming can be a potential solution. The reprogramming technique developed in our lab converts reactive astrocytes in the brain into functional neurons that can eventually integrate in neural networks and form synapses to compensate for the neuronal loss. Cell reprogramming can theoretically even reverse the pathology of neurodegenerative diseases. The reprogramming of reactive astrocytes to neurons is triggered by a neural transcription factor NeuroD1, which has been successfully used in our lab in stroke and Alzheimer's disease models. NeuroD1 binds to chromatin, thus facilitating binding of other transcription factors to promote neuronal development. Here we investigate the mechanism of reprogramming of astrocytes to neurons by determining the binding sites of NeuroD1. We determined that NeuroD1 binds to Hes6 promoter along with NeuroD1's own promoter through chromatin Immuno-precipitation assay (ChIP). We also cloned an HA-tagged version of NeuroD1 that can be used for a large scale analysis of NeuroD1 binding through ChIP-seq. Determining the binding sites of NeuroD1 would shed light of the mechanism of reprogramming, which can help us to better understand and potentially improve the method making it more suitable for clinical applications.

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## ACKNOWLEDGEMENTS

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# Chapter 1

# Introduction

## History of Reprogramming Technology & Impact on Neurodegenerative Diseases

Neurodegeneration is seen in many of the neurological diseases like Parkinson's and Alzheimer's disease and they are increasingly prevalent due to the increase in the elderly population over the years. These diseases lead to cerebral and cognitive impairments and also affect basic daily function (Gitler, Dhillon & Shorter, 2017). In the US alone, there are about 5.5 million people suffering from Alzheimer's disease. From the current 24 million people suffering from Alzheimer's worldwide, it is estimated that the numbers will increase to 81 million by 2040 (Alzheimer's Association, 2016). Scientists are actively searching for approaches to treat those brain disorders, including methods to introduce new neurons into the brain. Different sources of neurons were considered over the years with these various methods described in **Figure 1**.

Shinya Yamanaka won the Nobel Prize in 2007 after discovering that pluripotent stem cells can be induced from adult skin fibroblasts by using a set of transcription factors (Oct4, Sox2, Klf4 and c-Myc) (Takahashi et al., 2007). This has helped to signify the flexibility in changing the fate of a cell and helped to focus on neuronal regeneration using reprogramming technology. iPS technology has been used in cell therapy to perform clinical trials for neurological disorders like Parkinson's and Alzheimer's disease (Okano and Yamanaka, 2014) and some research also involved the use of transcription factors to convert closely related lineages to each other, which was from B-cells to macrophages in this case (Xie et al., 2004). These trans-differentiation-based studies formed the initial basis for neuronal reprogramming technology, including the finding that astroglial cells can be trans differentiated into neurons (Niu et al., 2013). The purpose of those studies was to regenerate neurons in vitro, so that they can be transplanted

into patients. Induced neurons were generated from fetal human fibroblasts, which were transplanted in rat brains with their identity being maintained for weeks post transplantation (Pereira, 2014).

At the same time, there are various problems with this procedure related to survival rate and potency of these methods. Specifically in the experiment mentioned above, immunosuppressants were administered. Unfortunately, the necessity for immuno-suppression presented some important concerns. The risk of immune rejection and tumor formation of these grafts from pluripotent and stem cells were quite significant. Although differentiation from stem cells or iPSCs seemed to be a better alternative with a lesser risk for tumorigenesis, it was difficult to store them for long term use (Goldman, 2016). This was not ideal for the application of this technology for disease treatment and a better alternative was needed to produce a viable therapeutic. Direct reprogramming presents a promising solution to this problem; the conversion happens directly in the brain and does not involve any implanted cells. Thus, there is no need for immunosuppressants and it does not require technically challenging transplantation procedures with low cell survival.

During transdifferentiation of astrocytes to neurons, the epigenetic landscape is altered and the transcription profile changes, which results in conversion of one cell type to another. This can be done with the help of pioneer transcription factors like NeuroD1 and Neurogenin-2, which are capable of binding to heterochromatin, making possible for the next wave of transcription factors to bind. The specific subtype of neurons that is obtained as a result depends on the primary cell and combination of transcription factors (Li & Chen, 2016).

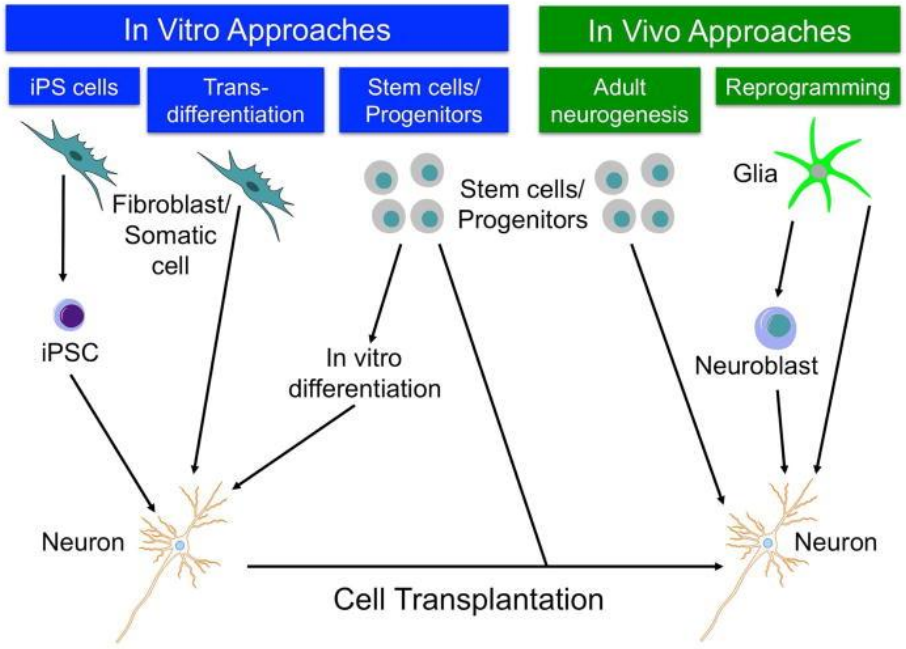


**Figure 1**: In-Vitro & In-Vivo Approaches to Neuronal Conversion (Li & Chen, 2016)

Direct in-vivo reprogramming has been an emerging technology over the years and our lab has been doing pioneering work in this field as well (Li & Chen, 2016). In-vivo reprogramming using transcription factors was first done in the pancreas with Ngn3, Pdx1 and MafA to produce insulin beta cells from pancreatic exocrine cells (Zhou et al., 2008). For functional reprogramming of astrocytes into functional neurons, our lab has used Ngn2 and NeuroD1 (Li & Chen, 2016).

## Neural Reprogramming with NeuroD1

NeuroD1- mediated reprogramming is a recent technology, which was developed in Dr. Gong Chen's lab, and has promising results for clinical applications. NeuroD1 is a transcription factor, which was shown to be key in reprogramming astrocytes into glutamatergic neurons. Gliosis is a common process when brain injuries occur; it results in scarring of nervous tissue due to proliferation of glial cells that fill up the injury site. The scarring caused by glial cells results in neuronal inhibition, which was

observed in different neurodegenerative disorders, such as Parkinson disease, Alzheimer disease, and stroke. Reprogramming of reactive astrocytes into neurons can be used to restore the lost neurons and reverse their inhibition. NeuroD1, a transcription factor which promotes neuronal differentiation, was expressed using retroviral vectors introduced via stab injury in cortical region of mice brain. The use of retroviruses allowed for a proper targeting of the reactive glial cells, which are proliferative after injury; the efficiency of astrocyte to neural conversion was found to very high (around 90%) within two weeks of expression. Reprogramming of human astrocytes resulted in glutamatergic neurons; electrophysiological studies showed integration of converted neurons into the neuronal circuits (Guo et al, 2014).

Other transcription factors have also contributed to the neuronal conversion process, in particular, Ascl1 induced the conversion of midbrain astrocytes into functional neurons (Liu et al., 2015). Studies have also shown that astrocytes can be indirectly reprogrammed into neurons by de-differentiating into neuroblast precursor cells. Sox2, which is expressed in neural stem cells, was used for producing these neuroblasts. (Niu et al., 2013). Apart from astrocytes, NG2 glia were converted into functional neurons in adult mouse brain upon expression of NeuroD1. These NG2 cells majorly converted into glutamatergic neurons with a small proportion of GABAergic neurons (Guo et al., 2014). Different other neuronal subtypes can also be directly obtained. One such example being ectopic expression of Fezf2, which led to reprogramming of cortical layer II/III neurons into layer V/VI neurons (Rouaux and Arlotta, 2013). Most promise in terms of the conversion process has been seen in Ngn2 and NeuroD1, which are members of the basic helix loop helix protein family and help in central nervous system development (Lee, 1997).

Small molecule-based reprogramming can be also be used for altering cell specific signaling pathways to result in neuronal conversion of human astrocytes grown in cell culture. This type of chemical reprogramming converted cultured human astrocytes into functional neurons with a small molecule cocktail with a conversion efficiency of 67%. These astrocytes were able to survive for at least 5 months in culture and transcription factors like NeuroD1 were upregulated upon chemical reprogramming as well, which is shown pictorially in **Figure 2** (Zhang et al., 2015).

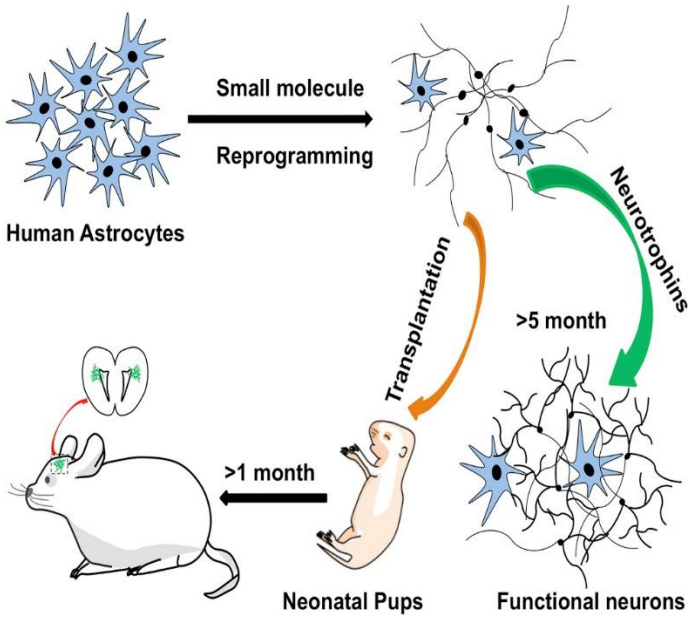


**Figure 2:** Overview of Chemical Reprogramming with Small Molecules (Zhang et al., 2015)

This reprogramming technology has become a valuable replacement to iPSCs due to direct conversion by avoiding the stem cell state, which is risky in terms of potential cancer development. The main advantage of this technology is its versatility in applying to any model of study, ranging from cancer to neurodegenerative diseases (Li & Chen, 2016). Glutamatergic neurons which release the excitatory neurotransmitter, glutamate, are lost in pathologies, such as Alzheimer's disease. Through the use of transcription factors, we are attempting to convert reactive astrocytes into functional glutamatergic neurons. Previous work in our lab has demonstrated the ability of a single transcription factor, NeuroD1, to directly reprogram astrocytes into functional neurons in-vivo in Alzheimer's model mice. This process is seen to occur in brain injury as well as human cultures (Guo et al, 2014). NeuroD1 is expressed during embryogenesis and into adulthood as it plays a vital role during embryogenesis (Lee, 1997). Further studies are being carried out to test the conversion of astrocytes into GABAergic and dopaminergic neurons. The main purpose of this project is to determine the mechanism by which NeuroD1 acts on astrocytes to result in reprogramming into glutamatergic neurons.

**Effect of NeuroD1 on the Transcriptional & Epigenetic Landscape of Neuronal Conversion**

NeuroD1 is a so-called pioneer transcription factor that can bind to heterochromatin and initiate the conversion process. It can bind to promoters and initiate transcription of other transcription factors, such as Hes6. Most previous studies focused on achieving the success of converting astrocytes into glutamatergic neurons. We analyze the mechanism of the conversion process by using immunoprecipitation; we are using HA-tagged NeuroD1 to determine which promoters it binds to. Apart from the regular reprogramming ability of NeuroD1 to convert reactive astrocytes into glutamatergic neurons, expressing Dlx2 together with NeuroD1 converts NG2 cells into GABAergic neurons. NeuroD1 alters the genome's epigenetic landscape and allows transcription of neuronal gene enhancers, which is characterized by removal of repressive histone methylation and promoting histone acetylation in promoter regions.

NeuroD1 accomplishes this epigenetic alteration by binding to regulatory elements of neuronal genes, which are silenced by epigenetic mechanisms. This results in heterochromatin to euchromatin conversion, leading to a better accessibility of chromatin. Neuronal migration is also initiated by NeuroD1 action as it induces genes in the epithelial to mesenchymal transition (Pataskar et al., 2016). In Xenopus and mouse models, core mediators of Ngn2 and NeuroD1 were found to be evolutionarily conserved and recognize targets with an enhancer signature of clustered consensus binding sites. Activation of a core set of transcription factors leads to formation of a network, which controls neurogenesis. In the context of microglia-neuronal conversion by NeuroD1, NeuroD1 initially occupies heterochromatin regions with H3K4me3 and H3K27me3 mark in microglia and in later stages of reprogramming, this changes to a singular H3K4me3 marker. Adding to this, NeuroD1 induces Scrt1 and Meis2, which are transcriptional repressors, and this leads to a decline in microglial gene expression with the respective epigenetic signature erased. NeuroD1 acts as a pioneering factor during the onset of neuronal property and loss of microglial identity (Matsuda et al., 2019).

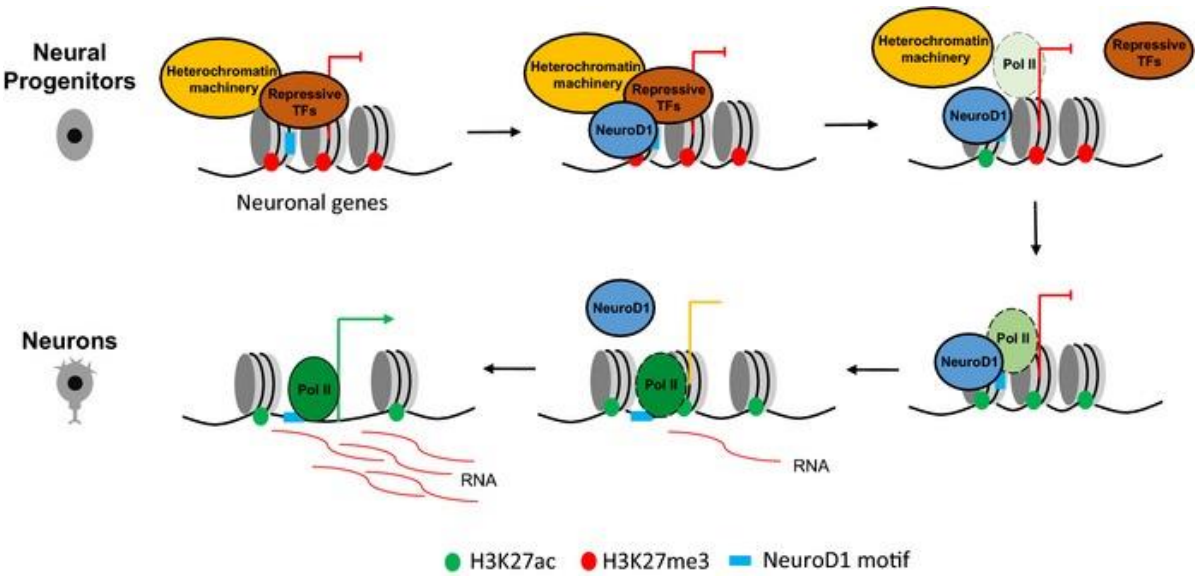


**Figure 3:** Changes in the Epigenetic Landscape Mediated by NeuroD1 (Pataskar et al., 2016)

To have a deeper understanding of NeuroD1 mediated changes, a ChIP-seq analysis-based study shows the association of ND1 effector genes and its impact on neuronal development. These studies further highlight the preferential binding of NeuroD1 to unmethylated CpG rich regions and access to closed chromatin showcasing epigenetic impact (Trudler & Lipton, 2019). Histone modifications were induced with around 20 transcription factors found, which contain H3K4 and H3K27 methylations. Namely, 3 transcription factors, Bhlhe22, Prdm8, and Myt1l, were upregulated and microglial transcription factors were downregulated (Matsuda et al., 2019). Interestingly, Hes6 genes, which are transcriptional repressors in Notch signaling, behave differently during neuronal differentiation. These are activated by the cascade expression of Ngn2 & NeuroD1 (Vilas-Boas & Henrique, 2010).

**Figure 3** gives us an idea of how epigenetic regulation mediated by NeuroD1 occurs. When NeuroD1 binds to promoters, there is a loss of the H3K27me3 mark and increase in H3K27ac as well as accessibility of chromatin. So, NeuroD1 activates enhances and promoters by targeting distal sites and a time course analysis showed earlier induction of genes associated with NeuroD1 compared to enhancer targets. This shows a varying cascade of mechanistic events occurring with regulatory elements targeted by NeuroD1. NeuroD1 targets transcription factors which repress neuronal gene expression like TBX3

and shuts them down. At the same time, inactive neuronal enhancers are activated and these enhancers are bound by MBD3. MBD3 is displaced by NeuroD1 binding, leading to an increase in H3K27ac expression and neuronal gene activation. This helps to show the induction of epigenetic memory at target sites post NeuroD1 expression and provide an insight into the genomic targets through which NeuroD1 acts (Pataskar et al., 2016).

It can be seen that neurogenic factors are brought into target sites by genetic mechanisms, which alter the epigenetic landscape and transient expression of transcriptional factors persist even after NeuroD1 has been expressed earlier through the mentioned epigenetic mechanisms (Pataskar et al., 2016). Here, we would like to draw a relation between these transcription factors and NeuroD1 expression through HA tagged ND1 retro-virus. ChIP studies will be used to offer an insight into the co-expression of these transcription factors post HA-ND1 retroviral infection in astrocytes.

Since the previous studies were focused on the conversion of mESC into neurons, we decided to perform our own analysis of the mechanism of conversion that triggers transdifferentiation of human astrocytes into neurons.

# Chapter 2

# Materials & Methods

## Plasmid Preparation & Cloning

### Cloning of the HA-tagged NeuroD1 in a plasmid for viral production.

A plasmid with the HA-tagged NeuroD1, pHABioA2loxNeuroD1, was kindly provided by Dr. Vijay Tiwary. First, HAND1 was cloned in pFUGWFoxA2 plasmid for the lentiviral production. pFUGWFoxA2, a derivative of pFUGW under control of UbC promoter (Lois et al., 2002).

HA-tagged NeuroD1 fragment was obtained with BamH1 digest of PCR of_pHABioA2loxNeuroD1 with primers 5'-CACACAGGATCCGCCGCCACCATGGGGTAC-3' and 5'-GCAGTCGATCCATCGTGAAAGATGGCATT-3'. It was ligated with dephosphorylated BamH1-digested pFUGWFoxA2 and introduced into E. coli XL-Blue strain by electroporation. Colonies were screened for the presence of the insert and for the correct orientation by HindIII digest.

Then, HAND1 was recloned in CAG-ND1-IRES-GFP for the retroviral production, under control of a CAG promoter, which is composed of CMV enhancer and chicken beta actin promoter with a part of the first exon. The Kozac sequence was also inserted at the 5' of the transcript for more efficient translation. For that, the HAND1 fragment was obtained by Age1, Xho1 digest of PCR of FUGWHAND1 with 5'-TCAGCTACCGTGCCACCATGGGGTAC-3' and 5'-TTCTAGGTCTCGAGGTCG-3'. CAG-ND1-IRES-GFP was also digested with Age1, and Xho1, dephosphorylated, and ligated to the fragment.

Cloning of the HAND1 was carried out with pCAG-GFP plasmid in strains of *E. coli*. NeuroD1 was fused with Hemagglutinin tag from flu and inserted into a retroviral vector pCAG-IRES-GFP to have generate pCAG-NeuroD1-IRES-GFP. Three major proteins encoded in the retroviral genome, Env, Gag

and Pol, that help in producing an envelope protein, forming the group antigen core structure and reverse transcriptase respectively for viral particle production were provided on separate other plasmids.

**Mammalian cell culture**

Human cortical astrocytes (HA1800) from ScienCell (California) were grown in a medium with DMEM/F12, 10% FBS, penicillin, 3.5 mM glucose supplemented with B27 and 10 ng/mL EFG & FGF2. Cell cultures were incubated under 5% CO2 at 37 degree Celsius (Zhang et al., 2015). Astrocytes cells were cultured in PDL coated coverslips (12 mm) at 50000 cells per coverslip density in 24 well plates.

Hek 293 T cells were grown in Dulbecco's Modified Eagle Medium (DMEM) and 10% fetal bovine serum (FBS) under 5% CO2 at 37 degree Celsius.

**Retroviral Production**

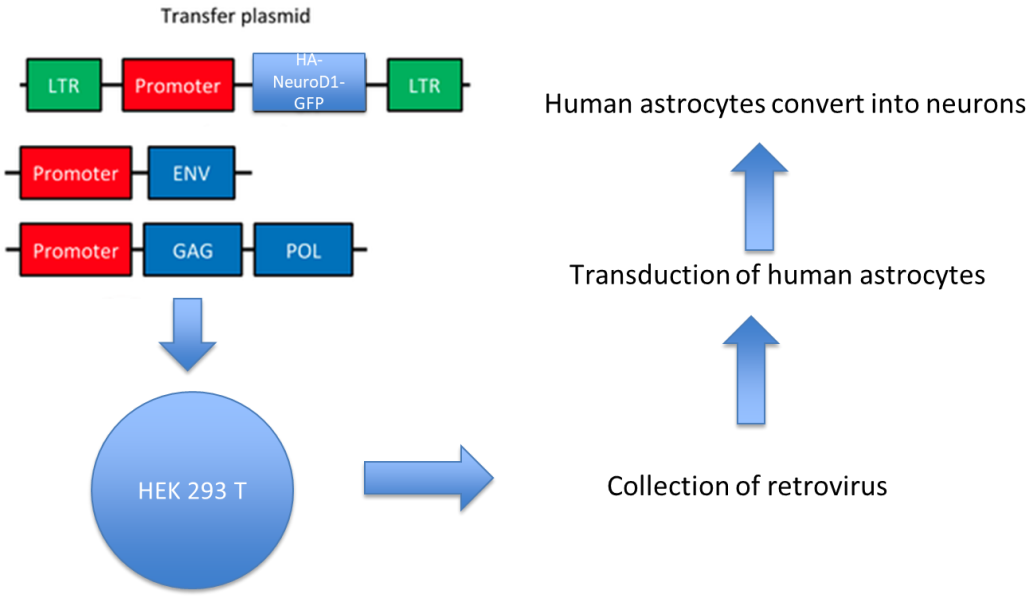


**Figure 4:** Protocol for Astrocyte Conversion into Glutamatergic Neurons (Addgene)

**Figure 4** gives us an overall view of the conversion process from astrocytes to neurons. Hek 293 cells were grown to about 70% confluency in a 15 cm diameter tissue culture dish and were transfected with a mixture of 18 μg pCAG-NeuroD1-IRES-GFP (transfer plasmid), 12 μg of CMVPG (GAG and Pol-containing plasmid), 6 μg of VSVG (envelope plasmid), and 130 uL PEI in 1 mL OptiMEM. The media was changed in 5 hours, and media with virus was collected in 48 hours post transfection. The titer of the virus was $10^7$ VP/ml. The specific viral particles were collected from media by centrifugation at 25,000 rpm for 2 hours with the pellet re-suspended in phosphate buffer. Cell cultures were incubated under 5% CO2 at 37 degree Celsius (Guo et al., 2014).

**Viral transduction**

After reaching 90% confluency, the virus was added to cells in the growth media supplemented with 10 μg/m. The medium was replaced after 24 hours, and then every other day for another week. (Guo et al., 2014). Neuron maturation occurred in about one week after infection.

**Immuno-Fluorescence Microscopy**

For cell culture staining, the coverslips were changed from the present cell media and fixed under 4% PFA for 12 mins at room temperature. This is followed by three washes with PBS under a normal rotation plate and this is followed by blocking for around an hour on a slow stirring plate. The blocking buffer comprises of 0.1% Triton in PBS and 0.05% Normal Donkey Serum. After this, the sample is incubated with the primary antibody necessary for the respective staining overnight in a cold room (4 degree Celsius). The sample is washed three times with PBS on a slow stirring plate and incubated with the secondary antibody (standard 1:800 dilution factor) for an hour on a slow stirring plate. The secondary antibody is conjugated to Alexa Fluor 488, 546 and 647. The sample is washed three times with PBS

again and the coverslips are mounted using polish on a glass slide with antifade & DAPI reagent added before placing the coverslip (Guo et al., 2014).

### Antibodies

Mouse NeuroD1, chicken GFP, and rabbit HA primary antibodies (Cell signaling) were used for immunostaining.

## Data and Statistical Analysis

Immunofluorescence microscopy images were taken by the Zeiss Apotome microscope and Olympus Confocal microscope. Cell counting was done and analyzed by using the ImageJ software with the number of total neurons being counted manually. The data for producing the graphical outputs from the QPCR result were done by compiling the data & producing graphs using Microsoft Excel.

**Chromatin Immunoprecipitation & qPCR Experiment (Adapted from Typed Protocol)**

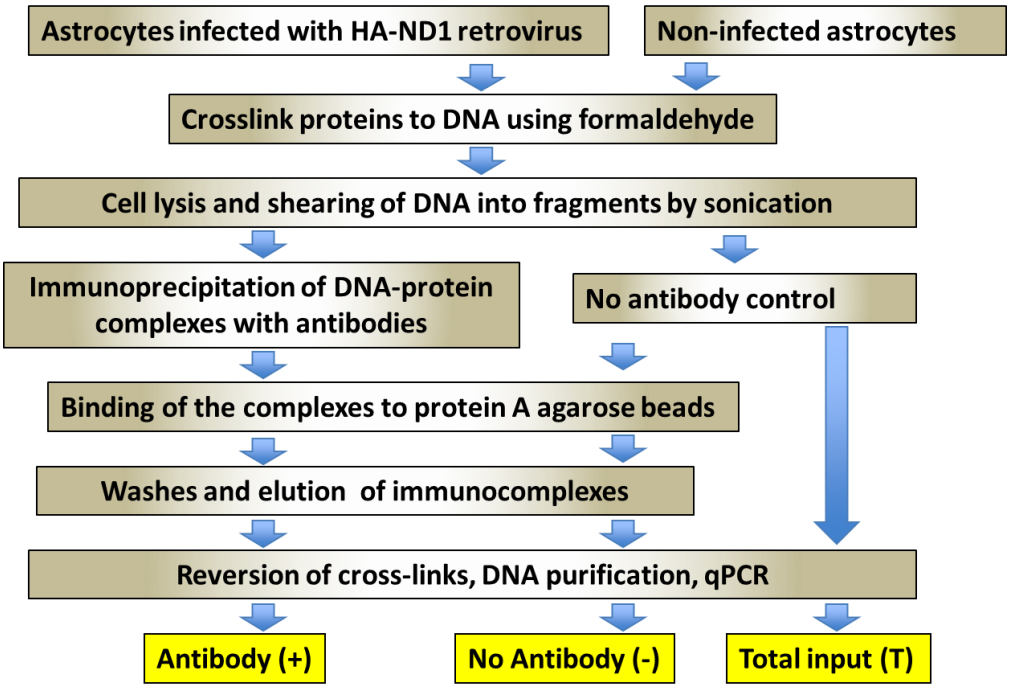


**Figure 5:** Scheme & Setup of ChIP-qPCR Experiment

| ChIP Primers | Primer Sequence |
|---|---|
| Hes6 F | AGGCAGCCTGTAGCCAATGAGAG |
| Hes6 R | CAAGAGAGAAGCCGGAGGTCACT |
| GAPDH F | CTCTGCTCCTCCCTGTTCC |
| GAPDH R | TCCCTAGACCCGTACAGTGC |
| NeuroD1-500F | GCCATATAAAAGCGGCTTCA |
| NeuroD1-500R | AGGAACTGGGAGAGGACGA |
| NeuroD1-250F | TGTTTTTACCCGCAGGAGAG |
| NeuroD1-250R | AGGCCACTCGCTCTGATCTA |
| NeuroD1-100F | CTGAGGGGCTAGCAGGTCTA |
| NeuroD1-100R | GGGAGAAGTGGGGAGGAG |
| NCAM F | TTAAGGAAGGCTGGGTAGCA |
| NCAM R | GCCGAACATCAAGGAGGTAA |

**Table 1:** List of Primers used in ChIP

**Figure 5** gives us a summarized view of the entire scheme of how the ChIP-seq was performed.

Lysed astrocytes after infection were incubated with 354 µL of 37% formaldehyde at room temperature

for 10 min to cross link proteins to DNA on rotating platforms for cos cells and neutralized with 885 µL of 2M glycine being added for 5 mins for 10 cm dishes. The medium was removed and washed thrice with cold 1X PBS. The cells were placed on ice and suspended in 0.5% Triton X-100 and 1 mL Nuclei Washing Buffer (0.32 M sucrose, 1mM PMSF, 10 Mm Tris-Cl (pH 8.0) and 5 mM MgCl2) with a 10 min incubation. The cells were watched for lysis under microscope as cells should remain intact, but skinnier in shape. Now, the cells were scraped into 1.7 mL Eppendorf tubes. The cells were pelleted by centrifugation for 2 mins (6000 rpm) at four degree Celsius with the supernatant being removed. The pellet was resuspended again in 1 mL of NWB followed by centrifugation and removal of supernatant. This was followed by another resuspension in 2 mL IP buffer (0.1 M NaCl, 66.7 mM Tris-Cl [pH 8.0], 5 mM EDTA, 0.33% SDS, 1.67% Triton X-100, 1 mM PMSF) followed by a 10 min incubation. The sample was sonicated with five cycles of 15 second pulses with 50% amplitude and 30 second rest intervals. This is followed by taking out 30 uL of sonicated sample.

The sample was centrifuged at 14000 rpm for 5 mins at 4 degree Celsius and the 60 microliters of 50% slurry protein A agarose/Salmon Sperm DNA was added to supernatant with rotation for an hour at 4 degree Celsius. The beads were pelleted at 4000 rpm for a minute at 4 degree Celsius with the supernatants being centrifuged at 14000 rpm for 15 mins at 4 degree Celsius. 5 microliter of the respective antibodies, HA and ND1, were added to 500 microliter of pre-cleared supernatant and the rest was preserved for negative control. These samples went through overnight incubation at 4 degree Celsius and were centrifuged the next day at 14000 rpm for 5 mins at 4 degree Celsius. A total input control was made with 30 microliters of no-antibody sample and 15 microliters of 50% slurry Protein A agarose beads were added to every sample apart from total input. This was followed by rotating the samples for an hour at 4 degree Celsius and pelleting the samples at 4000 rpm for a minute at 4 degree Celsius.

The supernatants were disposed and the pellets were washed using three 2 min washes in mixed micelle buffer (0.15 M NaCl, 20 mM Tris-Cl pH 8.0, 5 mM EDTA, 5.2% sucrose, 0.2% SDS, 1% Triton X-100, 1 mM PMSF) followed up by two 2 min washes in Buffer 500 (10 mM Tris-Cl pH 8.0, 50 mM

HEPES, 0.5 M NaCl, 1% Triton X-100, 0.1% sodium deoxycholate, 1 mM EDTA, 1mM PMSF), two 2 min washes in LiCl/detergent buffer (10 mM Tris-Cl pH 8.0, 250 mM LiCl, 0.5% NP40, 0.5% sodium deoxycholate, 10 mM EDTA, 1mM PMSF) and two 2 min washes in TE (10 mM Tris-Cl, pH 8.0, 1 mM EDTA, 1 mM PMSF). These washes were done with a 1 mL solution under mild rotation and these samples were repelleted at 4000 rpm for 1 min between washes at 4 degree Celsius.

The non-antibody control and non-control samples were resuspended in 300 microliters of SDS/bicarbonate buffer (0.1 M NaHCO3, 1% SDS) along with 1 microliter GADPH-2 post washing. The total input buffers were resuspended with 270 microliters of the same buffer and same amount of GAPDH-2. These samples were vortexed for 15 mins and spun at 4000 rpm for a min with the supernatants being collected. These samples were incubated overnight at 65 degree Celsius to be reverse cross-linked by covering with 200 uL Mineral Oil. The samples were then incubated with 3 microliters of RNase A for 30 min at 37 degree Celsius, which is followed by adding 5 microliters of Proteinase K for an hour at 55 degree Celsius for incubation and the mineral oil was removed. DNA purification was performed by doing the phenol/chloroform extraction twice and one chloroform extraction. DNA precipitation was done by adding up to 500 uL TE along with adding 30 uL of 3 M NaOAc, 1 microliter polyacryl carrier and 1.5 mL of 100% ethanol. These samples were pelleted at 14000 rpm for 5 mins at 4 degree Celsius and the supernatant was discarded. Pellets were dissolved in 300 microliters of TE (10 mM Tris-HCl pH 8.0, 1 mM EDTA) followed by adding 30 microliters of 3M NaOAc and vortexing the sample. 900 microliters of 100% ethanol were added and the sample was mixed by inverting 10 times. The sample was later incubated for 2 mins followed by pelleting at 14000 rpm for 5 mins at room temperature. The pellet was also washed twice with 70% ethanol and dried for 5 mins in Speedvac at low temperature before dissolving in 300 microliters of TE buffer in the vortex-multi-tube attachment.

This was followed by a ten-fold serial dilution using PerfeCTa SYBR Green Supermix on a reaction plate using the StepOnePlus real-time PCR system. The samples were denatured at 95 degree Celsius with incubation for 10 mins and then followed by forty 17 cycles of denaturation at 95 degree

Celsius for 30 secs. Annealing and extension was done at 60 degree Celsius for 30 secs. A melt curve was generated with the CT values being plotted against log dilution factor. There was a linear trend in the data with the slopes being used to calculate amplification factors & efficiency and **Figure 6** shows the amplification plot produced while hitting the threshold.

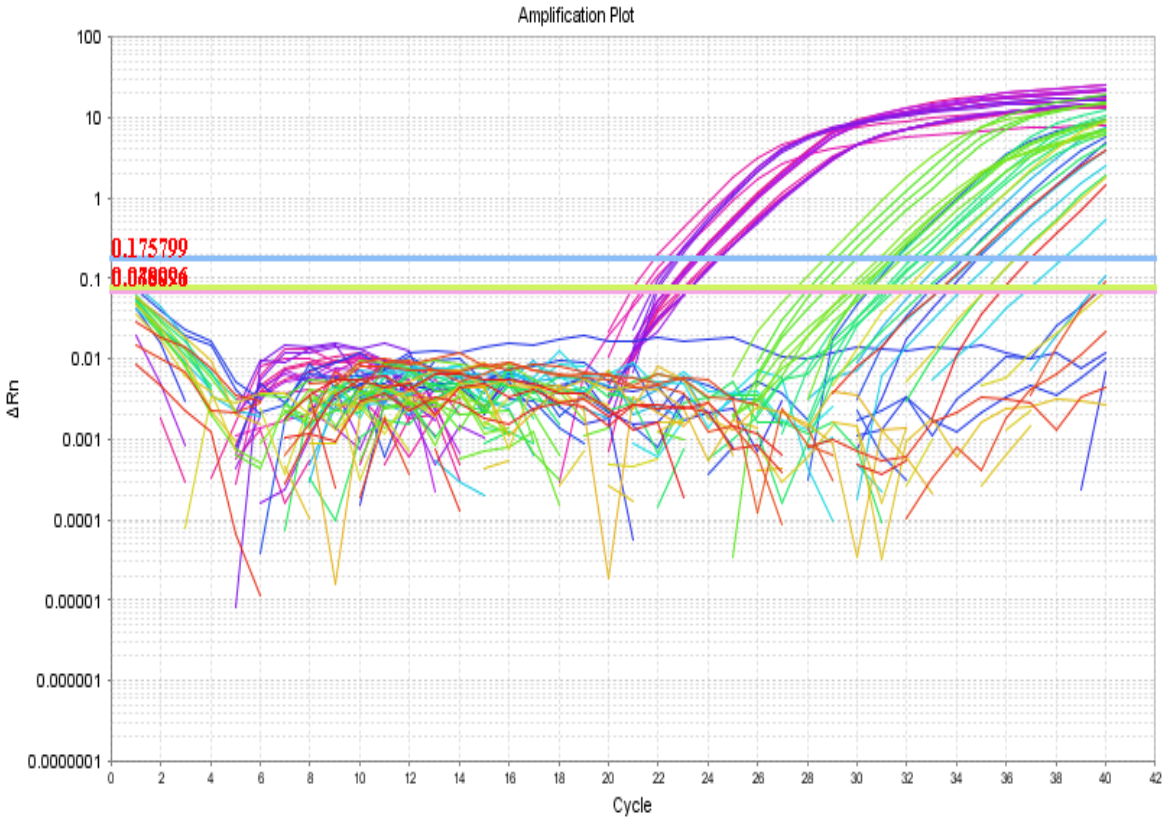


**Figure 6:** q-PCR Amplification Plot Showing the Time Hitting the Threshold

# Chapter 3

# Results

## Expression of HA-tagged NeuroD1

In order to successfully perform ChIP-seq, we wanted to express HA-tagged NeuroD1 instead of NeuroD1 transcription factor. HA-tagged antibodies were previously used in ChIP-seq; they are known to provide more reliable results than antibodies for the Neuro D1. For that purpose, we designed and cloned a new plasmid that contained HA-tag at the 5' end of the NeuroD1, mouse NeuroD1 coding sequence, and GFP gene separated from NeuroD1 with IRES, internal ribosomal entry site. We wanted to have a strong expression of NeuroD1, so the transcript was preceded by the Kozac sequence that improves the efficiency of translation. We also used the CAG promoter, which contains CMV enhancer from cytomegalovirus fused with a portion of chicken beta actin promoter and first exon for maximum activity. The GFP coding sequence was followed by the Woodchuck Hepatitis Virus (WHP) Posttranscriptional Regulatory Element (WPRE) that is known to adopt a structure improving the amount of transcript by stabilizing mRNA. This plasmid was made to be packaged as a viral genome, however, it did not contain functional gag and pol elements, so that the virus would not reproduce in the infected cells. GAG and Pol are supplied on different plasmids that can be used as a source of DNA for gene expression, but won't be a part of the viral genome. The final scheme of cloning the HAND1 retrovirus is shown in **Figure 7**.

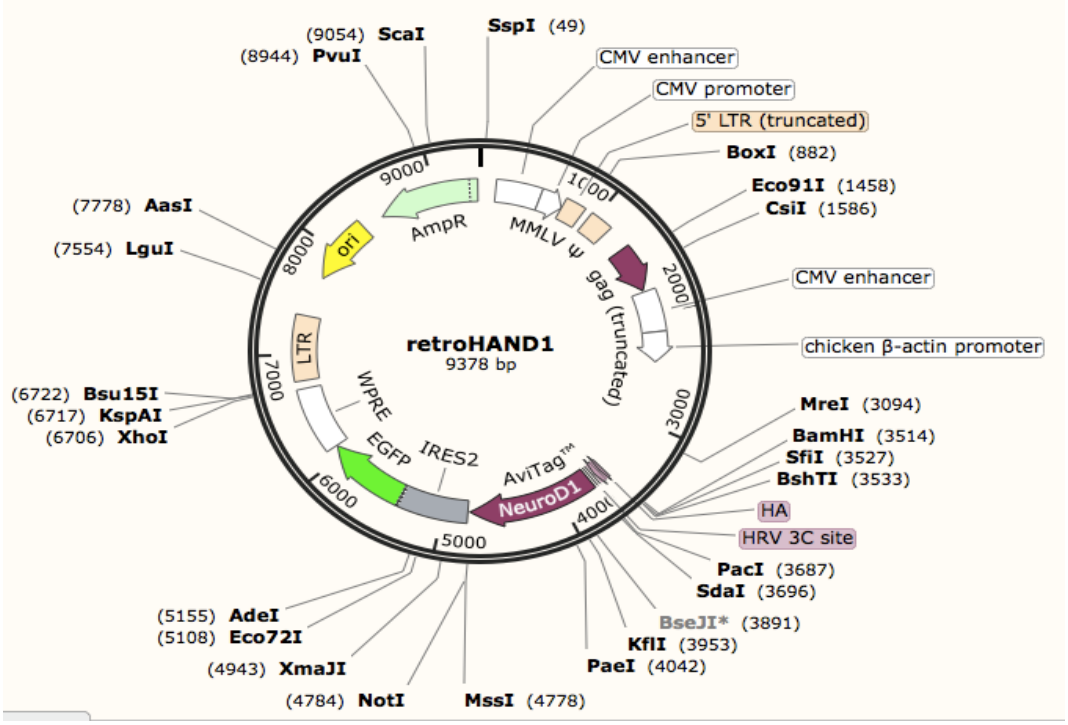


**Figure 7:** Scheme of Retroviral Plasmid with NeuroD1 Fused with HA Tag

## Reprogramming of Astrocytes into Neurons by HAND1

In order to eventually perform the ChIP-seq analysis, we cloned HA-tagged NeuroD1 for more reliable binding of the antibody in the immunoprecipitation. The purpose of this study was to see the efficacy of the virus and whether this virus can be used for ChIP based studies in terms of specificity and antibody binding.

Immunofluorescence based studies were done to test the effectiveness and viability of our HA-ND1 virus. It would also give us an insight into the conversion efficiency post viral infection and allow us to check for the presence of certain transcription factors, which are upregulated during neuronal generation. First, we needed to confirm that HA-tagged NeuroD1 is actually expressed from our plasmid. For that, we used antibodies to NeuroD1, HA tag, and GFP to confirm that all of them are present in the cells.

Immunofluorescence study was done on passage 18 cells infected with the HA-ND1 virus three days post infection, which is shown in **Figure 8**. We could visualize the presence of GFP (cy2), ND1 (Cy5) and the HA (Cy3) signals. We concluded that all three of those co-localized in our infected cells, however, HA antibody did not provide very strong signal, which can be associated with its localization within the protein.

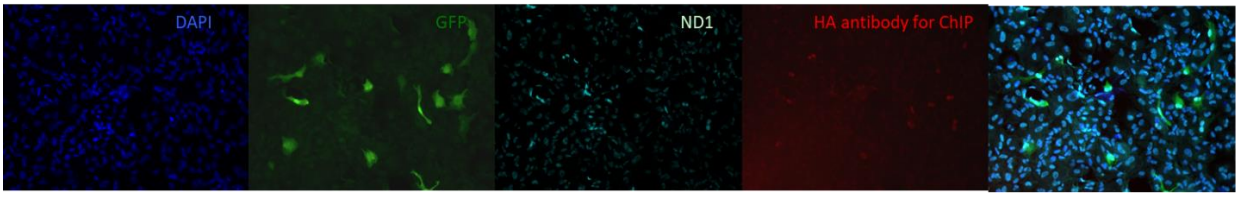


**Figure 8:** Immunostaining of HA-ND1 virus-infected cells 3 days post infection

Next, we decided to confirm that HA-ND1 protein remains functional in terms of reprogramming, and HA tag does not interfere with its normal activity. For this study, we infected astrocytes with a HA-tagged ND1 virus to analyze for the presence of neuronal markers 1 month post infection, which is seen in **Figure 9**. Since this virus also expressed GFP, we intended to look for the presence of GFP along with other neuronal markers and look for any co-localized signals. The two antibodies used were GFP (488) and NeuN (Cy3). Since one of the main objectives of this experiments is to see if our virus can reprogram astrocytes into glutamatergic neurons and check the efficiency of conversion, this was fulfilled when neuronal morphology was observed post treatment with presence of GFP signal, which is in line with the presence of the GFP tag. To depict neuronal morphology, NeuN marker was used to test this as this shows the presence of mature neurons and is present in the neuronal cell's nucleus. The NeuN marker was found to be present as well and co-localized with the GFP signal. A problem that was observed with this experiment was that the number of converted neurons was low, due to the low infection rates.

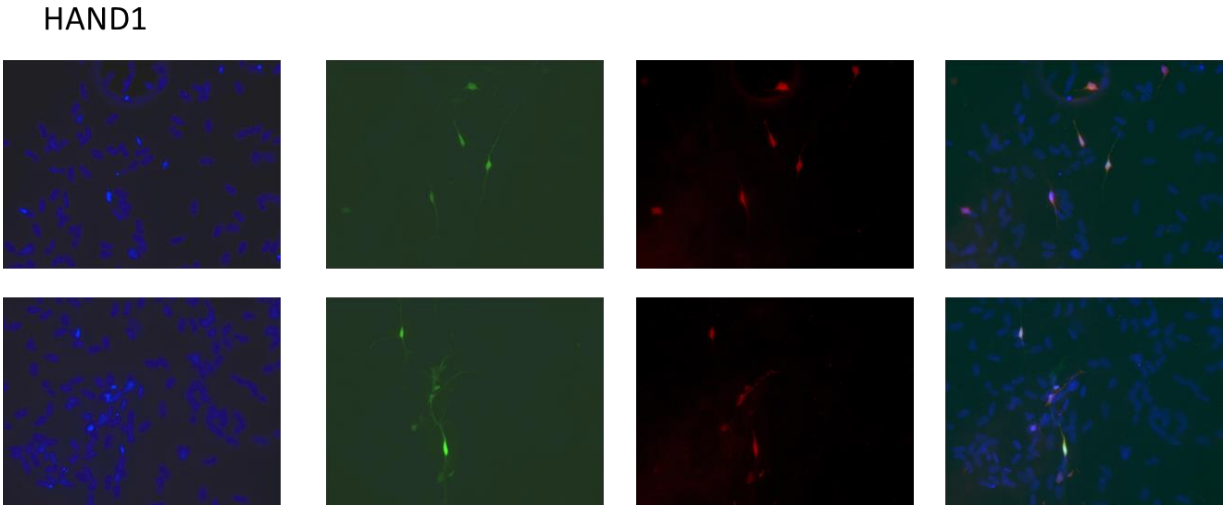


**Figure 9:** Astrocytes infected with a virus expressing GFP tagged ND1 and HA tagged ND1 show neuronal morphology. Blue-DAPI, green-GFP, red-NeuN

**Optimization of the Infection Condition**

Next, we needed to improve the efficiency of the virus infection to be suitable for use in immunoprecipitation essays. The efficiency of infection is a limiting factor for ChIP-seq essay since the quality of the sequencing is poor for the low number of infected cells that actually go through the reprogramming. We experimented with different additives that improve infection efficiency, such as polybrene and DEAE-dextran to select the best condition for the ChIP experiments. The polybrene reagent that was typically used in transduction did not give us sufficient infection. We have tried to experiment with three different passages of astrocytes, but they all were about 10% infected in **Figure 10**. So we decided to test if our human astrocytes would survive the treatment with DEAE-dextran, which is

an alternative to polybrene in the transduction essays, and if the infection rates would be higher.

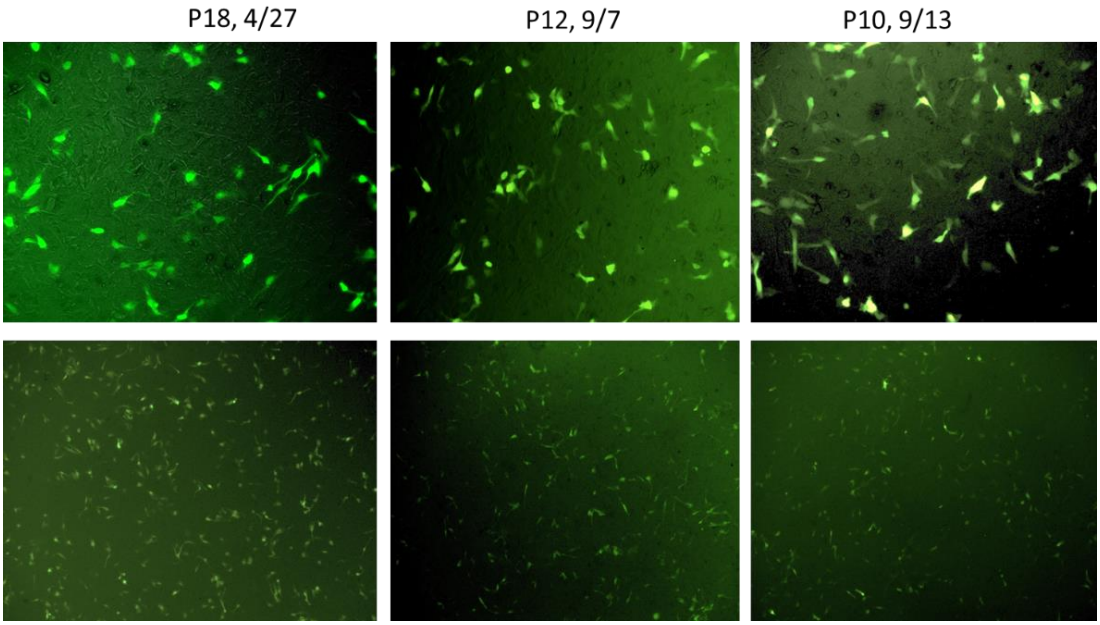


**Figure 10:** Comparison of HAND1 Infection in 3 Independent Cell Cultures - 3dpi. Green-GFP fluorescence. Upper panel – 20x, lower panel 10x magnification

First, 10 ug/mL dextran was tested in infections of the Hek 293T cells, which showed a decent infection rate and survived the treatment. So, 10 ug/mL dextran was used as the transduction reagent while infecting astrocytes. With the use of ImageJ software, we were able to quantify the number of neurons converted to the total number of cells, which gave us a 25-30% HAND1 retroviral infection efficiency in **Figure 11**. This was done by counting the GFP (488) signals, which gave us the total number of infected cells and comparing that with the total number of cells represented by the blue DAPI stain. The co-localization results were in accordance with the hypothesis as well.

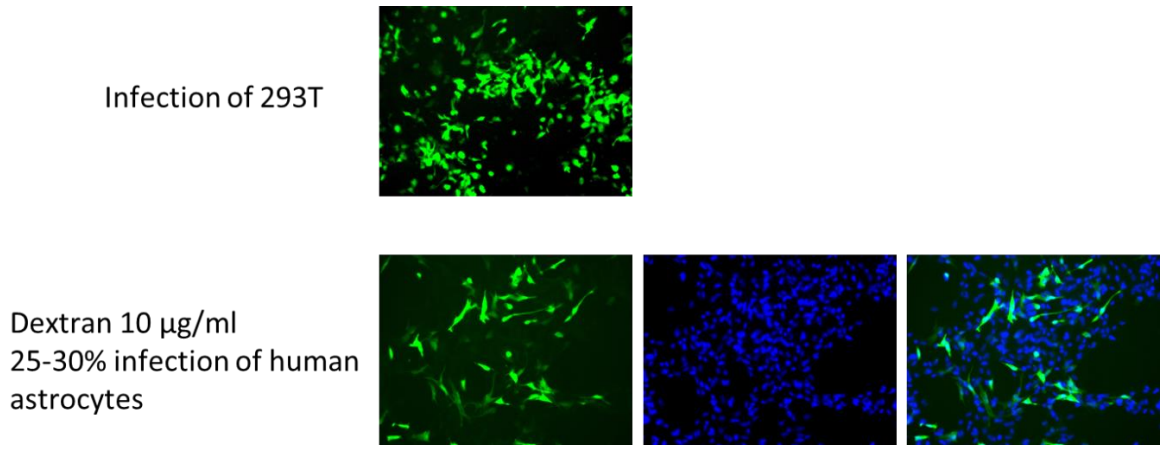


**Figure 11:** Virus Infects about 30% of Astrocyte Cells

We then reproduced this experiment, also comparing the infection rates to those with the previously used polybrene in **Figure 12**. Polybrene was the first and standard transduction reagent used in the previous experiments, which failed to produce substantial results. These infected cells were passage 12 astrocyte cells grown in flasks with 5 microliters of $10^8$ VP-ml titer HAND1 retrovirus being added. These cells were stained one day post infection. We were able to achieve a 10% infection rate using polybrene as a transduction reagent and after switching to dextran, the infection rate increased from 10 percent to around 25-30%. Also, additional experiments were done with a short time exposure to higher concentrations of dextran (1 mg/mL) compared to 10 ug/mL; dextran was removed, followed by addition of virus for 24 hours. This yielded similar results with a 25-30% percent infection rate being recorded. This increase in infection rate was determined by quantifying the GFP signal present with the total number of cells using ImageJ and the presence of GFP signal due to the tag helped us determine the number of cells infected with the HAND1 retrovirus.

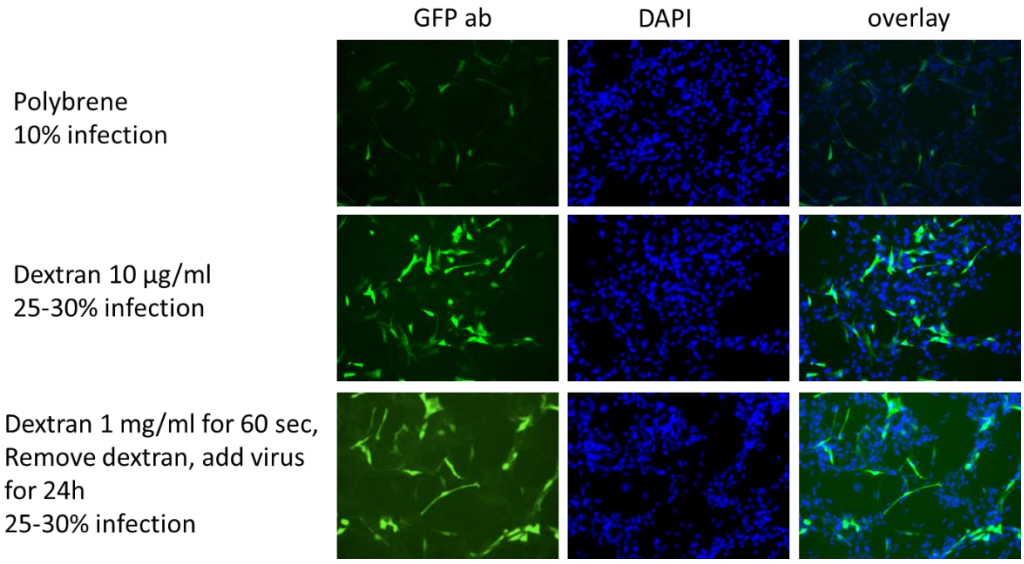


**Figure 12:** Infection of P12 cells stained 1dpi

Next, we wanted to confirm that the presence of dextran in infection does not interfere with the astrocytes to neuron conversion.

A comparison based study was done to see if there are differences in between neurons converted with polybrene and dextran used as the transduction reagents. For this study, we infected astrocytes with the HAND1 retrovirus and performed staining one month after infection when we expected the neurons to mature in **Figure 13**. The three antibodies used were GFP (488), NeuN (Cy3) and MAP2 (Cy5). MAP2 is a mature neuronal cytoskeleton marker, which can help in visualizing the elongating dendritic structures. We can visualize increased GFP & NeuN signals when observed 1 month post infection with extended neuronal projections indicated by MAP2 signal presence. Co-localization of these three signals was also observed.

Results show that we can use either of the two reagents, polybrene or dextran from the perspective of reprogramming as they produce neurons of similar morphology. In this case, the infection

efficiencies were similar, which may be associated with the use of more actively dividing astrocyte cells, which were also converting better.

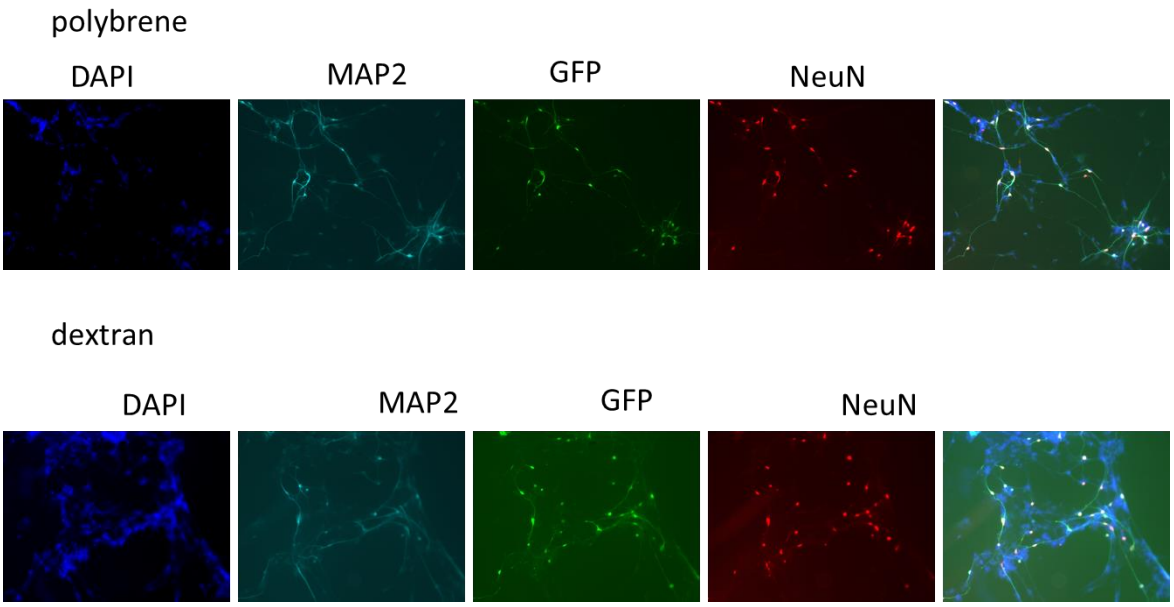


**Figure 13:** Reprogrammed Neurons by Retro HAND1 using Polybrene & Dextran (1 Month)

These results give us an overall view for the transfection and transduction stages of testing the virus to the actual application of the virus on astrocytes. We primarily used immunofluorescence based studies to determine the presence of signals, which might signify conversion of astrocytes into glutamatergic neurons. Also, this was done progressively with comparisons between cell generations as well as the difference when different transduction reagents are used (Polybrene & Dextran). Since we have visualized results which signify reprogramming, ChIP and q-PCR results can provide greater insight into the epigenetic landscape governing NeuroD1 binding to transcription factors and promoters, which constitutes the overarching theme of this work.

## Qualitative Assessment of NeuroD1 Binding by ChIP Study & q-PCR

In preparation to performing ChIP-seq on using HA antibody, we decided to first analyze immunoprecipitation with ChIP-qPCR to confirm that we can detect binding of NeuroD1 to promoters predicted in previous mouse studies. We performed immunoprecipitations with antibodies to NeuroD1, and then to HA tag to select what is best to use in the subsequent ChIP-seq essays. We used several promoters, such as Hes6, NCAM, and endogenous NeuroD1 that have been previously shown to recruit NeuroD1.

First, immunoprecipitations were performed using antibody to NeuroD1 with multiple batches of infected astrocytes. We used NeuroD1-infected astrocytes and GFP-infected as a control, and performed ChIP in 24 hours after infection. The lysates were subjected to sonication to shred genomic DNA into fragments. A typical sonication result is shown in **Figure 14** and it shows that the sonicated fragments were mostly in the range of 200 to 500 bp (left lane), as recommended for ChIP analysis.

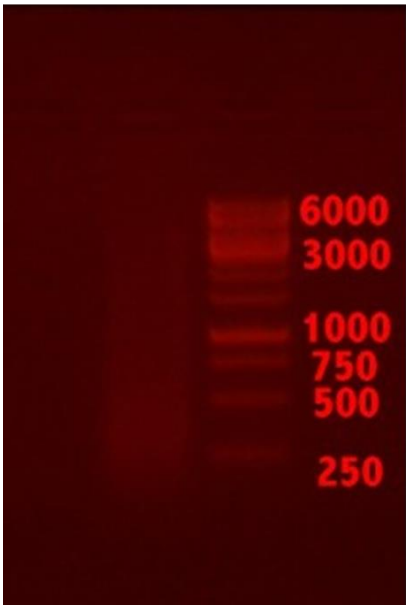


**Figure 14:** A typical fragment distribution obtained in our experiments upon sonication of DNA.

Our results in **Figure 15** showed that NeuroD1 was recruited to Hes6 and NCAM promoters. However, NCAM promoter showed a significant signal in GFP control sample. Since we do not expect

NeuroD1 to be expressed in GFP-infected control, we concluded that this was unspecific binding of the antibody to this promoter. We also analyzed binding of NeuroD1 to its own endogenous promoter. Since we didn't have information on NeuroD1 binding sites for this promoter, we covered several regions using ND00, ND250, and ND500 primers located at the transcription start site, 250 and 500 bp downstream the start site, accordingly. It seemed like there was a recruitment of NeuroD1 in both astrocytes batches to the region surrounding the transcription start site, but binding was much stronger for Hes6 and NCAM promoters. GADPH promoter was used as a negative control since it is not expected to bind NeuroD1, however it was slightly upregulated in the second experiment, which may be due to an unspecific antibody binding. It was equally upregulated in the GFP-infected control, which also attests to the unspecific nature of our NeuroD1 antibody.

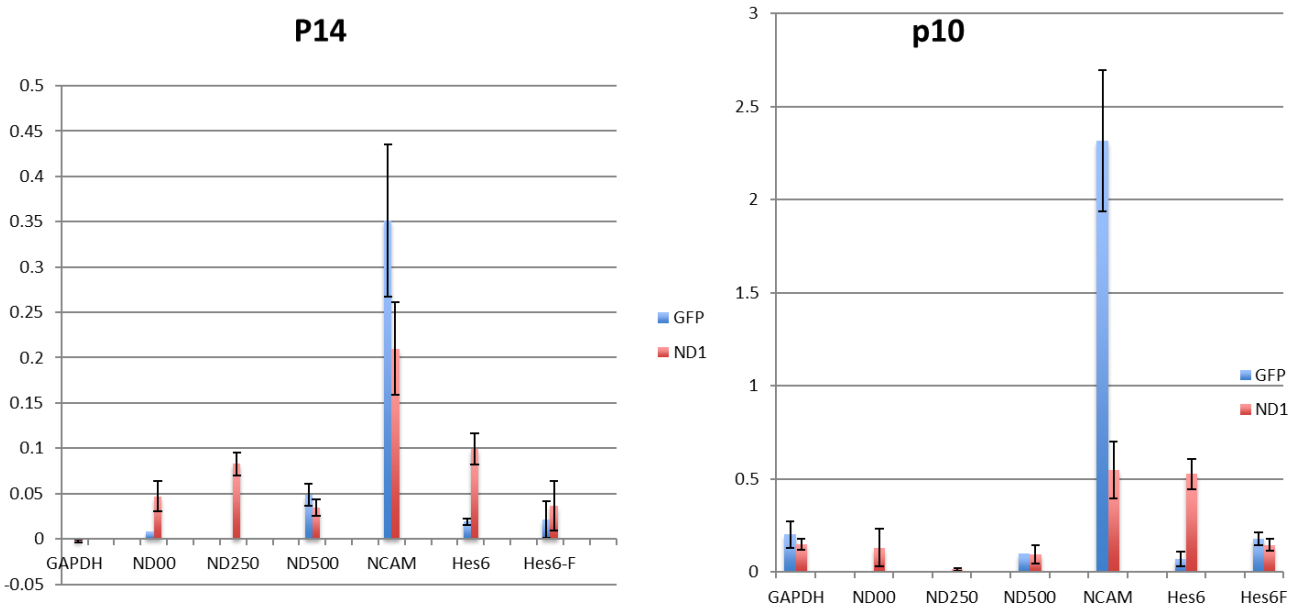


**Figure 15:** ChIP q-PCR Results with GFP and ND1 antibodies on P10 and P14 Cells

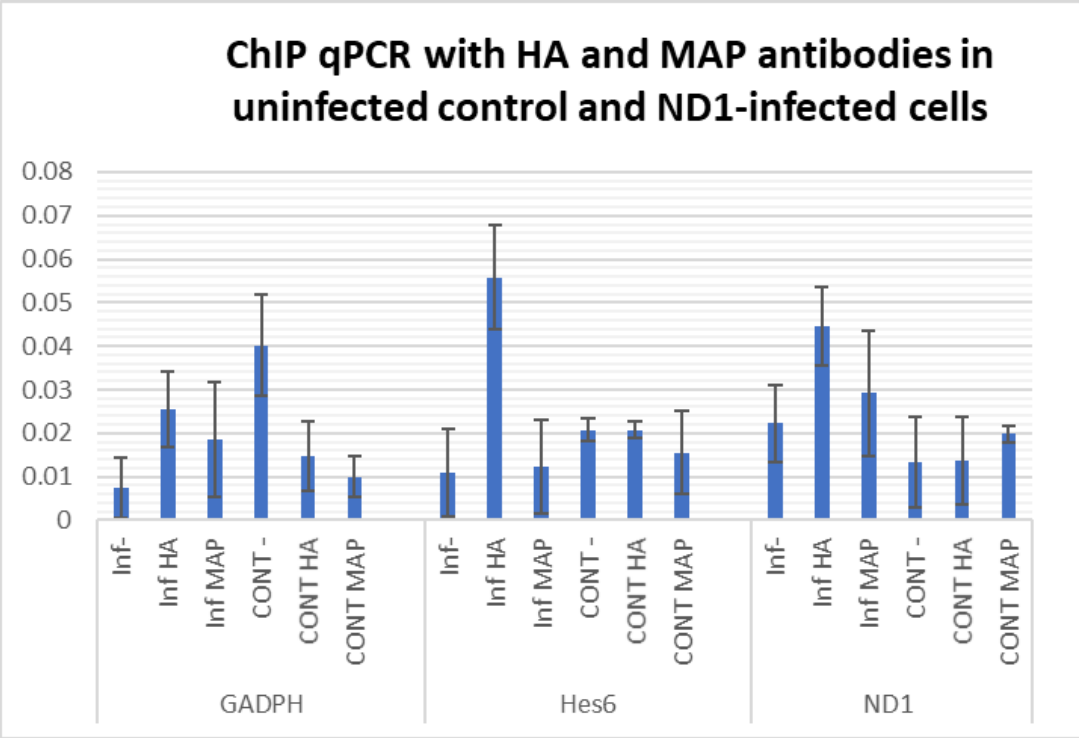


**Figure 16:** ChIP q-PCR Results with HA and ND1 antibody

Next, we performed ChIP q-PCR experiment with HA antibody in **Figure 16**. We selected Hes6 region and NeuroD1 transcriptional start site surrounding regions to check for the specific antibody binding, while GADPH region served as a negative control. We included an additional Map2 antibody precipitation as a negative control, including an antibody that is not supposed to bind to DNA. Map2 binds to microtubule-associated proteins and should not be binding to the genome. Such negative control is considered better than no antibody control by some sources since random antibody may block the beads to prevent random DNA binding.

Similar results to previous ChIP experiments were seen for the HA antibody. Hes6 promoter region showed the most pronounced recruitment of HA antibody, about three times higher than the control sample. NeuroD1 promoter showed about three times more recruitment than the uninfected control as well, although overall the signal was lower than for Hes6, as it was for NeuroD1 antibody. The background binding of DNA to the beads, and also precipitated by Map2 antibody was high in this

experiment, possibly due to overheating of the sample in immunoprecipitation, which may lead to partially denatured proteins and may cause random binding to random antibodies. This random binding was also present in GAPDH region that had HA and MAP antibody associations both in infected and uninfected samples, with comparable strengths. Also, the amount of DNA precipitated in this experiment was significantly lower than we have previously observed, which can be due to loss of some protein conformations in sonication caused by overheating. Low amount of DNA could lead to higher deviation in between technical replicas that was observed.

Overall, the result of this experiment was similar to the experiment with NeuroD1 antibody, however, further experiments needs to be repeated with milder sonication conditions. In particular, amplitude of sonication has to be lowered and compensated by higher number of sonication cycles. Upon repeating this experiment with less background, it may be possible to use the system developed here in ChIP-seq experiment.

# Chapter 4

# Discussion

## Reprogramming of Astrocytes into Neurons by HAND1

In this work, we make the first preparatory steps to determine the mechanism of reprogramming of astrocytes to neurons.

Chromatin immunoprecipitation methods are powerful in determining transcription factor binding sites and chromatin landscape. However, there are certain criteria that the system is supposed to meet for the ChIP-qPCR and ChIP-seq to provide meaningful results. The purpose of our study was to improve those parameters in our astrocytes to neural conversion to be able to successfully perform ChIP-seq.

One of the bottlenecks of the ChIP-seq procedure is the number of cells used. The starting cell culture should contain certain percentage of the infected cell for the sequencing to work. Initially, we started with 10% of infection with the cells that were currently available in the lab. With that rate of infection, we would need to provide 10 times the amount of cells for the experiment, which would require considerable deviation from standard ChIP-seq protocols. The generational age of the cells play a role in the infection rate as younger batch of cells from earlier generations tend to have a higher infection rate. Younger cells tend have epigenetic biomarkers associated with aging, which could potentially affect infection rates and ultimately, reprogramming efficiency as well (Huh et al., 2016). However, we had to adjust to the cell batches currently available in the lab that lost their ability to reprogram due to prolonged storage and occasional temperature changes. We experimented with different infection conditions to select the best transduction reagents for our current cells. The use of two different transduction reagents, polybrene and DEAE-dextran, showed differences in infection rate with the use of polybrene resulting in 10% infection and using dextran resulted in 25-30% infection. Positively charged polycations are known to reduce repulsive forces between a negative charged cell and a viral particle. An analysis by examining various transduction reagents like polybrene and dextran showed that dextran showed superior activity in

lenti-viral & retro-viral vectors compared to other poly cation reagents (Denning et al., 2013). 25-30% infection rates were suitable to move forward with chromatin precipitation essays.

The antibody quality is another limiting factor for chromatin immunoprecipitation. Unspecific binding of antibodies can obscure the data by adding many artifact binding sites that would prevent determining the true consensus of binding. In our assays, we encountered problems with unspecific binding of ND1 antibody that has been previously used for ChIP essays in mouse work done by other lab. We could observe the recruitment of this antibody to NCAM promoter region in the cells where NeuroD1 was exogenously expressed and without such expression. This is some artifact since NeuroD1 is not present in astrocyte culture, and infection with GFP should not result in elevated NeuroD1. This NeuroD1 antibody has also failed in ChIP-seq essay that we attempted (data not shown).

As a result of the unspecific nature of NeuroD1 antibodies, we decided to use an alternative approach and express a tagged Neuro D1, to use a well characterized and widely used HA antibody in our ChIP-seq essay.

Immunofluorescence-based studies gave us an idea about the effectiveness of the HA-ND1 virus and whether this virus is causing reprogramming of astrocytes into neurons with the expression of transcription factors, which suggest so. The first experiment on viral expression allowed us to confirm that cloning experiments were done correctly. This is evidenced by the presence of GFP, ND1 and HA staining with appropriate co-localization of the signals. We could even observe the tendency of the cells to elongate and form projections as an indication of early reprogramming steps. In mouse cortex, HAND1 retroviral injections showed many NeuroD1-GFP infected cells with bipolar morphology and immature neuronal markers three days post infection (Guo et al., 2014).

After confirming that the cloning was correct and all the necessary factors were expressed, we showed that dextran-converted astrocytes with improved infection rate are similar to polybrene-converted astrocytes. Astrocytes were infected with HA tagged NeuroD1 virus to check the conversion results. Our converted cells presented GFP and NeuN signals 1 month post infection along with co-localized signals.

A study conducted in the mouse cortex by a NeuroD1-GFP retrovirus showed presence of NeuN marker within a week of infection and extensive neurites were seen in 3 weeks with NeuN signal levels reaching the level of mature neurons. Also, the use of this virus in cultured mouse astrocytes showed reprogrammed neurons with strong NeuN signals (Guo et al., 2014). This can be attributed to the up regulatory nature of NeuroD1, which we will examine when analyzing ChIP q-PCR results. GFP, NeuN and MAP2 signals were observed with extended networks being formed with neurons and the signals were also found to be co-localized. Strong NeuN signals indicate maturity of neurons and the MAP2 signals are indicative of the neuronal dendritic extensions seen in properly differentiated neurons. Retroviruses allow for the specific targeting of glial cells and allow us to express target genes in dividing cells with high efficiency. A previous study shows that NeuroD1 converted neurons mature 1 month post infection and form synaptic connections with other neurons, which can be extrapolated from our results as well (Li & Chen, 2016).

Overall, we can see that our HA-ND1 virus has been produced with a 30% infection rate efficiency and good reprogramming efficiency as indicated by the presence of neuronal markers, which indicate neuronal growth and maturity. The HA tag primarily allows us to analyze these converted neurons through ChIP q-PCR and ChIP-seq studies, which will give us an insight into the epigenetic landscape behind the reprogramming process and how NeuroD1 binds to specific promoters. From our present results, we can say that NeuroD1 upregulation is causing the conversion of astrocytes into glutamatergic neurons.

## Qualitative Assessment of NeuroD1 Binding by ChIP Study & q-PCR

From the immunofluorescence data, we were able to observe the reprogramming efficiency and check the viability of the virus. ChIP q-PCR allows us to look at promoter and transcription factor binding with NeuroD1 and will provide insights into drawing associations for determining the mechanism

of NeuroD1. This study, where P14 and P10 cells were infected with GFP and NeuroD1 retroviruses show binding of NeuroD1 to various promoters like NeuroD1, NCAM and Hes6. The percent inputs also seem to vary between P14 and P10 cells with younger generation P10 cells having higher values. Intriguingly, NeuroD1 antibody seems to majorly bind to NCAM promoter in GFP-infected control. The highly linked association of NeuroD1 with NCAM can be attributed to NCAM being a downstream target of NeuroD1 and this over-expression has also been considered as a potential target for neuroendocrine tumors. So, NeuroD1 depletion has been linked to reduction in NCAM expression (Osborne et al., 2013). Ninjurin1 is a type of NCAM, which promotes axonal growth and this was found to be upregulated upon expression of NeuroD1 in glial cells (Kamath et al., 2005). However, the GFP control is not expected to have expression of NeuroD1, thus we attributed this result to unspecific binding of NeuroD1 antibody. NeuroD1 antibody also detected NeuroD1 at its own promoter. This can be expected since NeuroD1 was shown to be upregulated during conversions, however, NeuroD1 repressor was detected to be upregulated after exogenous NeuroD1 expression (Matsuda et al., 2019), which may result in relatively low level of recruitment of NeuroD1 observed in our immunoprecipitations

Interestingly, NeuroD1 also binds to Hes6 promoter strongly as well. A transcriptome analysis of Xenopus was done to find transcriptional targets and regulatory enhancers for NeuroD1. This showed that Hes6 is one of the primary transcription factor targets of NeuroD1, which was connected with a core transcriptional network regulating neurogenesis (Seo et al., 2007). In mouse ES cells where NeuroD1 levels were upregulated, H3K27ac acetylation marks were seen to be upregulated in Hes6 promoter and H3K27me3 methylation marks were seen to be downregulated. So, this shows the activation of transcription at the Hes6 promoter and how NeuroD1 is responsible for this upregulation of Hes6 (Pataskar et al., 2016).

As for the ChIP-qPCR experiments performed with HA-tagged antibody, the results were very close to what was obtained with antibody to NeuroD1. HA-tagged NeuroD1 shows great binding affinity to Hes6 and NeuroD1 promoter similar to the previous experiment, showing that the tagged version has a

similar binding pattern as the non-tagged one and in confirming that HA antibody is found where it is expected to be.

However, there is still a concern regarding the high background of HA antibody without infection and at the control GAPDH promoter, which is likely an artifact. This may be attributed to the antibody quality, so one potential solution is to acquire a new antibody to be used in the repeat experiment. However, the higher background may also indicate a problem with our ChIP-qPCR assay rather than the antibody quality. The high background was observed in both no antibody sample, and the one that used unrelated MAP2 antibody as a control. The similar issues occurred in associations with the GAPDH region showing high background in both infected and uninfected astrocytes. Map2 is a generic neuronal marker, which was showed to be highly expressed in neurons after NeuroD1 levels were upregulated in the periventricular region of mice brain (Boutin et al., 2019). However, MAP2 does not bind the DNA and it binds to microtubules in the cytosol, so binding of Map2 is indicative of a random binding of this antibody. Random binding may be due to partially denatured proteins, so improving conditions of our sonication and immunoprecipitation may be a potential solution.

Astrocytes to neuron conversion is a potential approach to treatment of the multiple brain disorders. In the course of conversion, multiple pathways are targeted and many other transcription factors are activated to orchestrate the changes in astrocyte cells resulting in neural differentiation. Conversion by NeuroD1 is only one of the potential approaches to trans-differentiation; there are also other methods including small molecules treatments and combinations of small molecules and viruses to be used to obtain not just glutamatergic, but other neural types, such as GABAergic and dopaminergic neurons. In our lab, we mostly used an intelligent approach to selection of the chemicals and viruses needed for obtaining this or another cell types. Knowing the mechanism of conversion, what factors and pathways are activated by NeuroD1, would help us to come up with more strategies and also improve conversion methods currently used.

## Future Work & Conclusion

Further repeat of ChIP q-PCR experiments will need to be done as there are antibody issues, which has resulted in antibody background in the GAPDH region and uninfected astrocytes. Also, we intended to eventually move on to analyze NeuroD1 binding sites with ChIP sequencing. From this study, we determined that NeuroD1 binds to Hes6 promoter along with NeuroD1's own promoter through ChIP q-PCR. To further our research, it is necessary to repeat the experiments with different batches of the treatment infected with the HAND1 retrovirus and this will allow us to see the reproducibility of our results. Our reprogramming research is intended for providing therapeutics for debilitating neurodegenerative diseases. Our work with determining factors, which can reprogram glial scars into functional glutamatergic neurons, shows promise as a potential therapeutic for Alzheimer's disease and other prevalent neurodegenerative diseases. This line of work is significant to neuroscience in order to further the understanding of neuronal reprogramming and the factors which can influence it for brain repair and regeneration.

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# Academic Vita
# Kailash Jayachandran

(908)-642-5573 | kxj5170@psu.edu |

## Education

**The Pennsylvania State University** **University Park, PA**
*Eberly College of Science* | Bachelor of Science (General Option) with Honors in BMB *Class of 2019*
**Dean's List:** All Seven Semesters for Excellent Academic Performance

## Work Experience

**Pennsylvania State University** **Research Experience**
*Undergraduate Researcher at Dr. Krasilnikova's & Dr. Chen's Lab* *October 2017 – Current*
- Working on a project to find the mechanism of NEUROD1 mediated reprogramming from astrocytes to neurons
- Contributing in a side project to perform NEUROD1 mediated reprogramming to convert astrocytes into GABAergic neurons

**Vellore Institute of Technology** **Summer Internship**
*Intern at the Department of Bio-technology* June 2016 - August 2016
- Learnt various molecular biological techniques like culture preparation, DNA isolation, PCR, Agarose Gel Electrophoresis, SDS PAGE under Dr. Gothandam
- Worked in the immunopathology lab to learn techniques like Immuno Fluorescence under Dr. Rasool

**Pennsylvania State University** **Research Experience**
*Researcher in the College of Engineering Research Initiative Program* Spring 2016 – Fall 2016
- Worked on measuring the bioaccumulation of radium in benthic macro-invertebrates like mussels in western PA streams under Dr. Nathaniel Warner
- Participated in the 2016 Poster Exhibition and submitted a Research Paper summarizing my project

## Leadership Experience

**International Student Council** **University Park, PA**
*Roles: Presidential Assistant & Vice President* *Spring 2017 – Spring 2019*
- Helped in organizing major cultural events (International Soccer Tournament & We Are The World) to emphasize the presence of Penn State's international student body and explore the campus' diversity
- Worked with leaders from various international student body groups to represent international students to address issues to make an inclusive environment, introduce diversity initiatives and build lasting relationships

**Learning Assistant** **University Park, PA**
*Penn State University, Department of Chemistry, Biology & BMB* *Spring 2017-Spring 2018*
- Taught CHEM 112 (Spring 2017), BIOL 141 & CHEM 110 (Fall 2017) and BMB 251 (Spring 2018)
- Was trained in different methods of teaching and explaining concepts, and implemented those methods throughout the semester in helping students better understand the material covered in class

**TriBeta National Honor Society** **University Park, PA**
*Public Relations Chair* *Fall 2018 – Spring 2019*
- Promoted the club's agenda and events on social media and was involved in recruiting new members
- Participated in events to enhance biological scientific knowledge and gave summary reports of meetings

**Finalist** University Park, PA
*Penn State University, Engineering Consulting Collaborative Case Competition* *Fall 2018*

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- Reached the Top 4 in the Case Competition with the objective of designing an online engineering program using EDSGN 100 as a model
- Designed ways to make Online Engineering Programs more accessible for students using augmented reality

**Semi-Finalist** University Park, PA
*Penn State University, Idea Makers Business Pitch Competition* *Spring 2019*

- Among the top 8 teams to make it to the semifinals in this competition to develop a business idea and make a pitch based on it.
- Worked on the idea of "Scan & Go" checkout through phones in stores based in small college towns and planned to integrate the technology with the store's security systems through IOT & location services

## Skills, Interests, and Awards

- **Skills:** Biochemistry, Genomics, Organic Chemistry, Immunology & Molecular Medicine
- **Techniques:** Agarose Gel Electrophoresis, DNA Isolation, PCR, Cloning, Cell Culture, Mice Handling & Surgery, Immunofluorescence Microscopy, Drug Treatment
- **Erickson Discovery Grant:** Awarded a sum of 3500 for conducting Neuroscience Research (Summer 2018)
- **Volunteer Hours:** Lifelink PSU (25 hours) & Madras ENT Research Foundation (100 hours)
- **Mentorship**: Engineering Orientation Network (Fall 2016), Science Lionpride (Spring 2018-Spring 2019), Schreyer Orientation Leader & Schreyer Ambassador (Fall 2018-Spring 2019)
- **Languages**: Fluent in English & Tamil, Limited Proficiency in Hindi and Basic Proficiency in Telugu & Japanese

## Papers & Presentations

- *"Study of Bioaccumulation of Radium in Mussels from Western Pennsylvania Streams due to Unconventional Oil & Gas Wastewater discharge"*
    - Spring 2016 Research Paper for the College of Engineering Research Initiative Program
- *"Investigating the Mechanism of NeuroD1 in Astrocyte Reprogramming by Chromatin Immunoprecipitation"*
    - Summer 2019 Schreyer Honors Thesis in Biochemistry & Molecular Biology
- *"Bioaccumulation of Radium in Benthic Macro-Invertebrates in Western Pennsylvania Streams"*
    - Research Poster Presented at the Spring 2016 Penn State Undergraduate Poster Exhibition
- *"Mechanisms and Subtype Identity of Transdifferentiated GABAergic Neurons for Applications in Brain Repair"*
    - Research Poster Presented at the Spring 2018 Penn State Undergraduate Poster Exhibition
- *"Investigating the binding sites of NEUROD1 through Chromatin Immuno-precipitation"*
    - Research Poster Presented at the Fall 2018 Penn State Undergraduate Poster Exhibition