

Apr 30, 2019

## Transcriptome profiling of brain and lung under Dip2a regulation

DOI

[dx.doi.org/10.17504/protocols.io.2drga56](https://dx.doi.org/10.17504/protocols.io.2drga56)

Rajiv Kumar Sah<sup>1</sup>, Yang Anlan<sup>1</sup>, Fatoumata Binta Bah<sup>1</sup>, Salah Adlat<sup>1</sup>, Ameer Ali<sup>1</sup>

<sup>1</sup>Northeast Normal University



Rajiv Kumar Sah

Northeast Normal University

OPEN  ACCESS



**DOI:** [dx.doi.org/10.17504/protocols.io.2drga56](https://dx.doi.org/10.17504/protocols.io.2drga56)

**Protocol Citation:** Rajiv Kumar Sah, Yang Anlan, Fatoumata Binta Bah, Salah Adlat, Ameer Ali 2019. Transcriptome profiling of brain and lung under Dip2a regulation. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.2drga56>

**License:** This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

**Protocol status:** Working

**We use this protocol and it's working**

**Created:** April 26, 2019

**Last Modified:** April 30, 2019

**Protocol Integer ID:** 22673

**Keywords:** Sequencing, DEG,



## Abstract

Total RNA from brain and lung of E19.5 *Dip2a*<sup>-/-</sup> and wildtype embryos was isolated and used as a input material for cDNA synthesis. The library preparation were send for sequenced and paired-ends were generated. Raw data of fastq format were processed and clean reads were obtained by removing adapter, reads containing poly-N and low quality reads. The clean reads were then mapped to mouse reference genome. Gene annotation were done beased upon Nt, Nr, KOG/COG , EggNOG, KO and GO database. Quantification of transcription expression level was presented by FPKM. In order to identify diferentially expressed genes between WT and *Dip2a*<sup>-/-</sup> embryos in brain and lung , DESeq from R package was used. DEG enrichment was done based upon KEGG , GO and TF database.

## Guidelines

All procedure were conducted following guidelines recommended in the guide of Care and Use of Laboratory Animals of National Institute of Health with approval of Institutional Animal Care and Use Comittee of Northeast Normal University (NENU/IUCAC, AP2013011) .

## Materials

### MATERIALS

⊗ RNAiso plus **Takara Bio Inc.**

⊗ NEBNext UltraTM RNA Library **New England Biolabs**

Total RNA was isolated by using RNAiso plus. cDNA libraries were generated by using NEBNext Ultra TM RNA library Preparation Kit.

## Safety warnings

! All mice were anesthetized before ethunasia with 1% pentobarbitol at a dose of 10mg/kg and all effort was made to minimize sufferings.

## Before start

*Dip2a* heterozygous mice (*Dip2a*<sup>+/-</sup>) were intercrossed, female mice checked for the presence of copulation plug (Vaginal plug) and designated as E0.5 day. At the age of E19.5 , pregnant dams were euthanized and embryos were collected on ice-cold 1X PBS. Brains and lungs were dissected out from each embryos and frozen immediately on liquid nitrogen



## Sample collection protocol

- 1 Dip2a heterozygous mice (Dip2a+/-) were intercrossed, female mice checked for presence of copulation plug (Vaginal plug) and designated as E0.5 day. At age of E19.5, pregnant dams were euthanized and embryos were collected on ice-cold 1X PBS. Brains and lungs were dissected out from each embryo and frozen immediately in liquid nitrogen and stored at -80oC.

## Nucleic acid extraction protocol

- 2 Total RNA was isolated using RNAiso plus reagent (Takara, Dalian) according to manufacturer's instruction. RNA integrity was assessed using the RNA Nano 6000 Assay Kit of the Agilent Bioanalyzer 2100 system (Agilent Technologies, CA, USA).

## Nucleic acid library construction protocol

- 3 Sequencing libraries were generated using NEBNext UltraTM RNA Library Prep Kit for Illumina (NEB, USA) following manufacturer's recommendations.

## Nucleic acid sequencing protocol

- 4 The clustering of samples was performed on a cBot Cluster Generation System using TruSeq PE Cluster Kit v4-cBot-HS (Illumia) according to the manufacturer's instructions. Library were sequenced on an Illumina HiseqTM 2500 platform.

## Normalization data transformation protocol

- 5 Raw read (clean reads) were aligned under Tophat2 software and assembled with Cufflink software. Gene quantification was done based upon FPKM for each sample.