Transcriptome profiling of brain and lung under Dip2a regulation

rajiv kumar sah[1], Yang Anlan[1], Fatoumata Binta Bah[1], Salah Adlat[1], Ameer Ali[1]

[1]Northeast Normal University

Apr 30, 2019

Working

dx.doi.org/10.17504/protocols.io.2drga56

rajiv kumar sah
Northeast Normal University

ABSTRACT

Total RNA from brain and lung of E19.5 Dip2a$^{-/-}$ 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 ouse 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$^{-/-}$ embryos in brain and lung , DESeq from R package was used. DEG enrichement was done based upon KEGG , GO and TF database.

GUIDELINES

All procedure were conducted following guidlines 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

NAME	CATALOG #	VENDOR	CAS NUMBER	RRID
RNAiso plus	View	Takarabio		
NEBNext UltraTM RNA Library	View	New England Biolabs		

MATERIALS TEXT

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 STARTING

Dip2a heterozygous mice (Dip2a$^{-/-}$) 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

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.