P435-1: DYRK1A Inhibition via Harmine

Date Requested: 01-18-2022
Date of Approval: 02-02-2022

Project Number: P435-1
Approval Acquired: Yes

Investigator: Bernard Khor, Khor Lab (BRI)
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Research Coordinator:

Additional Personnel

Investigator submitting samples

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Study Description: Chemical inhibition of DYRK1A (e.g. using harmine) inhibits Th17 and promotes Treg differentiation. To better understand the mechanisms by which DYRK1A regulates Th17 differentiation, we are comparing expression profiles of naïve CD4+ T cells during the course of Th17 differentiation in the presence or absence of harmine.

Experimental Information

Hypotheses: RNAseq comparison can identify pathways regulated by DYRK1A/harmine during Th17 differentiation.

Experimental Design: We MACS-isolated naïve CD4+ T cells from 4 mice on C57/Bl6 background. These were plated in pro-Th17 conditions (see below) in the presence or absence of 10 uM harmine. Cells were harvested at 0, 5, 24, 48, and 96 hours and RNA isolated by RNeasy Micro & Mini kits. pro-Th17 conditions: 2ug/mL Anti-Il-4 2ug/mL Anti-Il-12 2ug/mL Anti-IFN 20ng/mL Il-6 20ng/mL Il1 0.1ng/mL TGFB

Variables: - 4 separate mice - 5 different timepoints (0hrs, 5hrs, 24hrs, 48hrs, 96hrs) - No Harmine vs Harmine (10uM) during incubation.

Controls: No Harmine

Quick Summary of Services Requested: Library Construction, Sequencing

Do you need additional bioinformatics analysis beyond gene counts? Yes

Do you have specific deadlines for data generation or analysis? No

In addition to providing you with data for your analysis, we sometimes wish to compare data across studies. Is it okay with you for us to use your data for our internal analyses (we will never publish anything relating to your data without discussing it and getting your permission)? Yes

Sample Description

Species: Mouse
Sample origin (whole blood, PBMC, sorted cells, tissue, etc.): Splenocytes

Sample Type: Total RNA

Number of Samples: 36

Sample/Library Purification Method/kit: Qiagen RNeasy

For RNA samples, were they treated with DNase? Yes

Sample Storage/Elution Buffer: Water (RNAse-free)

Do samples contain known biohazards? No

Plate-Sorted Samples: Single Cell or Bulk

Sample Type Bulk

What volume of SMARTseq buffer did you sort into? half volume

Do you have multiple populations sorted onto the same plate?

10x-sorted samples: Single Cell

Number of samples:

How many cells do you want to target for capture in each sample:

Which kit did you want to use
Additional Kit Details:

What library types do you want to sequence
Additional Library Details:

What sequencing depth per cell are you targeting:

Next Generation Sequencing

Which molecule(s) are you studying: RNA

Library Preparation

RNA sequencing library options (Note: For 10x RNAseq, please use the earlier "10x section" to specify):
NexteraXT (STANDARD for low input cDNA - requires 0.5 ng total RNA)

Chromatin/Epigenetics sequencing library options:

DNA sequencing library options:

Analysis Platform Information

Sequencer Requested

- Illumina NextSeq 2000

Requested Run Type: Paired End
Requested Run Length: 59 nt
Indexing:

If your project needs to match sequencing parameters to previous projects or runs, check here and indicate the parameters and platform:

If your samples will be run across multiple lanes or flowcells, please indicate how you would like them to be

grouped or multiplexed:

Output and Data Reporting

Please list emails of all investigators who should receive data results report: bkhor@benaroyaresearch.org mmalueg@benaroyaresearch.org

Output Desired: FASTQ Files
Both Gene Counts & FASTQ Files

Do you want access to FASTQ files via BaseSpace? Yes

Please specify email address(es) for sharing on BaseSpace: bkhor@benaroyaresearch.org mmalueg@benaroyaresearch.org

Are you submitting custom libraries (libraries that you prepared yourself and handed off to the Core)?

If your project needs to match sequencing parameters to previous projects or runs, check here and indicate the parameters and platform:

If your samples will be run across multiple lanes or flowcells, please indicate how you would like them to be grouped or multiplexed:

Other Custom Requests

None provided