Hi Huitian,

Thanks for the link in previous e-mail. It works.

(1) I analyzed the differential ATAC-Seq data with some reference TFs including Prdm1, TCF1, LEF1 and E proteins (excel file attached). I think they meets criteria that you used in the Immunity paper (frequency in target sequences >15%, on-target frequency/background-frequency ratio ≥1.5, and pval ≤0.05). For example, Prdm1 was observed in Tfh Associated UP in comparison WT\_Tfh vs. WT\_Th1, indicating that Blimp1 binding to its TFBS potentially makes lower chromatin accessibility. Comparing ATAC-seq and Blimp-1 ChIP-Seq side by side can provide more information.

I think the differential ATAC-Seq analyses worked well in my experiment setting and is informative.

(2) I would like to know some details about definitions of followings:

(a) p-value: What is determinant for p-value?

(b) q-value

(c) # Target Sequences with motif

(d) % of Targets sequences with Motif: Comparison with what?

(e) # Background Sequences with Motif: What does consist the background?

(f) % of Background Sequences with Motif: The same with (d).

(3) It will be good for the figure if we get heat maps for following comparisons with grouped gene sets from current analyses (I attached a screenshot for this).

Heatmap:

 (a) For Group I target gene sets,

WT Tfh vs. Th1

DKO Th1 vs. Bcl6KO Th1

DKO Tfh vs. Prdm1KO Tfh

 (b) For Group II target gene sets,

WT Tfh vs. Th1

DKO Th1 vs. Bcl6KO Th1

DKO Tfh vs. Prdm1KO Tfh

 (c) For Group III target gene sets,

WT Tfh vs. Th1

DKO Th1 vs. Bcl6KO Th1

DKO Tfh vs. Prdm1KO Tfh

 (d) For Group IV target gene sets,

WT Tfh vs. Th1

DKO Th1 vs. Bcl6KO Th1

DKO Tfh vs. Prdm1KO Tfh

(4) I generated some candidate transcription factors which are potential Bcl6 targets and important for Tfh genetic program based on ATAC-Seq analysis and gene expression pattern analysis as below. The heat map I asked above can show better visuality for these candidates’ position. I wonder if you have any shRNA plasmids for above TFs.

|  |  |  |  |
| --- | --- | --- | --- |
| Atxn1 | Etv1 | Klf6 | Rxra |
| Bhlhe40 | Fli1 | Maf | Smad7 |
| Cbfb | Gata3 | Mxd1 | Snai3 |
| Cbx2 | Hopx | Mxd3 | Stat5a |
| E2f1 | Id2 | Pogk | Stat5b |
| E2f2 | Id3 | Rara | Tbx21 |
| E2f7 | Irf1 | Runx1 | Tcf19 |
| E2f8 | Irf7 | Runx2 | Trip13 |
| Ets1 | Klf2 | Runx3 | Zbtb32 |

Please let me know if you have any questions or if there is anything unclear. Thank you.

Best,

Jinyong

