



Data in Brief

Identifying type 1 diabetes candidate genes by DNA microarray analysis of islet-specific CD4 + T cells



Gregory J. Berry^a, Christine Friele^a, Robert M. Brucklacher^b, Anna C. Salzberg^c, Hanspeter Waldner^{a,*}

^a Department of Microbiology & Immunology, College of Medicine, Pennsylvania State University, Hershey, PA 17033, USA

^b Genome Sciences Facility, College of Medicine, Pennsylvania State University, Hershey, PA 17033, USA

^c Public Health Sciences, College of Medicine, Pennsylvania State University, Hershey, PA 17033, USA

ARTICLE INFO

Article history:

Received 4 May 2015

Accepted 10 May 2015

Available online 14 June 2015

Keywords:

Diabetes
Genetic susceptibility
Autoimmunity
T cells
Microarray

ABSTRACT

Type 1 diabetes (T1D) is a T cell-mediated autoimmune disease resulting from the destruction of insulin-producing pancreatic beta cells and is fatal unless treated with insulin. During the last four decades, multiple insulin-dependent diabetes (*Idd*) susceptibility/resistance loci that regulate T1D development have been identified in humans and non-obese diabetic (NOD) mice, an established animal model for T1D. However, the exact mechanisms by which these loci confer diabetes risk and the identity of the causative genes remain largely elusive. To identify genes and molecular mechanisms that control the function of diabetogenic T cells, we conducted DNA microarray analysis in islet-specific CD4 + T cells from BDC2.5 TCR transgenic NOD mice that contain the *Idd9* locus from T1D-susceptible NOD mice or T1D-resistant C57BL/10 mice. Here we describe in detail the contents and analyses for these gene expression data associated with our previous study [1]. Gene expression data are available at the Gene Expression Omnibus (GEO) repository from the National Center for Biotechnology Information (accession number GSE64674).

© 2015 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Specifications	
Organism/cell line/tissue	Mus musculus/islet-specific CD4 + T cells
Sex	Female
Sequencer or array type	Illumina MouseWG-6 v2.0 expression beadchip
Data format	Raw and processed
Experimental factors	Ex vivo and antigen-activated islet-specific CD4 + T cells
Experimental features	Analysis of gene expression levels in ex vivo and BDC2.5 mimotope-stimulated BDC2.5 CD4 + T cells containing insulin-dependent diabetes (<i>Idd</i>) locus 9 from diabetes-susceptible NOD or diabetes-resistant C57BL/10 mice
Consent	N/A
Sample source location	Hershey, PA, USA

1. Direct link to deposited data

<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE64674>.

* Corresponding author at: Department of Microbiology & Immunology, Pennsylvania State University College of Medicine, 500 University Dr., Hershey, PA 17033-2390, USA. Tel.: +1 717 531 0003.

E-mail address: huw10@psu.edu (H. Waldner).

2. Experimental design, materials and methods

2.1. Experimental design

Diabetes-free female BDC mice [2] and recently generated BDC-*Idd9.905* NOD mice [1] were used as donors of splenic islet-specific CD4 + T cells that were pooled from two mice per group (6–9 weeks old; n = 6/group). CD4 + T cells transgenic for the islet-specific BDC2.5 TCR (CD4 + TCRVβ4 +) were isolated by flow cytometry immediately (ex vivo) or following splenocyte stimulation (48 h) with BDC2.5 mimotope p79 (p79-stimulated) [3]. We used three independent biological replicates for each condition for DNA microarray analysis.

2.2. Materials and methods

2.2.1. RNA extraction

Total RNA was extracted from ex vivo or p79-stimulated CD4 + TCRVβ4 + BDC and BDC-*Idd9.905* T cells [1,2] using the RNeasy kit (Qiagen). RNA quality and concentration were assessed using an Agilent 2100 Bioanalyzer with RNA Nano LabChip (Agilent, Santa Clara, CA). Labeled cRNA (750 ng/sample) was synthesized by TotalPrep Amplification (Ambion, Austin, TX) and used for hybridization to MouseWG-6

v2.0 R3 Expression BeadChips for 18 h at 58 °C according to manufacturer's instructions. Following hybridization, Beadchips were washed and fluorescently labeled. Three independent replicate microarray hybridizations were processed per strain and per condition (12 hybridizations total). To collect intensity data, Beadchips were scanned with a BeadArray Reader (Illumina, San Diego, CA). A project was created with resultant scan data imported into GenomeStudio 1.0 (Illumina). Results were exported to GeneSpring Gx11 (Agilent Technologies).

2.2.2. Microarray data analysis

GeneSpring Gx11 software (Agilent Technologies, Santa Clara, CA) was used to analyze expression data, which was normalized to the median expression level of each gene. Expression of a transcript with detection p-value < 0.15 was considered present/marginal. Transcripts were subsequently filtered for signal level > 100 in at least 50% of the values in one of the six samples of each condition. Transcripts that failed to meet these requirements were excluded from further analysis. Differentially expressed genes were identified through volcano plots between non-averaged group comparison using fold-change of 1.4 or greater and asymptotic unpaired t-test p-value computation of $p < 0.05$ [4]. We

subsequently subjected significantly differentially expressed gene sets to bioinformatics analyses to cluster them according to their biological functions and to discover T cell-specific gene networks using Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.7 [5] and Ingenuity Pathway Analysis (IPA) 8.6 (Ingenuity Systems, Redwood City, CA) software, respectively. Heat maps and hierarchical clustering of significantly differentially expressed genes were generated with R using the heatmap.2 function of the gplots package. Raw and processed gene expression data were deposited in Gene Expression Omnibus repository at the National Center for Biotechnology Information and are accessible through the accession number GSE64674.

3. Discussion

We described here unique datasets of ex vivo and antigen (p79)-stimulated islet-specific CD4+ T cells containing either the *Idd9* from T1D-susceptible NOD mice or T1D-resistant C57BL/10 mice (Fig. 1). These datasets show genome wide gene expression determined using Illumina MouseWG-6 v2.0 expression beadchip platform. We identified 55 genes and 80 genes that were differentially expressed in ex vivo and p79-stimulated CD4+ T cells, respectively (Figs. 2, 3). The majority of these genes were unique to either experimental condition, whereas 18 genes were differentially expressed under both conditions (Fig. 2). Notably, gene sets were highly enriched in genes within the *Idd9* locus and *Idd11* locus, which partially overlaps *Idd9* (Tables 1, 2). Bioinformatics analyses revealed that islet-specific CD4+ T cells containing the T1D-resistant *Idd9* were most significantly enriched for genes associated with cellular growth and development/differentiation. Of these genes, *Eno1*, *Rbbp4* and *Mtor* are encoded by *Idd9*, suggesting that they contribute to *Idd9*-dependent T1D susceptibility by regulating the diabetogenic function of islet-specific CD4+ T cells. The provided datasets, together with our previous gene expression validation by RT-qPCR and functional analyses of BDC and BDC-*Idd9.905* CD4+ T cells [1], demonstrate the validity of using global gene expression analysis to discover T1D candidate genes and mechanisms that control T1D susceptibility.

Acknowledgments

We thank Dr. Willard Freeman at the PSU College of Medicine Genome Sciences Facility for advice with the DNA microarray analysis. This work was supported by funds from the Pennsylvania Department of Health (RFA#67-27a) to HPW.

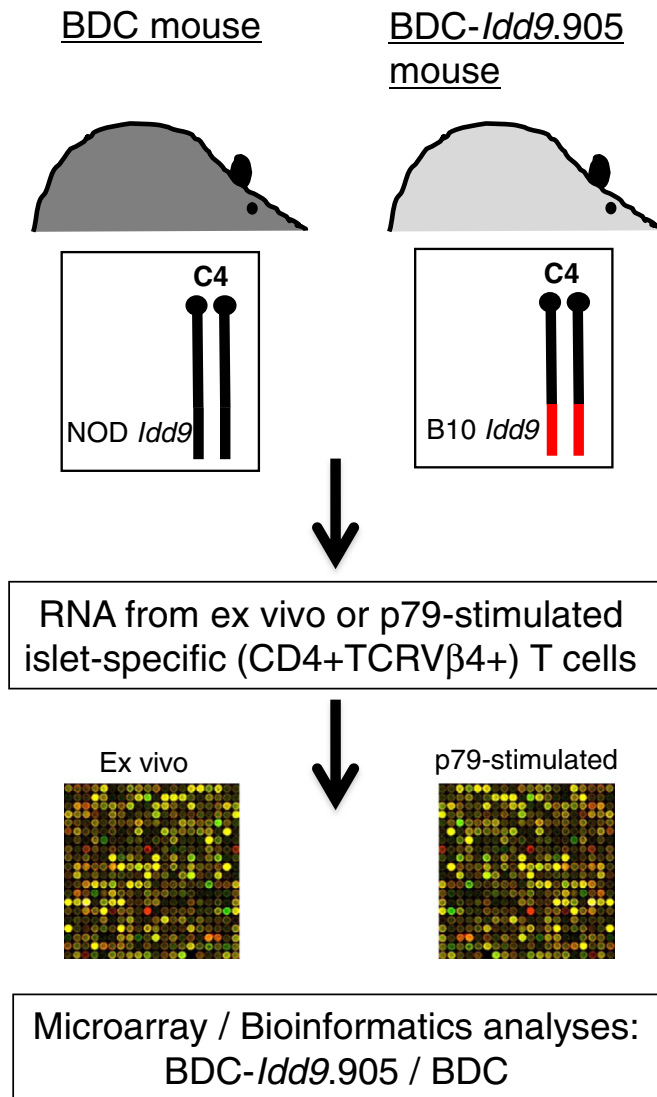
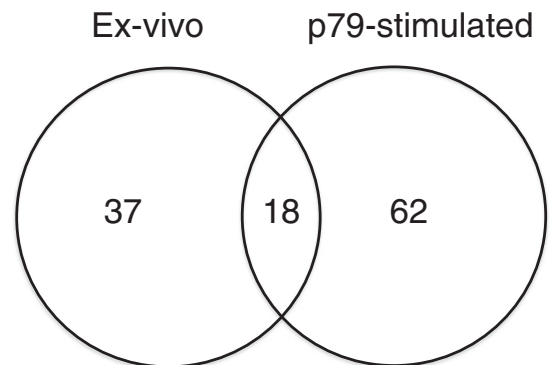


Fig. 1. Experimental design of gene expression analyses of islet-specific (CD4+TCRVβ4+) T cells containing NOD- or B10-derived *Idd9*.



BDC-*Idd9.905* vs. BDC CD4+ T cells

Fig. 2. Differentially expressed genes in BDC-*Idd9.905* versus BDC CD4+ T cells. Venn diagram showing the number of commonly or uniquely differentially expressed genes in ex vivo or p79-stimulated islet-specific CD4+ T cells from BDC-*Idd9.905* and BDC mice.

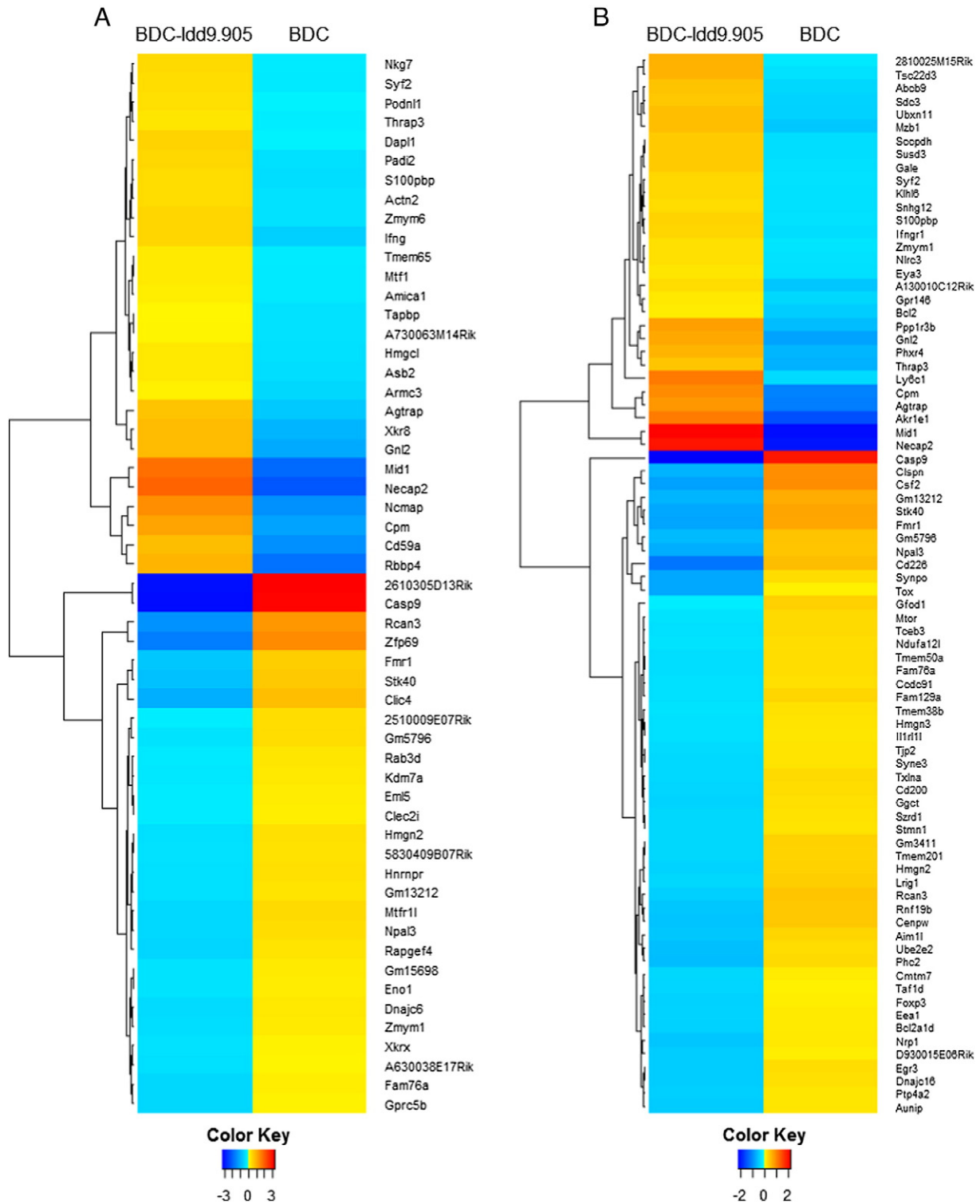


Fig. 3. Gene expression heat map and hierarchical clustering from microarray analysis. Columns show the expression patterns for significantly differentially expressed genes in ex vivo (A) or p79-stimulated (B) islet-specific CD4 + T cells from BDC-*Idd9.905* vs. BDC mice. The red and blue colors represent high and low gene expression values within the indicated ranges, respectively.

Table 1

Differentially expressed transcripts between ex vivo BDC-*Idd9.905* and BDC CD4 + TCRV β 4 + T cells detected by Illumina microarray probes.

Probe ID	Gene symbol	Gene ID ^a	Chromosome	Idd region	p-Value	Fold change ^b	Regulation
1570725	Rcan3	53902	4		2.47E-08	6.50	Down
7210706	Casp9	12371	4		1.33E-06	77.69	Down
1470608	2610305D13Rik	112422	4	Idd9.2	2.80E-06	78.00	Down
770133	Xkr8	381560	4	Idd11	1.18E-05	3.49	Up
5720706	Fmr1	14265	X		2.32E-05	2.53	Down

Table 1 (continued)

Probe ID	Gene symbol	Gene ID ^a	Chromosome	Idd region	p-Value	Fold change ^b	Regulation
4810746	Necap2 (synonym: 1110005F07Rik)	66147	4		3.57E−05	17.49	Up
1110349	Ncmap (synonym: A330049M08Rik)	230822	4		5.81E−05	7.04	Up
4490356	Zfp69	381549	4		1.28E−04	8.79	Down
160519	Tmem65	74868	15		3.27E−04	1.46	Up
5360181	Cpm	70574	10		3.27E−04	4.61	Up
610253	Eno1	13806	4	Idd9.3	8.39E−04	1.54	Down
3400491	Clic4	29876	4		0.00126461	3.54	Down
2190193	Gnl2	230737	4		0.001477347	3.87	Up
2850403	Mid1	17318	X		0.001548464	13.21	Up
610600	Hmgn2	15331	4		0.002030275	1.73	Down
270446	S100pbp	74648	4	Idd9.1	0.002319395	1.87	Up
1470278	Rab3d	19340	9		0.002501244	1.52	Down
5260433	Agtrap	11610	4	Idd9.2	0.002749308	2.71	Up
7160392	Rbbp4	19646	4	Idd9.1	0.003758136	6.69	Up
4890079	Padi2	18600	4		0.004021732	1.89	Up
3420601	Npal3	74552	4		0.004041954	1.87	Down
6660020	Zmym6 (synonym: 9330177P20Rik)	100177	4	Idd11	0.004275929	1.91	Up
5310274	Rapgef4	56508	2		0.005024596	1.85	Down
2030328	Hnrnp1 (synonym: 2610528B01Rik)	74326	4		0.005105765	1.77	Down
4850270	Gm15698	217066	11		0.005937492	1.56	Down
6660601	Hmgcl	15356	4		0.008095779	1.63	Up
7160132	Kdm7a (synonym: A630082K20Rik)	338523	6		0.0083255	1.53	Down
4480543	Mtf1	17764	4		0.009257403	1.47	Up
1990021	Gm5796 (synonym: ENSMUSG00000068790)	545007	14		0.009987115	1.75	Down
6940537	Dnajc6	72685	4		0.010992913	1.71	Down
1400685	Fam76a (BC008163)	230789	4	Idd11	0.015078585	1.67	Down
2030632	Stk40	74178	4	Idd11	0.015925948	2.75	Down
1440706	Armc3	70882	2		0.017203094	1.62	Up
6280477	lfng	15978	10		0.017631533	2.18	Up
430543	A730063M14Rik	100504703	10		0.017646367	1.44	Up
6200619	Xkrr	331524	X		0.019023608	1.46	Down
3360132	Syf2	68592	4		0.019146714	1.62	Up
2850398	Asb2	65256	12		0.0203302	1.66	Up
6980170	Podnl1	244550	8		0.023623548	1.50	Up
7200180	5830409B07Rik	76020	4		0.0246163	1.71	Down
430066	Gprc5b	64297	7		0.026957931	1.60	Down
1090441	Clec2i	93675	6		0.031066772	1.41	Down
870370	2510009E07Rik	72190	16		0.03157309	1.57	Down
1440019	Cd59a	12509	2		0.03529127	4.79	Up
1570544	Thrap3 (C730026O12Rik)	230753	4	Idd11	0.035571814	1.49	Up
460390	Mtfr11 (synonym: 2410166I05Rik)	76824	4		0.036052007	1.93	Down
1570288	Dapl1	76747	2		0.036334574	1.65	Up
6200386	Gm13212 (LOC433801)	433801	4	Idd9.2	0.03805429	1.67	Down
5390243	Zmym1	68310	4	Idd11	0.040785447	1.66	Down
1050678	Actn2	11472	13		0.04146113	1.79	Up
3850692	Amica1	270152	9		0.04214731	1.42	Up
6760736	Eml5	319670	12		0.0422112	1.42	Down
50255	A630038E17Rik	219065	14		0.04455469	1.47	Down
4040035	Nkg7	72310	7		0.04593347	1.65	Up
3140386	Tapbp	21356	17		0.04769256	1.42	Up

^a Fold change in expression of BDC-Idd9.905/BDC samples.^b Gene ID: Entrez database.

Table 2

Differentially expressed transcripts between p79-stimulated BDC-Idd9.905 and BDC CD4+TCRVβ4+ T cells detected by Illumina microarray probes.

Probe ID	Gene symbol	Gene ID ^a	Chromosome	Idd region	p-Value	Fold change ^b	Regulation
4810746	Necap2 (synonym: 1110005F07Rik)	66147	4		1.04E−05	15.57	Up
5720706	Fmr1	14265	X		1.50E−05	2.81	Down
6200386	Gm13212 (LOC433801)	433801	4	Idd9.2	4.05E−05	2.48	Down
2190193	Gnl2	230	4		1.23E−04	2.81	Up
5260433	Agtrap	11610	4	Idd9.2	2.04E−04	3.93	Up
7210706	Casp9	12371	4		2.50E−04	17.66	Down
5360181	Cpm	70574	10		2.53E−04	4.08	Up
5560131	Syne3 (synonym: 4831426I19Rik)	212073	12		4.21E−04	1.46	Down
3890682	Cenpw (synonym: 2610036L11Rik)	66311	10		4.84E−04	1.97	Down
130270	Ndufa12l	75597	13		5.54E−04	1.45	Down
2030632	Stk40	74178	4	Idd11	6.77E−04	2.83	Down
70019	Akr1e1	56043	13		8.21E−04	6.03	Up
3420601	Npal3	74552	4		9.22E−04	2.27	Down
1980608	Tmem201	230917	4		0.001167733	1.64	Down
610600	Hmgn2	15331	4		0.001190718	1.69	Down

(continued on next page)

Table 2 (continued)

Probe ID	Gene symbol	Gene ID ^a	Chromosome	Idd region	p-Value	Fold change ^b	Regulation
1850403	Mtor (synonym: Frap1)	56717	4	Idd9.2	0.001898694	1.47	Down
1570544	Thrap3 (C730026O12Rik)	230753	4	Idd11	0.001915356	2.23	Up
6370176	Csf2	12981	11		0.002062027	3.40	Down
3360132	Syf2	68592	4		0.002120117	1.50	Up
2450470	Txlna	109658	4	Idd9.1	0.002173837	1.56	Down
6250133	Ccdc91	67015	6		0.002950219	1.46	Down
7650139	Phc2	54383	4	Idd9.1	0.003565085	1.83	Down
6510647	Tceb3	27224	4		0.003816067	1.50	Down
3170619	Rnf19b	75234	4	Idd9.1	0.003891535	1.94	Down
270446	S100pbp	74648	4	Idd9.1	0.005145121	1.47	Up
1780445	Klhl6	239743	16		0.005396708	1.53	Up
4120709	Dnajc16 (synonym: 2900037O03Rik)	214063	4		0.005405837	1.62	Down
1570725	Rcan3	53902	4		0.006421643	1.91	Down
270139	Cd200	17470	16		0.00670664	1.49	Down
2850403	Mid1	17318	X		0.006896318	18.23	Up
2900139	Ube2e2	218793	14		0.007577249	1.79	Down
5820646	Ptp4a2	19244	4	Idd9.1	0.007635422	1.55	Down
3120392	Stmn1	16765	4		0.007748838	1.49	Down
4540626	Tmem38b	52076	4		0.00839877	1.41	Down
1990021	Gm5796 (synonym: ENSMUSG00000068790)	545007	14		0.009142185	2.11	Down
2650397	Ubxn11 (synonym: D4Bwg1540e)	67586	4		0.009179465	1.90	Up
520286	Phxr4	18689	9		0.009703344	2.36	Up
7160626	Il1rl11	17083	9		0.010156409	1.41	Down
5080600	Aim11	230806	4		0.01032208	1.79	Down
1690255	Susd3	66329	13		0.011247533	1.66	Up
4920564	Szrd1 (synonym: D4Ertd22e)	213491	4		0.011548025	1.50	Down
6350192	Snhg12 (synonym: 2310005L22Rik)	100039864	4	Idd11	0.011811014	1.48	Up
2940441	Nlrc3 (synonym: D230007K08Rik)	268857	16		0.012169471	1.43	Up
5820438	Cd226	225825	18		0.012342826	3.30	Down
5270575	Gale	74246	4		0.012346829	1.62	Up
5690348	Taf1d (synonym: 4930553M18Rik)	75316	9		0.015596034	1.40	Down
940274	A130010C12Rik	320211	8		0.015818644	1.74	Up
1030152	Ifngr1	15979	10		0.01632345	1.57	Up
1980768	Cmtm7	102545	9		0.016449802	1.42	Down
2480500	Zmym1 (4933412A02Rik)	68310	4	Idd11	0.01667966	1.41	Up
7210672	Mzb1 (synonym: 2010001M09Rik)	69816	18		0.01843534	2.00	Up
5570358	Bcl2	12043	1		0.019133182	1.52	Up
7380113	Ggct (synonym: A030007L17Rik)	110175	6		0.019302472	1.55	Down
5670634	Synpo	104027	18		0.021028133	2.04	Down
5360608	Tjp2	21873	19		0.02300658	1.44	Down
6510286	Tmem50a	71817	4		0.0230189	1.49	Down
3800300	LOC100041569 (Gm3411)	100041569	14		0.023209462	1.65	Down
1980370	Gpr146	80290	5		0.024589665	1.45	Up
5720017	Eea1	216238	10		0.024702739	1.46	Down
1400685	Fam76a (BC008163)	230789	4	Idd11	0.025232932	1.50	Down
990671	D930015E06Rik	229473	3		0.026054276	1.50	Down
6760762	Sdc3	20970	4	Idd9.1	0.028066363	1.80	Up
5310431	Fam129a	63913	1		0.031280726	1.54	Down
3370196	Gfod1 (A1850995)	328232	13		0.032448232	1.41	Down
4210152	2810025M15Rik	69953	1		0.033680663	1.80	Up
3180408	Bcl2a1d	12047	9		0.036864646	1.49	Down
360743	Nrp1	18186	8		0.0391383	1.63	Down
2230132	Eya3	14050	4	Idd11	0.040735032	1.40	Up
6980315	Clspn	269582	4	Idd11	0.040747937	3.01	Down
5080634	Ppp1r3b	244416	8		0.043392483	2.59	Up
1570241	Foxp3	20371	X		0.044076834	1.45	Down
1090008	Abcb9	56325	5		0.045361392	1.79	Up
5340288	Scppdh	109232	1		0.04552452	1.65	Up
2070328	Aunip (synonym: 2610002D18Rik)	69885	4		0.046391055	1.59	Down
6840382	Tsc22d3	14605	X		0.046913423	1.88	Up
670133	Hmgn3	94353	9		0.046961725	1.43	Down
3130020	Lrig1	16206	6		0.04753622	1.68	Down
20612	Egr3	13655	14		0.047545053	1.65	Down
5550671	Ly6c1	17067	15		0.049469374	2.70	Up
5690424	Tox	252838	4		0.049562268	1.85	Down

^a Fold change in expression of BDC-Idd9.905/BDC samples.

^b Gene ID: Entrez database.

References

- G.J. Berry, C. Frielle, T. Luu, A.C. Salzberg, D.B. Rainbow, L.S. Wicker, H. Waldner, Genome-wide transcriptional analyses of islet-specific CD4+ T cells identify Idd9 genes controlling diabetogenic T cell function. *J. Immunol.* 194 (2015) 2654–2663.
- J.D. Katz, B. Wang, K. Haskins, C. Benoist, D. Mathis, Following a diabetogenic T cell from genesis through pathogenesis. *Cell* 74 (1993) 1089–1100.
- V. Judkowsky, C. Pinilla, K. Schroder, L. Tucker, N. Sarvetnick, D.B. Wilson, Identification of MHC class II-restricted peptide ligands, including a glutamic acid decarboxylase 65 sequence, that stimulate diabetogenic T cells from transgenic BDC2.5 nonobese diabetic mice. *J. Immunol.* 166 (2001) 908–917.
- I.V. Yang, E. Chen, J.P. Hasseman, W. Liang, B.C. Frank, S. Wang, V. Sharov, A.I. Saeed, J. White, J. Li, N.H. Lee, T.J. Yeatman, J. Quackenbush, Within the fold: assessing differential expression measures and reproducibility in microarray assays. *Genome Biol.* 3 (2002) (research0062).
- W. Huang da, B.T. Sherman, R.A. Lempicki, Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat. Protoc.* 4 (2009) 44–57.