Maintained mouse lines
(Updated Jan 2012)

RAG-1\(^{0}/\)N
Recombination Activating Gene 1 Knock Out

Development
A neomycin resistance cassette was inserted in the RAG-1 gene, resulting in a 1356 bp deletion in the 5’ end of the coding sequence into 129-derived AB1 ES cell line. The C57BL/6J strain was generated by backcrossing mice carrying the Rag-10 mutation 10 times to C57BL/6J inbred mice. (Mombaerts P et al, Papaioannou VE., 1992). Rag-1 deficient mice were transferred to the NOD/LtSz strain background. (Carnaud C. et al, Bendelac A., 1999; Shultz et al Greiner, 2000). The line is currently at the 18th backcross to NOD.

Description
RAG-1 deficient mice are viable and fertile. They are unable to initiate V(D)J recombination in Ig and TCR genes and lack functional T and B lymphocytes. Although RAG-1 expression has been reported in the central nervous system of the mouse, no obvious neuroanatomical or behavioral abnormalities have been found in the RAG-1-deficient mice. (Mombaerts P et al, Papaioannou VE., 1992).

On the NOD background, NOD.Rag10 mice are devoid of mature T or B cells. NOD/LtSz-Rag10 recipients of adoptively transferred spleen cells from diabetic NOD/Lt+/+ mice rapidly develop diabetes. (Carnaud C. et al, Bendelac A., 1999; Shultz et al Greiner, 2000).

Tcra \(^{0}/\)N
T-cell receptor alpha chain Knock Out
NOD.129P2(C)-Tcratm1Mjo/DoiJ

Development
The Tcra\(^{a}\) targeted mutation was generated in GK129 ES cells (derived from substrain 129P2/OlaHsd) via homologous recombination. The neomycin resistance gene was inserted into the first constant region of the Tcra gene. (Philpott KL et al, Owen MJ. 1992.) The Tcratm1Mjo allele on the C;129 mixed background was then transferred to the NOD background (Katz JD et al, Mathis D., 1995). The line is currently at the 12th backcross to NOD/LtJ (2004).

Description
The mutation prevents expression of any endogenous TCR alpha chains and thereby blocks differentiation of alpha beta T cells. NOD mice homozygous for the Tcra targeted mutation lack alpha beta T cells and are completely protected from diabetes development. Because of the complete elimination of alpha beta T cells, these mice are useful in adoptive transfer experiments or in crosses to TCRab transgenic lines. (Katz JD et al, Mathis D., 1995; Hoglund et al, Mathis D., 1999).

μMT\(^{0}/\)N
Immunoglobulin IgM Knock out
NOD.129S2(B6)-Igh-6tm1Cgn/DoiJ

Development
The membrane exons of the gene encoding the mu-chain constant region were disrupted by insertion of a neomycin resistant gene (Kitamura et al, Rajewsky K, 1991). The mutation was then transferred from the original chimeric stock with a mixed 129/Sv and C57 BL/6 genome onto the NOD/Lt background (Serreze DV et al, Schultz LD, 1996). The line is currently at the 14th backcross to NOD/LtJ (2005).
**Description**

Heterozygous mice are normal and fertile. In homozygous animals however, B-cell development is stopped at the stage of pre-B-cell maturation and B cells are therefore absent. (Kitamura et al, Rajewsky K, 1991). No IgM is present on the pre B cell surface.

NOD.μMT-deficient mice have normal numbers of T cells but are free of overt Diabetes and insulitis resistant. The frequency of disease in the B lymphocyte intact segregants is equivalent to that of standard NOD mice (Serreze DV et al, Schultz LD, 1996). Homozygous μMT knock out animals display a high incidence of lymphoma of both T- and B-cell origin compared with these mutations on other genetic backgrounds. The lymphoma incidence in both strains is greater in females, reflecting the greater incidence of autoimmune type 1 diabetes in NOD females than in males. (Chiu PP et al., Danska JS., 2002).

### IL4^0/N

**Interleukin 4 Knock Out**

NOD.129P2(B6)-Il4tm1Cgn/DvsJ

**Development**

A neomycin resistance cassette was used to disrupt the first exon of IL4 into E14-1 embryonic stem cells (derived from 129P2/OlaHsd), Kuhn R et al, Muller W, 1991). The mutation was maintained on a B6:129 segregating background until it was transferred to NOD/Lt using a marker assisted protocol (Wang et al, Mathis D, 1998). The line is currently at the >9th backcross to NOD/LtJ.

**Description**

Mice homozygous for the IL4 targeted mutation are viable and fertile. T and B cell development is normal but IgG1 and IgE levels and the ability of homozygous mutant mice to produce Th2-derived cytokines are significantly reduced. On the NOD background, the IL-4-null mutation does not accelerate or intensify insulitis in regular NOD and has no effect on the timing or frequency of the transition to diabetes. (Wang et al, Mathis D, 1998; Serreze DV et al, Rabinovitch A, 2001). Coxsackievirus B4 (CVB4) infection in NOD mice leads to acceleration of Type 1 diabetes. When NOD.IL4^-/- mice are infected with CVB4 at 12 weeks of age, the onset of diabetes is accelerated in NOD.IL4^-/- mice compared to NOD. IL4^+/+ mice. In the absence of an insulitic threshold, CVB4 infection leads to long-term disease protection. This protective mechanism is not affected by the disruption of IL4 gene. (Serreze DV et al, Atkinson MA., 2005).

### IL10^0/N

**Interleukin 10 Knock Out**

NOD.Cg-Il10tm1Cgn/DvsJ

**Development**

The first exon of IL10 was disrupted after the fourth codon, a neomycin resistance cassette, and an additional termination codon in the third exon, was used for homologous recombination in E14-1 ES cells (129P2/OlaHsd-derived) (Kuhn R et al, Muller W, 1993). The mutation was then transferred to the NOD/Lt background using a marker assisted protocol (Serreze DV et al, Rabinovitch A, 2001). NOD/Lt mice heterozygous for this IL10^0 allele and homozygous for diabetes susceptibility loci (Idd) were intercrossed to develop mice homozygous for IL10tm1Cgn and all Idd loci. The line is currently at the 11th backcross to NOD/LtJ (2005).

**Description**

Mice homozygous for the IL10 targeted mutation are viable and fertile when housed under SPF conditions. This mutant develops type 1 diabetes at the same rate as the NOD/Lt parental strain. The II10 mutation also renders this line susceptible to colitis (although not as severe as other strains of Il10 deficient mice when maintained under standard housing conditions. (Serreze DV et al, Rabinovitch A, 2001).

### IFN-γ^0/N

**Gamma Interferon Knock Out**

NOD.129S7(B6)-Ifngtm1Ts/DvsJ

**Development**

The targeting vector has a neomycin resistance gene inserted into exon 2, which introduces a terminaison codon after the first 30 amino acids of the mature IFN-γ protein. The targeting vector was transferred into AB-1 embryonic stems. (Dalton DK et al, Stewart T, 1993). The mutation was then transferred to the NOD background (Wang B et al, Mathis D, 1997). The line is currently at the 9th backcross in NOD/LtJ.

**Description**

Deficient mice develop normally and are healthy in the absence of pathogens. However, mice deficient for IFN-γ have impaired production of macrophage antimicrobial products and reduced expression of macrophage major histo-compatibility complex class II antigens (Dalton DK et al, Stewart T, 1993). NOD Mice homozygous for the IFN-γ KO targeted mutation are viable and fertile. The genetic absence of IFN-γ does not prevent either insulitis or diabetes in the NOD mice, but increases the time to onset. Splenocytes taken from IFN-γ deficient...
diabetic mice are fully capable of transferring diabetes to naive recipients (Hultgren B et al, Stewart TA., 1996). In both NOD, IFN-γ -/- and NOD, IFN-γ -/+ mice, IL-12 administration generates a massive and destructive insulitis and increases the number of pancreatic CD4(+) cells. (Trembleau S et al, Adorini L, 2003). NOD IFN-γ -/- mice display increased acinar cell apoptosis and abnormal salivary protein expression, typically observed in parental NOD mice prior to Sjogren's syndrome-like autoimmune exocrinopathy. (Cha S et al, Peck AB., 2004). When NOD, IFN-γ -/- mice are infected with Coxsackievirus B4, Insulitis or diabetes development is delayed by several weeks compared to NOD mice. When mice are infected at 12 weeks of age, neither acceleration nor long-term protection is elicited in NOD, IFN-γ -/- mice. (Serreze DV et al, Atkinson MA., 2005).

NY8.3 TCR/N
NY8.3 TCRalpha/beta transgene
NOD.Cg-Tg(TcraTcrbNY8.3)1Pesa/DvsJ

Development
Development Functional VDJbeta and VJalpha rearrangements were isolated from the beta cell cytotoxic CD8(+) H-2Kd-restricted T cell clone NY8.3 (Verdaguer et al, 1997). Transgenic constructs bearing the functional VDJb and VJa rearrangements of NY8.3, upstream regulatory sequences, and the TCRb enhancer (for the TCRb construct) or the TCRa or IgH enhancers (for the TCRa construct) were co-injected into fertilized (SJL3 C57BL/6) F2 eggs. Two transgenic founder mice expressing the transgenes were backcrossed with NOD/Lt. In 2005, the Type 1 Diabetes Resource received congenic NOD hemizygous transgenic mice backcrossed at least 14 generations to NOD; these were cryopreserved, and the line passed to the CITDH’s Core and backcrossed at least 6 more times.

Description
NY8.3 TCR/N expresses rearranged Tcra and Tcrb transgenes derived from the pancreatic beta cell-cytotoxic CD8+ T cell clone NY8.3. CD4-CD8+ thymocytes and lymph derived T cells are skewed toward VB8.1/2+ expression when compared to wild type controls (Verdaguer et al, 1997). Transgenic mice exhibit accelerated diabetes (onset between 21 and 26 days of age. The cumulative diabetes incidence and kinetics of disease are remarkably similar the their wild type cohort (Verdaguer et al, 1997). Transgenic animals bearing both TCR transgenes offer a source of CTL precursors useful in examining the diversity of beta cell peptides recognized by the autoreactive CD8+ T lymphocytes contributing to the earliest phase of IDDM development.

Foxp3DTR/N
Foxp3-Human DTR-eGFP transgene

Development
Transgene-directed expression of the human Diptheria Toxin Receptor (DTR) can be used for lineage ablation studies, to determine the role of particular cell types. Mouse cells are resistant to DT for lack of a receptor, but targeted expression of DTR as a transgene renders DTR-expressing cells sensitive to DT treatment in vivo (Saito et al., 2001). Mice expressing the human DTR under the control of foxp3 transcriptional control elements were generated by BAC transgenesis. The BAC construct spanned from 150 kb upstream to 70kb downstream of the Foxp3 transcription start site. A DTR-eGFP cDNA with a stop codon was inserted between the first and second codon of the Foxp3 open reading frame. (Feuerer et al, 2009).

Description
Heterozygous NOD.Foxp3.DTR + transgenic mice show no obvious phenotype. The eGFP component can be used for identification of Treg cells, although the fluorescence intensity is lower than that of common reporter lines. DT administration into young adults results in 80%-90% depletion of Foxp3+ T cells in secondary lymphoid organs after a few days. Treg cell pools recover quickly after the end of DT treatment, and Treg cell numbers recovered to 60% of normal by three days after the last injection. (Feuerer et al, 2009). The pancreatic islet of the DT-injected mice showed a strong immune infiltrate compared to littermate control when DT is injected every other day in young adult for 9 days. Very mild infiltrate can be detected in lung and liver of Foxp3 DTR transgenic mice after such a regimen. (Feuerer et al, 2009).

FoxP3-I-GFP/N
Forkhead Box P3 - IRES-GFP Knock-in

Development
FoxP3-I-GFP mice were generated in the Kuchroo laboratory (Betteli et al, 2006). An enhanced fluorescent protein reporter (EGFP) was introduced by recombination into the Foxp3 locus, creating a bi-cistronic transcript that expresses both Foxp3 and GFP proteins. An IRES-EGFP derived from pMSCV-IRES-EGFP was subcloned into a TKPbs-LoxP-Neo cassette with an SV40 polyadenylation sequence. A 5.3 kb fragment containing the 3’ untranslated region and a 1.7 kb fragment containing exons 11, 12 and part of the exon 13 of C57BL/6 Foxp3 genomic DNA were generated by PCR amplification using a BAC
clone (RP23-54C14) as a template, then inserted into the targeting construct. Targeted Bruce4 ES cells were injected into BALB/c blastocysts and male chimeras were initially bred with female C57BL/6. The line was subsequently backcrossed onto the NOD/MrkTac in the Kuchroo lab over 12 times, intercrossed for 6 generations, then 3 generations in NOD/Ltj in the T1 Resource (ongoing).

Description

Knock-in mice express a functional chimeric GFP protein only in cells where the Foxp3 gene is transcriptionally active (Betteli et al, 2006, Xu et al, 2010). FoxP3 is expressed in Treg cells, which constitutes about 13% of CD4 alpha/beta T cells in NOD mice. The GFP fluorescent protein is readily detectable by flow cytometry, providing an easy identification of Treg cells in lymphoid organs and pancreas and spleens of NOD mice, useful for tracking or purification (Betteli et al, 2006, Xu et al, 2010).

B2M0/N
Beta-2 microglobulin Knock Out

NOD.129P2(B6)-B2mtm1Unc/J

Development

The β2Mo mutant strain was generated by a targeted disruption of the β2M gene into 129-derived E14TG2a ES cell line (Koller BH et al, Smithies O. 1990). The NOD/LtJ strain was produced by backcrossing the mutation 10 times to NOD/LtJ inbred mice (Christianson et al, Roopenian , 1996). The line is currently at the >12th backcross generation on NOD/LtJ.

Description

Mice homozygous for the β2M targeted mutation have little if any MHC class I protein expression on the cell surface. There are few CD8+ cytotoxic T-cells and under some circumstances a compensatory increase in CD4+ cytotoxic T-cells. Immune responses involving CD8+ T-cells are severely deficient (Koller BH et al, Smithies O., 1990; Christianson et al, Roopenian , 1996). The elimination of cell surface MHC class I expression blocks both insulitis and autoimmune diabetes in NOD/Lt mice. (Katz J et al, Mathis D., 1993; Wicker et al, Peterson LB., 1994; Wang et al, Mathis D.,1996)

Ealpha16/N Tg
E Alpha 16 Transgene

Development

A cloned Ek alpha MHC Class II gene was introduced by microinjection into (C57BL/6 x SJL) F1 mouse oocytes. These strains, as NOD mice, are generally deficient in the MHC II E complex because of a large deletion in the promoter of the Ealpha gene. The transgene thus restore proper expression of the E alpha E beta complex, and immune reactivities linked to it (Le Meur et al, Mathis D., 1985). The line is currently at the >99th backcross generation in NOD/LtJ (Bohme J et al, Mathis D, 1990).

Description

Transgenic mice are viable and fertile. Ealpha 16 abundance in the spleen of all transgenic mice is equivalent to that in BALB/c and B10-BR controls. E alpha 16 expresses the I-E complex on all cells that normally display class II molecules. On the NOD genetic background, the transgene E alpha 16 confers almost complete protection from insulitis by preventing lymphocyte infiltration into the pancreatic islets of 10 week old mice (Bohme J et al, Mathis D, 1990).

B6.H-2g7

Development

B6.H2g7 is a congenic line developed by H Kikutani. This strain carries the NOD-derived MHC class II g7, haplotype, located on Chromosome 17, but has all other genes from the B6 strain. The line is currently at the >20th generation.

Description

B6-H2g7 mice are viable, fertile, and present with no particular phenotype. They show no insulitis or diabetes (Luhder F et al, Mathis D, 2000). When crossed with BDC2.5 transgenic line, BDC2.5/B6g7 mice develop typical diabetes in most features, but, in contrast to the disease in BDC2.5/N or regular NOD animals, there is a quick transition from initial islet infiltration to massive destruction of the insulin–producing beta cells (Gonzalez A et al, Mathis D, 1997).

CTLA40/N
CTLA 4 Knock Out

Development

A neomycin expression cassette was inserted into exon 3 of Ctl4 (cloned from 129/Sv) and transfected into R1 (129X1/SvJ x 129S1/Sv)F1-Kitl+ embryonic stem cells. These ES cells were injected into C57BL/6 background. (Chambers CA et al, Allison JP, 1997). Luhder F et al subsequently backcrossed this mutation to NOD/LtJ. The line is currently at the 31th backcross (2005).
Description
Ctla-4 deficient mice are viable, fertile, and normal in size but develop a fatal lymphoproliferative disorder. All homozygous mice on various backgrounds, including C57BL/6, BALB/c, and 129SV die at 3-4 weeks of age due to massive polyclonal expansion of T-cells and massive lymphocyte infiltration into non-lymphoid organs, such as heart, liver, lung and pancreas. On NOD/LtJ background, CTL4 deficient mice die around 3 weeks of age of massive lymphocyte proliferation and infiltration into multiple organs, or of hyperacute diabetes when crossed to a pancreas-reactive TCR transgene. (Luhder F et al, Mathis D, 2000).

CD40<sup>0/N</sup>
CD40 Knock Out

Development
The CD40 gene was disrupted by replacing exon 3 by the neomycin resistance gene (Kawabe et al, Kikutani H, 1994). Mice carrying the CD40 null mutation were transferred to the NOD background (Korganow AS et al, Mathis D, 1999). The line is currently at the >13th backcross to NOD/LtJ.

Description
Homozygous mutant mice show a deficiency in T cell activation. The mutation causes a significant reduction of CD23 expression on mature B cells and relatively decreased number of IgM bright and IgD dull B cells. The mutant mice mount IgM responses but no IgG, IgA, and IgE responses to thymus dependent antigens. However, IgG as well as IgM responses to thymus-independent antigens are normal (Kawabe et al, Kikutani H, 1994).

Homozygous CD40<sup>0</sup> mice on the NOD background are protected from diabetes (Poirot et al, unpublished).

MIP-GFP/N
Mouse insulin promoter (MIP), human Growth Hormone/EGFP transgene

Development
This transgenic allele expresses Enhanced Green Fluorescent Protein fused to a 2.1kb fragment of human Growth Hormone under the control of Mouse Insulin Promoter 1. The transgenic construct was injected directly into NOD/LtJ oocytes. (Hara et al, 2003). The line was backcrossed onto the NOD/LtJ background.

Description
Transgenic mice develop normally and behave similarly to controls with respect to glucose tolerance and pancreatic insulin content. Histology confirms transgenic mice have normal islet architecture with co-expression of insulin and GFP. (Hara et al, 2003). The enhanced GFP reporter allows the beta cells to be easily identified and purified for further studies. Studies completed at The Jackson Laboratory (Leiter EH et al 2007) indicate there is strong non-mosaic expression of green fluorescent protein in NOD/LtJ-Tg(Ins1-EGFP/GH1)14Hara/HaraJ islets. 100% of homozygous Ins1-EGFP transgenic males and females, identified by qPCR, become diabetic by 9 weeks of age. Pancreatic histopathology of homozygous mice shows beta cell loss without insulitis.

Hemizygous Ins1-EGFP mice are viable and are used for breeding. A 30-week incidence study comparing NOD/LtJ controls with mice hemizygous for the Ins1-EGFP transgene shows severely depressed diabetes incidence in these hemizygotes, with males atypically at greater risk than females. Glucose tolerance in prediabetic, Ins1-EGFP transgenic hemizygous 8-week-old males is selectively impaired compared to wild type controls. Although these 8-week-old Ins1-EGFP transgenic and wild type males did not differ in plasma insulin content; a significant decline was noted in hemizygous Ins1-EGFP transgenic males compared to normoglycemic wildtype males when sampled at 30 wk. Adoptive transfer of highly diabetogenic CTL (A14 TCR Tg Rag) CD8<sup>+</sup> T cells produced diabetes within 8 days post-injection into NOD/LtJ females, but failed to produce any diabetes or even home to islets weeks after injection into hemizygous females. Pancreatic histopathology of 31 week old, non-diabetic, hemizygous Ins1-EGFP transgenic males and females indicate peri and intra islet fibrosis, peri-insulitis and depleted beta cell granulation in 70% of the animals, while only 30% of the mice have intra islet insulitis.

BDC2.5/N
TCR alpha /beta transgene

Development
BDC2.5/N mice carry both rearranged TCR alpha and beta genes from the diabetogenic H2-Ag7 restricted BDC2.5 CD4<sup>+</sup> T cell clone BDC-2.5. The BDC2.5 TCR<sup>α</sup> and TCR<sup>β</sup> sequences were co-injected into (B6xSJL)F2 eggs. To achieve the natural expression of Tcra, the rearranged V<sup>α</sup> J<sup>α</sup> sequence from BDC2.5 was cloned into the cassette vector generated by Kouskoff et al (1996), which contains both the 5’ upstream promoter and the 3’ downstream enhancer regions of the Tcra gene. The integration site for these BDC2.5 Tcr transgenes was localized to chromosome 13 near the D13mit125 marker (Katz et al, Mathis D. 1993). The line is currently at the >99 backcross generation to NOD/LtJ.
On the NOD background, mice carrying the transgenes have a reduced incidence of diabetes relative to NOD/LtJ controls (12% incidence at age 30 weeks). BDC2.5/N animals show a generalized and very extensive islet infiltration after a few weeks of age, but most remain free of overt diabetes for long periods or develop forms of insulitis that does not cause beta cells destruction. When coupled with the homozygous Rag1 knock Out, mice develop diabetes extremely early (mean age of 25 days). (Katz et al Mathis D. 1993, Gonzalez et al, Benoist C., 2001, Mombaerts et al, Papaioannou VE., 1992).

**BDC2.5/N Thy1.1**

**TCR alpha/beta transgene, Thymus Cell Antigen 1a variant**

**Development**
The BDC2.5 TCR alpha and beta sequences were co-injected into (B6xSJL)F2 eggs. The integration site for these BDC2.5 TCR transgenes was localized to chromosome 13 near the D13mit125 marker (Katz et al., Mathis D. 1993). BDC2.5/N Thy1.1 mice are created by crossing BDC2.5/N with NOD.Thy1a (NOD.NON-Thy1a/1LtJ). Thy1.1 is an allogenic marker from PL/J backcrossed onto NOD. (Fabien N et al, Thivolet C. 1995). The BDC2.5/N Thy1.1 line is currently at the N22 F6 backcross generation.

**Description**
On the NOD background, mice carrying the transgenes have a reduced incidence of diabetes relative to NOD/LtJ controls (12% incidence at age 30 weeks). BDC2.5/N animals show a generalized and very extensive islet infiltration after a few weeks of age, but most remain free of overt diabetes for long periods or develop forms of insulitis that does not cause beta cells destruction. The BDC2.5/N Thy1.1 congenic strain carries the T lymphocyte specific Thy1a (Thy1.1) allele. Donor T cells can be easily distinguished from recipient T cells by both flow cytometric and histological analysis.

**BDC2.5/B6H2g7**

**TCR alpha/beta transgene**

**Development**
The BDC2.5 TCR alpha and TCR beta sequences were co-injected into (B6xSJL)F2 eggs. The integration site for these BDC2.5 Tcr transgenes was localized to chromosome 13 near the D13mit125 marker (Katz et al., Mathis, 1993). Transgenes have been transferred on B6.H2g7 background, a congenic line developed by H Kikutani. This strain carries the NOD-derived MHC class II g7 haplotype, located on Chromosome 17, but has all other genes from the B6 strain (Gonzalez A et al, Mathis D, 1997). The BDC2.5/B6g7 line is currently at the >20th backcross generation.

**Description**
On the B6.H2g7 background, mice carrying the BCD2.5 transgenes show a generalized and very extensive islet infiltration after a few weeks of age, and a rapid onset diabetes that affects 40-70% of mice by 8 weeks of age (Gonzalez A et al, Mathis D, 1997; Poirot L. et al, Mathis D., 2004).

**NOD.Nk1.1**  Please note: This line is temporarily unavailable

**NK1.1 cell surface receptor**

**Development**
The surface marker NK1.1 is a receptor of the NKR-P1 family normally expressed in the C57BL/6 (B6) strain but not in the NOD strain. The NK.1 cell surface receptor has been bred onto NOD mice, which carry a reported 10 cM of Chr. 6 from C57BL/6. A homozygous line was initiated from the progeny of the intercross. (Carnaud et al, Herbelin A, 2001). The line is currently at the >50th backcross to NOD/LtJ (2005).

**Description**
NK cells are B6-like and can be differentiated from NOD-like NK cells. NOD.NK1.1 mice express the NK1.1 marker selectively on the surface of their NK and NKT cell subsets. In addition, the mice may manifest slightly reduced disease incidence and improved NK and NKT cell performance, as compared with wild-type NOD mice.