Whole Exome Sequencing to Estimate Alloreactivity Potential between Donors and Recipients in Stem Cell Transplantation

Supplementary Materials

Glossary

Exome- part of the genome comprised of exons, coding for all the RNA

Common positions – chromosomal positions sequenced with high-quality read coverage in both the recipient and donor.

Single nucleotide polymorphisms – variation at a single position in a DNA sequence across individuals.

Synonymous mutation – a single base mutation in an exon of a protein-coding gene which does not alter the amino acid produced for that protein.

Nonsynonymous mutation – a single base mutation in an exon of a protein-coding gene which alters the amino acid produced for that protein.

Nonsynonymous, conservative mutation – a single base mutation in an exon of a protein-coding gene which alters the amino acid produced for that protein. However, the physiochemical properties of the amino acid remain the same; less likely to produce conformational change in the resulting oligopeptide.

Nonsynonymous, nonconservative mutation – a single base mutation in an exon of a protein-coding gene which alters the amino acid produced for that protein. The physiochemical properties of the amino acid are different; more likely to produce conformational change in the resulting oligopeptide.

Stop- a mutation resulting in either gain or loss of a stop codon

Read coverage – the average number of times any given nucleotide position is represented in a sequence read.

Supplementary Table 1. Donor-Recipient characteristics and patient outcomes observed in patients transplanted using ATG+450 cGy TBI.

D-R Pair	D/R age	D/R sex	D/R race	Donor match	Diagnosis§	GVHD	Relapse and Survival Status
2	59/64	M/M	AA/ AA	MRD*	MM	No	R, D
3	32/44	M/F	M/C	URD	NHL	No	A
4	52/46	F/F	AA/ AA	MRD	MM	No	A
5	36/58	M/M	C/C	URD	CLL	Yes [A]	D
7	46/56	M/F	C/C	URD	NHL	No	A
8	40/57	M/M	C/C	URD	PLL	Yes [C]	D
10	24/57	M/F	C/AA	URD	MM	Yes [A, C]	R, D
16	65/62	M/M	C/C	MRD	PLL	No	R, D
23	58/55	F/F	C/C	MRD	NHL	No	A

AA indicates African American; M, Multiracial; C, Caucasian; MRD, matched related donor; URD, unrelated donor; *, 7/8 HLA mismatch (A antigen); MM- Multiple myeloma; NHL- Non-Hodgkin Lymphoma; CLL- Chronic lymphocytic leukemia; PLL- Pro-lymphocytic leukemia; §- Disease status at SCT was complete remission in NHL and CLL patients and very good partial remission in MM; [A], acute GVHD; [C], chronic GVHD; R, relapsed; D, deceased; A, alive; NA, not available.

Supplemenary Table 2. HLA type of the donor-recipient pairs

D-R	HLA-A	HLA-A	HLA-B	HLA-B	HLA-C	HLA-C	DRB1	DRBI
2	02:01*	24:02	15:16	27:05	02:02	17:01	0101	0102
3	03:01	11:01	07:02	55:01	03:03	07:02	14:01/54	1501
4	23:01	30:02	15:03	440301	02:10	07:18	0301	0701
5	01:01	03:01	570101	07:02	07:02	07:01	0401	1501
7	01:01	02:01	44:02	55:01	03:03	05:01	0101	0401
8	01:01	24:02	07:02	55:01	03:03	07:02	1454	1501
10	01:01	03:01	080101	40:01	03:04	07:01	0301	1501
16	01:01	26:01	13:02	27:05	02:02	06:02	0701	1501
23	03:01	240201	07:02	570101	060201	07:02	0701	1501

* Recipient HLA 23:01

Supplementary Table 3. Sample Statistics for the nine D-R pairs sequenced. The numbers in the parentheses denote percentage with respect to the number of HQ reads.

Sample ID	*No. of reads (10^7)*	*Total Bases (MB)*	*No. of HQ reads (10^7)*	*Total Bases in HQ reads (MB)*	*No. of reads Aligned (10^7)*	*No. of reads with duplicates (10^7)*	*No. of unique reads (10^7)*	*Total Bases in unique reads (MB)*	*X Coverage of the Exome*
10D	9.31	9307	7.53	7532	6.94 (92%)	1.65 (22%)	5.29 (78%)	5295	84
10R	11.61	1161	9.30	9297	8.47 (91%)	2.17 (23%)	6.29 (77%)	6291	100
16D	10.93	1093	8.92	8921	8.14 (91%)	1.96 (22%)	6.18 (78%)	6181	98
16R	9.67	9668	7.91	7911	7.19 (91%)	1.62 (21%)	5.57 (79%)	5568	88
23D	10.15	1015	8.38	8379	7.75 (93%)	1.85 (22%)	5.91 (78%)	5907	94
23R	10.97	1097	9.04	9042	8.32 (92%)	2.14 (24%)	6.18 (76%)	6177	98
2D	10.43	1043	8.37	8369	7.67 (92%)	1.93 (23%)	5.74 (77%)	5738	91
2R	10.95	1094	8.82	8822	8.08 (92%)	2.09 (24%)	5.99 (76%)	5988	95
3D	5.82	5819	4.96	4959	4.80 (97%)	2.35 (47%)	2.45 (53%)	2446	39
3R	6.90	6901	5.86	5860	5.67 (97%)	2.92 (50%)	2.75 (50%)	2749	44
4D	15.17	1517	9.53	9535	9.02 (95%)	5.39 (57%)	3.63 (43%)	3631	58
4R	20.13	2013	13.03	13034	12.32	7.93 (61%)	4.39 (39%)	4390	70
5D	5.98	5976	5.01	5010	4.82 (96%)	2.56 (51%)	2.26 (49%)	2261	36
5R	5.32	5323	4.49	4492	4.33 (96%)	2.18 (49%)	2.15 (52%)	2150	34
7D	3.92	3918	3.32	3321	3.20 (96%)	1.43 (43%)	1.77 (57%)	1766	28
7R	4.39	4385	3.72	3717	3.59 (97%)	1.68 (45%)	1.91 (55%)	1914	30
8D	6.40	6401	5.36	5363	5.17 (97%)	2.71 (51%)	2.47 (50%)	2465	39
8R	5.23	5229	4.40	4396	4.24 (97%)	2.12 (48%)	2.12 (52%)	2118	34

Supplementary table 4. Median normalized SNP/Kbp for whole exome and for the 6p22.1-21.2 (MHC) locus in the graft versus host, host versus graft and direction independent SNPs. The Mann-Whitney U test was used to compare the degree of donor-recipient sequence difference between HLA matched related and unrelated D-R pairs.

	Exome			**MHC locus**		
	MRD	**URD**	***p*-value**	**MRD**	**URD**	***p*-value**
Graft versus Host Direction						
Synonymous	0.065	0.16	0.016	0.03	0.11	0.016
Nonsynonymous	0.06	0.15	0.016	0.07	0.17	0.063
Conservative	0.02	0.05	0.016	0.02	0.05	0.032
Nonconservative	0.04	0.1	0.016	0.05	0.11	0.063
Host versus Graft Direction						
Synonymous	0.065	0.16	0.016	0.04	0.1	0.032
Nonsynonymous	0.065	0.15	0.016	0.04	0.15	0.032
Conservative	0.02	0.05	0.016	0.015	0.04	0.111
Nonconservative	0.045	0.1	0.016	0.025	0.09	0.032
Direction Independent						
Synonymous	0.115	0.19	0.016	0.04	0.13	0.032
Nonsynonymous	0.11	0.18	0.016	0.06	0.15	0.063
Conservative	0.035	0.06	0.016	0.025	0.05	0.063
Nonconservative	0.07	0.12	0.016	0.04	0.1	0.063

Matched related and unrelated donor SNP/Kbp distributions compared using the Mann-Whitney U Test.

MRD, matched related donor; URD, unrelated donor

Supplementary table 5. Total SNP counts / functional polymorphism for each D-R pair over the entire exome.

Graft vs. Host

	Raw Counts						Normalized Counts				
Pair	**Syn**	**Nonsyn**	**Cons**	**Noncons**	**Stop**	**Common Positions**	**Syn**	**Nonsyn**	**Cons**	**Noncons**	**Stop**
5R-5D	5497	5036	1737	3299	62	32992114	0.17	0.15	0.05	0.10	0.00
8R-8D	5497	5013	1754	3259	56	33461071	0.16	0.15	0.05	0.10	0.00
10R-10D	8218	7434	2534	4900	87	56634149	0.15	0.13	0.04	0.09	0.00
3R-3D	5575	5166	1775	3391	66	37333820	0.15	0.14	0.05	0.09	0.00
7R-7D	5485	5184	1779	3405	62	27267979	0.20	0.19	0.07	0.12	0.00
2R-2D	3989	3838	1370	2468	40	57112850	0.07	0.07	0.02	0.04	0.00
4R-4D	4099	3801	1338	2463	60	39689979	0.10	0.10	0.03	0.06	0.00
16R-16D	3111	2845	956	1889	32	57757544	0.05	0.05	0.02	0.03	0.00
23R-23D	3405	3151	1094	2057	38	57699413	0.06	0.05	0.02	0.04	0.00

Host vs. Graft

	Raw Counts						Normalized Counts				
Pair	**Syn**	**Nonsyn**	**Cons**	**Noncons**	**Stop**	**Common Positions**	**Syn**	**Nonsyn**	**Cons**	**Noncons**	**Stop**
5R-5D	5440	4935	1674	3261	61	32992114	0.16	0.15	0.05	0.10	0.00
8R-8D	5554	5126	1784	3342	57	33461071	0.17	0.15	0.05	0.10	0.00
10R-10D	6137	5676	1899	3777	65	56634149	0.11	0.10	0.03	0.07	0.00
3R-3D	5608	5264	1832	3432	52	37333820	0.15	0.14	0.05	0.09	0.00
7R-7D	5408	4888	1694	3194	48	27267979	0.20	0.18	0.06	0.12	0.00
2R-2D	4224	3905	1301	2604	51	57112850	0.07	0.07	0.02	0.05	0.00
4R-4D	3845	3540	1216	2324	49	39689979	0.10	0.09	0.03	0.06	0.00
16R-16D	3065	3003	1030	1973	49	57757544	0.05	0.05	0.02	0.03	0.00
23R-23D	3393	3187	1101	2086	55	57699413	0.06	0.06	0.02	0.04	0.00

Direction Independent

	Raw Counts						Normalized Counts				
Pair	**Syn**	**Nonsyn**	**Cons**	**Noncons**	**Stop**	**Common Positions**	**Syn**	**Nonsyn**	**Cons**	**Noncons**	**Stop**
5R-5D	6121	6024	2095	3929	82	32992114	0.19	0.18	0.06	0.12	0.00
8R-8D	6125	6087	2145	3942	75	33461071	0.18	0.18	0.06	0.12	0.00
10R-10D	11789	10972	3764	7208	136	56634149	0.21	0.19	0.07	0.13	0.00
3R-3D	6772	6786	2365	4421	82	37333820	0.18	0.18	0.06	0.12	0.00
7R-7D	5151	5297	1827	3470	67	27267979	0.19	0.19	0.07	0.13	0.00
2R-2D	7191	6868	2406	4462	80	57112850	0.13	0.12	0.04	0.08	0.00
4R-4D	5099	5129	1822	3307	74	39689979	0.13	0.13	0.05	0.08	0.00
16R-16D	5465	5179	1771	3408	76	57757544	0.09	0.09	0.03	0.06	0.00
23R-23D	6019	5668	1993	3675	79	57699413	0.10	0.10	0.03	0.06	0.00

Syn – synonymous SNPs; Nonsyn – nonsynonymous SNPs; Cons – nonsynonymous, conservative SNPs; Noncons – nonsynonymous, nonconservative SNPs; Stop – (stop gain/loss) SNPs.
MUD SCT- 3, 5, 7, 8, 10. Gender mismatched donor- 3, 7, 10. African American recipients-2, 4 and 10. HLA-A mismatched donor-2

Supplementary table 6. Total SNP counts / functional polymorphism for each D-R pair for the 6p22.1-21.2 (MHC) locus.

Graft vs. Host

	Raw Counts						Normalized Counts				
Pair	Syn	Nonsyn	Cons	Noncons	Stop	Common Positions	Syn	Nonsyn	Cons	Noncons	Stop
5R-5D	41	62	19	43	0	369261	0.11	0.17	0.05	0.12	0.00
8R-8D	64	62	23	39	1	369450	0.17	0.17	0.06	0.11	0.00
10R-10D	38	67	25	42	1	564287	0.07	0.12	0.04	0.07	0.00
3R-3D	28	26	8	18	0	397080	0.07	0.07	0.02	0.05	0.00
7R-7D	72	95	32	63	1	305280	0.24	0.31	0.10	0.21	0.00
2R-2D	22	48	13	35	0	563415	0.04	0.09	0.02	0.06	0.00
4R-4D	17	34	7	27	0	396481	0.04	0.09	0.02	0.07	0.00
16R-16D	9	30	9	21	1	573016	0.02	0.05	0.02	0.04	0.00
23R-23D	13	20	6	14	0	573415	0.02	0.03	0.01	0.02	0.00

Host vs. Graft

	Raw Counts						Normalized Counts				
Pair	Syn	Nonsyn	Cons	Noncons	Stop	Common Positions	Syn	Nonsyn	Cons	Noncons	Stop
5R-5D	37	56	16	40	0	369261	0.10	0.15	0.04	0.11	0.00
8R-8D	34	54	19	35	0	369450	0.09	0.15	0.05	0.09	0.00
10R-10D	30	33	8	25	0	564287	0.05	0.06	0.01	0.04	0.00
3R-3D	50	45	17	28	0	397080	0.13	0.11	0.04	0.07	0.00
7R-7D	55	96	26	70	1	305280	0.18	0.31	0.09	0.23	0.00
2R-2D	46	49	15	34	1	563415	0.08	0.09	0.03	0.06	0.00
4R-4D	18	17	5	12	0	396481	0.05	0.04	0.01	0.03	0.00
16R-16D	16	25	11	14	0	573016	0.03	0.04	0.02	0.02	0.00
23R-23D	11	15	4	11	0	573415	0.02	0.03	0.01	0.02	0.00

Direction Independent

	Raw Counts						Normalized Counts				
Pair	Syn	Nonsyn	Cons	Noncons	Stop	Common Positions	Syn	Nonsyn	Cons	Noncons	Stop
5R-5D	48	80	26	54	0	369261	0.13	0.22	0.07	0.15	0.00
8R-8D	28	31	12	19	0	369450	0.08	0.08	0.03	0.05	0.00
10R-10D	60	86	29	57	0	564287	0.11	0.15	0.05	0.10	0.00
3R-3D	58	54	18	36	0	397080	0.15	0.14	0.05	0.09	0.00
7R-7D	82	111	36	75	1	305280	0.27	0.36	0.12	0.25	0.00
2R-2D	61	83	26	57	1	563415	0.11	0.15	0.05	0.10	0.00
4R-4D	15	24	7	17	0	396481	0.04	0.06	0.02	0.04	0.00
16R-16D	20	36	15	21	0	573016	0.03	0.06	0.03	0.04	0.00
23R-23D	21	25	7	18	0	573415	0.04	0.04	0.01	0.03	0.00

Syn – synonymous SNPs; Nonsyn – nonsynonymous SNPs; Cons – nonsynonymous, conservative SNPs; Noncons – nonsynonymous, nonconservative SNPs; Stop – (stop gain/loss) SNPs.

Figure S1. WES to quantify SNPs between SCT D-R pairs. Whole exome demonstrates extensive variation between donors and recipients independent of HLA matching. **Actual D-R pairs (green bars and red *) depict exome variation between recipient and their actual HLA-matched donors, while simulated pairs (blue bars) consist of the recipients from each pair compared with a donor from other D-R pairs. Y axis depicts SNP in the pairs. X-axis depicts the actual and simulated pairs.** D-R pairs 3, 5, 7, 8 and 10 underwent MUD SCT (solid line), and 2, 4, 16 and 23 MRD SCT (dashed line).

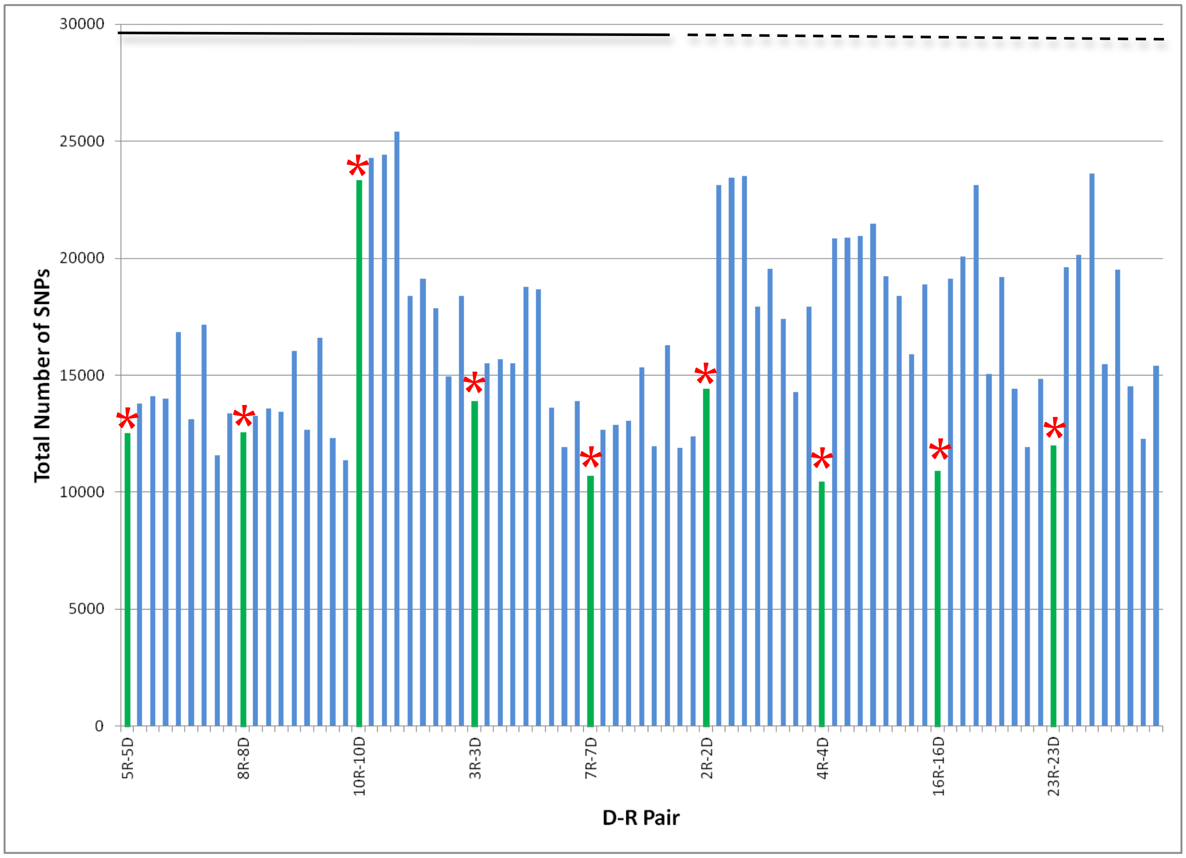
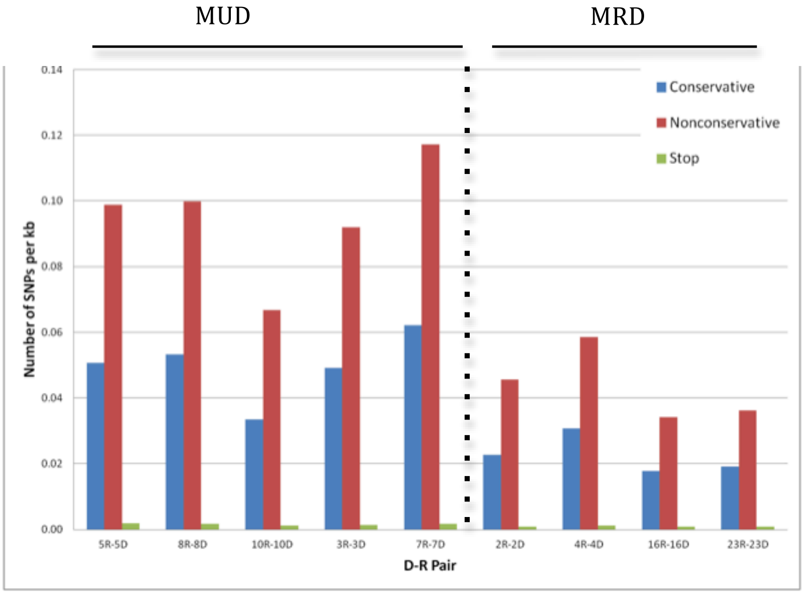
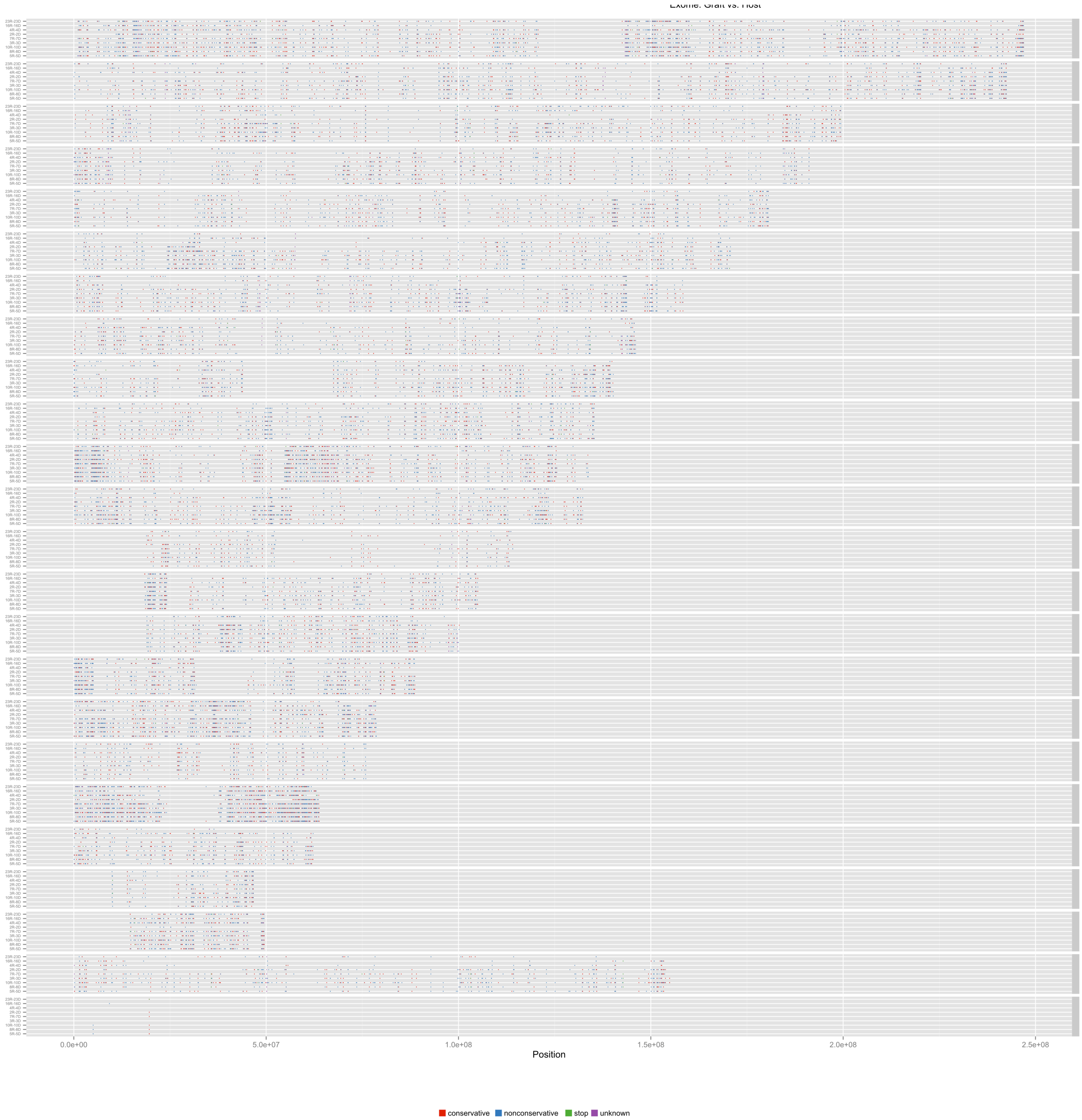


Figure S2. (A) Alloreactivity potential vectors depicting conservative vs. nonconservative, nonsynonymous nucleotide variation in D-R pairs across the whole exome in the host versus graft direction, variants absent in the recipient and present in the donor. (B) Nonsynonymous SNPs in the graft versus host direction mapped on to individual chromosomes in all the D-R pairs, demonstrate the genomic location of polymorphisms along the length of the chromosome in each D-R pair. All nine D-R pairs depicted on the y-axis for each chromosome; SNP coordinates (location) along the length of each chromosome depicted on the x-axis. (C) Nonsynonymous SNPs in the host versus graft direction mapped onto each chromosomes (data presented in Figure 1D)

A.



B.


Exome: Graft vs. Host
23R-23D
16R-16D
4R-4D
2R-2D
7R-7D
3R-3D
10R-10D
8R-8D
5R-5D
Donor-Recipient Pairs
0.0e+00
5.0e+07
1.0e+08
1.5e+08
2.0e+08
2.5e+08
Position
🟥 conservative
🟦 nonconservative
🟩 stop
🟪 unknown

C.


Exome: Host vs. Graft
23R-23D
16R-16D
4R-4D
2R-2D
7R-7D
3R-3D
10R-10D
8R-8D
5R-5D
0.0e+00
5.0e+07
1.0e+08
1.5e+08
2.0e+08
2.5e+08
Position
conservative
nonconservative
stop
unknown