Report for IGHV

23 August, 2017

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Table 1: Overview of the *Brezinschek1995_2nd* primer set.

ID	Sequence	Coverage	Mismatches	Position	T_m[°C]
B1995|VH1-FR|1_	ggtgcagctggt*r*cagtctggggctg	53.9%	[2,3]	+1 to +26 (100%)	70.65
B1995|VH2-FR|2_	cag*r*tcaccttga*r*ggagtctggtcc	13.8%	[0,0]	-1 to +24 (100%)	64.46
B1995|VH3-FR|3_	gagt*k*cagctggtggagtctggggg	53.9%	[2,4]	0 to +24 (100%)	67.99
B1995|VH4-FR|4_	ggtgcagctgcaggagt*s*gggc*s*cagg	62.5%	[0,5]	+1 to +27 (100%)	74.25
B1995|VH5-FR|5_	agctggtgcagtctggagcagagg	19.7%	[1.25,2]	+6 to +29 (100%)	65.16
B1995|VH6-FR|6_	tacagctgcagcagtcaggtccaggac	31.6%	[4,4.25]	+3 to +29 (100%)	65.85

Table 2: Overview of the *Glas1999* primer set.

ID	Sequence	Coverage	Mismatches	Position	T_m[°C]
G1999|IGHV4-59*01|1_	tccatcagtagttactactgg	30.3%	[2,6]	+80 to +100 (52%)	48.75
G1999|IGHV4-61*02|3	tccatcagcagtggtagttac	21.1%	[1,5]	+80 to +100 (100%)	52.64
G1999|IGHV4-61*01|4	tccgtcagcagtggtagttac	21.1%	[2,6]	+80 to +100 (100%)	55.36
G1999|IGHV3-30-3*01|5	gcagttatatcatatgatggaagc	5.3%	[1,3]	+143 to +166 (100%)	49.63
G1999|IGHV3-11*01|6	agtgactactacatgagctgg	36.8%	[4,6]	+86 to +106 (73%)	52.12
G1999|IGHV3-11*01|7	agtggtagtaccatatactac	11.8%	[1.5,4]	+158 to +178 (61%)	45.47
G1999|IGHV4-55*01|8	gaaatccatcatagtgggagc	31.6%	[2,5]	+152 to +172 (46%)	51.71
G1999|IGHV4-34*01|9	gaaatcaatcatagtggaagc	10.5%	[0,2]	+146 to +166 (56%)	47.07
G1999|IGHV4-39*01|1	agtatctattatagtgggagc	25%	[3,4]	+152 to +172 (58%)	45.86
G1999|IGHV4-59*01|11	tatatctattacagtgggagc	25%	[1,3]	+152 to +172 (58%)	46.12
G1999|IGHV4-61*02|1	cgtatctataccagtgggagc	25%	[4,6]	+152 to +172 (58%)	53.20
Gl1999|IGHV|14_	tcagctattggtgctggtggt	8.6%	[3,6]	+143 to +163 (100%)	57.78
G1999|IGHV3-47*	tcagctattggtactggtggt	8.6%	[4,5]	+143 to +163 (100%)	54.11
G1999|IGHV4-30-2*01|1	tacatctatcatagtgggagc	30.9%	[2,4]	+152 to +172 (47%)	48.95
G1999|IGHV4-4*0	gaaatctatcatagtgggagc	30.9%	[2,4]	+152 to +172 (47%)	48.37
G1999|IGHV4-31*01|1	tacatctattacagtgggagc	25%	[1,4]	+152 to +172 (58%)	49.39
G1999|IGHV3-53*	agtagcaactacatgagctgg	41.4%	[4,7]	+86 to +106 (67%)	52.93
G1999|IGHV3-23*01|21	tcagctattagtggtagtggt	7.9%	[0,4]	+143 to +163 (100%)	50.70
G1999|IGHV3-15*	cgtattaaaagcaaaactgat	2%	[0,0]	+146 to +166 (100%)	44.05
G1999|IGHV4-28*01|2	tacatctattatagtgggagc	25%	[1,3]	+152 to +172 (58%)	46.12
G1999|IGHV3-23*01|24	agcagctatgccatgagctgg	33.6%	[4,6]	+86 to +106 (98%)	59.33
G1999|IGHV3-30*02|25	agtagctatggcatgcactgg	29.6%	[2,6]	+86 to +106 (100%)	56.18
G1999|IGHV3-23*01|26	gtgaagggccggttcaccatc	53.3%	[1,5]	+188 to +208 (51%)	60.56
G1999|IGHV3-30-3*01|2	ggaagcaataaatactacgca	15.8%	[5.75,6]	+161 to +181 (71%)	48.40
G1999|IGHV3-30*02|28	ggaagtaataaatactatgca	9.2%	[0,5.75]	+161 to +181 (100%)	42.03
G1999|IGHV3-23*01|29	ggtggtagcacatactacgca	31.6%	[1,5]	+161 to +181 (85%)	55.72
G1999|IGHV3-23*01|31	tacgcagactccgtgaagggc	30.9%	[0,2.5]	+176 to +196 (72%)	61.31
G1999|IGHV3-30*01|3	ggaagtaataaatactacgca	21.7%	[1,5]	+161 to +181 (79%)	44.81
G1999|IGHV3-23*01|34	agctgttggagtctgggggag	77%	[1,6]	+6 to +26 (100%)	60.87
G1999|IGHV3-33*	gcagttatatggtatgatgga	5.9%	[2,2]	+143 to +163 (100%)	47.21
G1999|IGHV3-74*	agtagctactggatgcactgg	22.4%	[3,5]	+86 to +106 (100%)	55.31
G1999|IGHV3-13*	agtagctacgacatgcactgg	27.6%	[3,4.75]	+86 to +106 (100%)	55.71

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ID	Sequence	Coverage	Mismatches	Position	T_m[°C]
G1999|IGHV4-30-2*01|3	ggtggttactcctggagctgg	39.5%	[2,6]	+86 to +106 (50%)	59.35
G1999|IGHV3-20*	gatgattatggcatgagctgg	35.5%	[7,7]	-58 to -38 (50%)	52.30
G1999|IGHV3-30*01|4	gtccgccaggctccaggcaag	76.3%	[1,5]	+107 to +127 (58%)	66.05
G1999|IGHV4-31*01|4	agtggtggttactactggagc	25%	[1,4]	+89 to +109 (58%)	55.21
G1999|IGHV4-39*01|42	agtagtagttactactggggc	23%	[2.5,5]	+83 to +103 (43%)	51.36
G1999|IGHV4-28*01|4	agcagtagtaactggtggggc	12.5%	[2,4.5]	+86 to +106 (53%)	58.43
G1999|IGHV4-55*01|4	agcagtggtaactggtgaatc	6.6%	[4,4]	+86 to +106 (100%)	53.40
G1999|IGHV4-30-4	agtggtgattactactggagt	21.1%	[2,6]	+89 to +109 (56%)	50.65
G1999|IGHV4-34*01|4	agtggttactactggagctgg	32.2%	[1,3]	+86 to +106 (53%)	54.98
G1999|IGHV4-39*01|47	aagccttcggagaccctgtcc	32.2%	[0,2]	+35 to +55 (100%)	61.05
G1999|IGHV4-61*01|5	ggtagttactactggagctgg	32.2%	[1,3]	+86 to +106 (53%)	53.06

Table 3: Overview of the *Ippolito2012* primer set.

ID	Sequence	Coverage	Mismatches	Position	T_m[°C]
Ippl2012|VH1|1_	caggtccagct*k*gt*r*cagtctgg	63.8%	[2,4]	-1 to +21 (100%)	64.48
Ip2012|VH157|2_	caggtgcagctggtg*sa*rtctgg	94.7%	[1,3]	-1 to +21 (100%)	65.08
Ippl2012|VH2|3_	cag*r*tcaccttgaaggagtctg	21.7%	[0,7]	-1 to +20 (100%)	56.46
Ippl2012|VH3|4_	gaggtgcagctg*k*tggag*wcy*	84.9%	[0,4]	-1 to +19 (99%)	62.66
Ippl2012|VH4|5_	caggtgcagctgcaggagtc*sg*	81.6%	[1,4]	-1 to +20 (100%)	65.31
I2012|VH4-DP63|	caggtgcagctacagcagtggg	75%	[3,7]	-1 to +20 (99%)	62.68
Ippl2012|VH6|7_	caggtacagctgcagcagtca	47.4%	[3,4]	-1 to +19 (100%)	59.01
Ipp2012|VH3N|8_	tcaacacaacggttcccagtta	0%			55.11

Table 4: Overview of the *Kueppers1993* primer set.

ID	Sequence	Coverage	Mismatches	Position	T_m[°C]
Kppr1993|VH1|1_	ctcagtgaaggt*y*tcctgcaaggc	16.4%	[0,1]	+46 to +69 (100%)	61.49
Kppr1993|VH2|2_	gtcctgcgctggtgaaa*s*ccacac	13.8%	[0,1]	+21 to +44 (100%)	65.10
Kppr1993|VH3|3_	gggtccctgagactctcctgtgcag	34.2%	[0,1]	+44 to +68 (100%)	64.86
Kppr1993|VH4|4_	cctgtccctcacctgc*r*ctgtc	32.2%	[0,0]	+49 to +70 (100%)	64.88
Kppr1993|VH5|5_	aaaaagcccggggagtctctga*r*ga	3.3%	[0,0]	+32 to +56 (100%)	63.46
Kppr1993|VH6|6_	ctgtgccatctccggggacagtg	2.6%	[0,7]	+199 to +221 (50%)	64.29

Table 5: Overview of the *Lim2010* primer set.

ID	Sequence	Coverage	Mismatches	Position	T_m[°C]
Lm2010|VH1|1_fw	caggtccagct*k*gt*r*cagtctgg	63.8%	[2,4]	-1 to +21 (100%)	64.48
Lm2010|VH157|2_	caggtgcagctggtg*sa*rtctgg	94.7%	[1,3]	-1 to +21 (100%)	65.08
Lm2010|VH2|3_fw	cag*r*tcaccttgaaggagtctg	21.7%	[0,7]	-1 to +20 (100%)	56.46
Lm2010|VH3|4_fw	gaggtgcagctg*k*tggag*wcy*	84.9%	[0,4]	-1 to +19 (99%)	62.66
Lm2010|VH4|5_fw	caggtgcagctgcaggagtc*sg*	81.6%	[1,4]	-1 to +20 (100%)	65.31
L2010|VH4-DP63|	caggtgcagctacagcagtggg	75%	[3,7]	-1 to +20 (99%)	62.68
Lm2010|VH6|7_fw	caggtacagctgcagcagtca	47.4%	[3,4]	-1 to +19 (100%)	59.01
Lm2010|VH3N|8_f	tcaacacaacggttcccagtta	0%			55.11

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Table 6: Overview of the *Marks1991* primer set.

ID	Sequence	Coverage	Mismatches	Position	T_m[°C]
M1991|IGHV1-69*	caggtgcagctggtgcagtctgg	84.9%	[1,4]	-1 to +21 (100%)	65.08
M1991|IGHV2-70*	caggtcaacttaagggagtctgg	21.7%	[3,7]	-1 to +21 (100%)	56.18
M1991|IGHV3-35*	gaggtgcagctggtggagtctgg	83.6%	[1,4]	-1 to +21 (100%)	64.26
M1991|IGHV4-*01	caggtgcagctgcaggagtcggg	76.3%	[0.75,4]	-1 to +21 (100%)	67.25
M1991|IGHV5-51*	gaggtgcagctgttgcagtctgc	76.3%	[3,6]	-1 to +21 (100%)	63.03
M1991|IGHV6-1*0	caggtacagctgcagcagtcagg	36.2%	[3,4]	-1 to +21 (100%)	62.01

Table 7: Overview of the *Murugan2015_1st* primer set.

ID	Sequence	Coverage	Mismatches	Position	T_m[°C]
M2015|5'L-VH1|1	acaggtgcccactcccaggtgcag	76.3%	[5,6]	-16 to +7 (100%)	68.06
M2015|5'L-VH3|2	aaggtgtccagtgtga*r*gtgcag	73.7%	[0,7]	-15 to +7 (100%)	60.96
M2015|5'L-VH4/_	cccagatgggtcctgtcccaggtgcag	38.8%	[0,1]	-19 to +7 (100%)	69.00
M2015|5'L-VH5|4	caaggagtctgttccgaggtgcag	30.3%	[7,7]	-16 to +7 (100%)	61.38
M2015|VH3_L-A|_	taaaaggtgtccagtgt	37.5%	[0,1]	-18 to -2 (98%)	44.93
M2015|IGHV1,7-X	atggactggacctggag	30.3%	[0,6]	-58 to -42 (54%)	52.07
M2015|IGHV1-X1_	tcctctttgtggtggcagcagc	42.8%	[3,5]	-39 to -18 (100%)	62.07
M2015|IGHV2-X1-	tccacgctcctgct*r*ctgac	30.3%	[0,6]	-43 to -24 (100%)	62.25
M2015|RM-IGHV4-	atgaaacacctgtggttcttcc	30.9%	[0,0]	-58 to -37 (100%)	53.88

Table 8: Overview of the *openPrimeR2017* primer set.

ID	Sequence	Coverage	Mismatches	Position	T_m[°C]
O5_1_IGHV|122|7	cacctgtggttcttcctcctcc	30.9%	[0,1]	-52 to -31 (100%)	59.05
O5_2_IGHV|104|7	cacctgtggttcttcctcctgc	30.9%	[0,1]	-52 to -31 (100%)	59.73
O5_3_IGHV|100|1	atggagtttgggctgagctgg	34.2%	[0,1]	-58 to -38 (100%)	59.28
O5_4_IGHV|50|1:	atggagttggggctgagctg	34.2%	[1,1]	-58 to -39 (100%)	59.30
O5_5_IGHV|87|2:	tggagttttggctgagctggg	34.2%	[1,2]	-57 to -37 (100%)	59.43
O5_6_IGHV|27|9:	actttgctccacgctcctgc	29.6%	[1,7]	-50 to -31 (100%)	60.07
O5_7_IGHV|1|1:2	atggactggacctggagcatc	16.4%	[1,2]	-58 to -38 (100%)	58.30
O5_8_IGHV|18|1:	atggactggacctggaggttcc	16.4%	[1,2]	-58 to -37 (100%)	60.38
O5_9_IGHV|23|1:	atggactgcacctggaggatc	15.8%	[1,2]	-58 to -38 (100%)	58.30
O5_10_IGHV|13|1	atggactggacctggagggtcttc	16.4%	[2,3]	-58 to -35 (100%)	61.95
O5_11_IGHV|154|	tctgtctccttcctcatcttcctgc	1.3%	[0,0]	-55 to -31 (50%)	59.26
O5_12_IGHV|17|3	ggactggatttggagggtcctcttc	16.4%	[3,4]	-56 to -32 (100%)	60.55
O5_13IGHV|63|12	gctccgctgggttttccttg	29.6%	[2,3]	-47 to -28 (100%)	58.65
O5_14IGHV|152|2	tggggtcaaccgccatcc	2.6%	[0,0]	-57 to -40 (100%)	60.17
O5_15IGHV|151|1	ggcctctccacttaaacccagg	0.7%	[0,0]	-50 to -29 (100%)	59.41
O5_16IGHV|26|2:	tggacacactttgctacacactcc	2%	[1,2]	-57 to -34 (100%)	58.28

Table 9: Overview of the *Scheid2011* primer set.

ID	Sequence	Coverage	Mismatches	Position	T_m[°C]
S2011|VH1_LEADER-A	atggactggacctggaggat	15.8%	[0,1]	-58 to -39 (100%)	56.53
S2011|VH1_LEADER-B	atggactggacctggagcat	15.1%	[1,2]	-58 to -39 (100%)	57.32
S2011|VH1_LEADER-C	atggactggacctggagaat	15.1%	[1,2]	-58 to -39 (100%)	53.95

ID	Sequence	Coverage	Mismatches	Position	T_m[°C]
S2011|VH1_LEADER-D	ggttcctctttgtggtggc	10.5%	[1.5,3]	-42 to -24 (100%)	55.49
S2011|VH1_LEADER-E	atggactggacctggagggt	15.8%	[1,2]	-58 to -39 (100%)	59.59
S2011|VH1_LEADER-F	atggactggatttggaggat	13.2%	[2,3]	-58 to -39 (100%)	51.53
S2011|VH1_LEADER-G	aggttcctctttgtggtggcag	41.4%	[4,6]	-43 to -22 (100%)	58.89
S2011|VH3_LEADER-A	taaaaggtgtccagtgt	32.2%	[0,1]	-18 to -2 (100%)	44.93
S2011|VH3_LEADER-B	taagaggtgtccagtgt	32.2%	[1,2]	-18 to -2 (100%)	47.17
S2011|VH3_LEADER-C	tagaaggtgtccagtgt	32.2%	[1,2]	-18 to -2 (100%)	47.17
S2011|VH3_LEADER-E	tacaaggtgtccagtgt	32.2%	[1,2]	-18 to -2 (100%)	47.80
S2011|VH3_LEADER-F	ttaaagctgtccagtgt	32.2%	[2,3]	-18 to -2 (100%)	45.33
S2011|VH4_LEADER-D	atgaaacatctgtggttctt	30.3%	[1,1]	-58 to -39 (100%)	46.97
S2011|VH5_LEADER-A	ttctccaaggagtctgt	2.6%	[0,0]	-21 to -5 (100%)	47.17
S2011|VH3_LEADER-D	gctatttttaaaggtgtccagtgt	32.2%	[1,3]	-25 to -2 (100%)	51.64
S2011|VH4_LEADER-A	atgaaacacctgtggttcttcc	30.9%	[0,0]	-58 to -37 (100%)	53.88
S2011|VH4_LEADER-B	atgaaacacctgtggttctt	30.9%	[0,0]	-58 to -39 (100%)	49.89
S2011|VH4_LEADER-C	atgaagcacctgtggttctt	30.9%	[1,1]	-58 to -39 (100%)	52.68
S2011|VH5_LEADER-B	cctccacagtgagagtctg	0.7%	[0,0]	-24 to -6 (100%)	53.44
S2011|VH6_LEADE	atgtctgtctccttcctcatc	1.3%	[0,0]	-58 to -38 (50%)	52.19
S2011|VH7_LEADE	ggcagcagcaacaggtgccca	15.1%	[2,2]	-26 to -6 (100%)	65.90

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Primer Set Summary

Table 10: Summary of the primer sets. Brackets indicate inter-quartile ranges. (continued below)

Primers	Template Coverage	Nbr Primers	Binding Range fw	Nbr of mismatches fw
Brezinschek1995_2nd	152 of 152 (100%)	6	[+1,+26]	[2,4]
Glas1999	152 of 152 (100%)	43	[+86,+161]	[1,5]
Ippolito2012	152 of 152 (100%)	8	[-1,+20]	[1,4]
Kueppers1993	152 of 152 (100%)	6	[+44,+69]	[0,1]
Lim2010	152 of 152 (100%)	8	[-1,+20]	[1,4]
Marks1991	152 of 152 (100%)	6	[-1,+21]	[2,4]
Murugan2015_1st	152 of 152 (100%)	9	[-24,+7]	[0,6]
openPrimeR2017	152 of 152 (100%)	16	[-57,-35]	[1,2]
Scheid2011	128 of 152 (84.21%)	21	[-43,-18]	[1,2]

Table 11: Table continues below

coverage model	Tm range	Self dimers	Structures	Coverage	Specificity
[0,0]	[65.33,69.98]	[-3.65,-2.4]	[-0.42,0]	[34.5,82]	[0,0]
[0,0.02]	[48.58,55.72]	[-3.19,-1.15]	[-1.53,-0.08]	[21.5,48.5]	[0,0]
[0,0.01]	[58.37,64.63]	[-6.43,-4.37]	[0,0]	[62.25,125.25]	[0,0]
[0,0]	[63.67,64.88]	[-3.34,-1.45]	[-1.09,-0.02]	[9,43]	[0,0]
[0,0.01]	[58.37,64.63]	[-6.43,-4.37]	[0,0]	[62.25,125.25]	[0,0]
[0,0.04]	[62.27,64.88]	[-5.45,-3.73]	[-1.91,0]	[70.25,124.25]	[0,0]
[0,0.01]	[53.88,62.25]	[-1.51,-0.69]	[-0.34,0]	[46,65]	[0,1]
[0,0]	[58.95,60.09]	[-2.11,-0.61]	[-0.46,0]	[19,47]	[1,1]
[0,0.01]	[47.17,55.49]	[-2.51,-0.8]	[-0.18,0]	[23,49]	[1,1]

Tm deviation	Cross dimers	Length	GC clamp	GC ratio	Runs	Repeats
[6.8,9.62]	[-4.94,-3.64]	[25.25,26.75]	[2,2.75]	[0.6,0.67]	[2.25,4]	[1.25,2]
[13.68,18.91]	[-6.62,-4.74]	[21,21]	[1,2]	[0.4,0.52]	[2,3]	[1,2]
[7.57,10.03]	[-6.66,-5.02]	[21.75,22.25]	[0.75,2.25]	[0.56,0.63]	[2,2.25]	[1,2]
[2.94,3.55]	[-5.1,-3.82]	[23.25,24.75]	[1,1.75]	[0.58,0.65]	[3,3.75]	[2,2]
[7.57,10.03]	[-6.66,-5.02]	[21.75,22.25]	[0.75,2.25]	[0.56,0.63]	[2,2.25]	[1,2]
[7.16,10.53]	[-6.31,-5.84]	[23,23]	[2,2]	[0.61,0.65]	[2,2.75]	[1,1]
[16.45,23.13]	[-5.71,-5.38]	[20,24]	[2,2]	[0.54,0.62]	[2,3]	[1,2]
[2.26,3.39]	[-3.78,-2.85]	[20.75,22.5]	[2,2]	[0.57,0.59]	[2,3.25]	[1,2]
[12.46,18.73]	[-6.73,-5.49]	[17,20]	[0,0]	[0.45,0.55]	[2,3]	[2,2]

Coverage

Table 13: Percentage of covered template sequences per primer set and group of template sequences.

Run	Total	IGHV1	IGHV2	IGHV3	IGHV4	IGHV5	IGHV6	IGHV7
Brezinschek1995_2nd	100%	100%	100%	100%	100%	100%	100%	100%
Glas1999	100%	100%	100%	100%	100%	100%	100%	100%
Ippolito2012	100%	100%	100%	100%	100%	100%	100%	100%
Kueppers1993	100%	100%	100%	100%	100%	100%	100%	100%
Lim2010	100%	100%	100%	100%	100%	100%	100%	100%
Marks1991	100%	100%	100%	100%	100%	100%	100%	100%
Murugan2015_1st	100%	100%	100%	100%	100%	100%	100%	100%
openPrimeR2017	100%	100%	100%	100%	100%	100%	100%	100%
Scheid2011	84.2%	100%	0%	94.2%	100%	100%	100%	100%

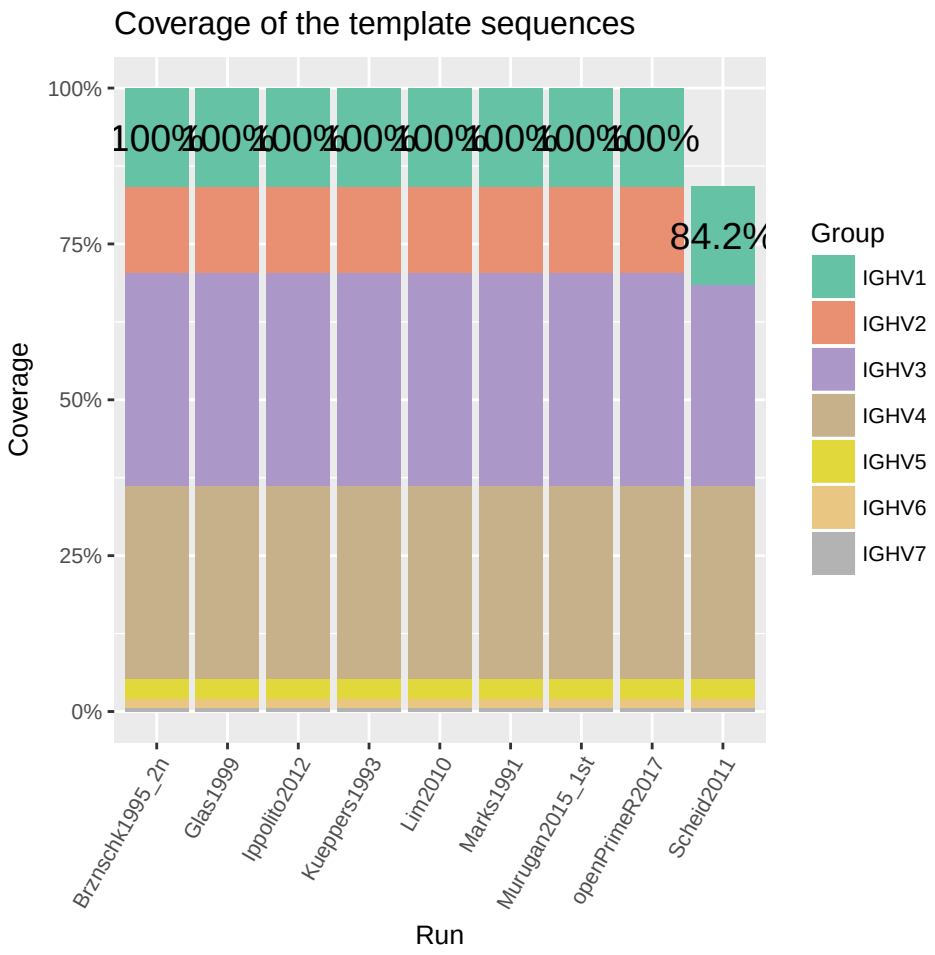

Figure 1: Coverage of each primer set.

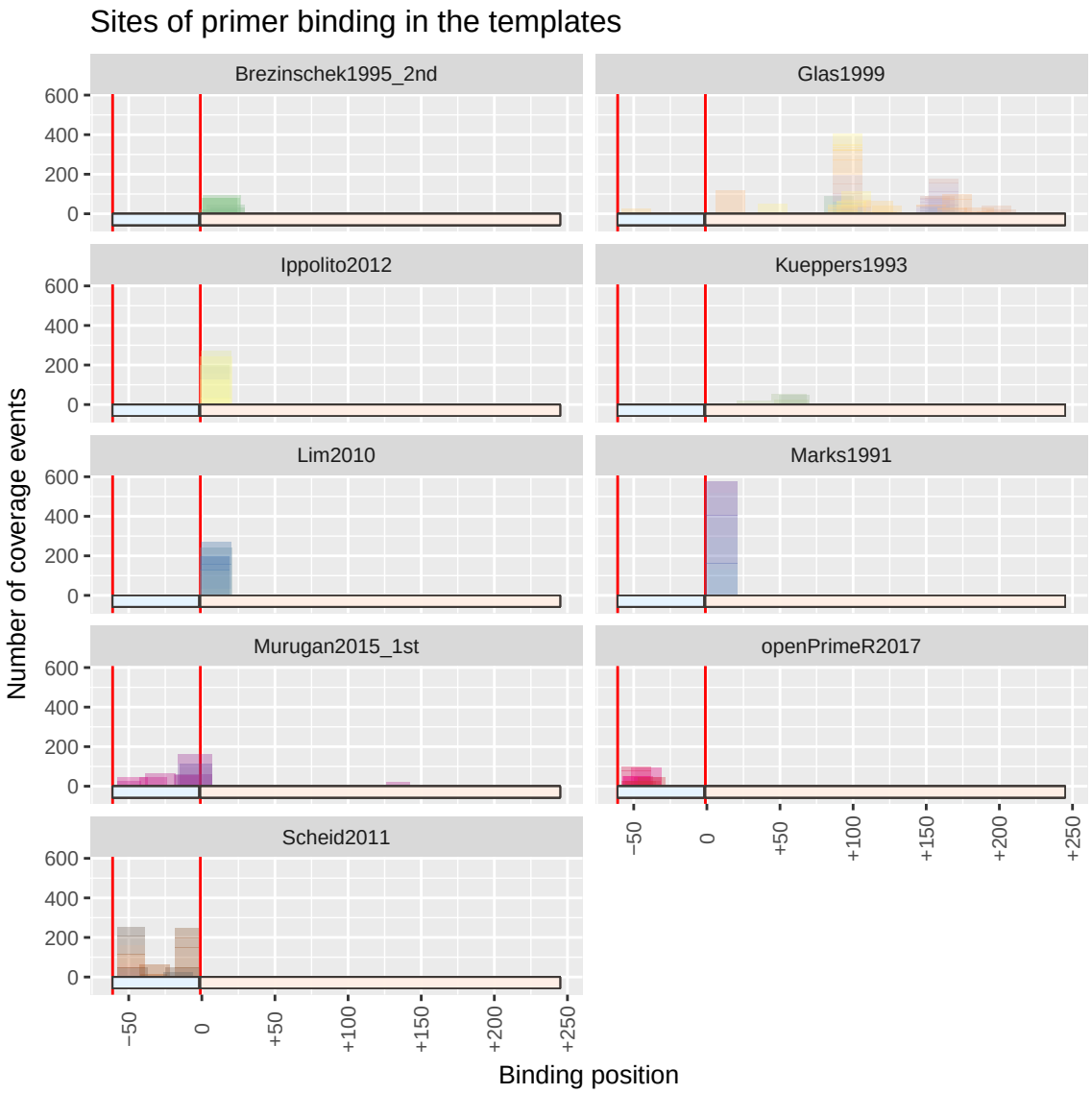

Figure 2: The binding position of the primers relative to the target binding region of forward primers. The rectangles stretching along the x-axis indicate the target binding region of forward primers and the following region, respectively.

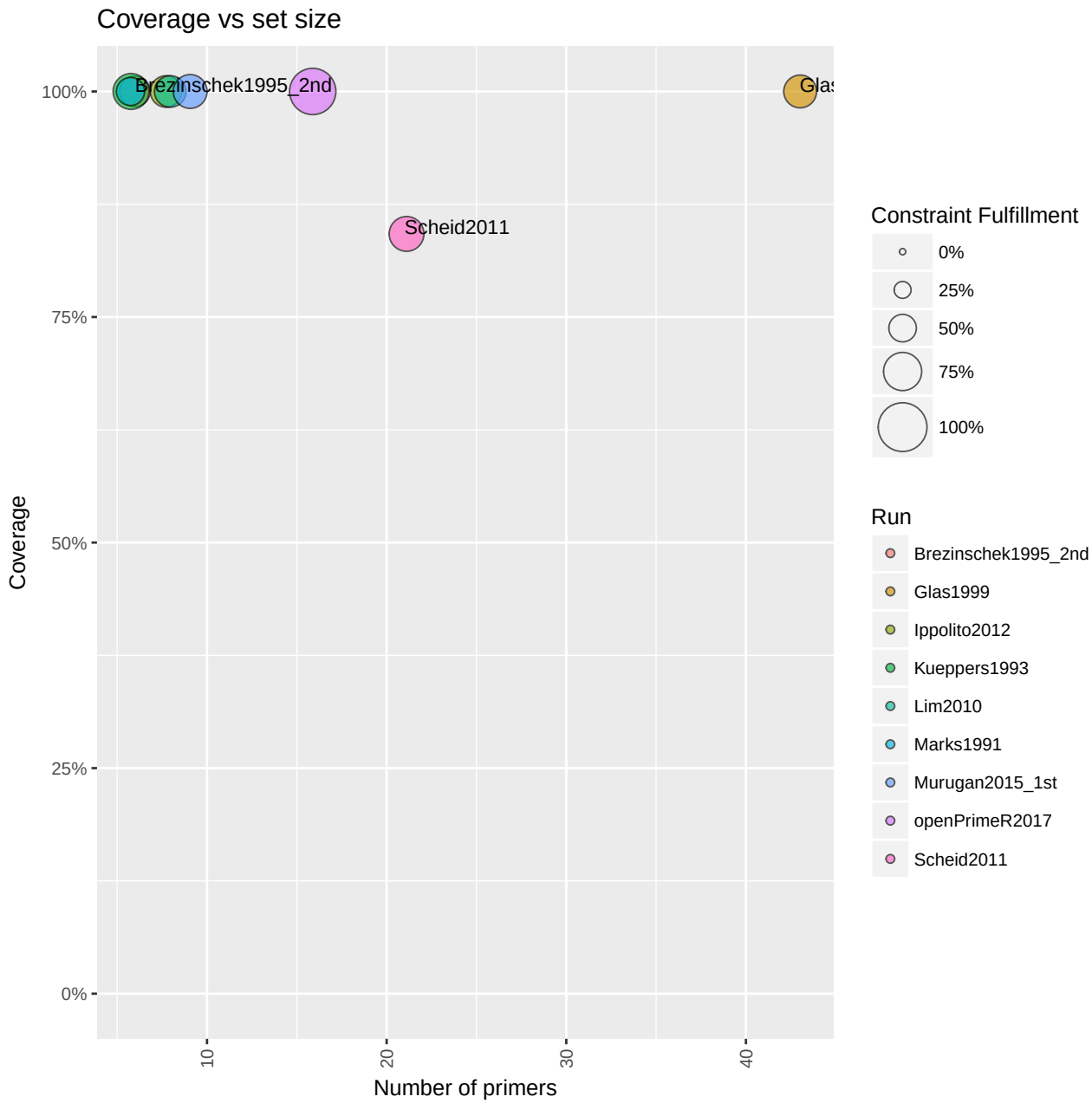

Figure 3: Coverage versus number of primers. Primer sets offering a good trade-off between coverage and set size will appear in the top left corner of the plot. The radius of each dot is correlated with the ratio at which the corresponding primer set fulfills the constraints.

Physicochemical properties

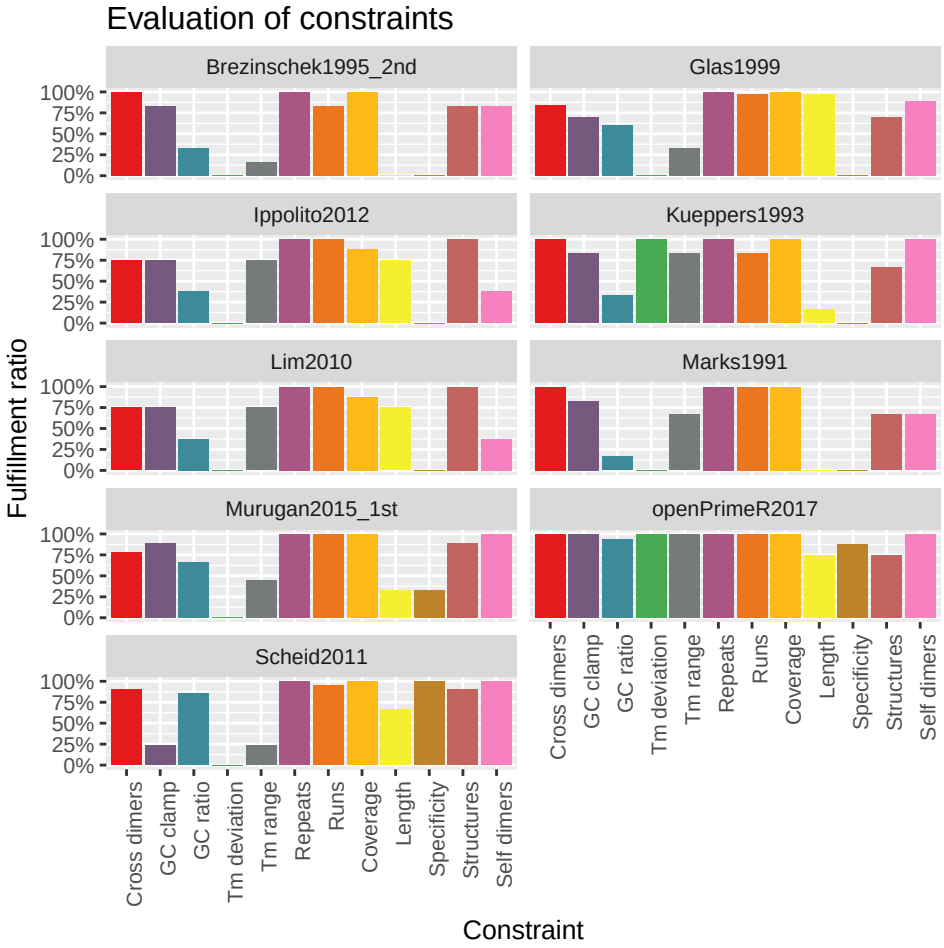

Figure 4: Percentage of constraints that are fulfilled by each primer set.

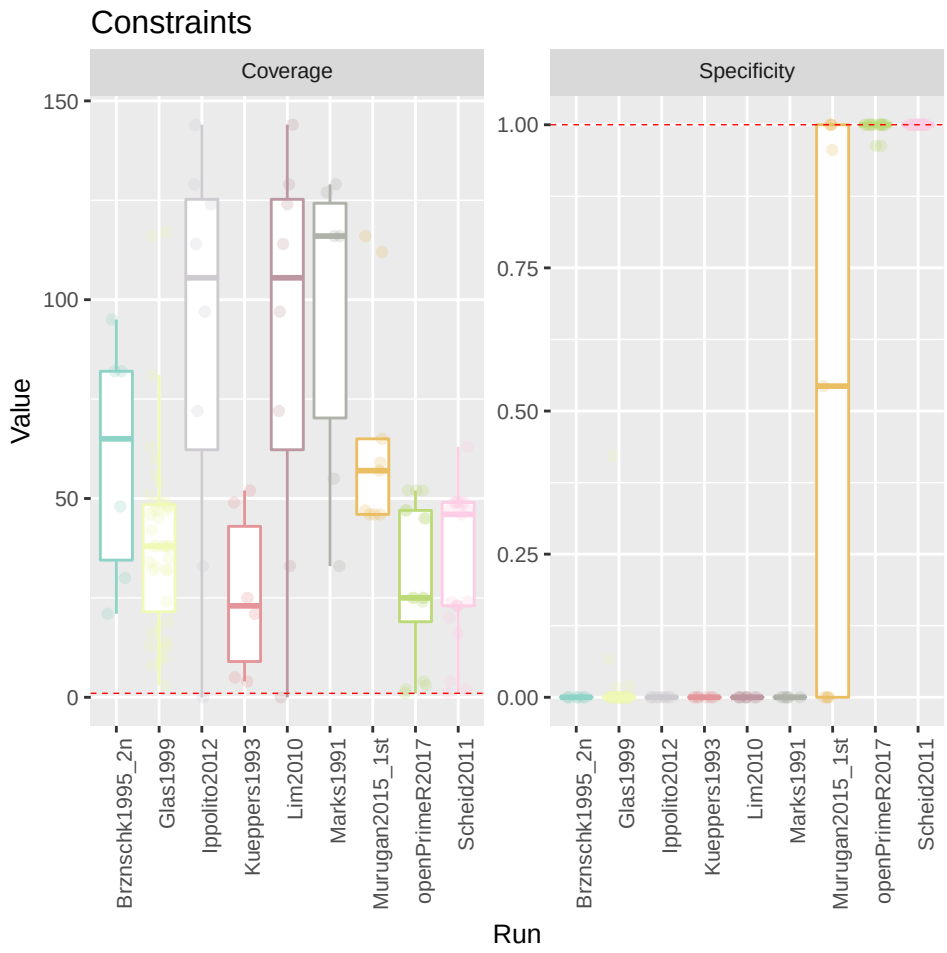

Figure 5: Binding properties of the primers. The horizontal dashed lines indicate the desired property value ranges.

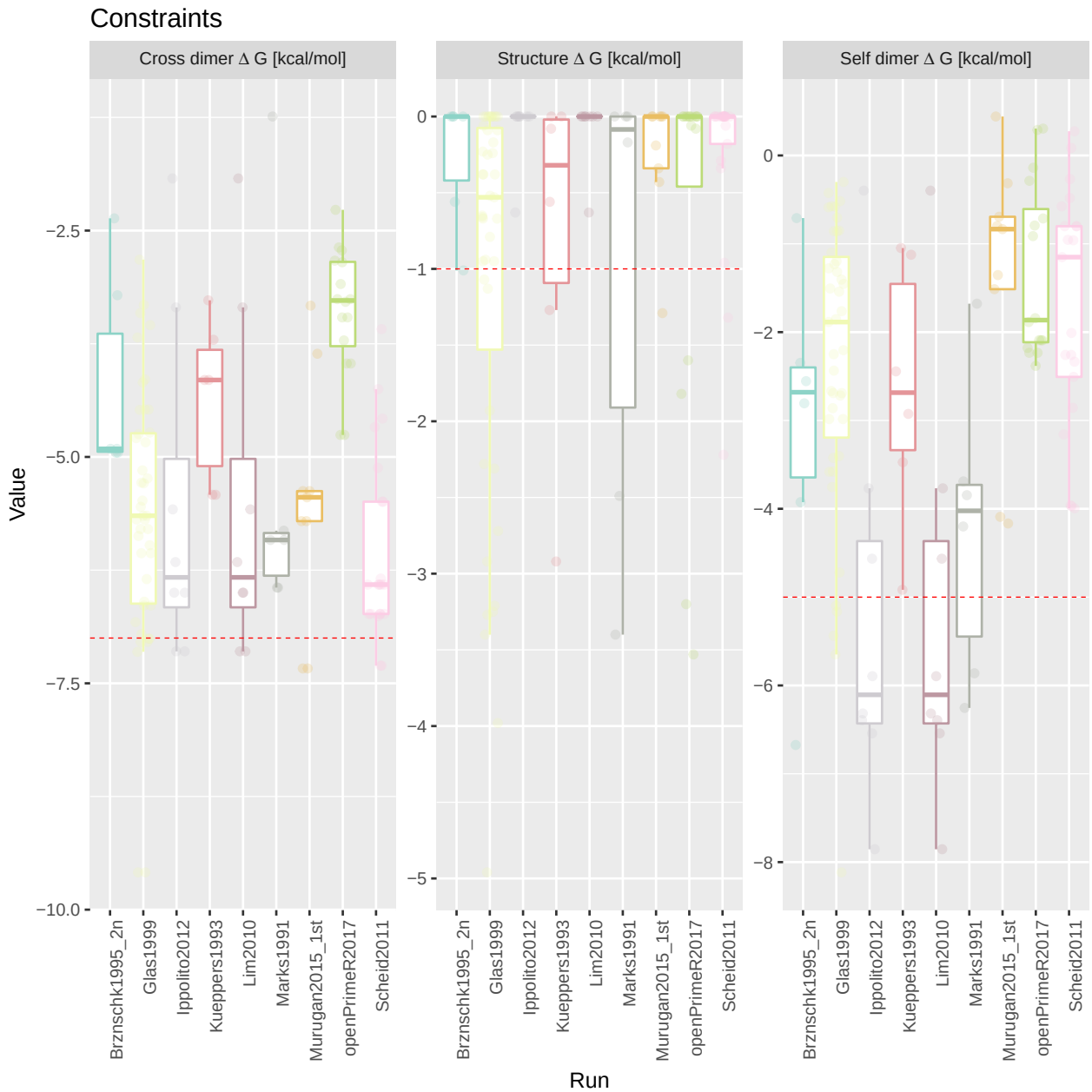

Figure 6: Structural properties of the primers. The horizontal dashed lines indicate the desired property value ranges.

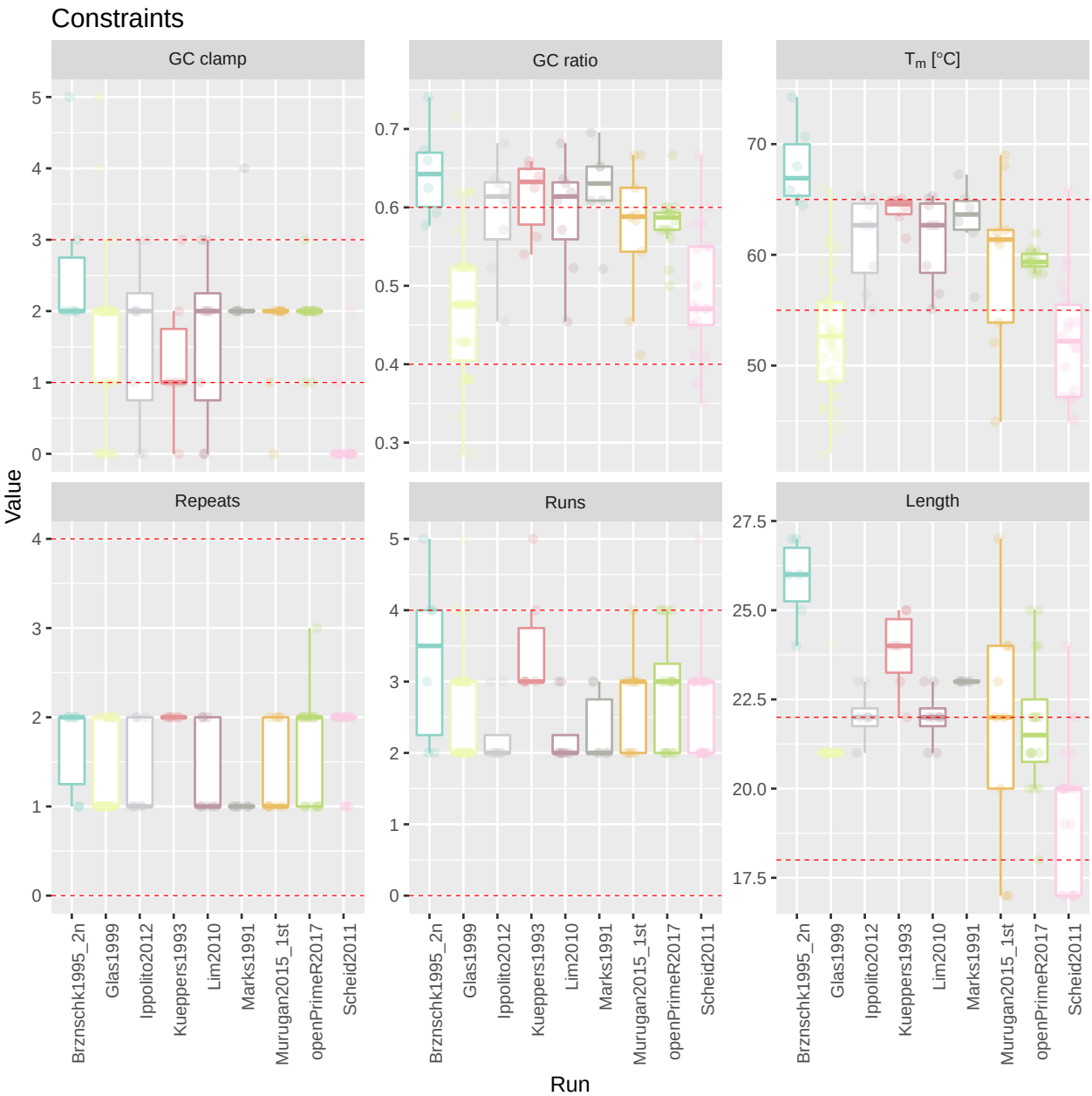

Figure 7: Properties relating to the nucleotide composition of the primers. The horizontal dashed lines indicate the desired property value ranges.

Analysis settings

Please verify that the analysis was performed with the desired settings!

Table 14: Constraints on the physicochemical properties of primers. The column *Target range* summarizes the user-specified constraints.

Constraint	Target range
Length	[18, 22]
GC clamp	[1, 3]
GC ratio	[0.4, 0.6]
Runs	[0, 4]
Repeats	[0, 4]
T_m range [°C]	[55, 65]
Self dimer $\Delta G[\frac{kcal}{mol}]$	[-5, ∞]
Structure $\Delta G[\frac{kcal}{mol}]$	[-1, ∞]
Coverage	[1, ∞]
Specificity	[1, 1]
T_m deviation [°C]	[0, 5]
Cross dimer $\Delta G[\frac{kcal}{mol}]$	[-7, ∞]

Table 15: Options for the active constraints. *Allowed mismatches* refers to the maximal allowed number of mismatches between a primer and a template. *Allowed off-target binding ratio* indicates the ratio of primers that are allowed to bind to non-target regions. *Binding region definition* defines whether primers shall bind within the target region (*within*) or are only required to overlap with the target region (*any*).

Option	Setting
Allowed mismatches	5
Allowed off-target binding ratio	1
Binding region definition	within

Table 16: The conditions for primer coverage. *Stop codons* indicates whether mismatch binding events are allowed to induce stop codons (*1*) or not (*0*). *Efficiency* indicates the required amplification efficiency of the primers. *Annealing* gives the required free energy of annealing. *3' Mismatch Position* indicates the positions in the primers (from the 3' end such that *1* indicates the last position) for which mismatches are allowed.

Constraint	Target range
Coverage Model FPR	[$-\infty$, 0.05]
Stop codons	[0, 1]
Substitutions	[0, 1]

Table 17: The experimental conditions that were used to evaluate the primers.

Condition	Setting
Taq polymerase	TRUE
$[Na^+]$ [M]	0

Condition	Setting
[Mg^{2+}] [M]	0.0015
[K^{+}] [M]	0.05
[Tris buffer] [M]	0
[Primer] [M]	2e-07
[Template] [M]	1.28e-11
PCR cycles	25