Report for Ippolito (IGHV)

23 August, 2017

openPrimeR is a research tool - we do not guarantee for any result!

Table 1: Overview of the analyzed primers. If forward and reverse primers are present, primers are paired such as to maximize the coverage. Ambiguous bases are shown in italics. The *Coverage* column indicates the percentage of template sequences that are covered by each primer. *Mismatches* gives the percentage of coverage events that are subject to a certain number of mismatches. *Position* indicates the major binding mode of a primer relative to the end of the target binding region. Negative values indicate binding upstream of the specified region, while positive positions indicate binding downstream of the target region.

ID	Sequence	Coverage	Mismatches	Position	T_m[°C]
Ippl2012|VH1|1_	caggtccagct*k*gt*r*cagtctgg	53.1%	[1.25,3]	0 to +22 (100%)	64.48
Ip2012|VH157|2_	caggtgcagctggtg*sa*rtctgg	84.4%	[1,3]	0 to +22 (100%)	65.08
Ippl2012|VH2|3_	cag*r*tcaccttgaaggagtctg	14.3%	[0,1]	0 to +21 (100%)	56.46
Ippl2012|VH3|4_	gaggtgcagctg*k*tggag*wcy*	76.9%	[0,4]	0 to +20 (100%)	62.66
Ippl2012|VH4|5_	caggtgcagctgcaggagtc*sg*	76.9%	[0,4]	0 to +21 (100%)	65.31
I2012|VH4-DP63|	caggtgcagctacagcagtggg	39.5%	[3,4]	0 to +21 (100%)	62.68
Ippl2012|VH6|7_	caggtacagctgcagcagtca	47.6%	[3,4]	0 to +20 (100%)	59.01
Ipp2012|VH3N|8_	tcaacacaacggttcccagtta	0%			55.11

Coverage

Table 2: Number of covered template sequences per group. In case that primers for both directions are present, the *Coverage (fw)* and *Coverage (rev)* columns refer to the individual coverage of forward and reverse primers, respectively. If primers of both directions are present, the total coverage is determined by the intersection of coverage events from primers of both directions. If, however, only primers of a single direction are present, the coverage solely depends on the primers of the given direction.

Group	Coverage
Total	146 of 147 (99.32%)
IGHV1	24 of 24 (100%)
IGHV2	21 of 21 (100%)
IGHV3	48 of 49 (97.96%)
IGHV4	47 of 47 (100%)
IGHV5	3 of 3 (100%)
IGHV6	2 of 2 (100%)
IGHV7	1 of 1 (100%)

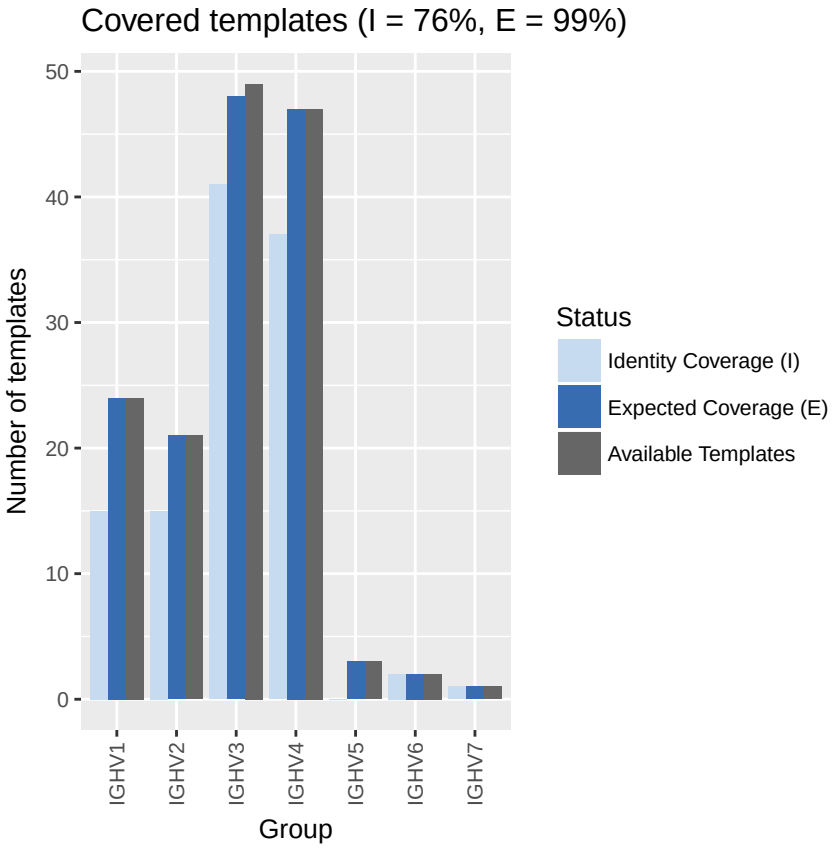

Figure 1: The number of covered and available template sequences per group of templates. *Identity Coverage* refers to the number of template sequences for which fully complementary primers exist. *Expected Coverage* indicates the expected number of covered templates, which is determined by applying the coverage constraints to the set of potential coverage events for the specified number of maximal mismatches. *Available Templates* shows the total number of available template sequences per group.

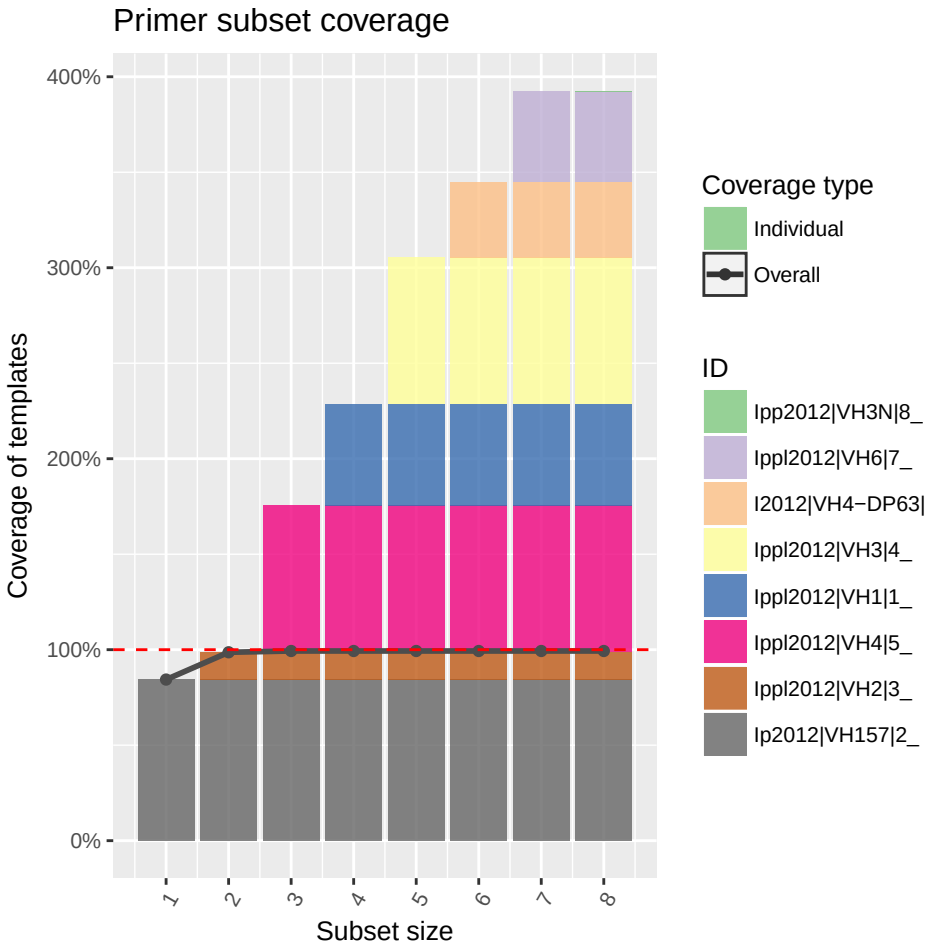

Figure 2: The coverage of optimal subsets of the input primer set. Here *optimal* refers to the fact that the subsets were selected such as to maximize the coverage. The line plot indicates the total percentage of covered template sequences, while the bars indicate the percentage of covered templates for individual primers. The cumulative coverage of the bars can exceed 100 percent because different primers may cover the same template redundantly. The target coverage ratio is indicated by the dashed horizontal line.

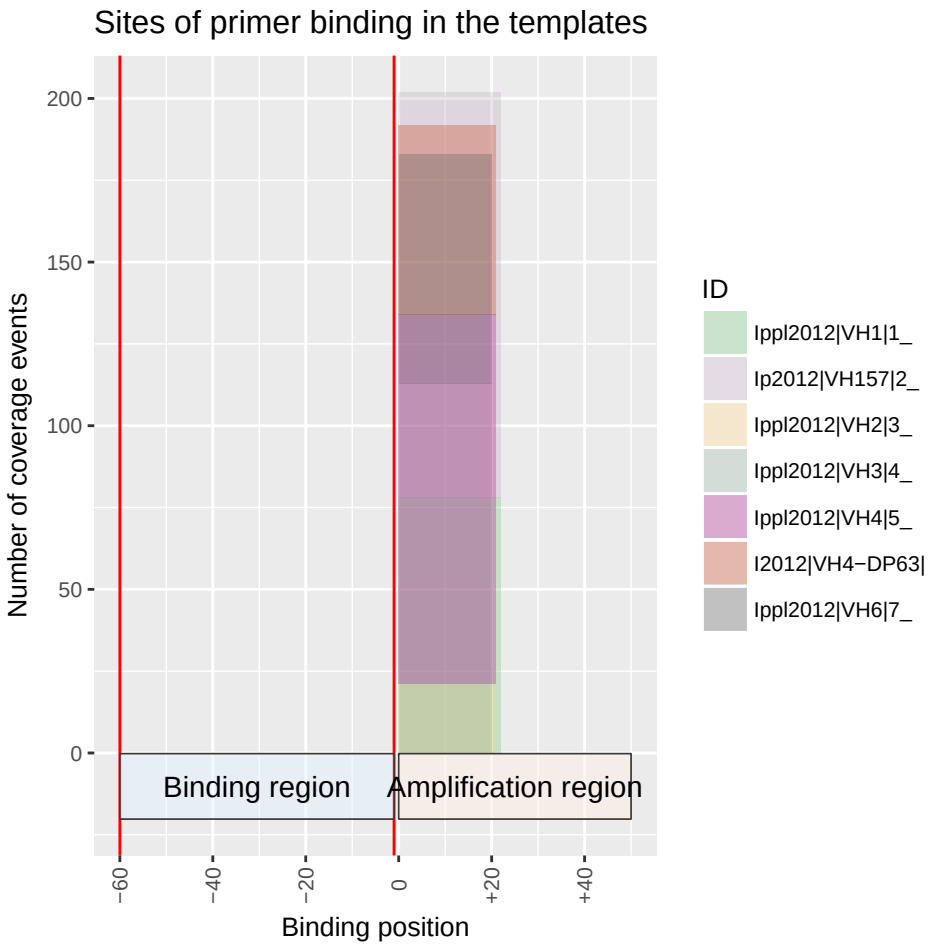

Figure 3: The binding positions of the primers relative to the target binding region of forward primers. Here, *Binding region* indicates the region where the forward primers should bind, while *Amplification region* indicates the following region.

Physicochemical properties

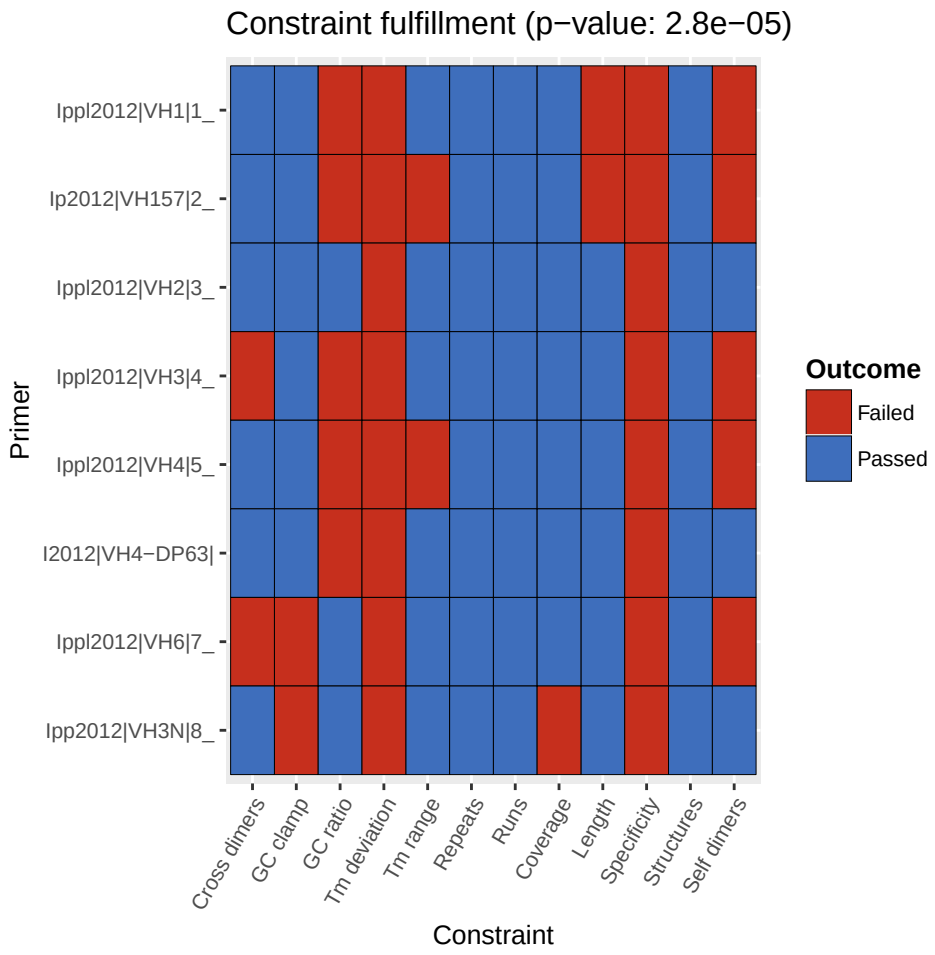

Figure 4: Failed and passed constraints on physicochemical properties for every primer. Constraints that are failed by a primer are colored in red, while constraints that are fulfilled are shown in blue. The provided p-value is an indicator of the overall quality of the primer set: significant p-values indicate primer sets fulfilling more constraints than reference primer sets from the literature.

Figure 5: Deviation of physicochemical properties from the desired value range. Positive deviations indicate that the upper bound of a property was exceeded, while negative deviations indicate values below the lower bound. Each dot corresponds to the deviation of a primer for an individual constraint. The boxes indicate the 1st, 2nd (median), and 3rd quartiles from top to bottom.

Analysis settings

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

Table 3: 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 4: 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 5: 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 6: 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