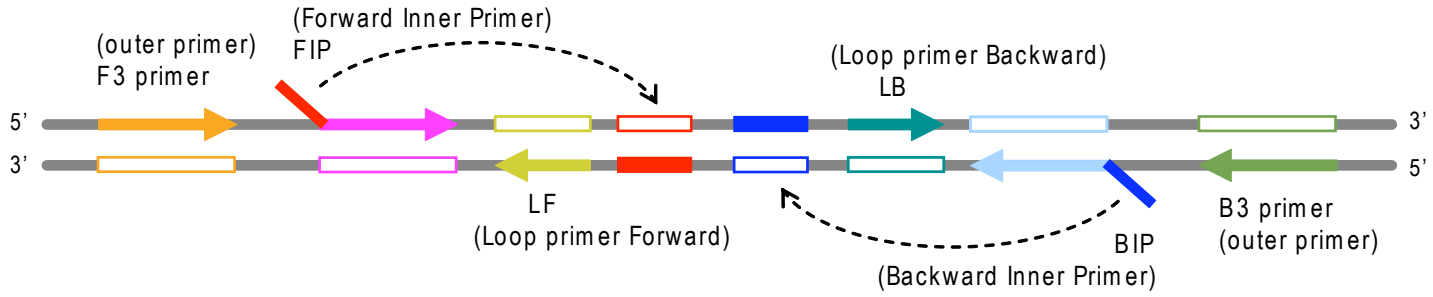
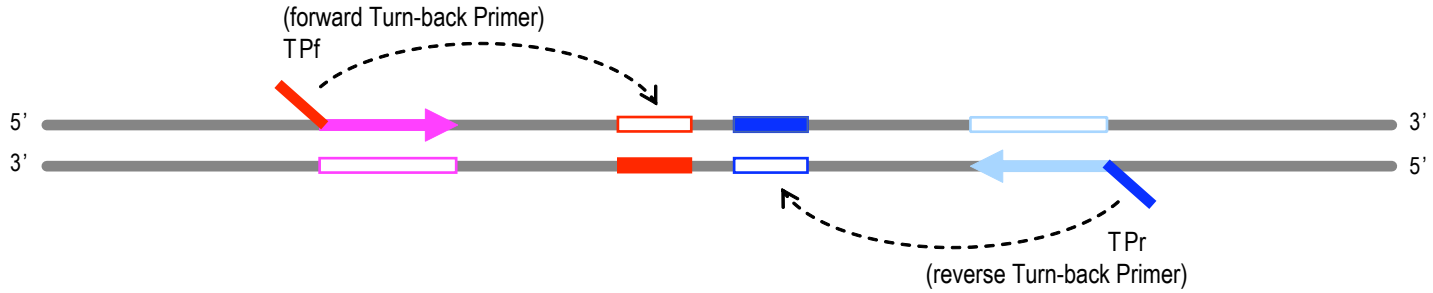
Supplemental FigS1. Isothermal amplification systems

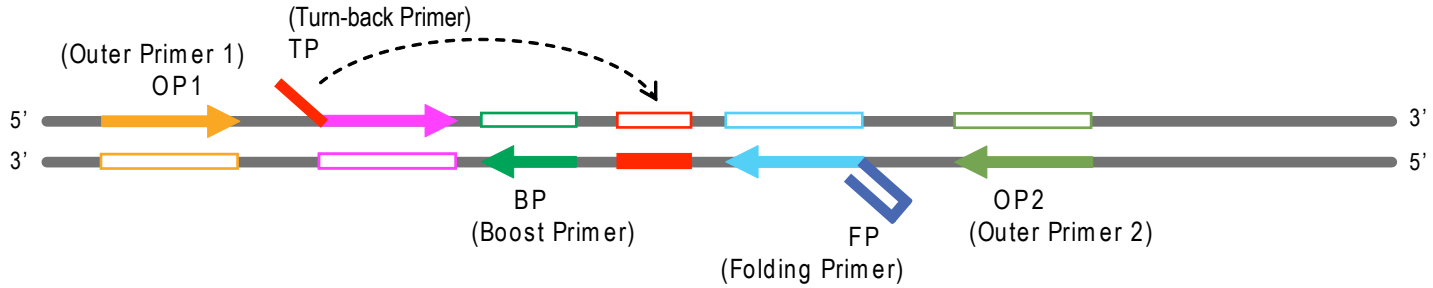
A. The LAMP system



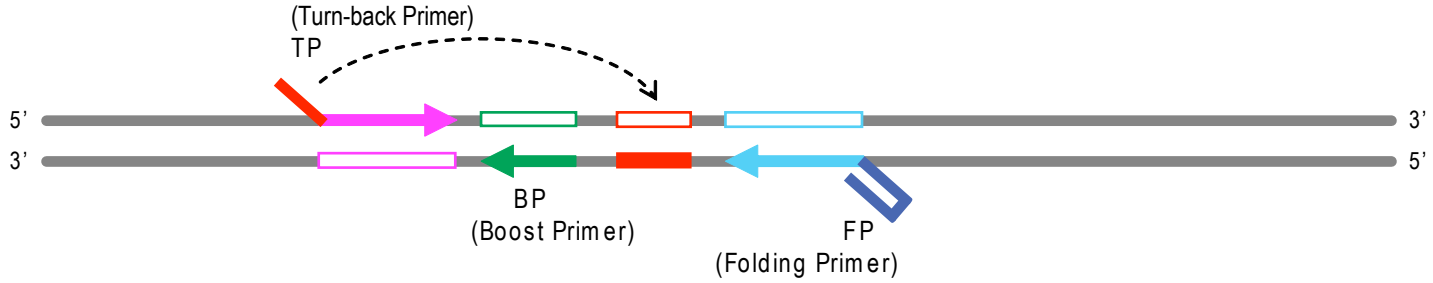
B. The TP-TP system



C. The SmartAmp system



D. The TP-FP-BP system



Supplemental Fig S2. TP-TP amplification pathway

Since the amplification pathway of each strand is symmetric, here we show an example derived from one particular strand.

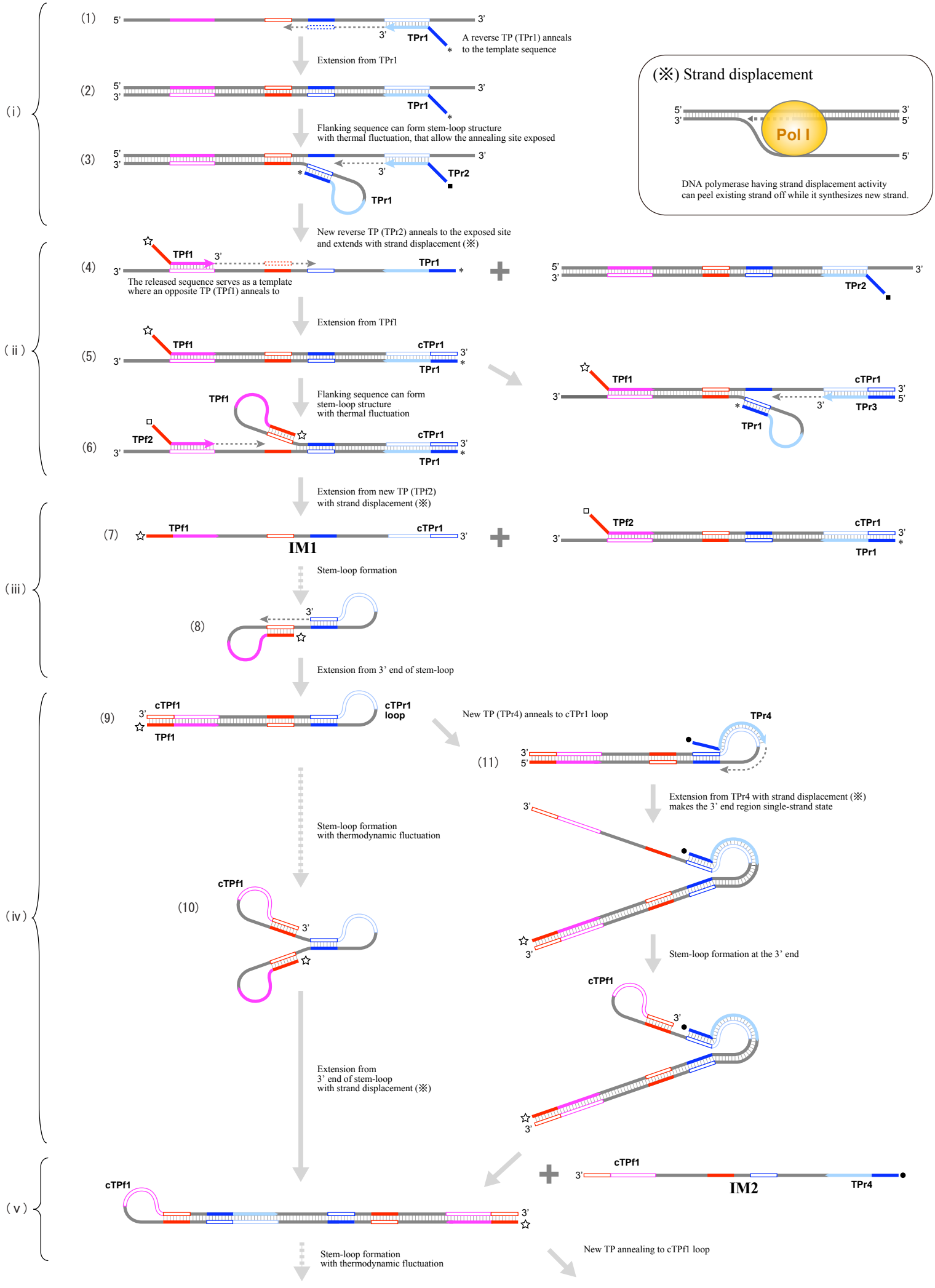
i. First, a reverse trun-back primer (TPr1) hybridizes to target DNA sequence (1) and followed DNA extension mediated by DNA polymerase creates DNA fragment flanked by TPr1 (2). Both ends of newly synthesized strand can be denatured partly and forming a stem-loop structure with thermal fluctuation. Such condition allows another reverse TP (TPr2) to hybridize to the exposed target sequence and extends new strand which peels the existing strand off by strand displacement activity of the DNA polymerase (3).

ii. The released DNA strand becomes a template for the next step as a forward TP (TPf1) can hybridize there (4). Extension from TPf1 creates DNA fragment flanked by TPf1 and complement of TPr1 (cTPr1) (5), which can be peeled off by the same mechanism as described above for TPr1 (6).

iii. We named this peeled fragment flanked by TPf1 and cTPr1 as Intermediate product (IM1) (7). The 3' end of the IM1, cTPr1, is designed to form a stem-loop structure and DNA extension can be initiated at the 3' end by self-priming mechanism (8).

iv. This event generates in total two different pathways (9). The one is self-priming from the complement of TPf1 (cTPf1) after stem-loop formation with thermal fluctuation (10). The other one occurs at the cTPr1 loop in the middle of the IM1 where another reverse TP (TPr4) can hybridize there (11).

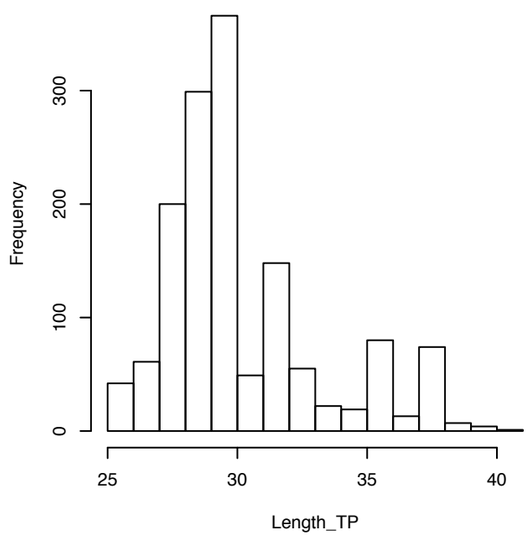
v. Products are continuously amplified by the same mechanism as described above.


(i)
(1)
5'
3'
TPr1
A reverse TP (TPr1) anneals to the template sequence
Extension from TPr1
(2)
Flanking sequence can form stem-loop structure with thermal fluctuation, that allow the annealing site exposed
(3)
TPr2
(※) Strand displacement
Pol I
DNA polymerase having strand displacement activity can peel existing strand off while it synthesizes new strand.
(ii)
New reverse TP (TPr2) anneals to the exposed site and extends with strand displacement (※)
(4)
TPf1
The released sequence serves as a template where an opposite TP (TPf1) anneals to
Extension from TPf1
(5)
cTPr1
Flanking sequence can form stem-loop structure with thermal fluctuation
(6)
TPf2
TPr3
(iii)
Extension from new TP (TPf2) with strand displacement (※)
(7)
IM1
Stem-loop formation
(8)
Extension from 3' end of stem-loop
(iv)
(9)
cTPf1
cTPr1 loop
New TP (TPr4) anneals to cTPr1 loop
(11)
TPr4
Extension from TPr4 with strand displacement (※) makes the 3' end region single-strand state
Stem-loop formation with thermodynamic fluctuation
(10)
Stem-loop formation at the 3' end
Extension from 3' end of stem-loop with strand displacement (※)
(v)
IM2
Stem-loop formation with thermodynamic fluctuation
New TP annealing to cTPf1 loop

Supplemental Fig S3. The distributions of all parameters in the Training data set.

The distributions of 78 parameters examined in this study (see Supplemental Table S1 for details) and the observed reaction speeds in the training data. The training data set was comprised of 24 target regions, 420 TPs, and 1,344 TP-TP combinations assayed under the Aac reaction conditions. The numbers on top of the figures correspond to the numbers in Supplemental Table S1.

1
Length_TP



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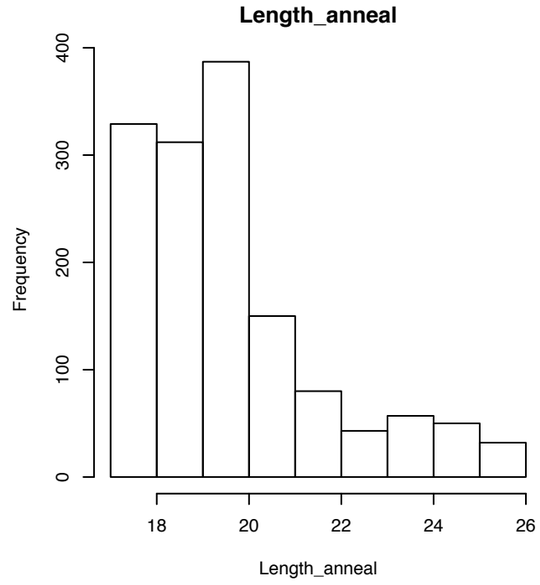
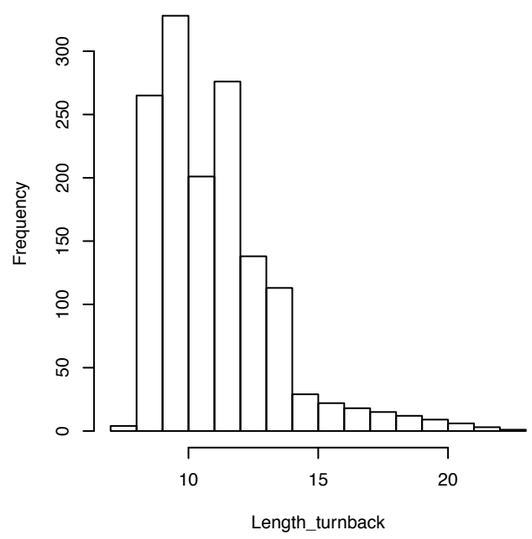
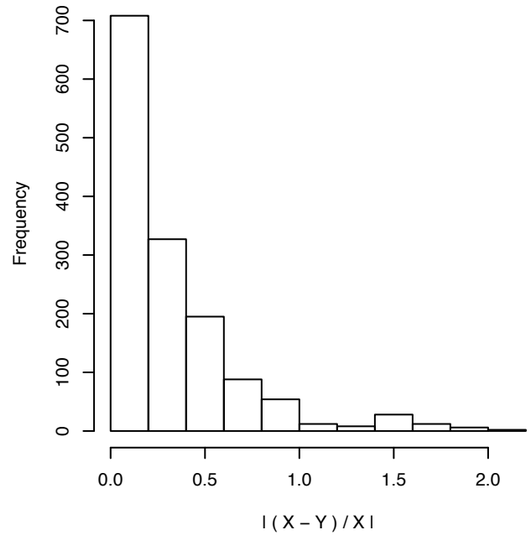
Length_anneal



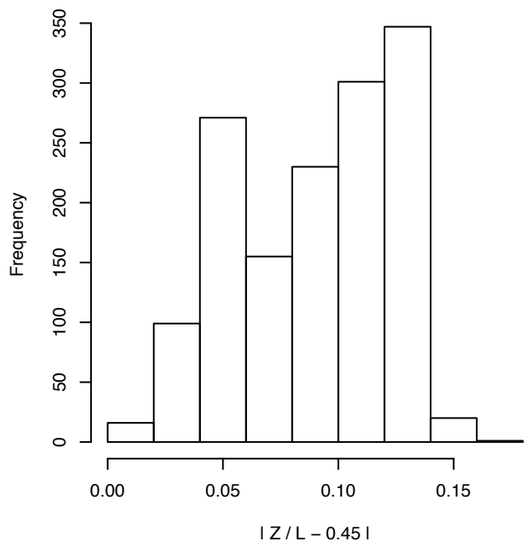
3
Length_turnback



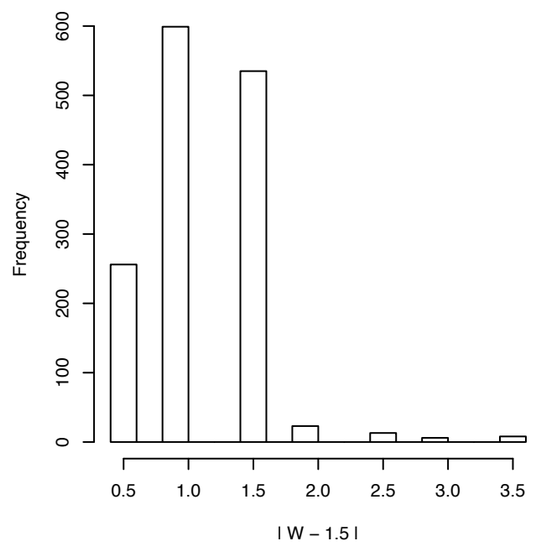
4
l (X – Y) / X l



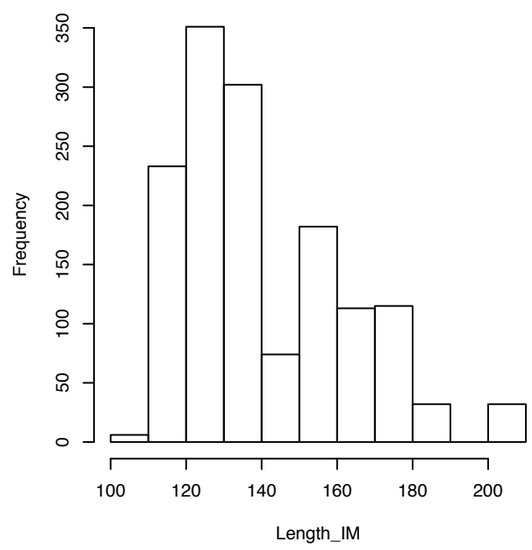
5
l Z / L – 0.45 l



6
l W – 1.5 l



7
Length_IM



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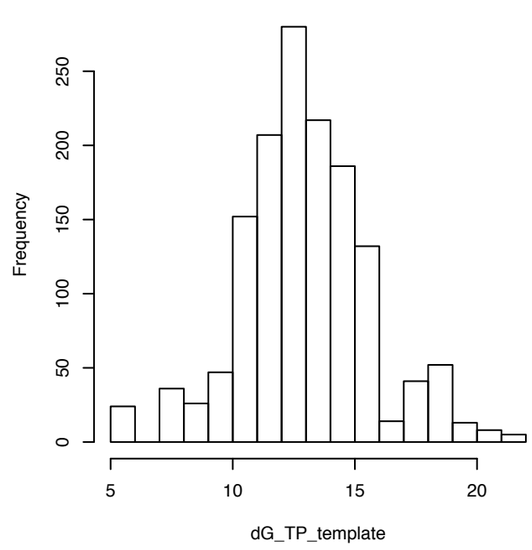

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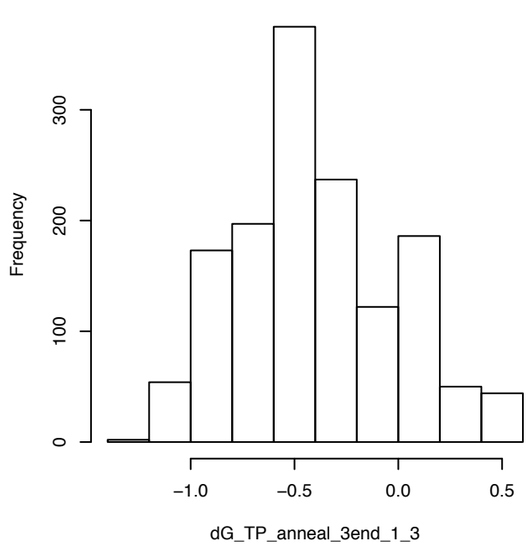

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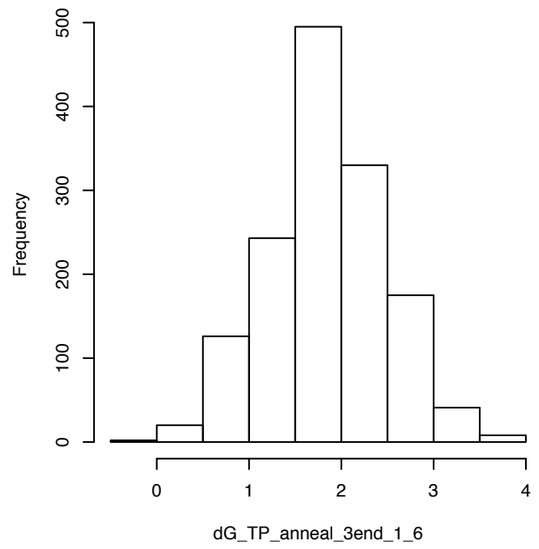

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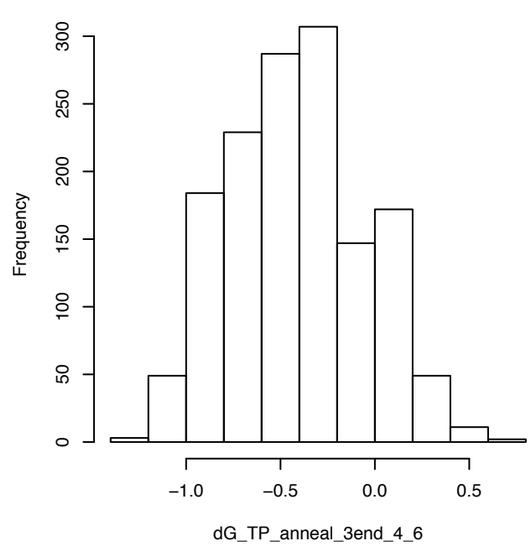

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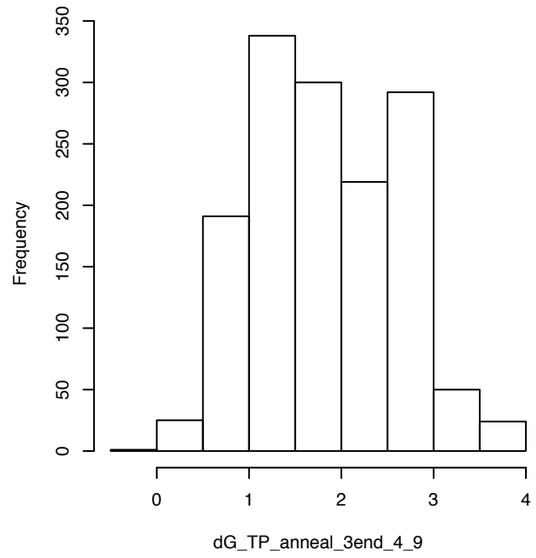

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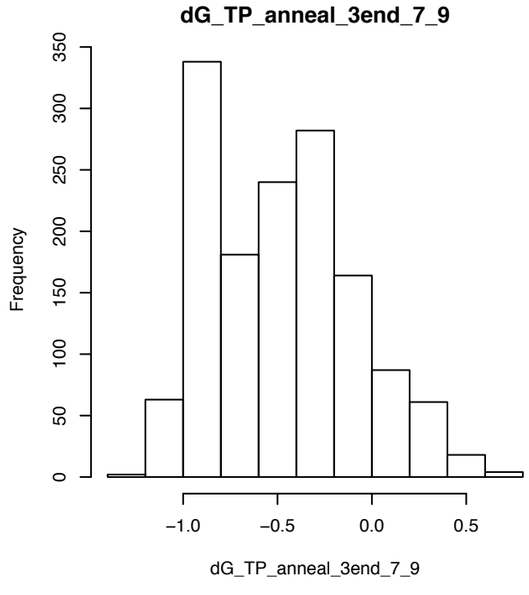

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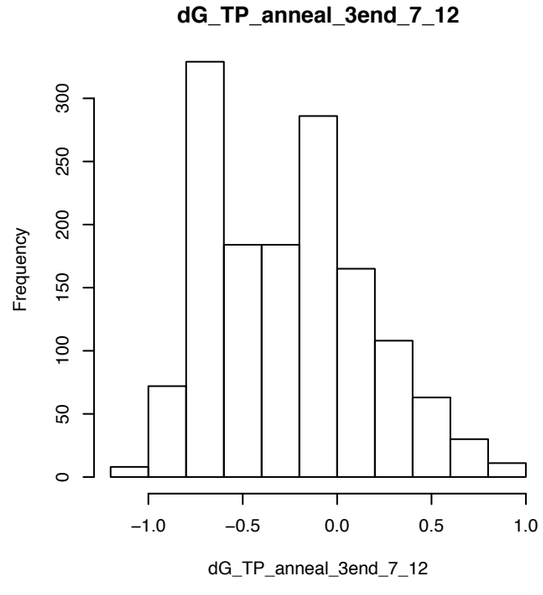

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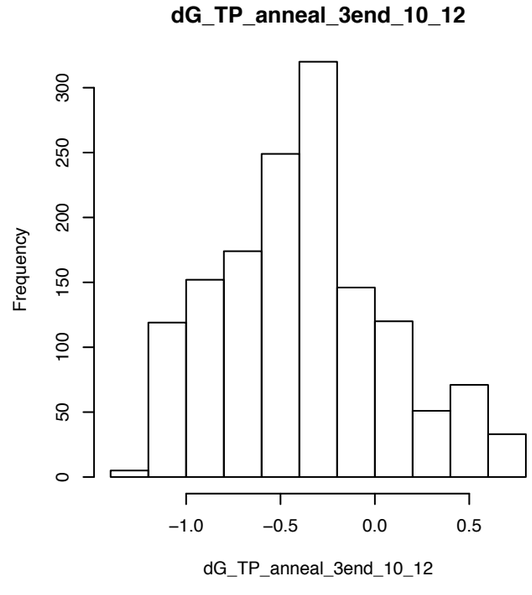

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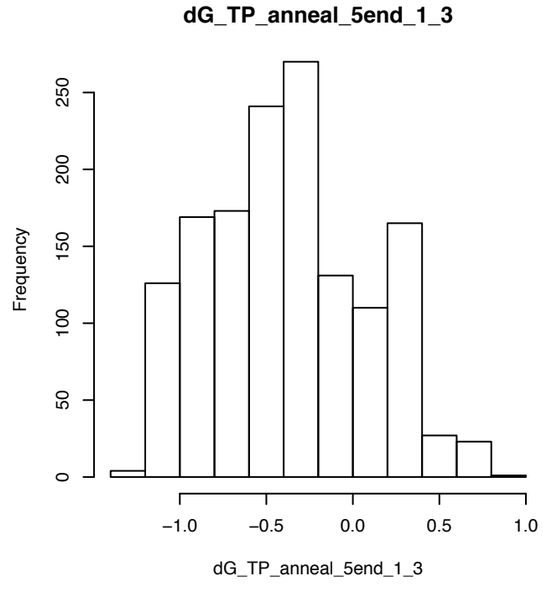

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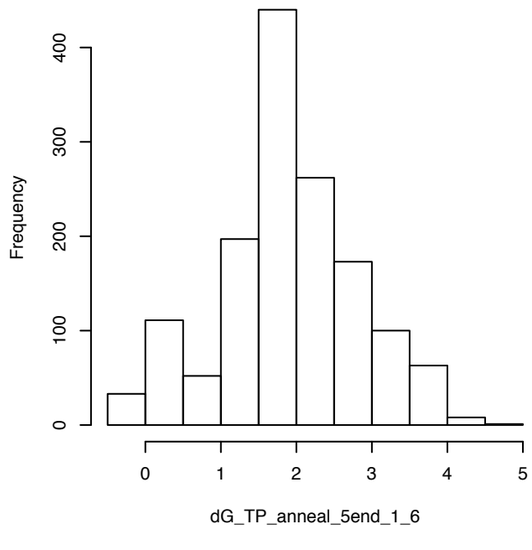

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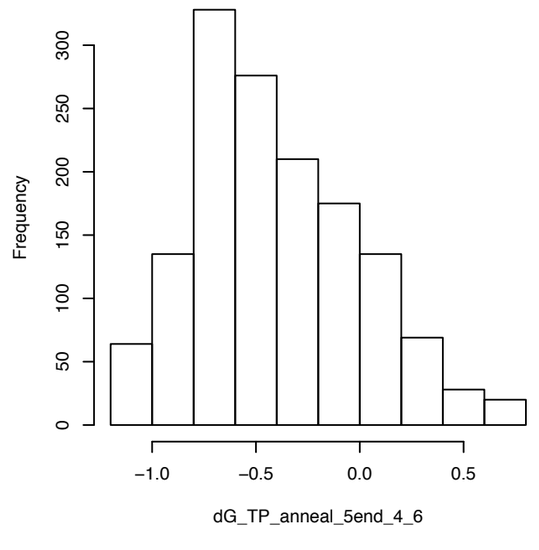

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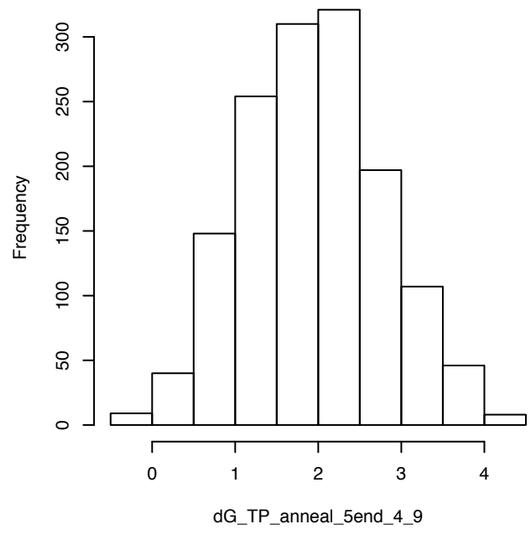

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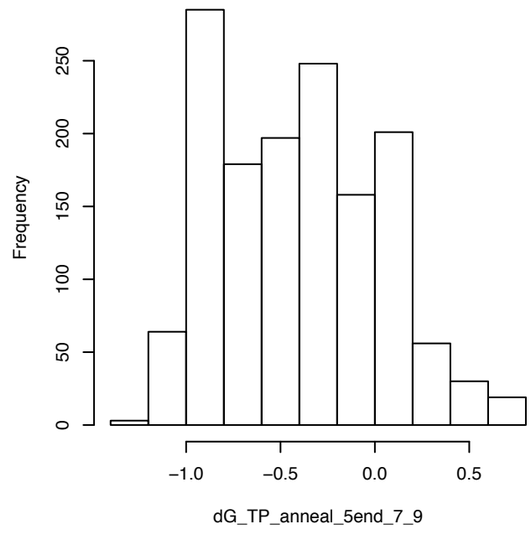

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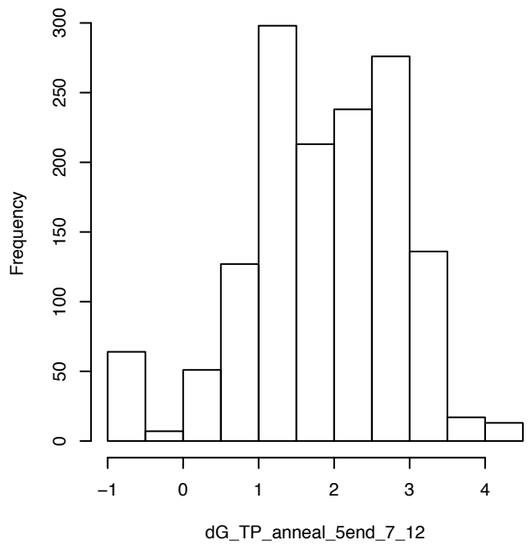

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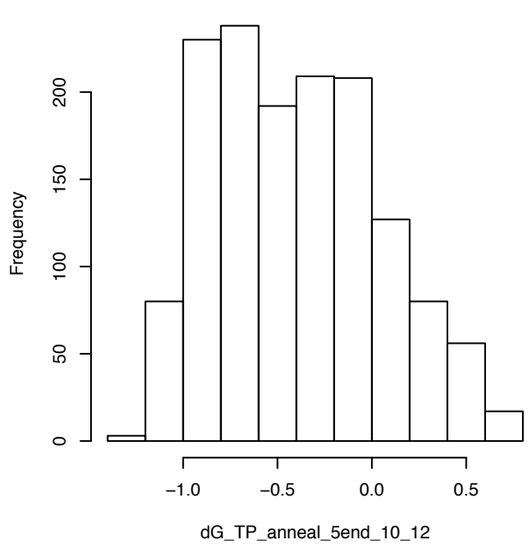

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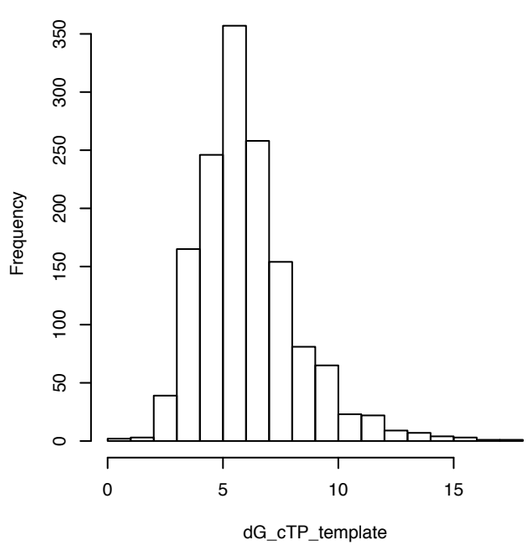

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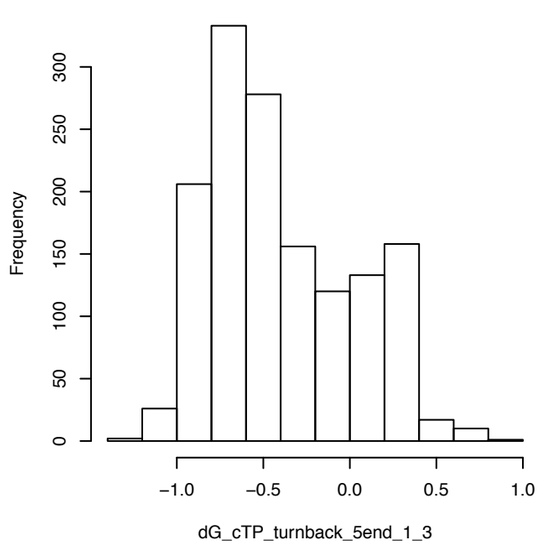

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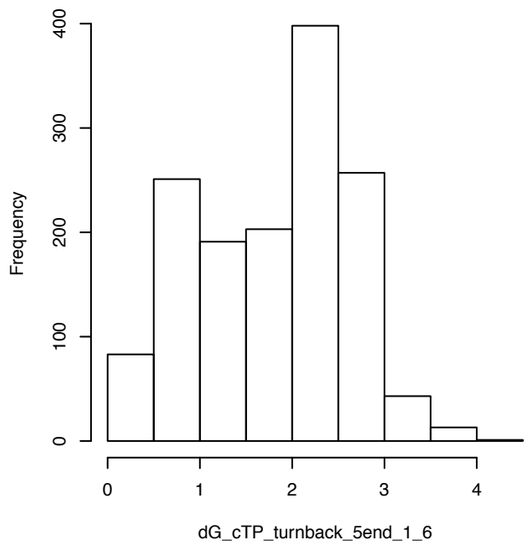

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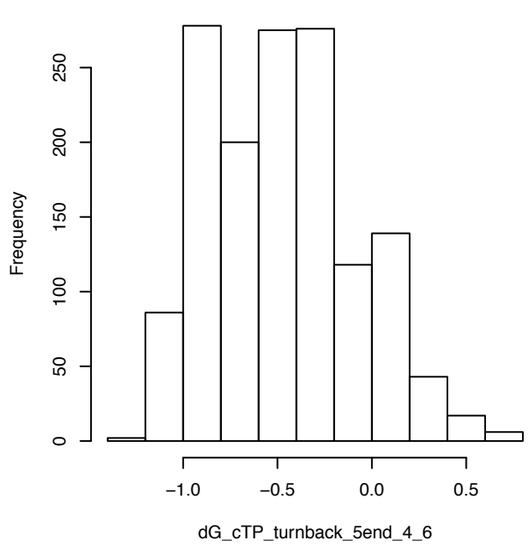

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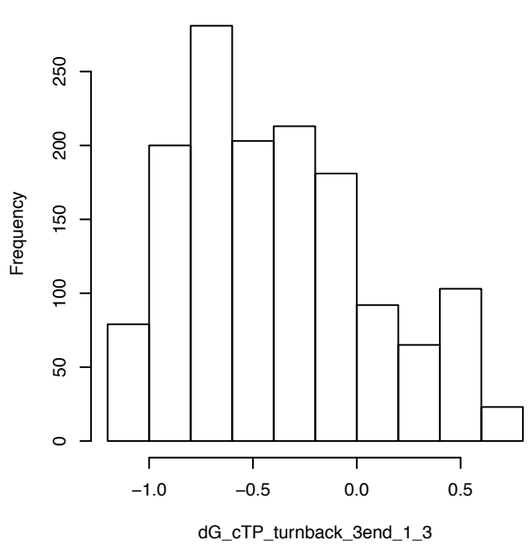

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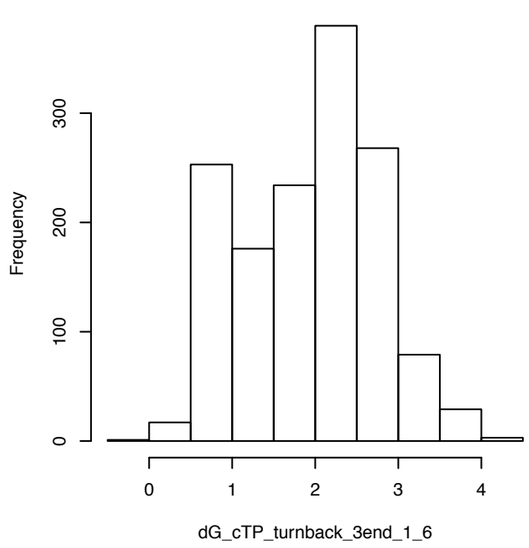

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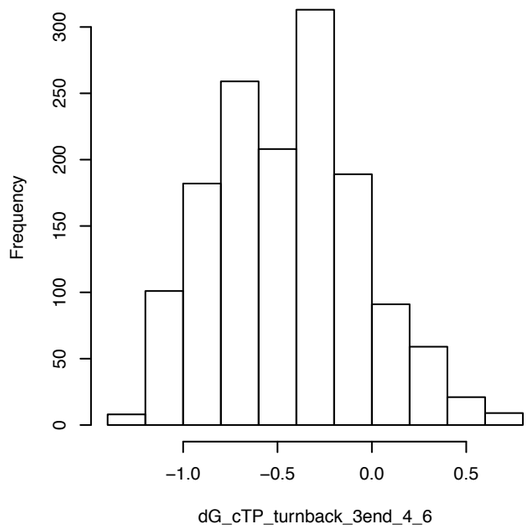

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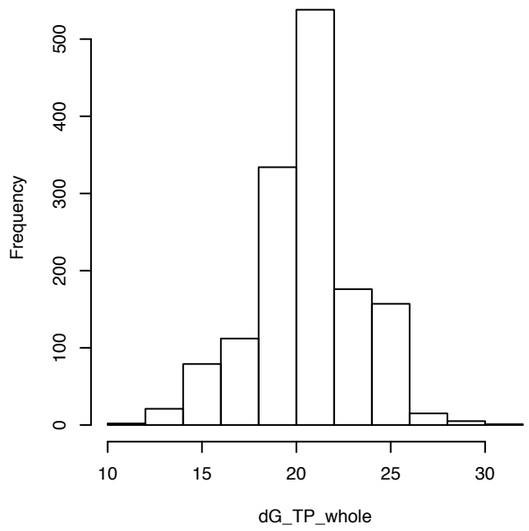
dG_TP_whole



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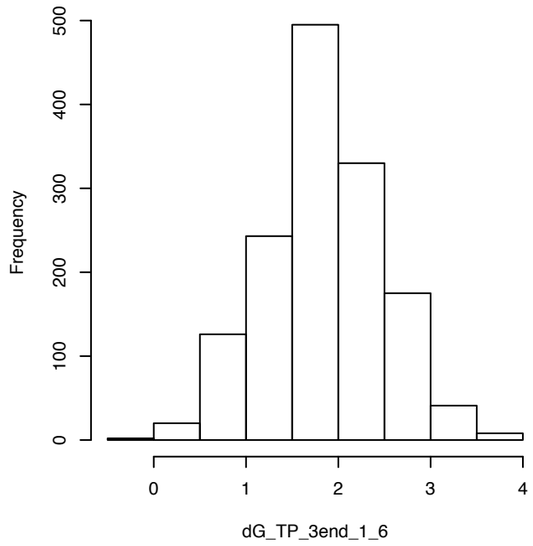

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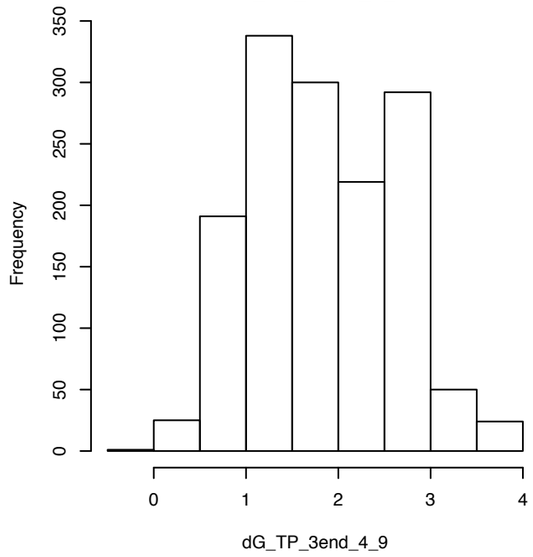

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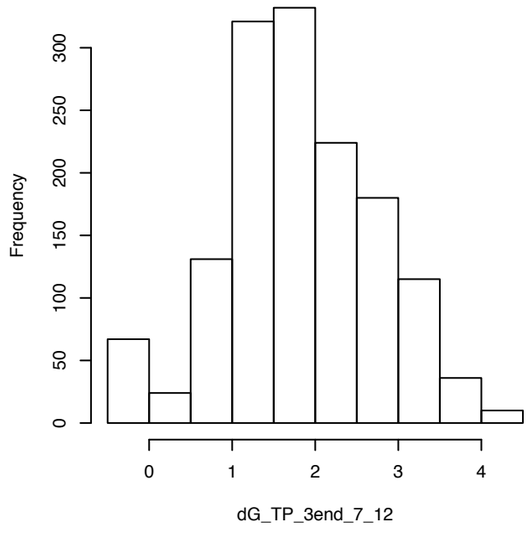

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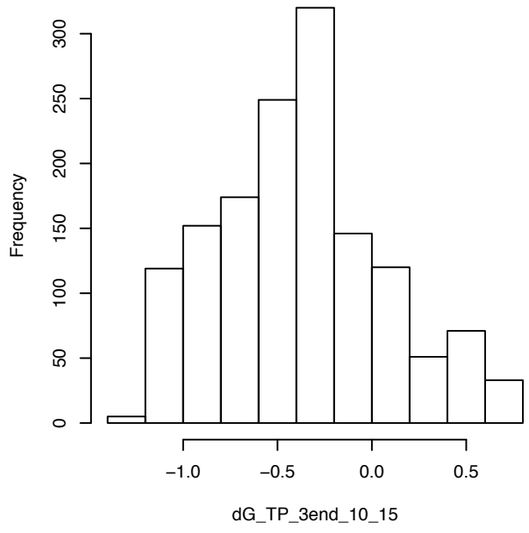

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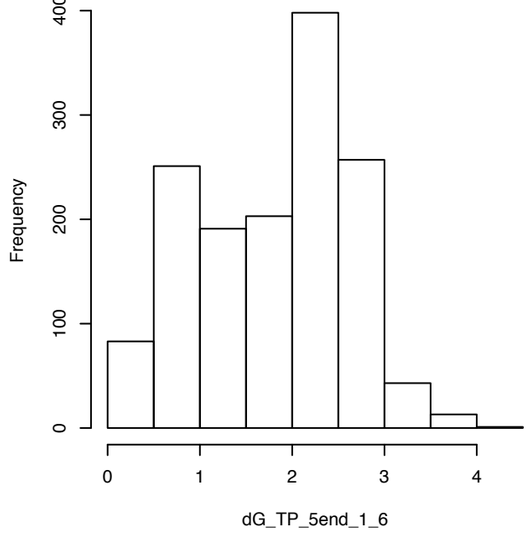

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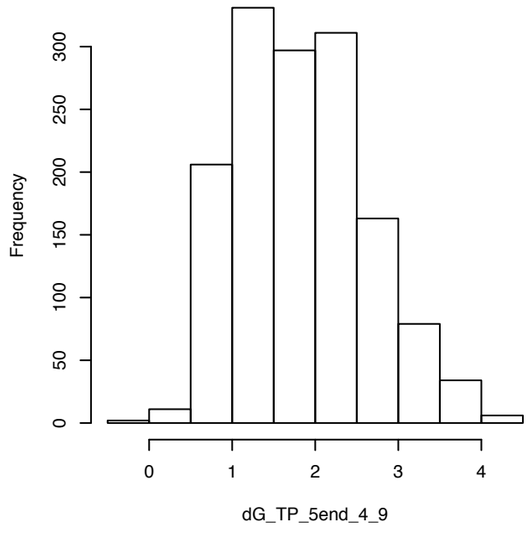


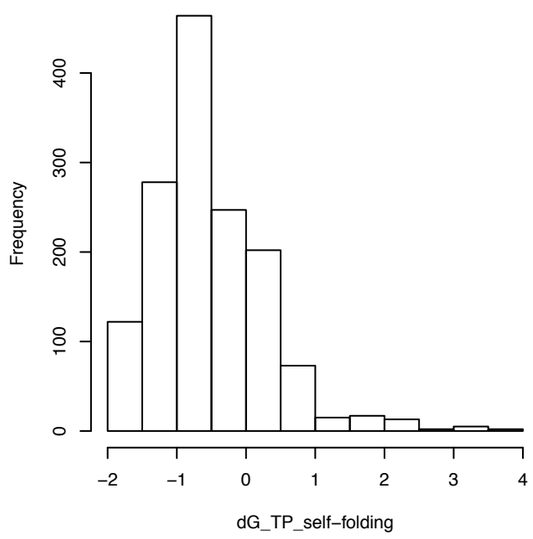
37
dG_TP_5end_7_12

38
dG_TP_5end_10_15

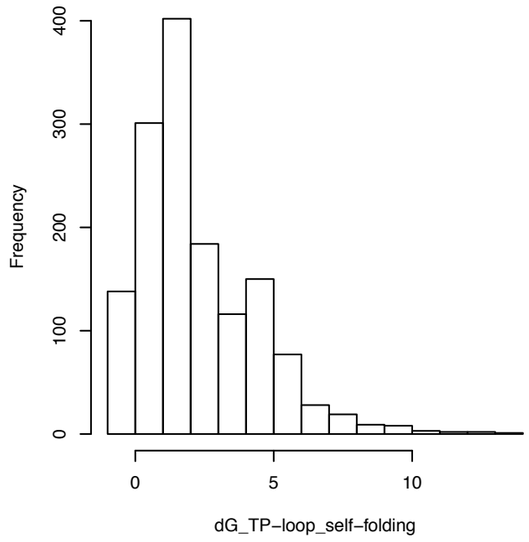
39
dG_TP_homodimer

40
dG_TP_heterodimer

41
dG_TP_self−folding



42
dG_TP−loop_self−folding



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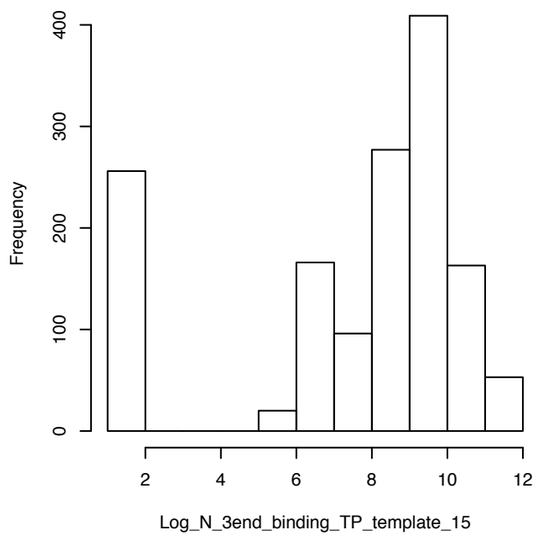
Log_N_3end_binding_TP_template_15



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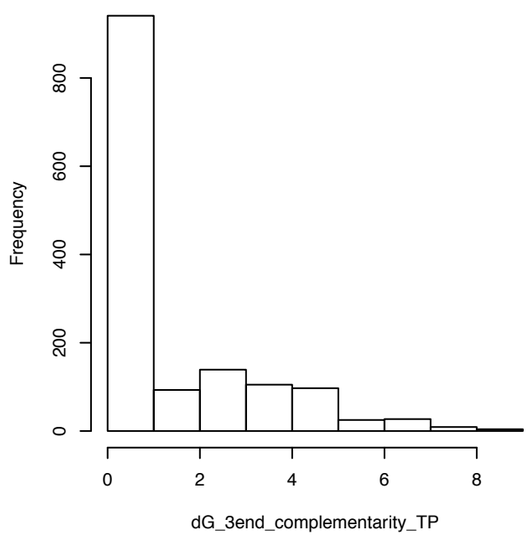
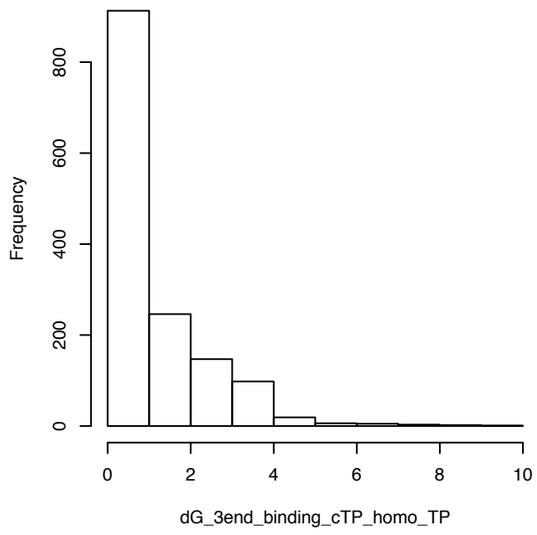
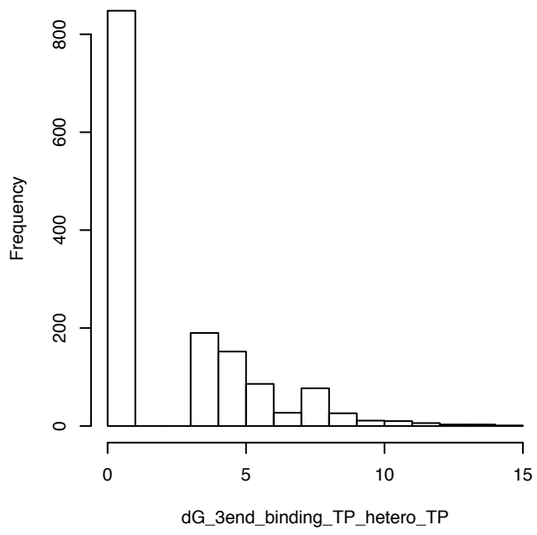
dG_3end_complementarity_TP



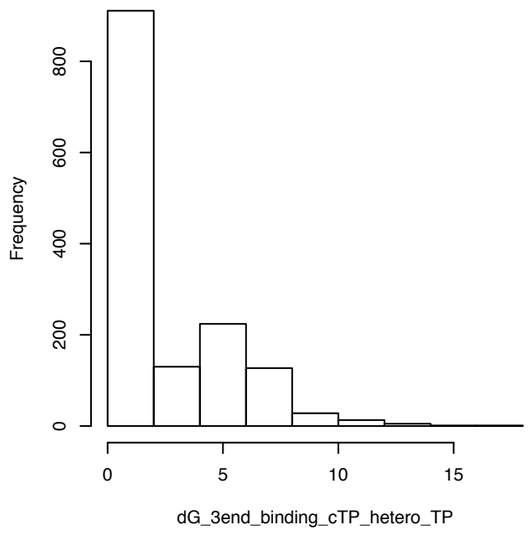
45
dG_3end_binding_cTP_homo_TP



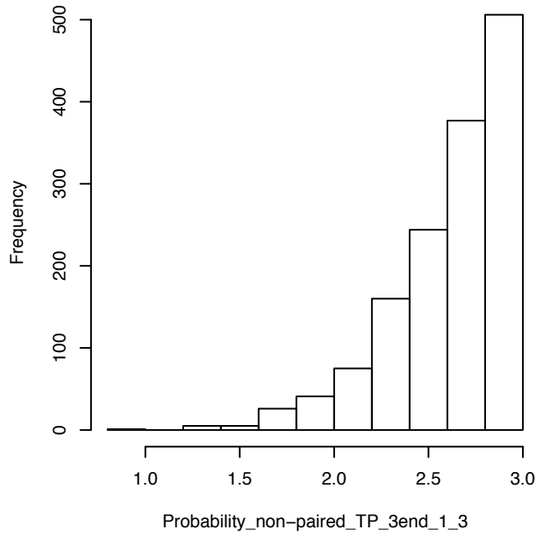
46
dG_3end_binding_TP_hetero_TP



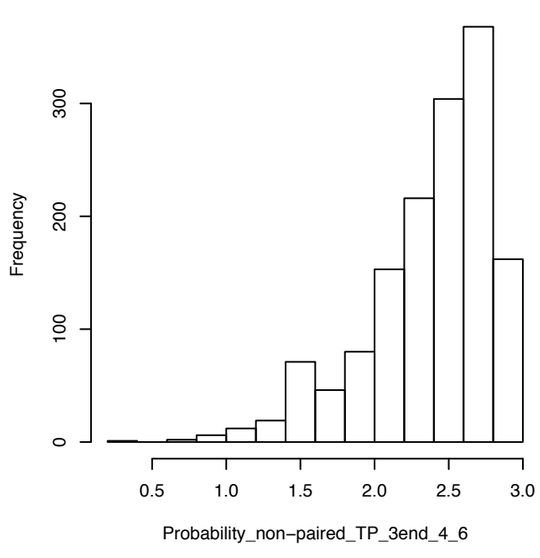
47
dG_3end_binding_cTP_hetero_TP



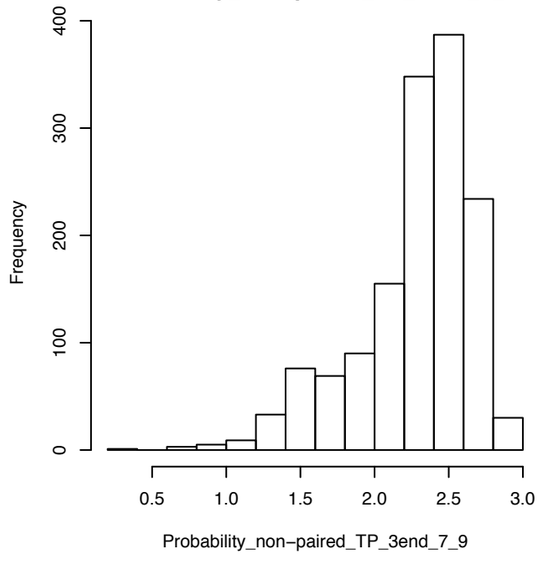
48
Probability_non−paired_TP_3end_1_3



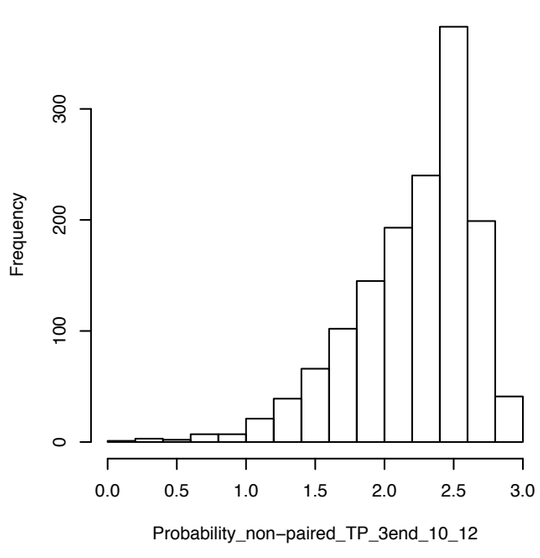
49
Probability_non−paired_TP_3end_4_6



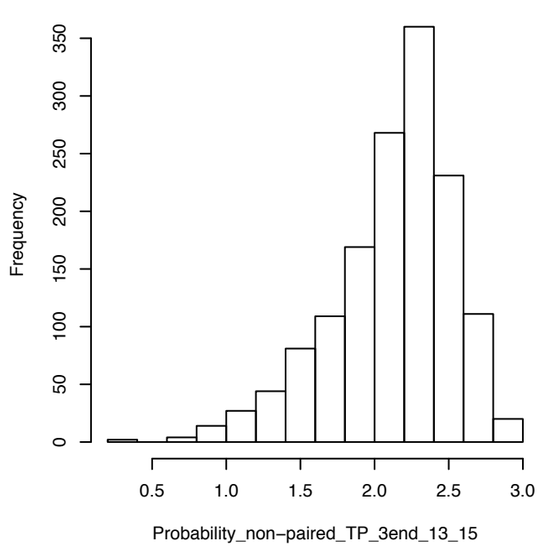
50
Probability_non−paired_TP_3end_7_9



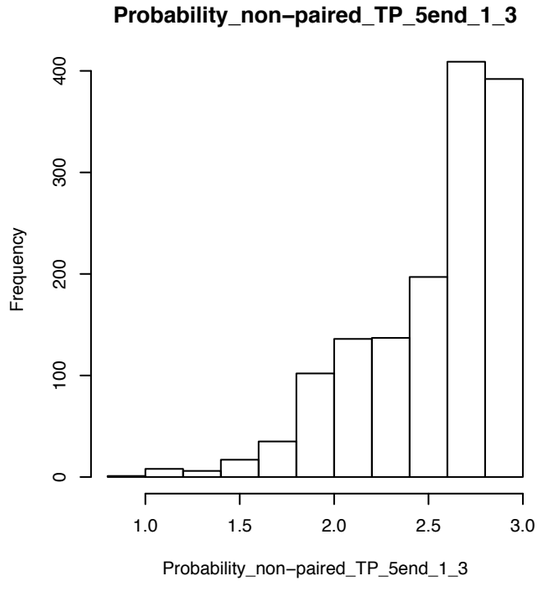
51
Probability_non−paired_TP_3end_10_12



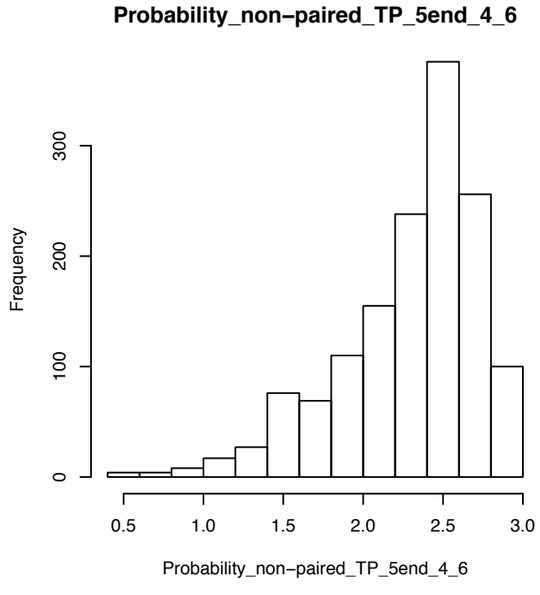
52
Probability_non−paired_TP_3end_13_15



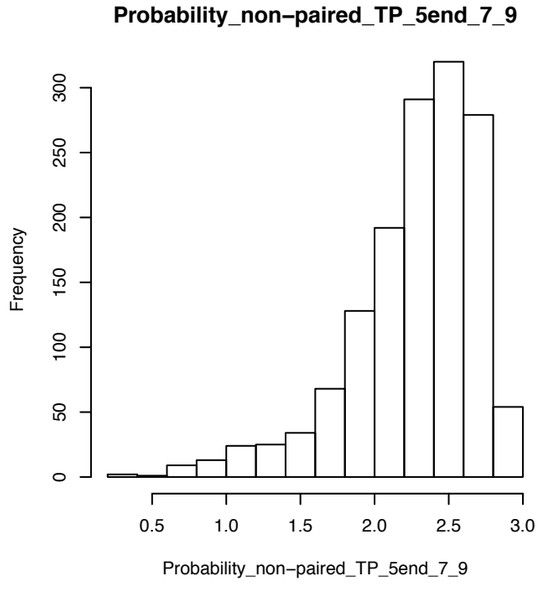
53
Probability_non−paired_TP_5end_1_3



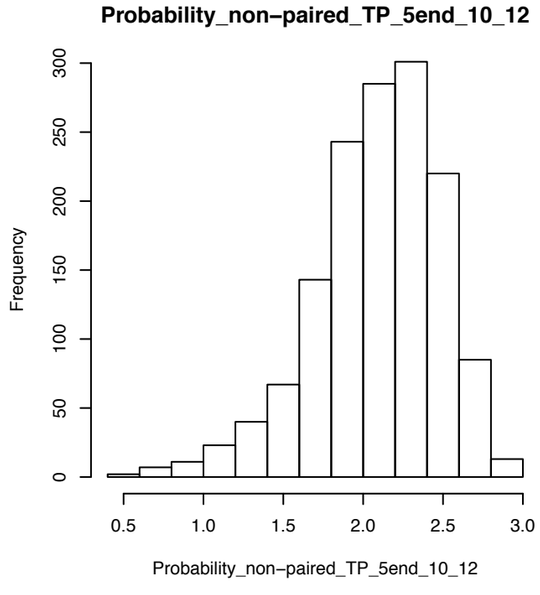
54
Probability_non−paired_TP_5end_4_6



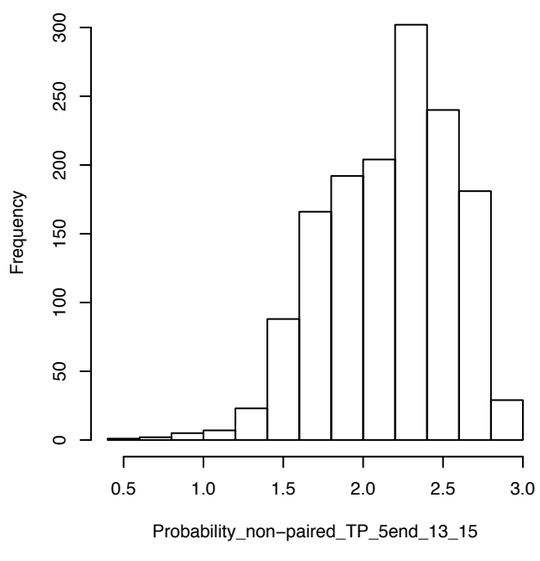
55
Probability_non−paired_TP_5end_7_9



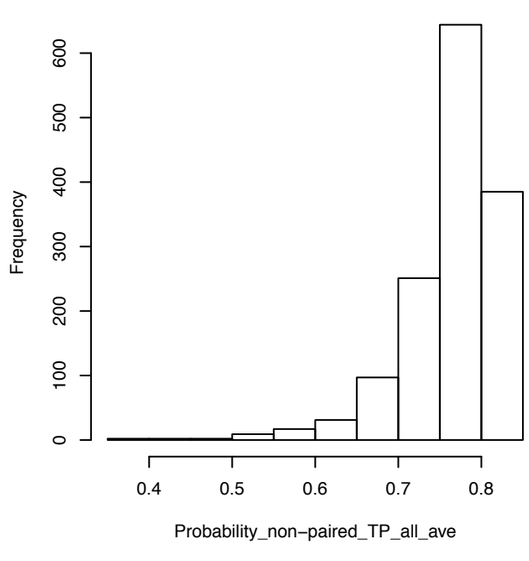
56
Probability_non−paired_TP_5end_10_12



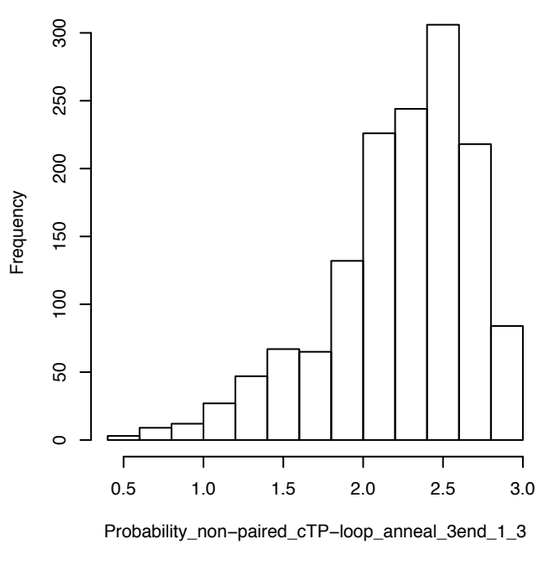
57
Probability_non−paired_TP_5end_13_15



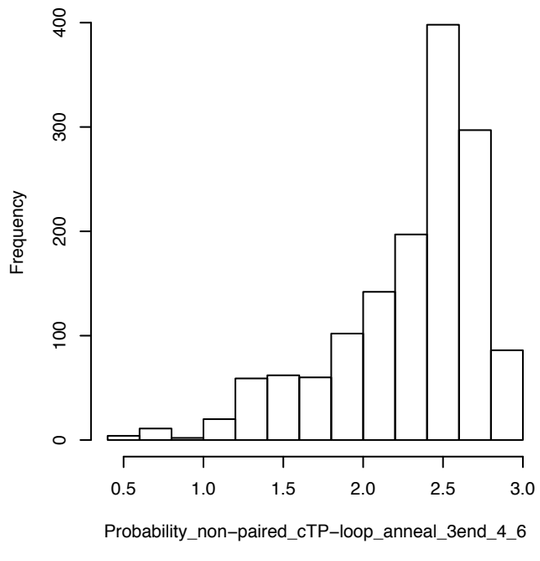
58
Probability_non−paired_TP_all_ave



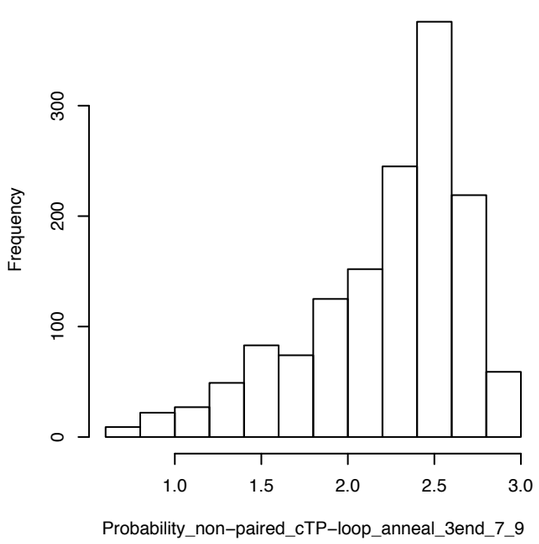
59
Probability_non−paired_cTP−loop_anneal_3end_1_3



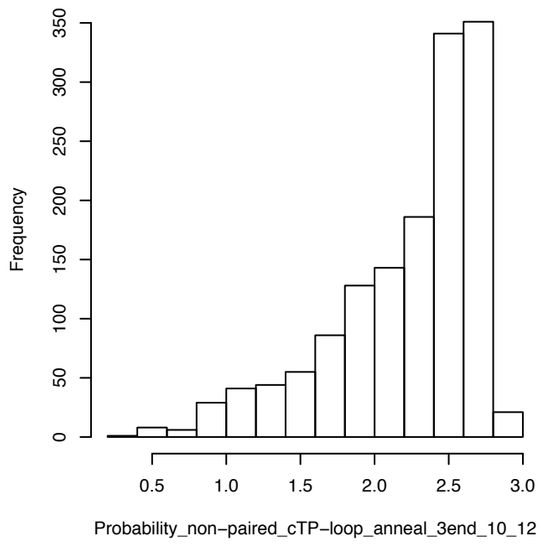
60
Probability_non−paired_cTP−loop_anneal_3end_4_6



61
Probability_non−paired_cTP−loop_anneal_3end_7_9



62
Probability_non−paired_cTP−loop_anneal_3end_10_12



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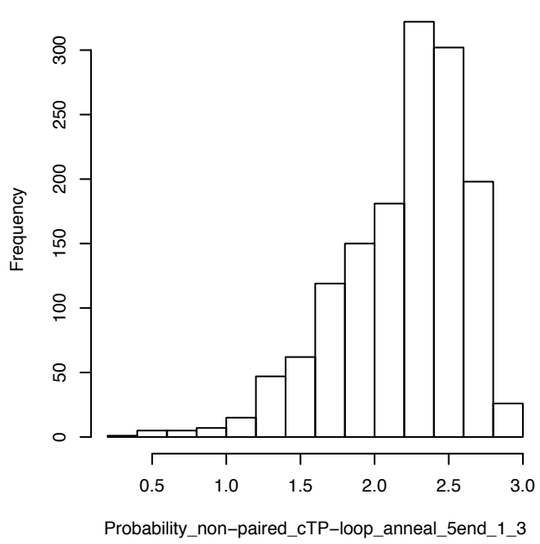
Probability_non−paired_cTP−loop_anneal_5end_1_3



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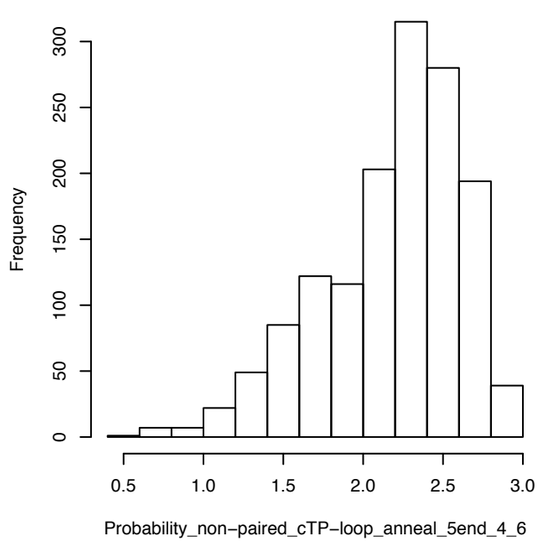
Probability_non−paired_cTP−loop_anneal_5end_4_6



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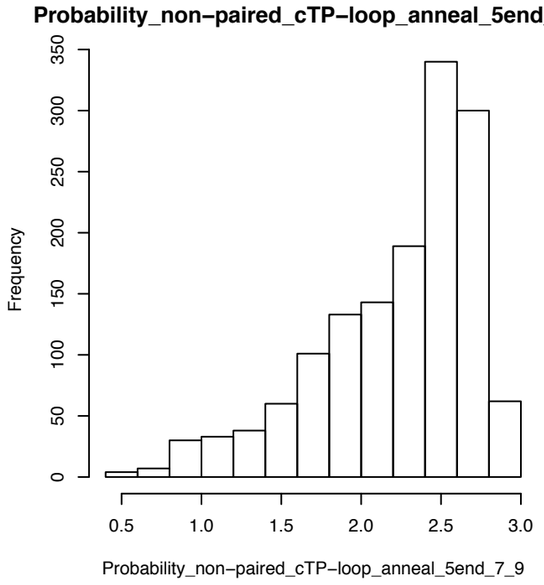
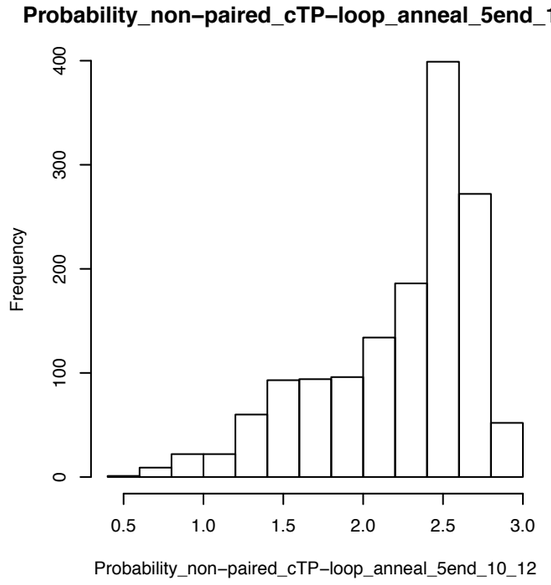
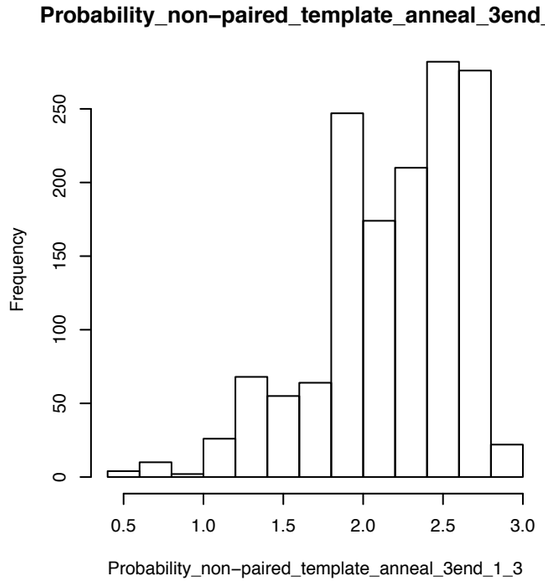
Probability_non−paired_cTP−loop_anneal_5end_7_9



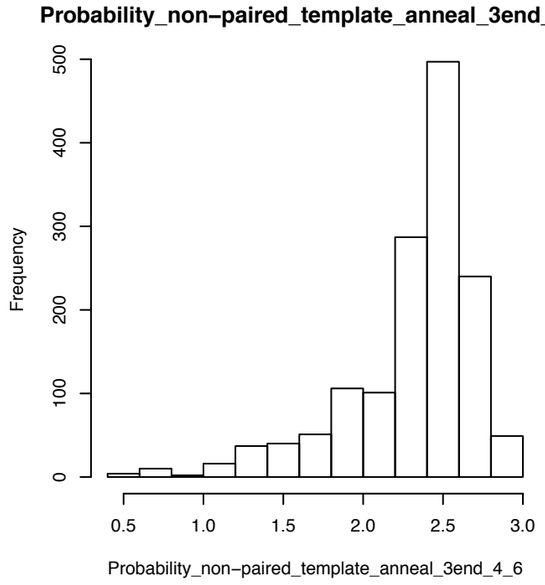
66
Probability_non−paired_cTP−loop_anneal_5end_10_12



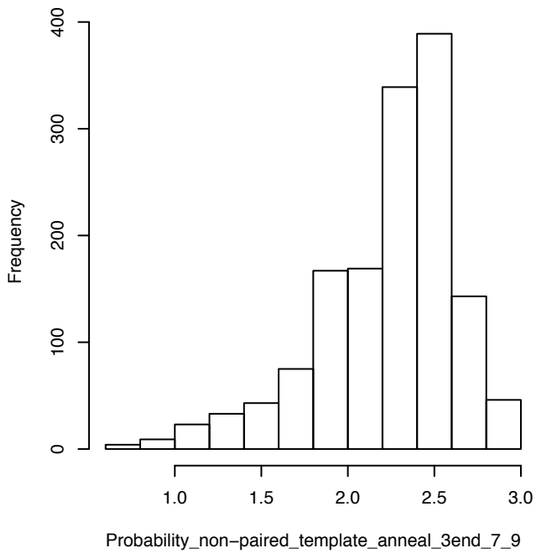
67
Probability_non−paired_template_anneal_3end_1_3



68
Probability_non−paired_template_anneal_3end_4_6



69
Probability_non−paired_template_anneal_3end_7_9



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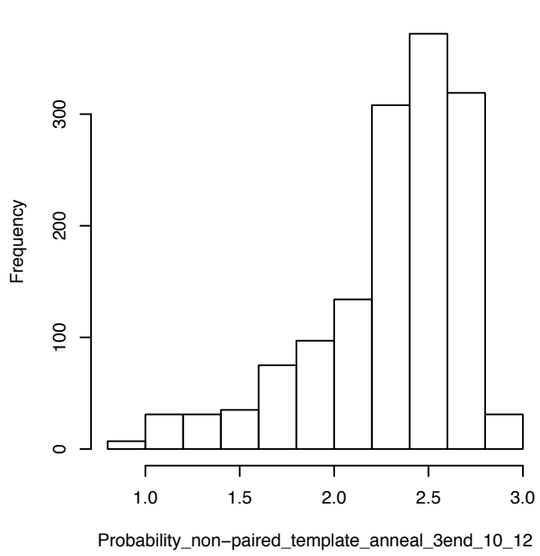
Probability_non−paired_template_anneal_3end_10_12



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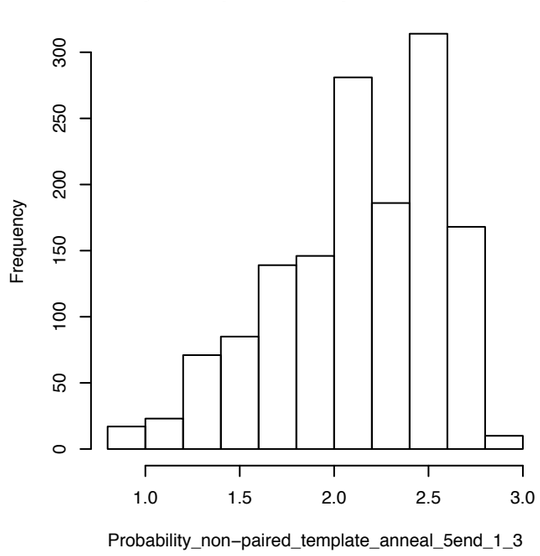
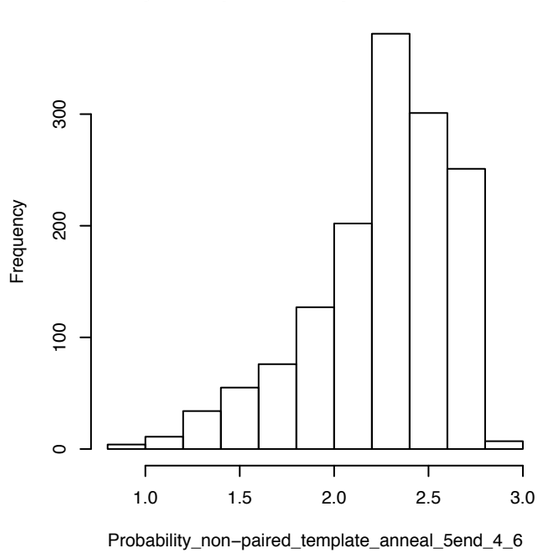
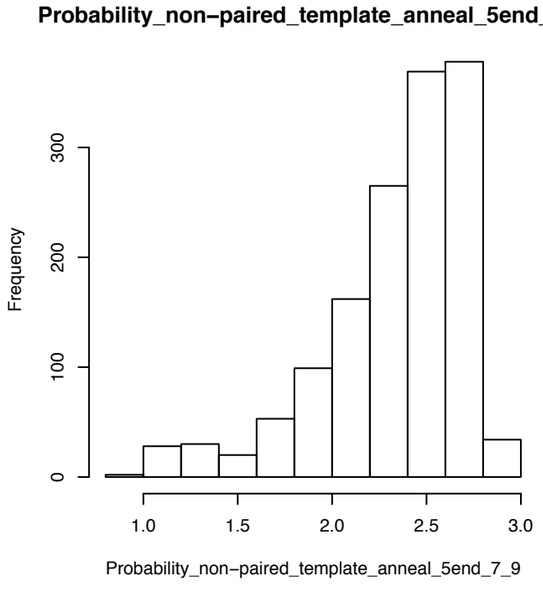
Probability_non−paired_template_anneal_5end_1_3



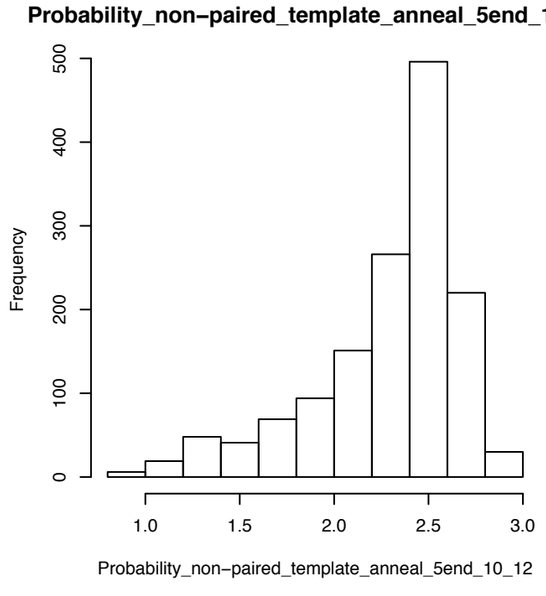
72
Probability_non−paired_template_anneal_5end_4_6



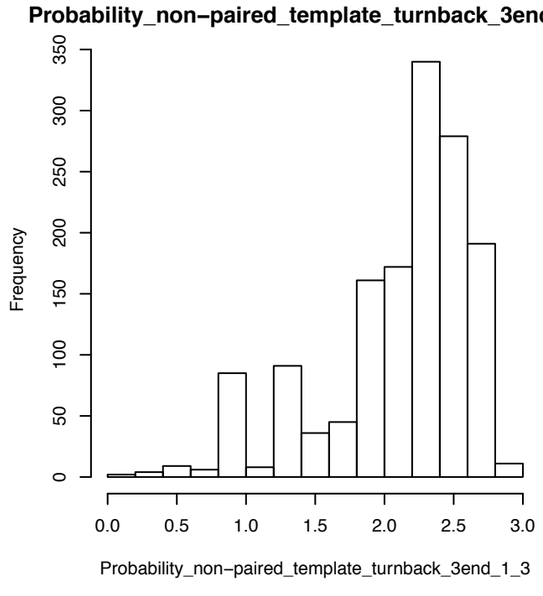
73
Probability_non−paired_template_anneal_5end_7_9



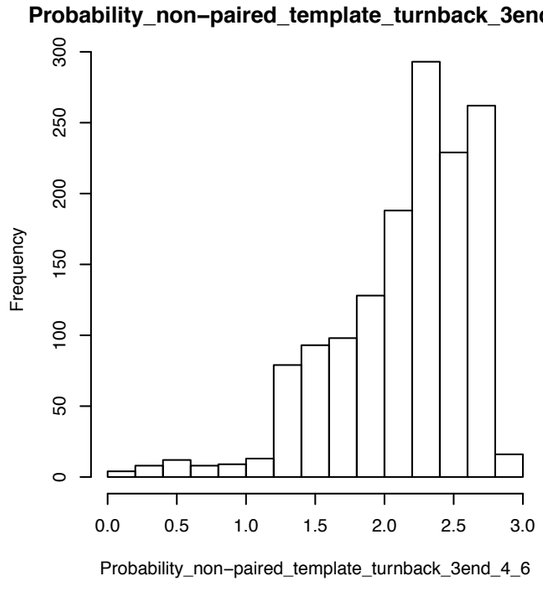
74
Probability_non−paired_template_anneal_5end_10_12



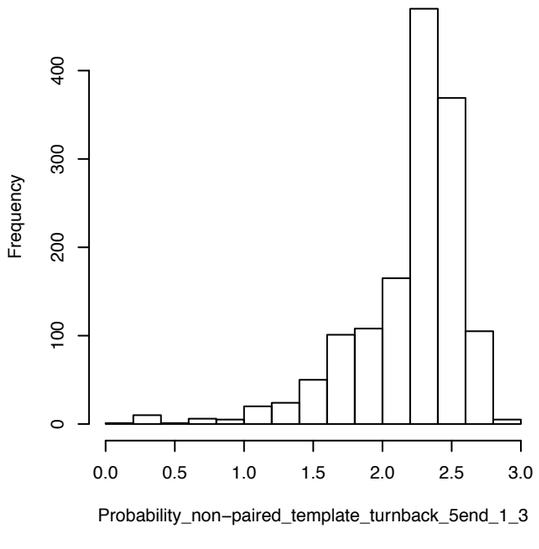
75
Probability_non−paired_template_turnback_3end_1_3



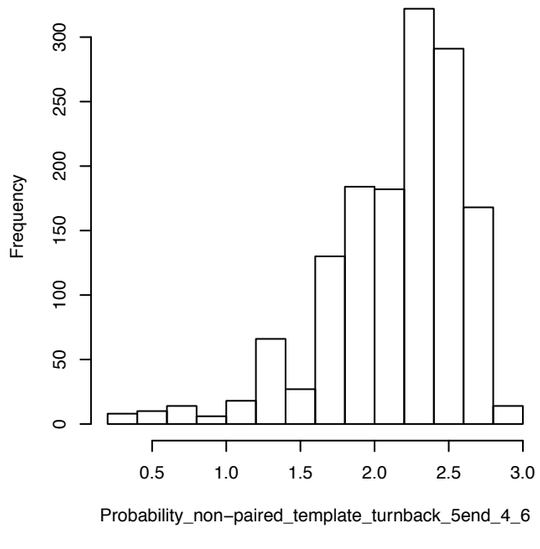
76
Probability_non−paired_template_turnback_3end_4_6



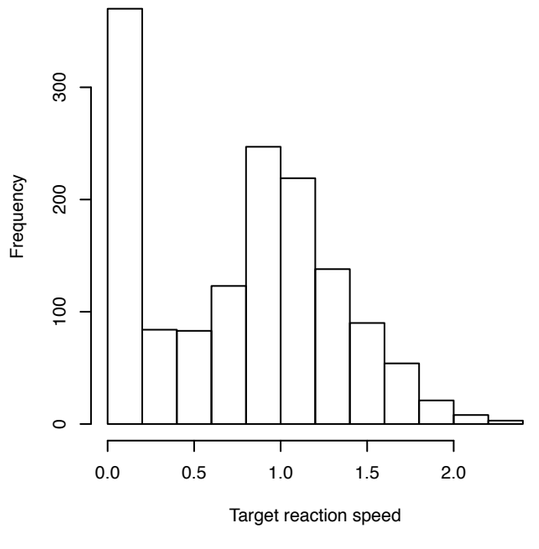
77
Probability_non−paired_template_turnback_5end_1_3



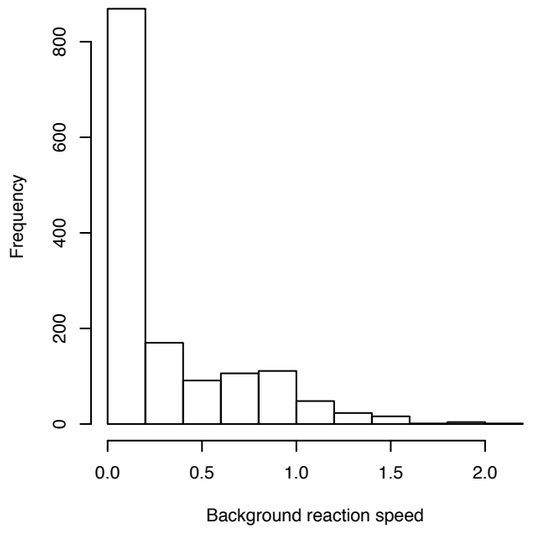
78
Probability_non−paired_template_turnback_5end_4_6



Target reaction speed



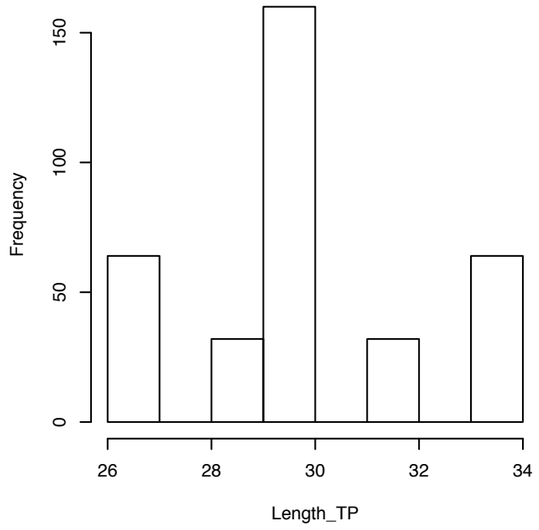
Background reaction speed



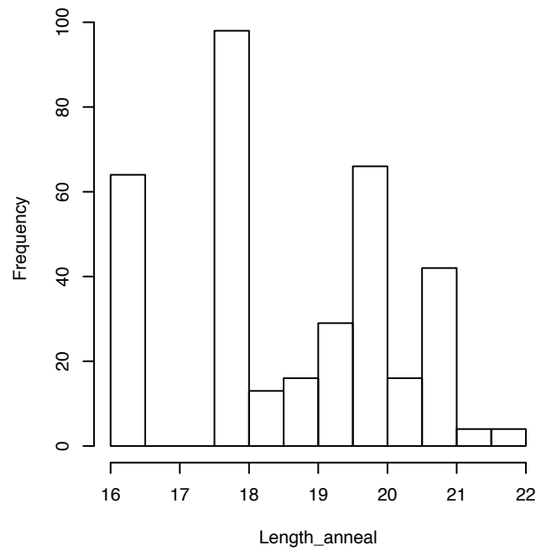
Supplemental Fig S4. The distributions of all parameters in the Test data set of the TP-TP system.

The distributions of 78 parameters examined in this study (see Supplemental Table S1 for details) and the observed reaction speeds in the test data. The test data set was comprised of 6 target regions with 100 TPs, and 352 TP-TP combinations assayed under the Aac reaction conditions. The numbers on top of the figures correspond to the numbers in Supplemental Table S1.

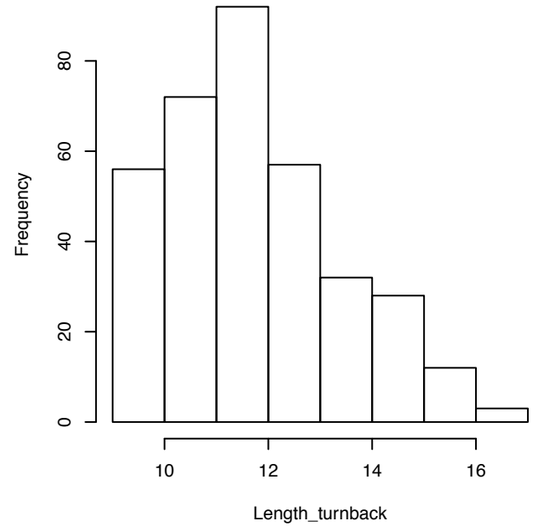
1
Length_TP



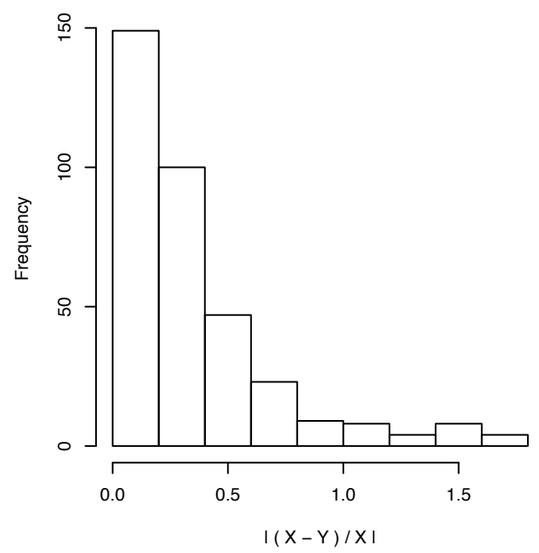
2
Length_anneal



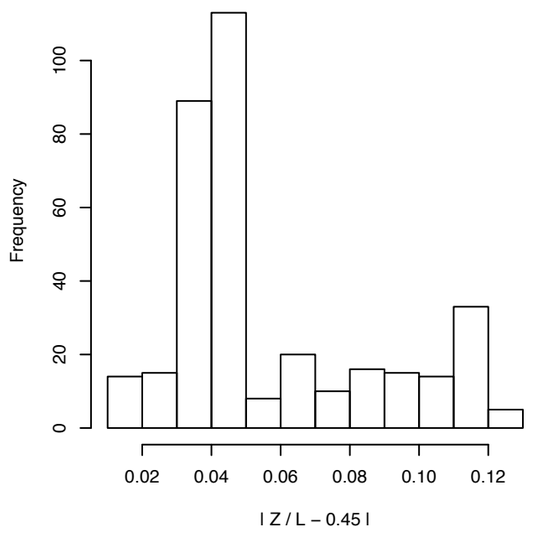
3
Length_turnback



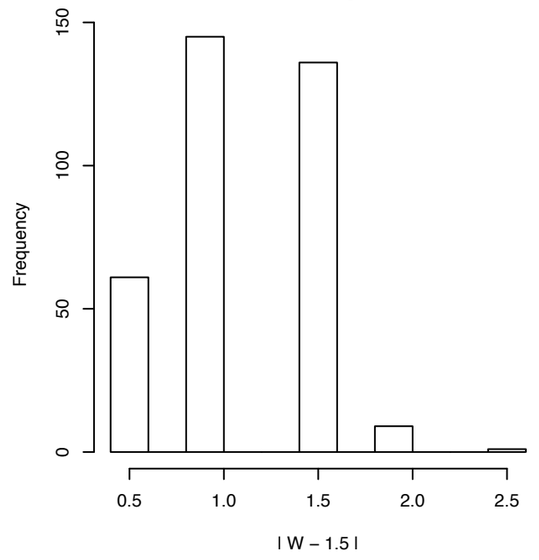
4
I (X – Y) / X I



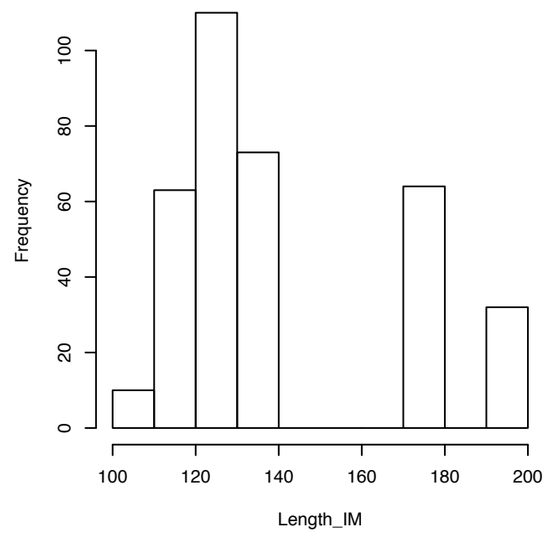
5
I Z / L – 0.45 I



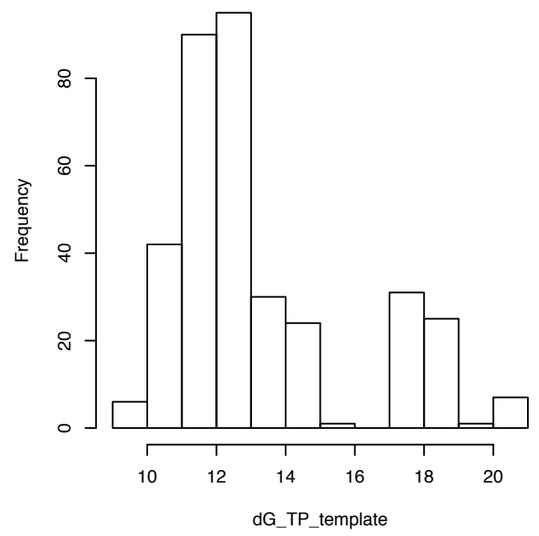
6
I W – 1.5 I



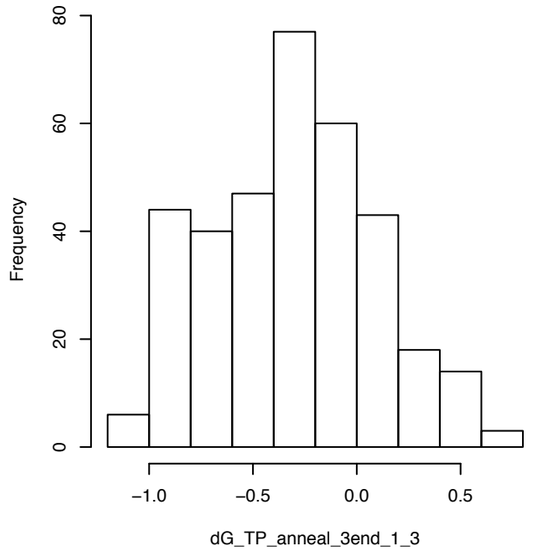
7
Length_IM



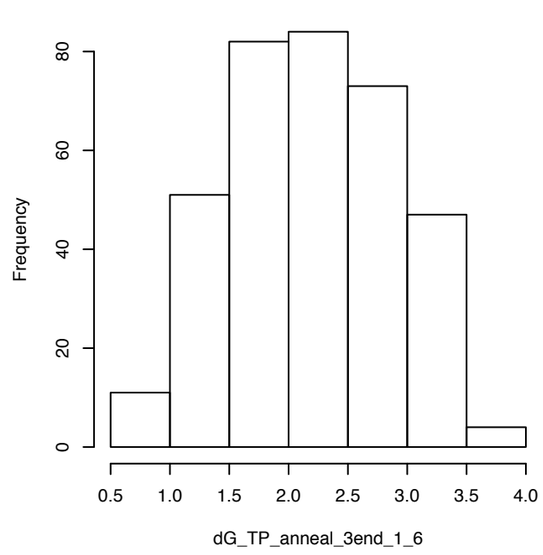
8
dG_TP_template



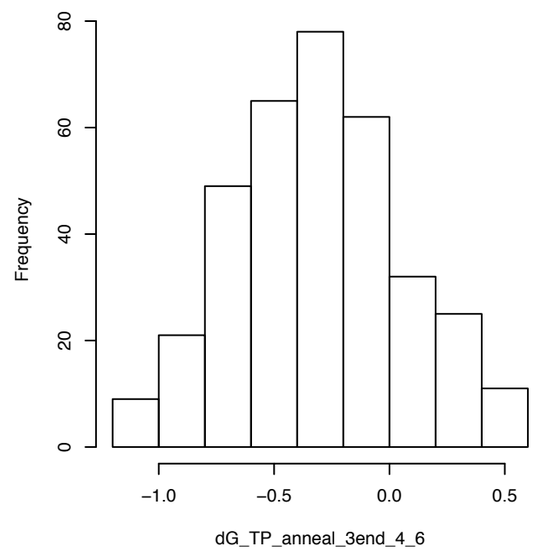
9
dG_TP_anneal_3end_1_3



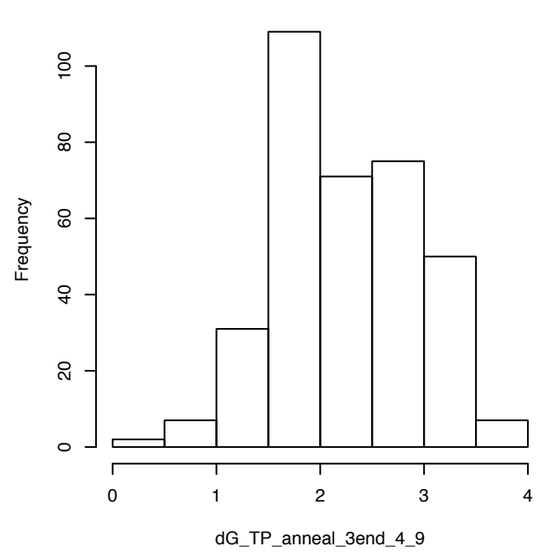
10
dG_TP_anneal_3end_1_6



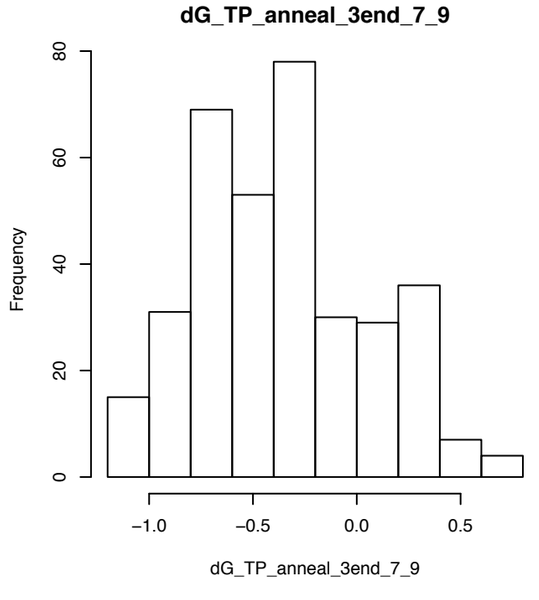
11
dG_TP_anneal_3end_4_6



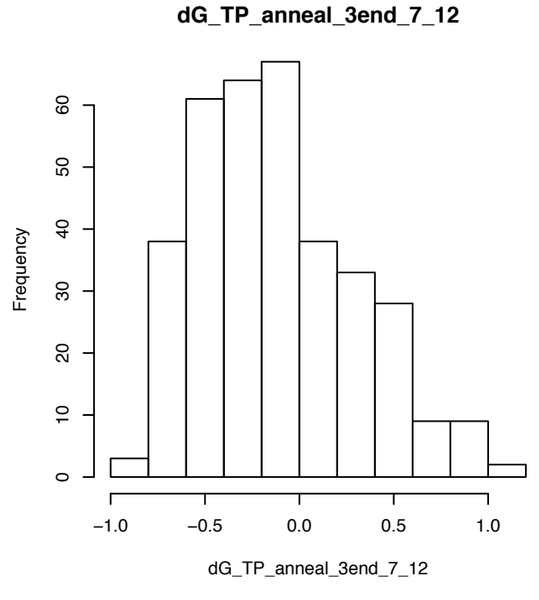
12
dG_TP_anneal_3end_4_9



13
dG_TP_anneal_3end_7_9



14
dG_TP_anneal_3end_7_12



15
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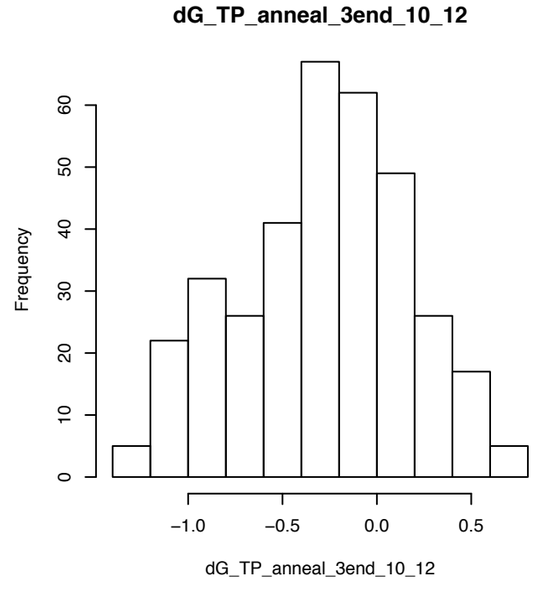
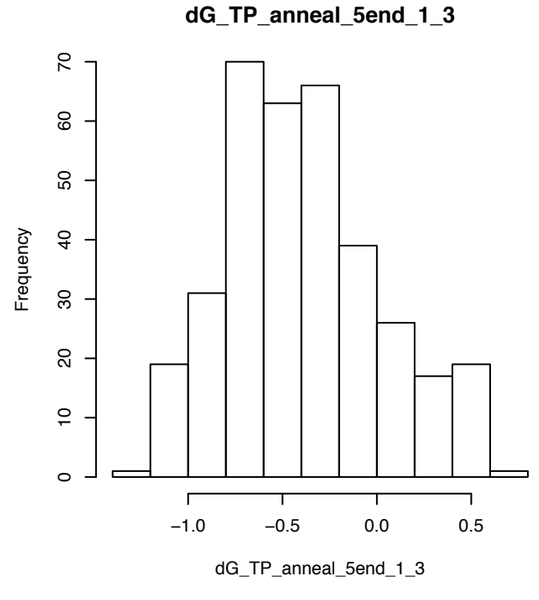
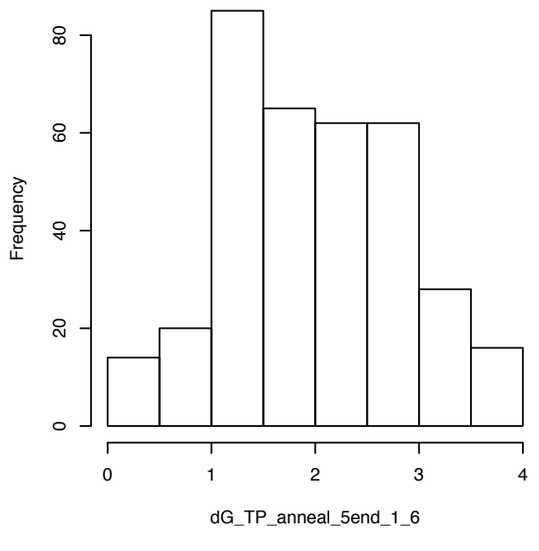


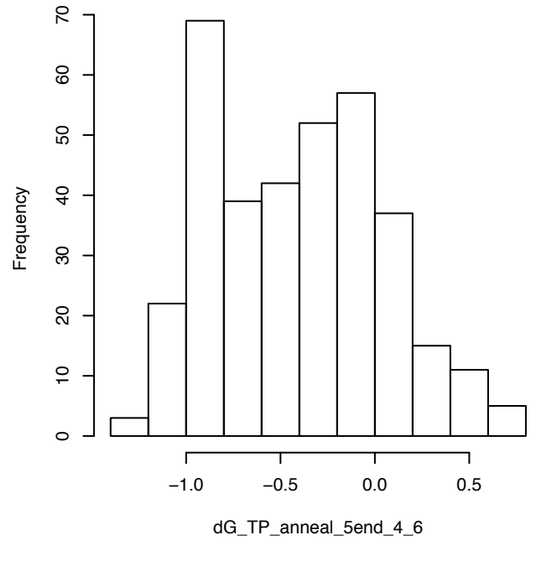
16
dG_TP_anneal_5end_1_3



17
dG_TP_anneal_5end_1_6



18
dG_TP_anneal_5end_4_6



19
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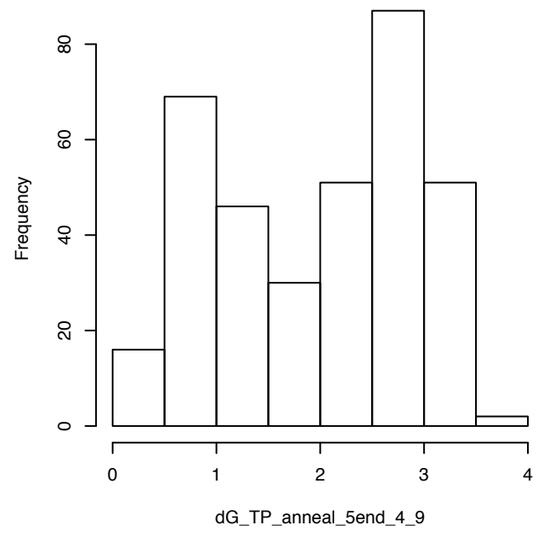
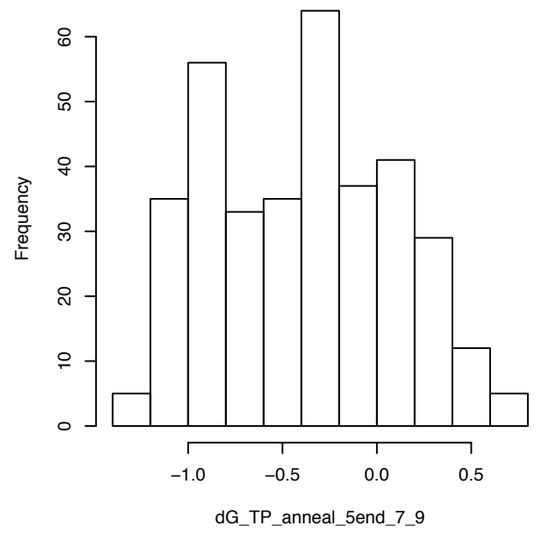
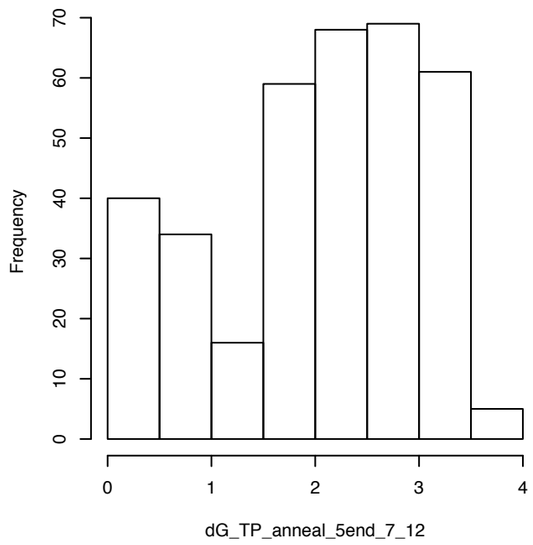


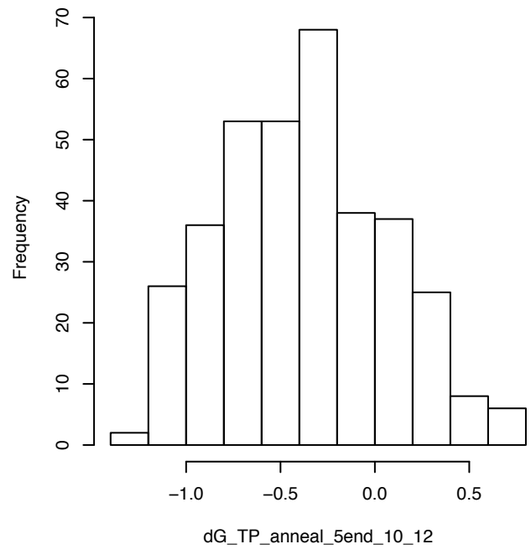
20
dG_TP_anneal_5end_7_9



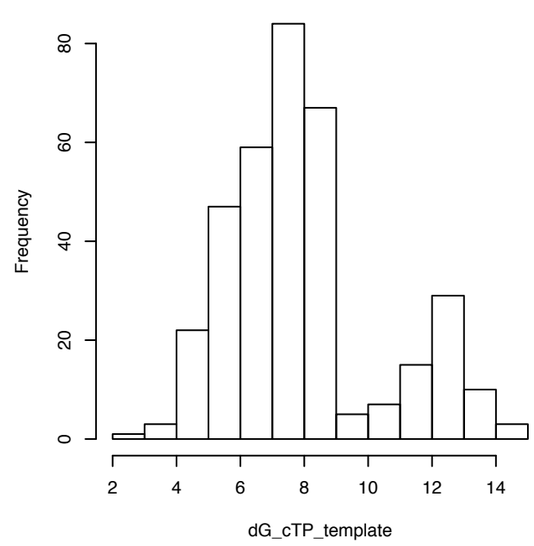
21
dG_TP_anneal_5end_7_12



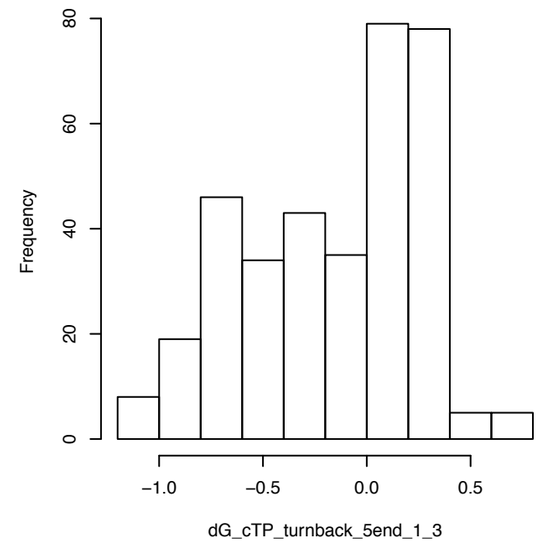
22
dG_TP_anneal_5end_10_12



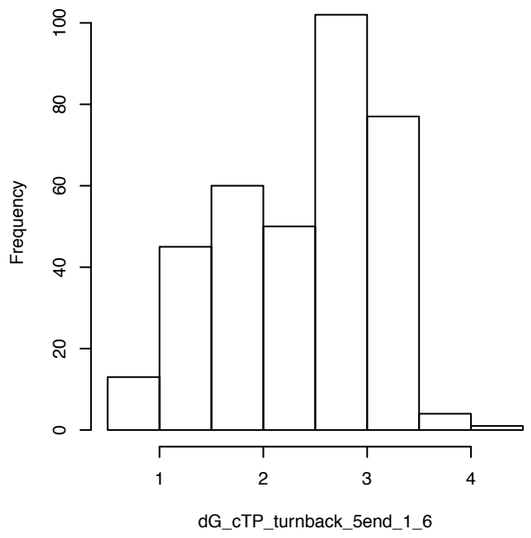
23
dG_cTP_template



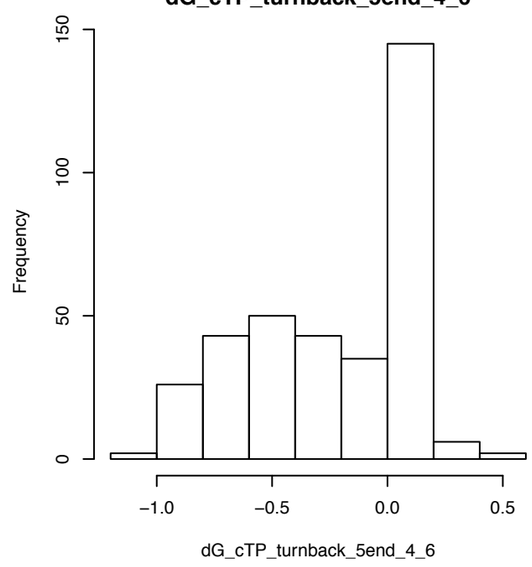
24
dG_cTP_turnback_5end_1_3



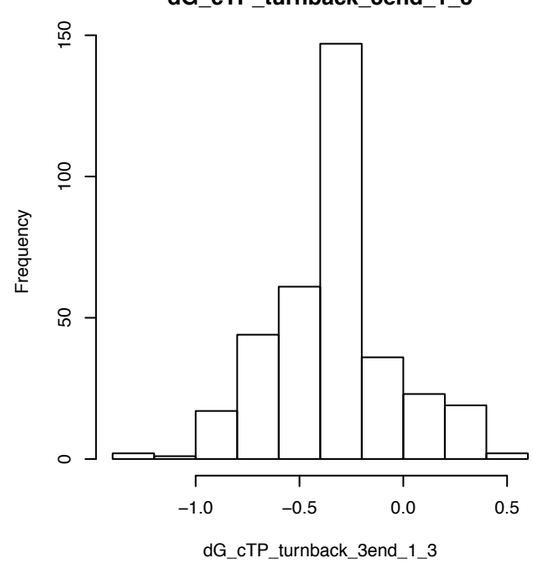
25
dG_cTP_turnback_5end_1_6



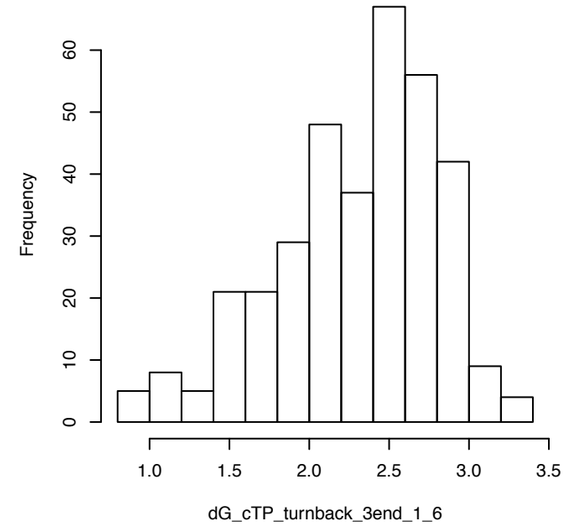
26
dG_cTP_turnback_5end_4_6



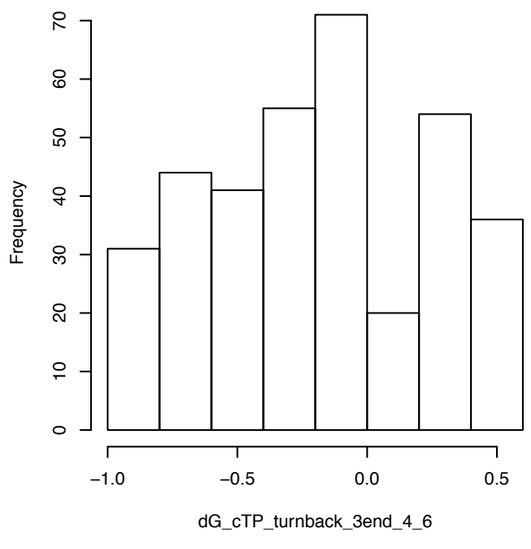
27
dG_cTP_turnback_3end_1_3



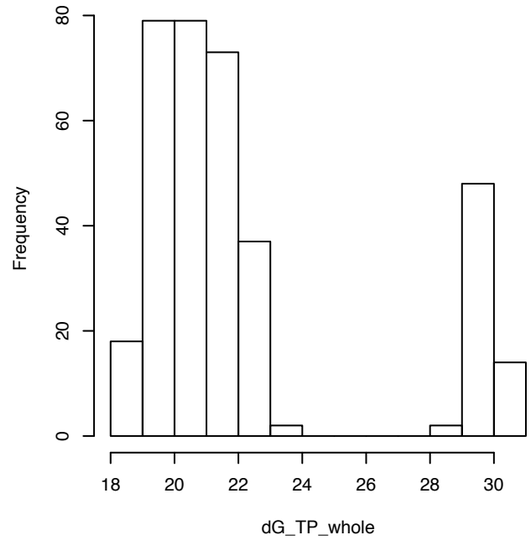
28
dG_cTP_turnback_3end_1_6



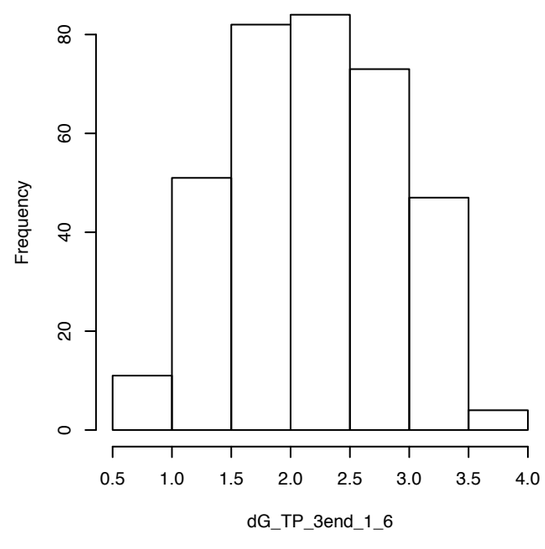
29
dG_cTP_turnback_3end_4_6



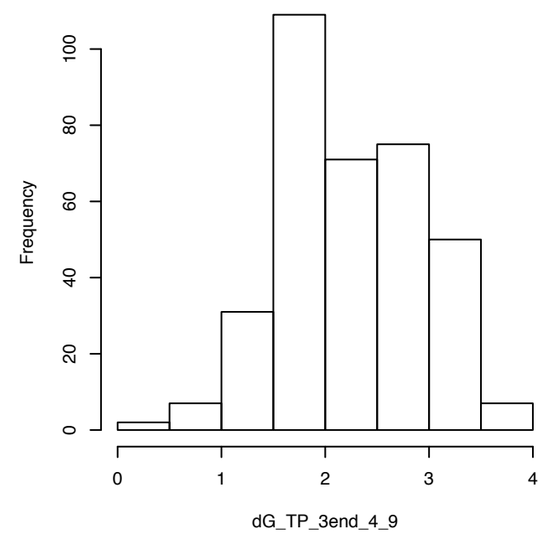
30
dG_TP_whole



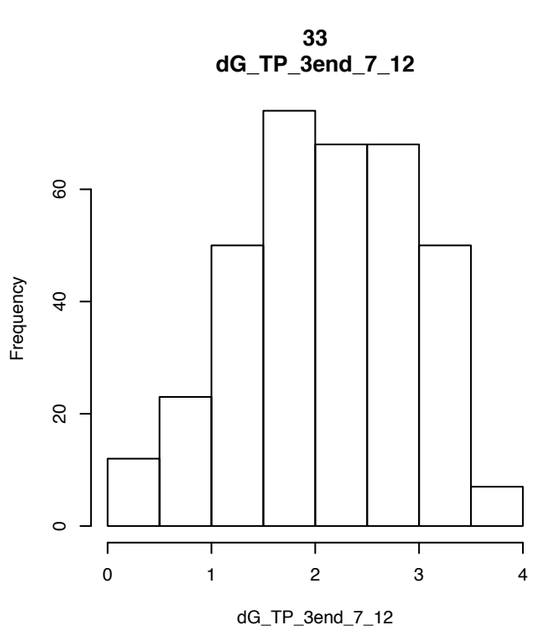
31
dG_TP_3end_1_6



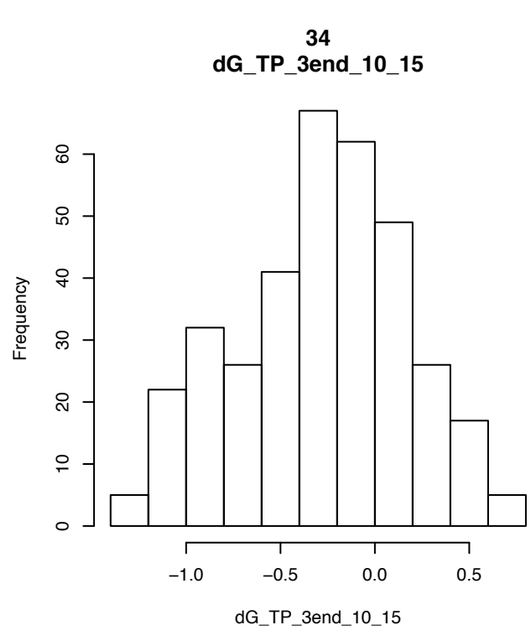
32
dG_TP_3end_4_9



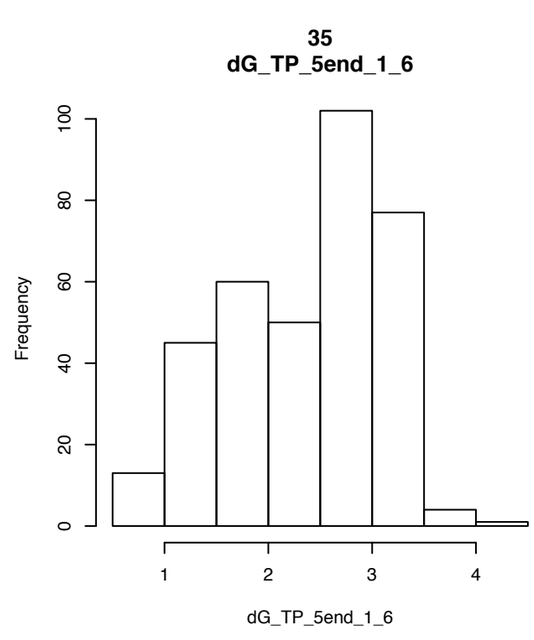
33
dG_TP_3end_7_12



34
dG_TP_3end_10_15



35
dG_TP_5end_1_6



36
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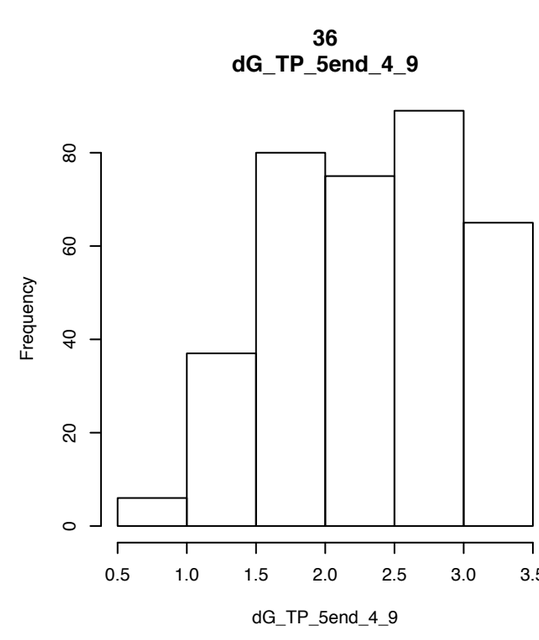
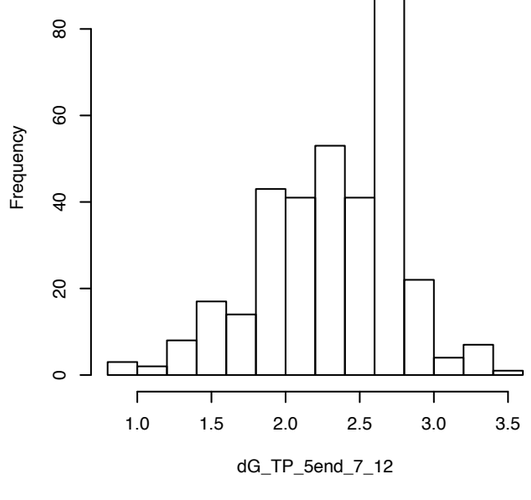
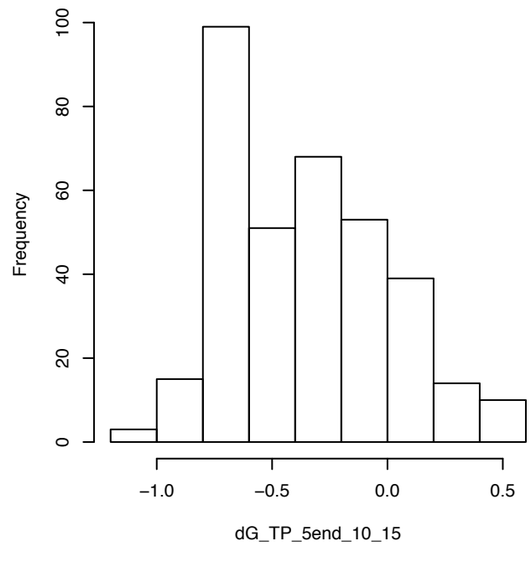


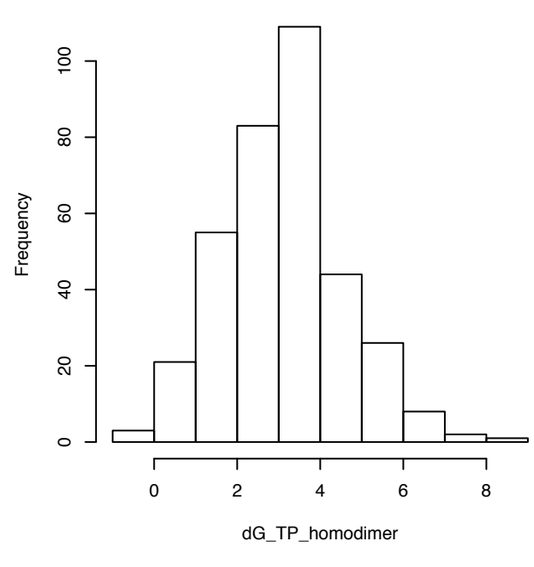
37
dG_TP_5end_7_12



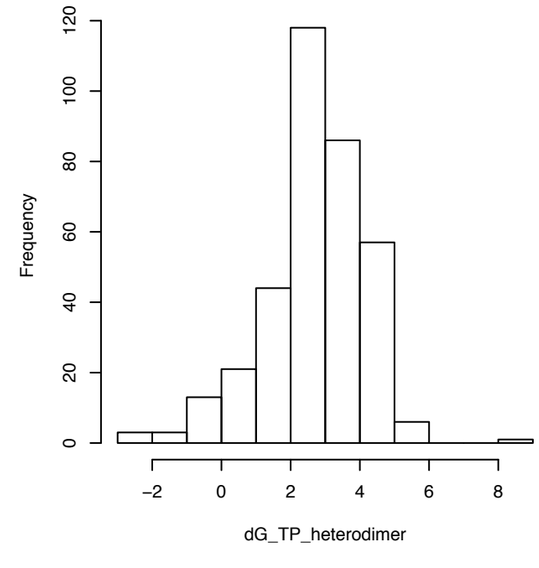
38
dG_TP_5end_10_15



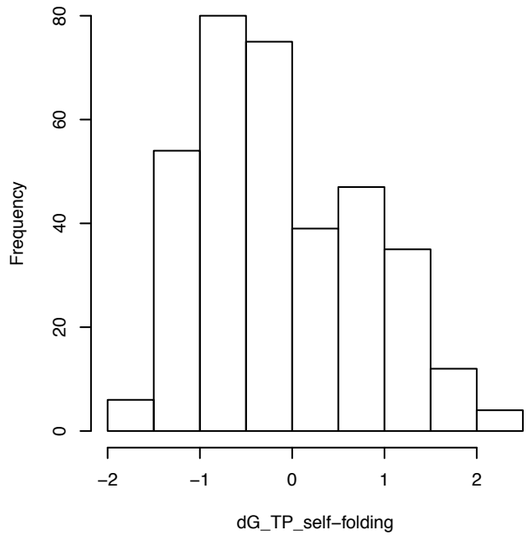
39
dG_TP_homodimer



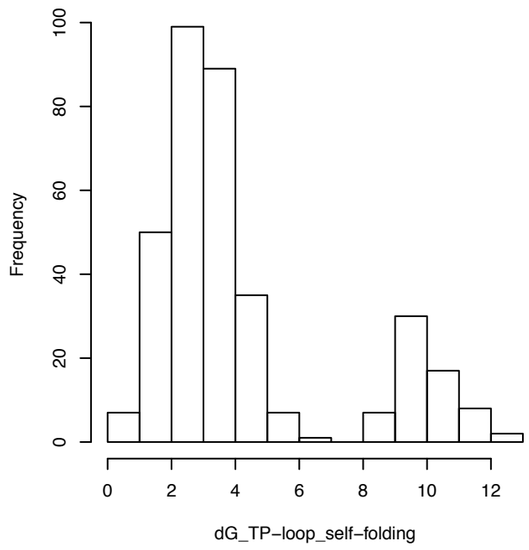
40
dG_TP_heterodimer



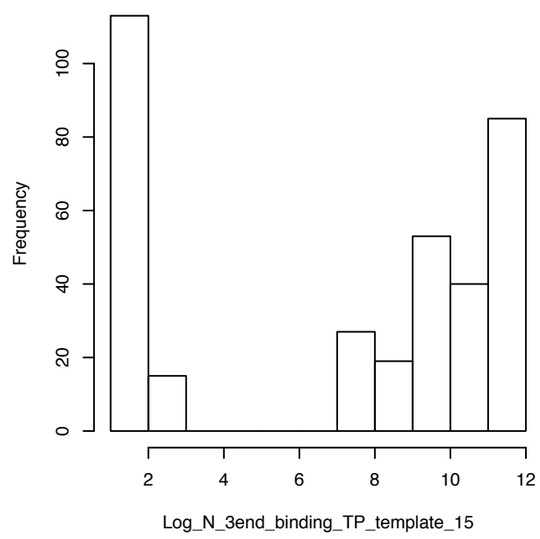
41
dG_TP_self−folding



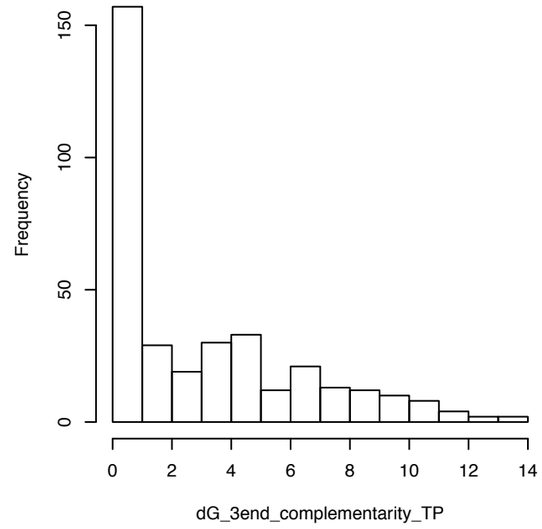
42
dG_TP−loop_self−folding



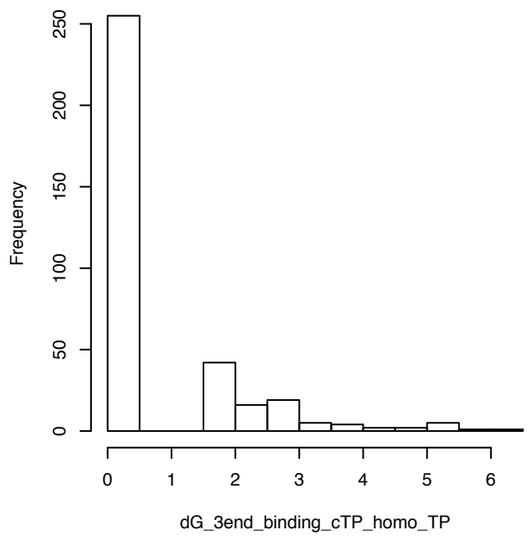
43
Log_N_3end_binding_TP_template_15



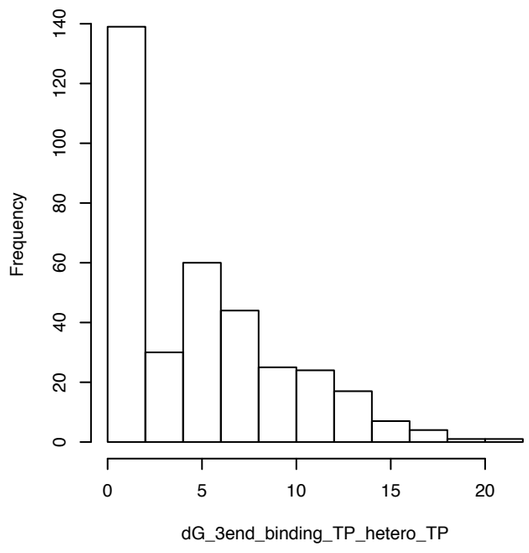
44
dG_3end_complementarity_TP



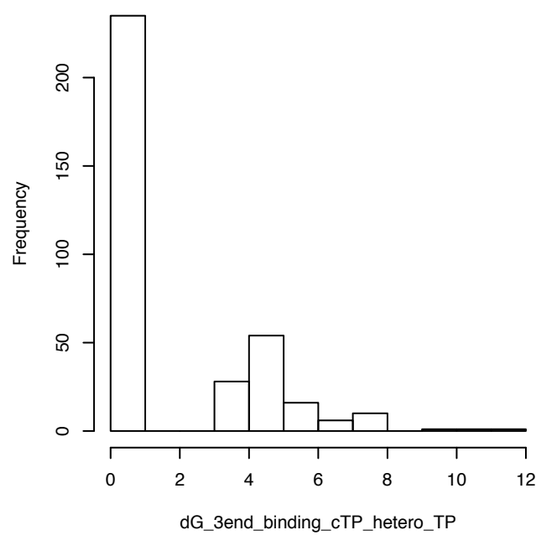
45
dG_3end_binding_cTP_homo_TP



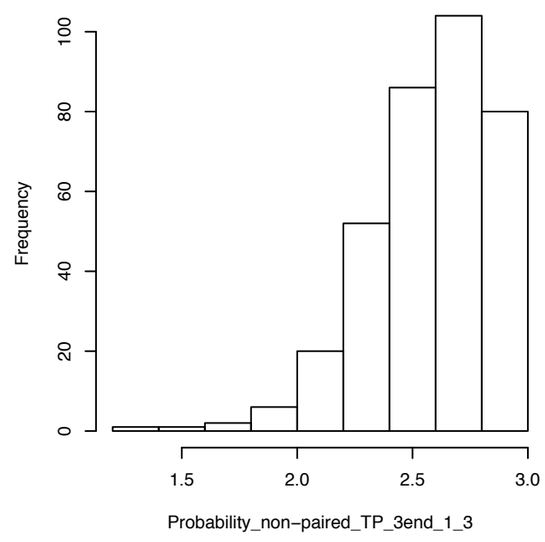
46
dG_3end_binding_TP_hetero_TP



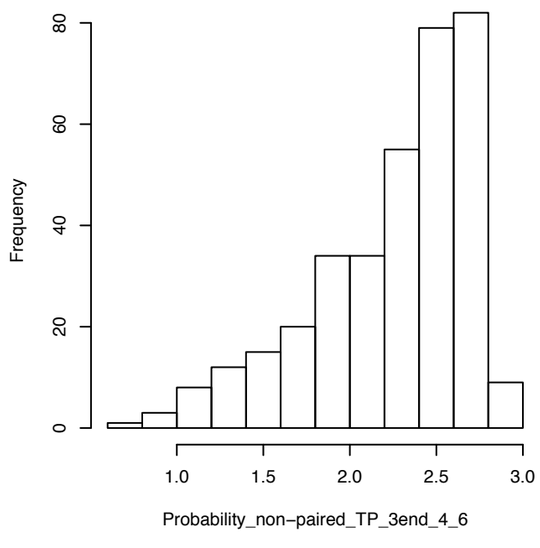
47
dG_3end_binding_cTP_hetero_TP



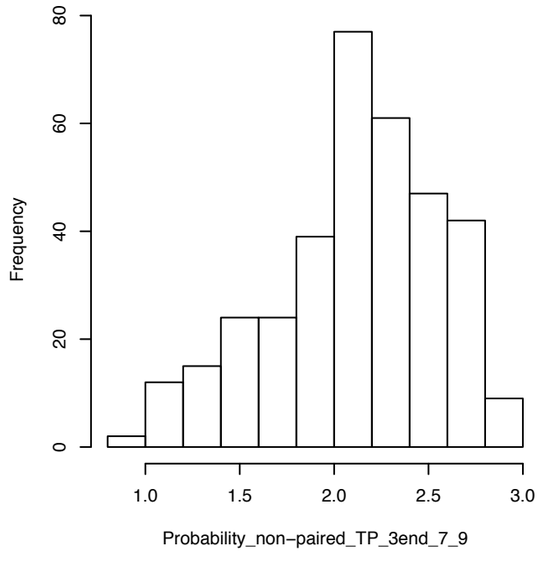
48
Probability_non−paired_TP_3end_1_3



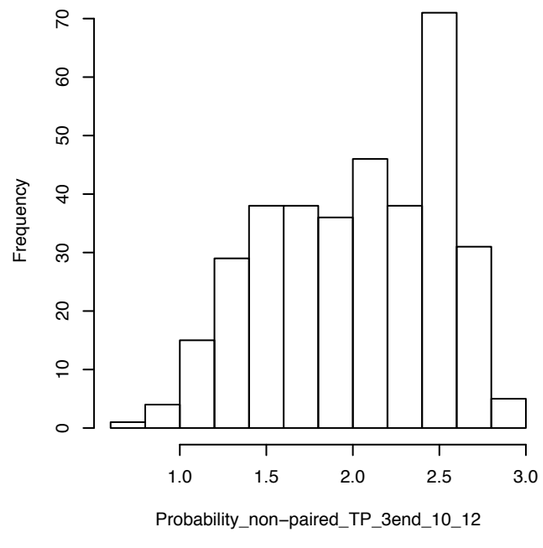
49
Probability_non−paired_TP_3end_4_6



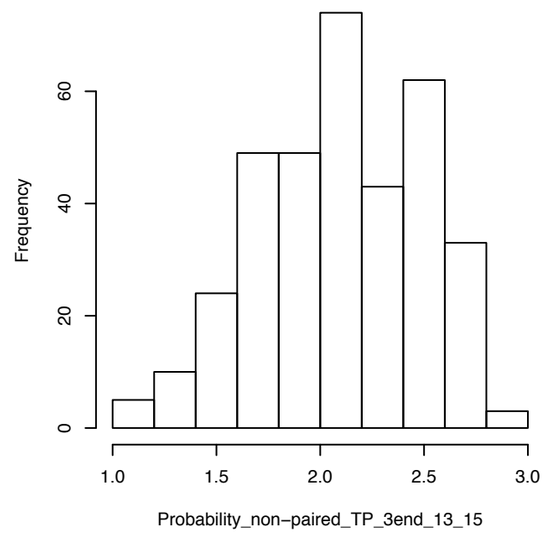
50
Probability_non−paired_TP_3end_7_9



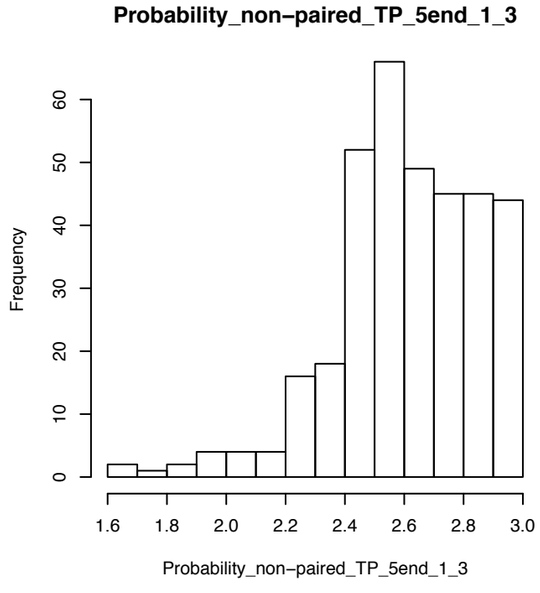
51
Probability_non−paired_TP_3end_10_12



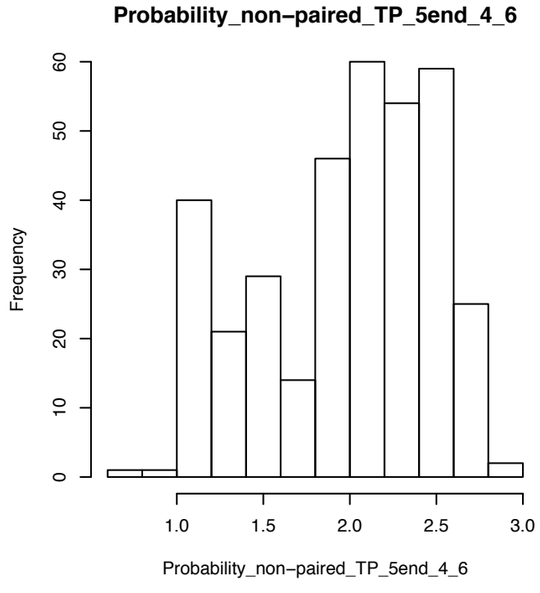
52
Probability_non−paired_TP_3end_13_15



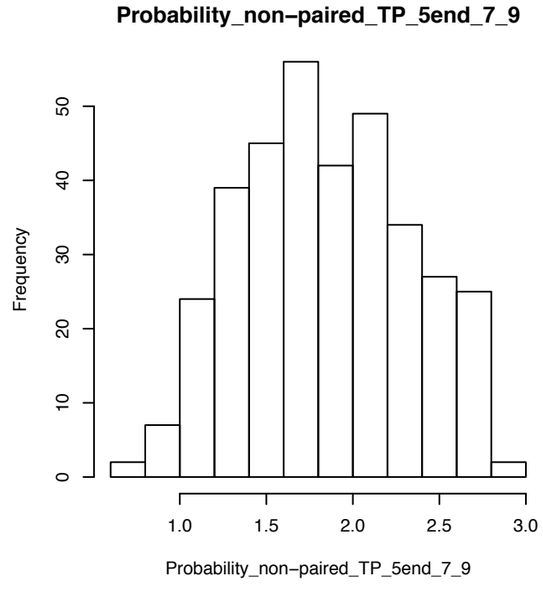
53
Probability_non−paired_TP_5end_1_3



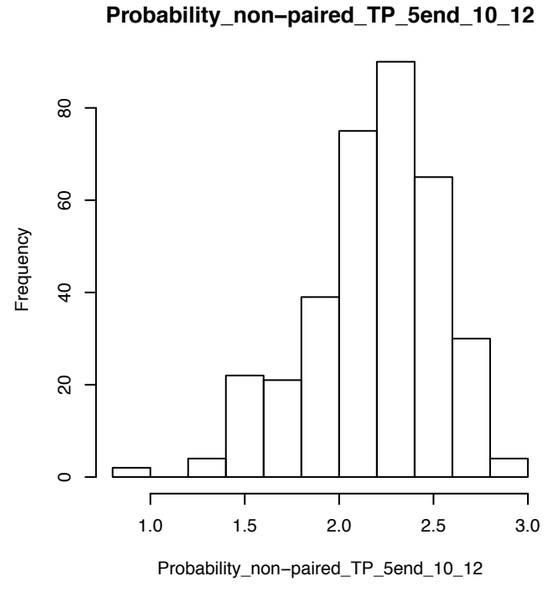
54
Probability_non−paired_TP_5end_4_6



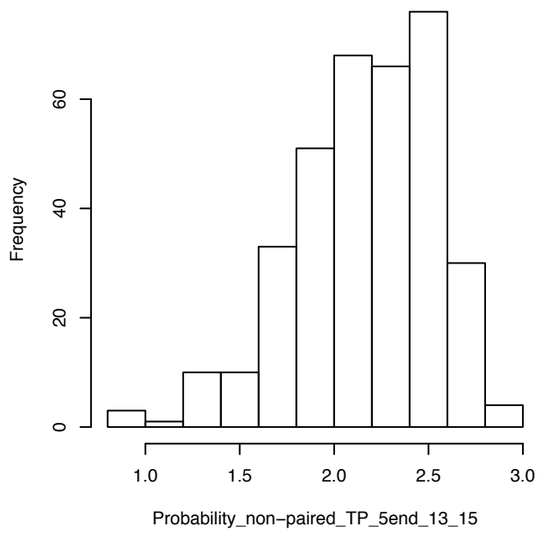
55
Probability_non−paired_TP_5end_7_9



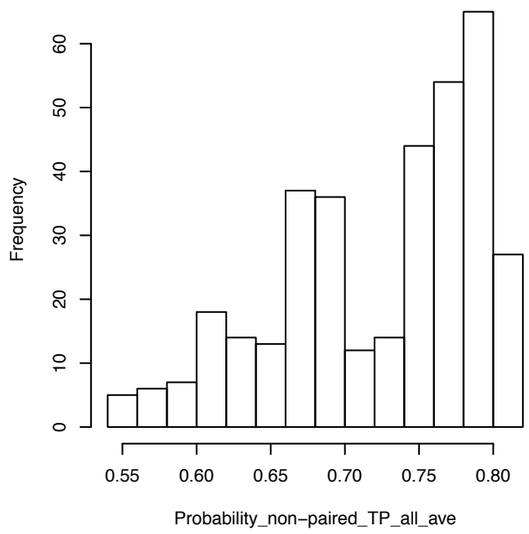
56
Probability_non−paired_TP_5end_10_12



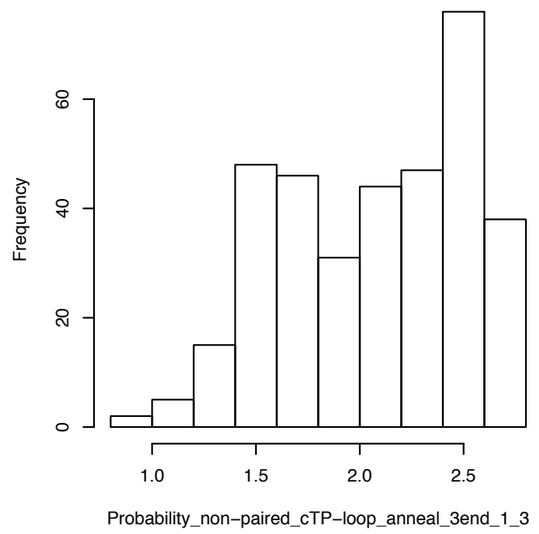
57
Probability_non−paired_TP_5end_13_15



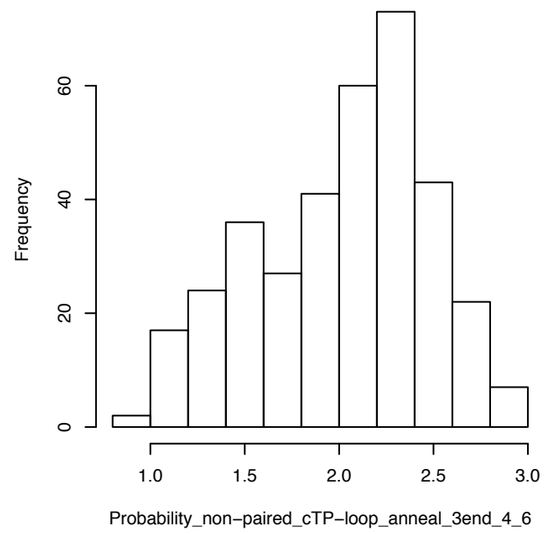
58
Probability_non−paired_TP_all_ave



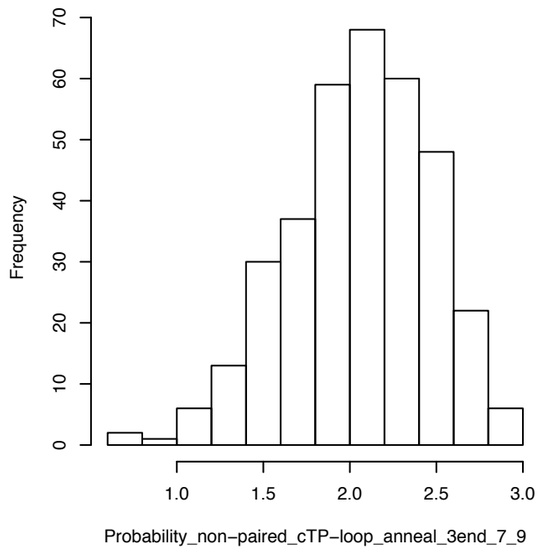
59
Probability_non−paired_cTP−loop_anneal_3end_1_3



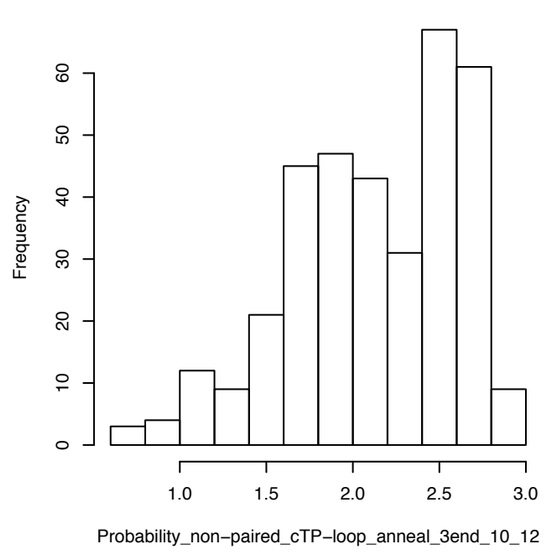
60
Probability_non−paired_cTP−loop_anneal_3end_4_6



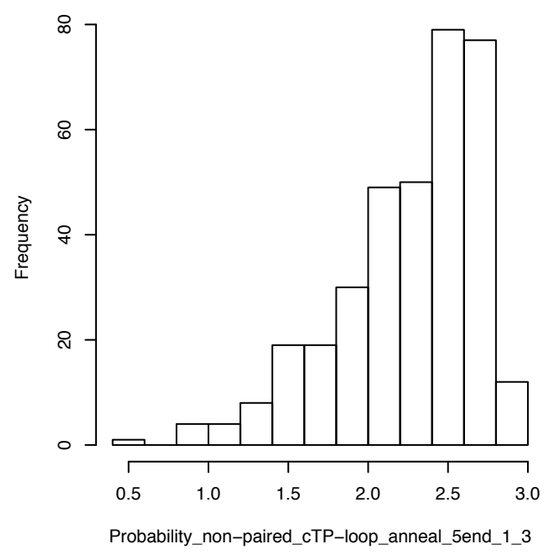
61
Probability_non−paired_cTP−loop_anneal_3end_7_9



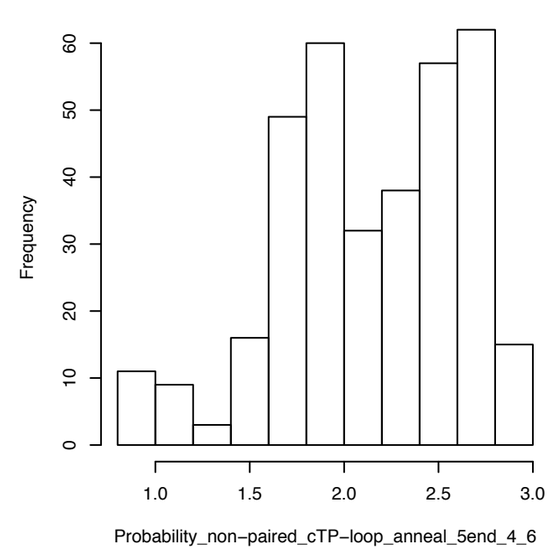
62
Probability_non−paired_cTP−loop_anneal_3end_10_12



63
Probability_non−paired_cTP−loop_anneal_5end_1_3



64
Probability_non−paired_cTP−loop_anneal_5end_4_6



65
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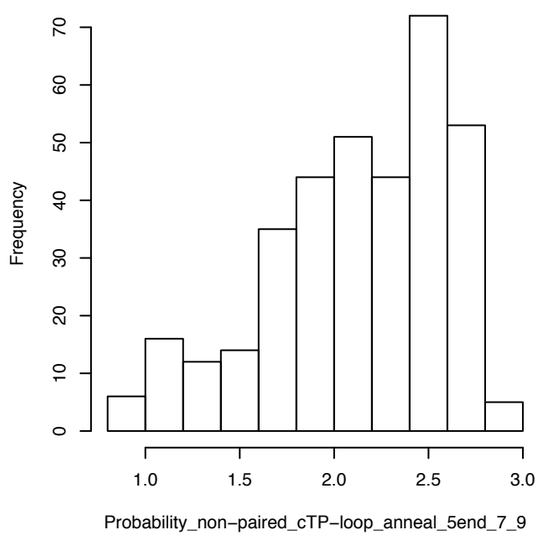

66
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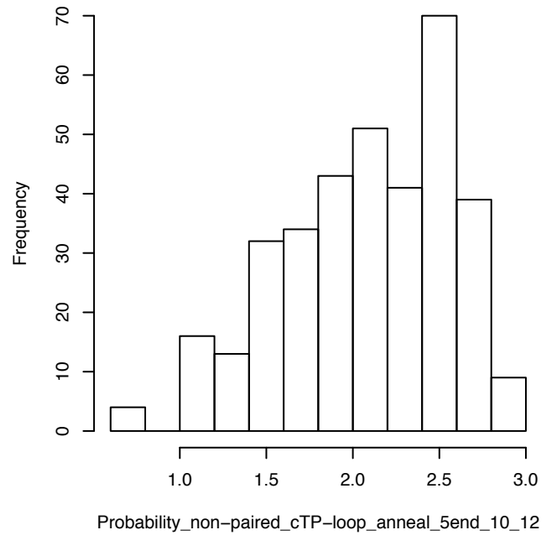
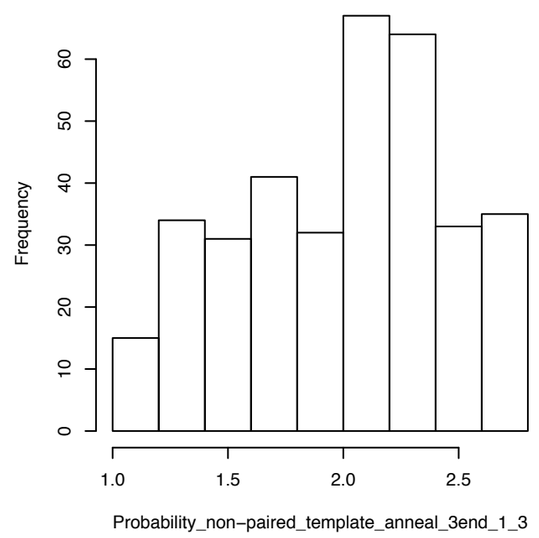
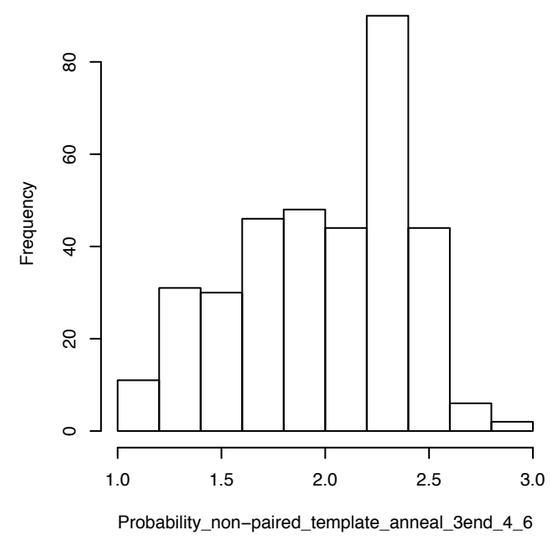


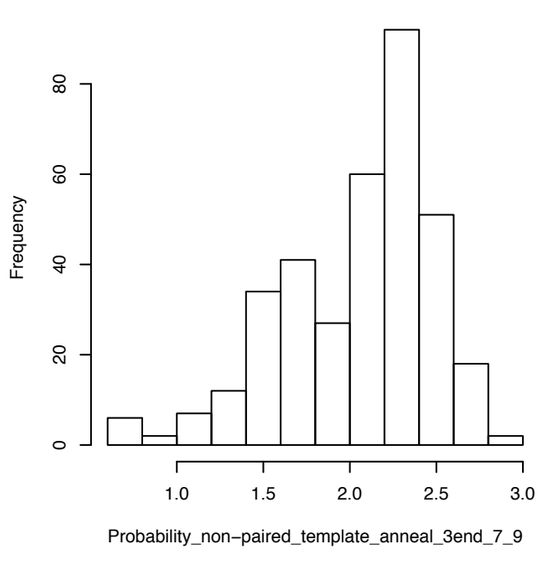
67
Probability_non−paired_template_anneal_3end_1_3



68
Probability_non−paired_template_anneal_3end_4_6



69
Probability_non−paired_template_anneal_3end_7_9



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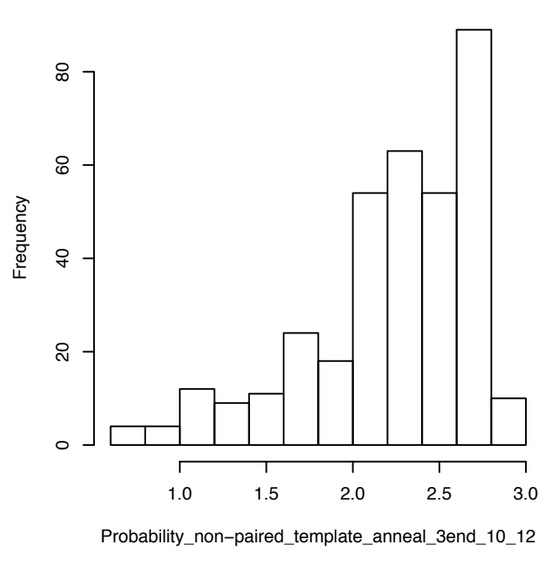

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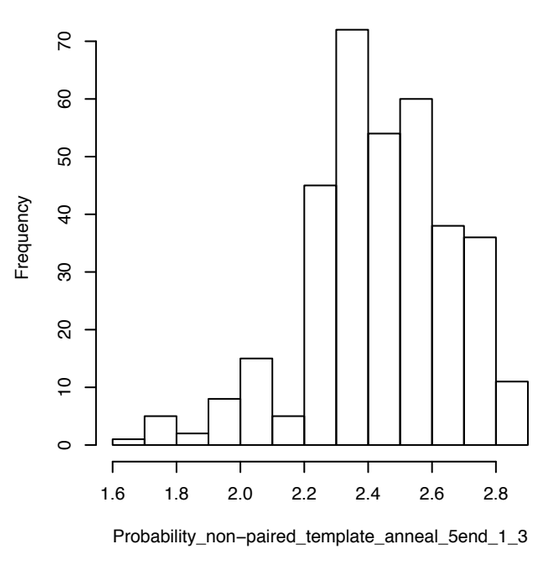

72
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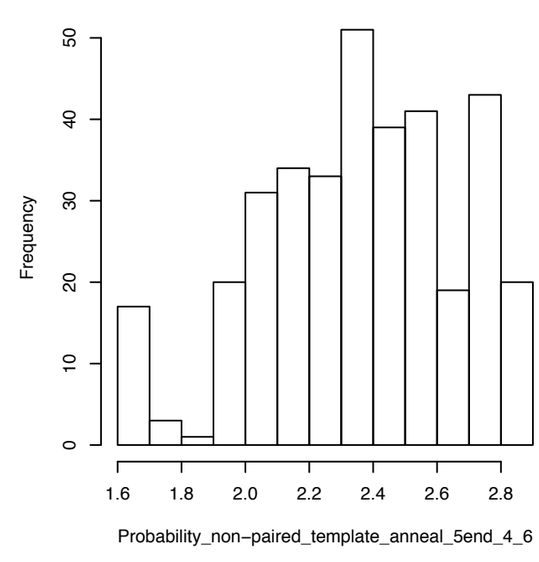

73
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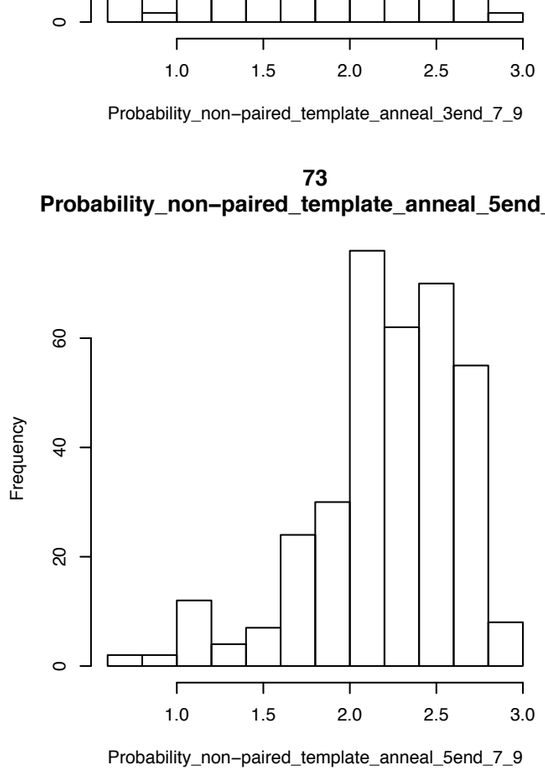

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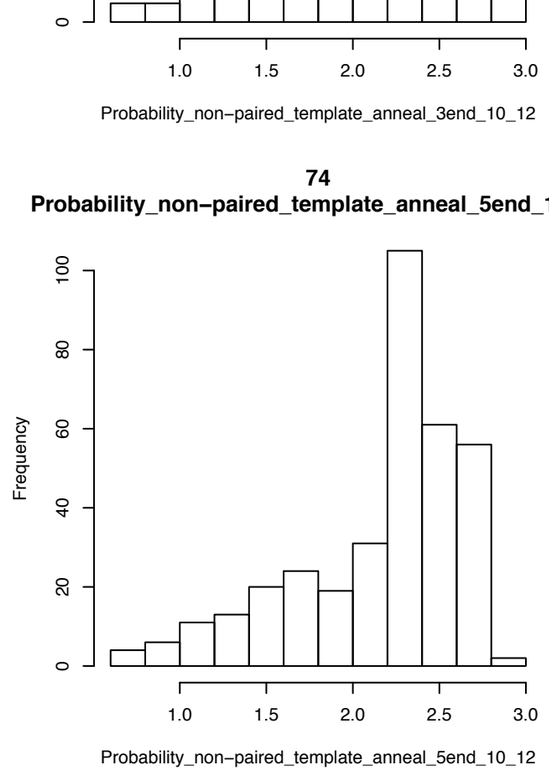
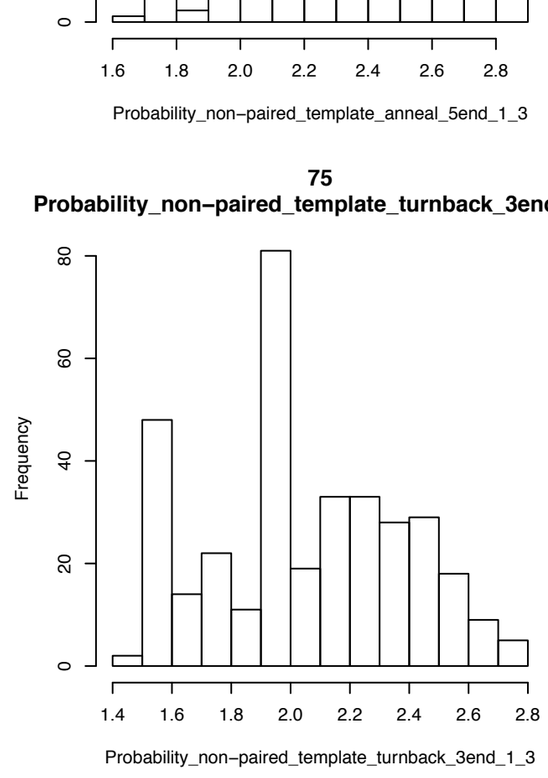
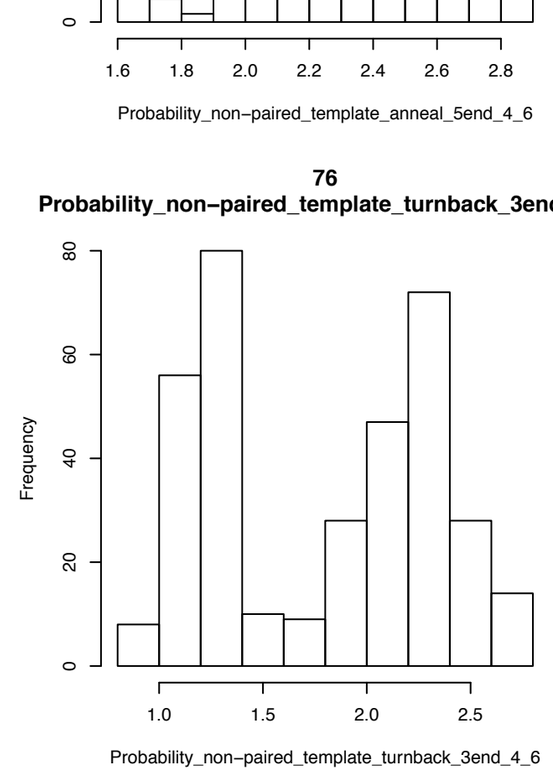


75
Probability_non−paired_template_turnback_3end_1_3



76
Probability_non−paired_template_turnback_3end_4_6



77
Probability_non−paired_template_turnback_5end_1_3

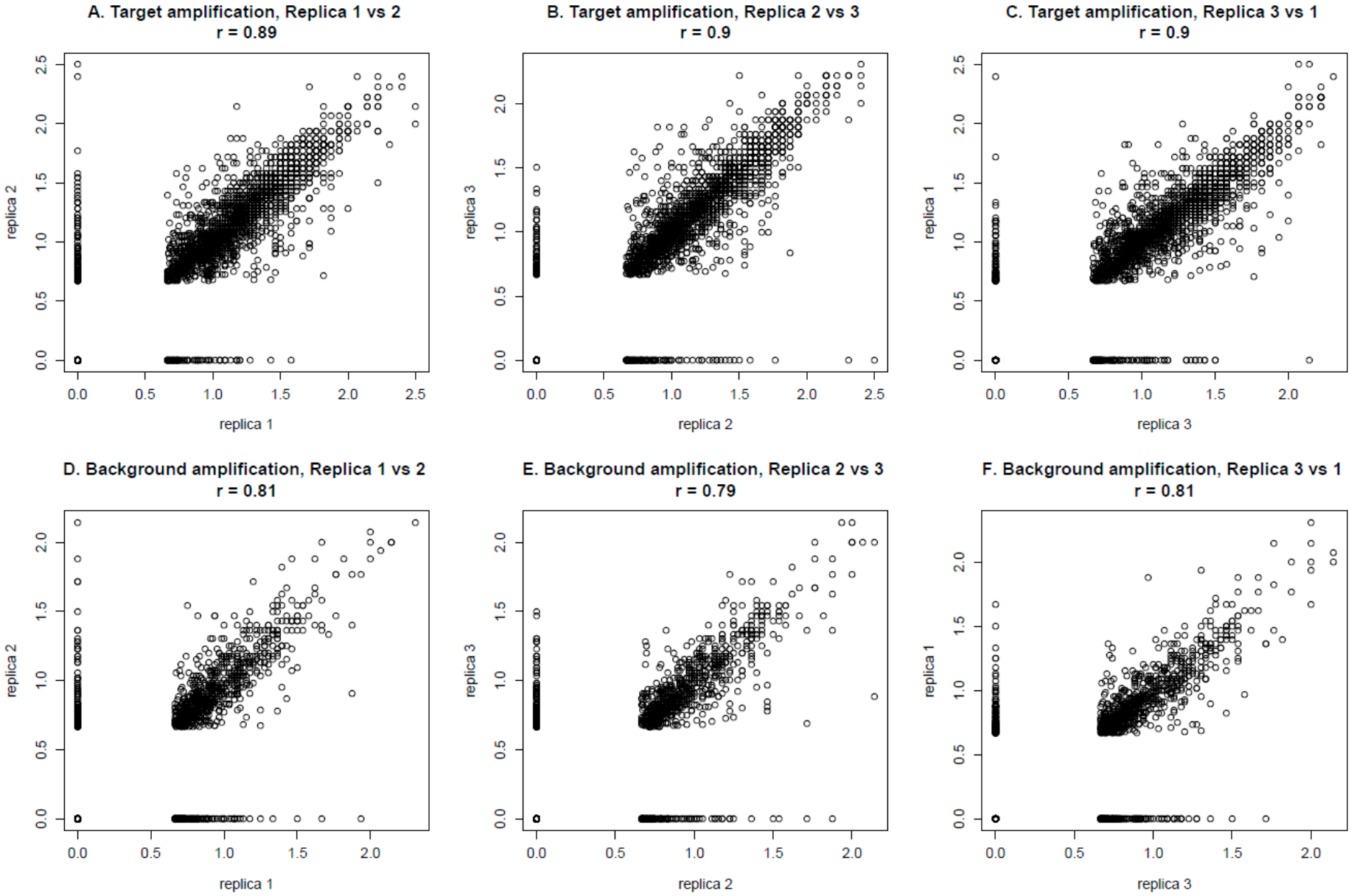
78
Probability_non−paired_template_turnback_5end_4_6

Target reaction speed

Background reaction speed

Supplemental Fig S5. Reaction speed variation between replicates

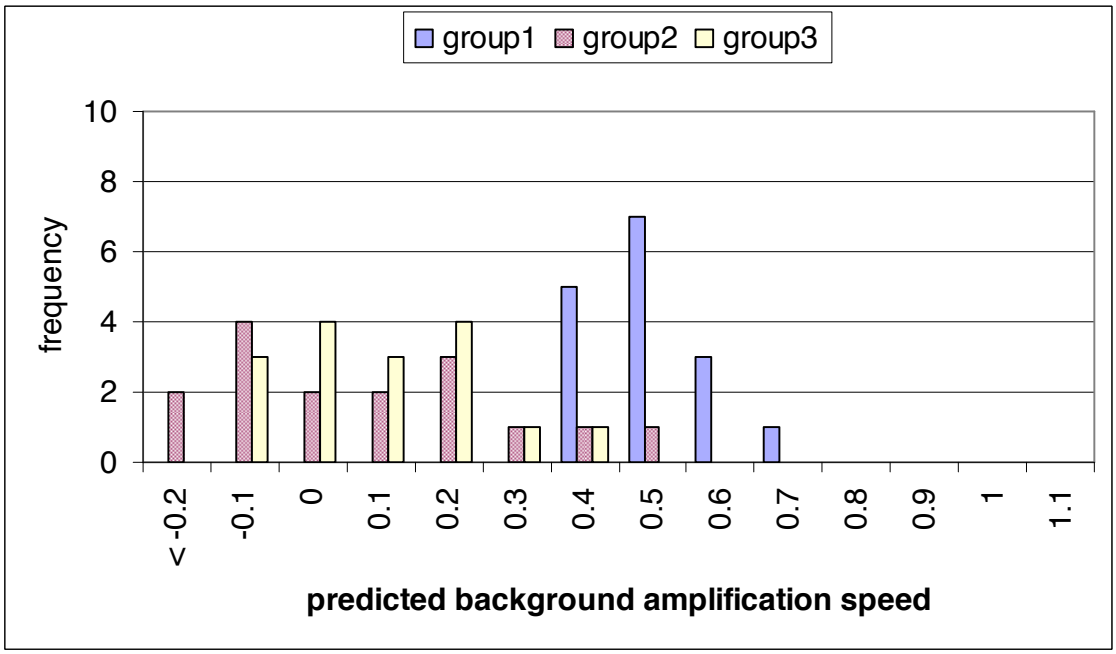
A, B and C show variation between replicates in the target amplification speed. D, E and F show variation in replicates in the background amplification speed.



Supplemental Fig S6. Predicted reaction speeds of TPs used for Evaluation in the TP-FP-BP system

The distribution of the predicted background amplification speeds (A) and target amplification speeds (B). Group 1 having a high predicted background amplification, group 2 having a low background and a low target amplification, and group 3 having low predicted background but high predicted target amplification.

A. The distribution of the predicted background amplification speeds.



B. The distribution of the predicted target amplification speeds.