Experiments to Learn Primer Sequence Bias of Reverse Transcriptase

Overview

The purpose of this set of experiments is to learn the reverse transcriptase priming efficiency of all 7mers. This will be done with primers that contain degenerate nucleotides (e.g. Random_P5 = GATCTCCGAGTTGCNNNNNNN), and the goal is to evaluate the priming efficiency of *all* 4^7 combinations of NNNNNNN. Unfortunately, strong primer hairpins will form for certain NNNNNNN, and these NNNNNNN will not prime RT and will falsely indicate very poor RT priming efficiency. To overcome this problem, there are two sets of experiments ("RT Experiments 1" and "RT Experiments 2", below) distinguished by having different "random primers" that have been designed such that the hairpins that form for one random primer will not form in the other random primer. The combined results of the two experiments will be used to quantify the RT priming efficiency of all 7mers.

Primer Sequences

Random_P5 = GATCTCCGAGTTGCNNNNNNN
Random_P7 = CGATCCGCTCAACTNNNNNNN

Common_P5 = CGACGCTCTTCCGATCT ACTCTGTCCGATGT CCTGCCTTGCTTTTGGTCCG
Common_P7 = GTGTGCTCTTCCGATC ACTCTGTCCGATGT CCTGCCTTGCTTTTGGTCCG

Univ_P5+ = CGACGCTCTTCCGATCTCCGAG
Univ_P7+ = GTGTGCTCTTCCGATCCGCTC

Primer Tm's

Random_P5 = 60-70C (for NNNNNNN = AAAAAAA,...,GGGGGGG)
Random_P7 = 60-70C (for NNNNNNN = AAAAAAA,...,GGGGGGG)

Common_P5 = 60C
Common_P7 = 60C

Univ_P5+ = 30C (for partial hybridization)
62C (for complete hybridization)
Univ_P7+ = 38C (for partial hybridization)
= 60.5C (for complete hybridization)

Universal primers (Used to construct Univ_P5+ and Univ_P7+; included here to confirm correct sequences used)

>UDTD5
ACACTCTTTCCCTACACGACGCTCTTCCGATCT
Universal_P5 CGACGCTCTTCCGAT

>UDTD7
GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC
Universal_P7 GTGTGCTCTTCCGAT

RT Experiments 1

Experiments to Perform

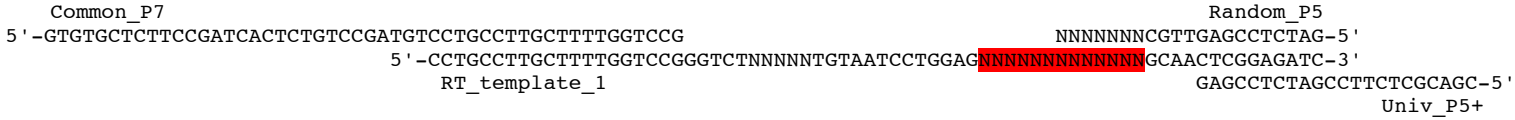
1.1) RT_Experiment_1.Back:

Standard PCR using RT_template_1 as input DNA and the two primers Univ_P5+ and Common_P7

1.2) RT_Experiment_1.Bias

RT-qSeq using RT_template_1 as "input RNA" and the two primers Random_P5 and Common_P7

Template and Primers



Note: RNA bases (ribonucleotides) are indicated in red.

POTENTIAL PROBLEM: RNAse treatment in the RT-qSeq for RT_Experiment_1.Bias would only partially degrade the "input RNA" RT_template_1. This could cause problems. Possible solutions: 1) include other RNA bases so that template is degraded to smaller pieces that would be removed in PCR cleanup; 2) no RNAse treatment.

RT_Experiment 1.Back

"Background" product after PCR with Univ_P5+ & Common_P7 primers is 112bp:

```
5'-GTGTGCTCTTCCGATCACTCTGTCCGATGTCCTGCCTTGCTTTTGGTCCGGGTCTNNNNNTGTAATCCTGGAGNNNNNNNNNNNNNNGCAACTCGGAGATCGGAAGAGCGTCG-3'
3'-CACACGAGAAGGCTAGTGAGACAGGCTACAGGACGGAACGAAAACCAGGCCCAGANNNNNNACATTAGGACCTCNNNNNNNNNNNNNNCGTTGAGCCTCTAGCCTTCTCGCAGC-5'
```

RT Experiment 1.Bias

"Bias" product after "Qiagen 1-Step" step of RT-qSeq with Random_P5 & Common_P7 primers is 100bp:

```
5'-GTGTGCTCTTCCGATCACTCTGTCCGATGTCCTGCCTTGCTTTTGGTCCGGGTCTNNNNNTGTAATCCTGGAGNNNNNNNNNNNNNNGCAACTCGGAGATC-3'
3'-CACACGAGAAGGCTAGTGAGACAGGCTACAGGACGGAACGAAAACCAGGCCCAGANNNNNNACATTAGGACCTCNNNNNNNNNNNNNNCGTTGAGCCTCTAG-5'
```

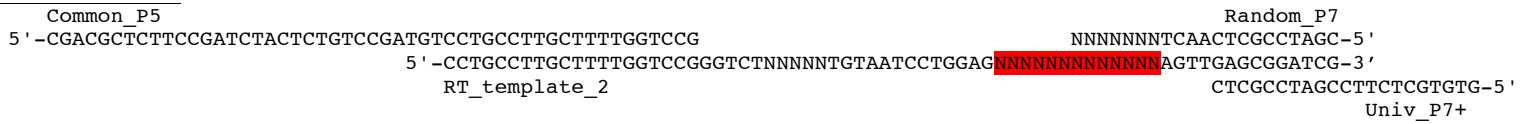
RT Experiments 2

Experiments to Perform

1) RT_Experiment_2.Back:
Standard PCR using RT_template_2 as input DNA and the two primers Univ_P7+ and Common_P5

2) RT_Experiment_2.Bias
RT-qSeq using RT_template_2 as "input RNA" and the two primers Random_P7 and Common_P5

Template and Primers



Note: RNA bases (ribonucleotides) are indicated in red.

POTENTIAL PROBLEM: RNAse treatment in the RT-qSeq for RT_Experiment_1.Bias would only partially degrade the "input RNA" RT_template_1. This could cause problems. Possible solutions: 1) include other RNA bases so that template is degraded to smaller pieces that would be removed in PCR cleanup; 2) no RNAse treatment.

RT Experiment 2.Back

"Background" product after PCR with Univ_P7+ & Common_P5 primers is 112bp:

```
5'-CGACGCTCTTCCGATCTACTCTGTCCGATGTCCTGCCTTGCTTTTGGTCCGGGTCTNNNNNTGTAATCCTGGAGNNNNNNNNNNNNNNAGTTGAGCGGATCGGAAGAGCACAC-3'
3'-GCTGCGAGAAGGCTAGATGAGACAGGCTACAGGACGGAACGAAAACCAGGCCCAGANNNNNACATTAGGACCTCNNNNNNNNNNNNNNTCAACTCGCCTAGCCTTCTCGTGTG-5'
```

RT Experiment 2.Bias

"Bias" product after "Qiagen 1-Step" step of RT-qSeq with Random_P7 & Common_P5 primers is 101bp:

```
5'-CGACGCTCTTCCGATCTACTCTGTCCGATGTCCTGCCTTGCTTTTGGTCCGGGTCTNNNNNTGTAATCCTGGAGNNNNNNNNNNNNNNAGTTGAGCGGATCG-3'
3'-GCTGCGAGAAGGCTAGATGAGACAGGCTACAGGACGGAACGAAAACCAGGCCCAGANNNNNACATTAGGACCTCNNNNNNNNNNNNNNTCAACTCGCCTAGC-5'
```