Experiments to Learn Primer Sequence Bias of DNA Polymerase

Overview

The purpose of this set of experiments is to learn the DNA polymerase (DNAPol) 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 DNAPol and will falsely indicate very poor priming efficiency. To overcome this problem, there are two sets of experiments ("DNAPol Experiments 1" and "DNAPol 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 DNAPol priming efficiency of all 7mers.

Primer Sequences

bkgrnd_P5 = CGACGCTCTTCCGATCTGGTTTGTCTACGAGGAGG
bkgrnd_P7 = GTGTGCTCTTCCGATCCCCCGTCAAGTAGGAGG

Common_P5 = CGACGCTCTTCCGATCTACTCTGTCCGATGTCCTGCCTTGCTTTTGGTCCG
Common_P7 = GTGTGCTCTTCCGATCACTCTGTCCGATGTCCTGCCTTGCTTTTGGTCCG

Random_P5 = GATCTCCGAGTTGCNNNNNNN
Random_P7 = CGATCCGCTCAACTNNNNNNN

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)

bkgrnd_P5 = 58C (for the initial hybridization region)
bkgrnd_P7 = 60C (for the initial hybridization region)

Univ_P5+ = 35C (for partial hybridization)
= 67C (for full hybridization)
Univ_P7+ = 42C (for partial hybridization)
= 67C (for full hybridization)

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

>UDTD5
ACACTCTTTCCCTACACGACGCTCTTCCGATCT
P5_1 CGACGCTCTTCCGAT

>UDTD7
GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC
P7_1 GTGTGCTCTTCCGAT

Protocol Outline for all experiments

1) For each of the experiments below, do only 1 cycle of PCR with specified primers.
2) PCR cleanup with Ampure beads
3) 15 cycles of PCR with the augmented universal primers Univ_P5+ and Univ_P7+.
4) PCR with Illumina sequencing adapters.

DNAPol Experiment 1

Experiments to Perform

1.1) DNAPol_Experiment_1.Back

-1 cycle PCR with DNAPol_template_1 as input DNA and the two primers bkgrnd_P5 & Common_P7

-PCR Cleanup

-Standard PCR with the primers Univ_P5+ and Univ_P7+

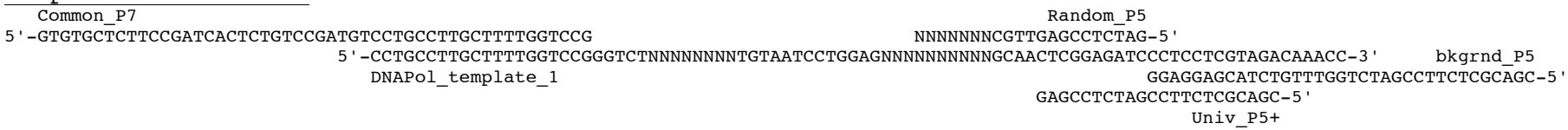
1.2) DNAPol Experiment 1.Bias

-1 cycle PCR with DNAPol_template_1 as input DNA and the two primers bkgrnd_P5 & Common_P7

-PCR cleanup

-Standard PCR with the primers Univ_P5+ and Univ_P7+

Template and Primers



DNAPol Experiment 1.Back

"Background" product after PCR with bkgrnd_P5 & Common_P7 primers is 135bp:

5'-GTGTGCTCTTCCGATCACTCTGTCCGATGTCCTGCCTTGCTTTTGGTCCGGGTCTNNNNNNNNTGTAATCCTGGAGNNNNNNNNNNGCAACTCGGAGATCCCTCCTCGTAGACAAACCAGATCGGAAGAGCGTCG-3'
3'-CACACGAGAAGGCTAGTGAGACAGGCTACAGGACGGAACGAAAACCAGGCCCAGANNNNNNNNNACATTAGGACCTCNNNNNNNNNNCGTTGAGCCTCTAGGGAGGAGCATCTGTTTGGTCTAGCCTTCTCGCAGC-5'

DNAPol Experiment 1.Bias

"Background" product after PCR with bkgrnd_P5 & Common_P7 primers is 100bp:

5'-GTGTGCTCTTCCGATCACTCTGTCCGATGTCCTGCCTTGCTTTTGGTCCGGGTCTNNNNNNNNTGTAATCCTGGAGNNNNNNNNNNGCAACTCGGAGATC-3'
3'-CACACGAGAAGGCTAGTGAGACAGGCTACAGGACGGAACGAAAACCAGGCCCAGANNNNNNNNNACATTAGGACCTCNNNNNNNNNNCGTTGAGCCTCTAG-5'

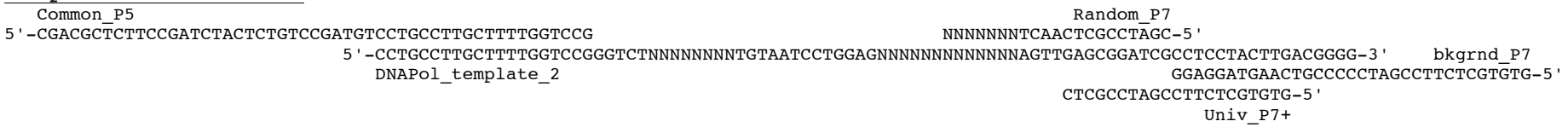
DNAPol Experiment 2

Experiments to Perform

2.1) DNAPol_Experiment_2.Back
- -PCR with DNAPol_template_2 as input DNA and the two primers bkgrnd_P7 & Common_P5
- -PCR cleanup
- -Standard PCR with the primers Univ_P5+ and Univ_P7+

2.2) DNAPol Experiment 2.Bias
- -PCR with DNAPols_template_2 as input DNA and the two primers Random_P7 & Common_P5
- -PCR cleanup
- -Standard PCR with the primers Univ_P5+ and Univ_P7+

Template and Primers



DNAPol_Experiment_2.Back

"Background" product after PCR with bkgrnd_P7 & Common_P5 primers is 137bp:

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5'-CGACGCTCTTCCGATCTACTCTGTCCGATGTCCTGCCTTGCTTTTGGTCCGGGTCTNNNNNNNNNTGTAATCCTGGAGNNNNNNNNNNNNNNAGTTGAGCGGATCGCCTCCTACTTGACGGGGGATCGGAAGAGCACAC-3'
3'-GCTGCGAGAAGGCTAGATGAGACAGGCTACAGGACGGAACGAAAACCAGGCCCAGANNNNNNNNNNACATTAGGACCTCNNNNNNNNNNNNNNTCAACTCGCCTAGCGGAGGATGAACTGCCCCCTAGCCTTCTCGTGTG-5'
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DNAPol_Experiment_2.Bias

"Bias" product after 1 cycle of PCR with Random_P7 & Common_P5 primers is 104bp:

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5'-CGACGCTCTTCCGATCTACTCTGTCCGATGTCCTGCCTTGCTTTTGGTCCGGGTCTNNNNNNNNNTGTAATCCTGGAGNNNNNNNNNNNNNNAGTTGAGCGGATCG-3'
3'-GCTGCGAGAAGGCTAGATGAGACAGGCTACAGGACGGAACGAAAACCAGGCCCAGANNNNNNNNNNACATTAGGACCTCNNNNNNNNNNNNNNTCAACTCGCCTAGC-5'
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