

Lab Notebook

Ethan Holleman

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1 Restriction digest part II

After digest on Friday indicated that the plasmid we thought was pMal-hRNASEH1 actually seems to be pcDNA3-hRH1 doing a follow up digest to confirm that.

1.1 Protocol

All units are in micro liters.

Reagent Name	Control	Digest 1	Digest 2
DNA	1.17	1.17	1.17
10x 2.1 buffer	2	2	2
NdeI	0	0.5	0
HindIII	0	0.5	0
EcoRI	0	0	0.5
RstI	0	0	0.5
H2O	16.83	15.83	15.83

Incubate all samples 1 hr at 37 C. Run on 0.8 % gel for 1 hr at 90 v. 1 ul DNA ladder. Samples loaded with 4 ul purple loading dye .

1.2 Expected results

MW: 1 kb DNA Ladder

1: pcDNA3-hRH1
1. 6212 bp

2: pcDNA3-hRH1
EcoRI + PstI
1. 4045 bp
2. 2167 bp

3: pcDNA3-hRH1

HindIII + NdeI

1. 5807 bp
2. 405 bp

1.3 Gel image

Yep looks like its pcDNA3-hRH1, seeing expected bands at 2 and 4 kb with the EcoRI and Pst digest.