Perfect PAGE III

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1 Procedure Purpose

This experiment was run to determine whether DNA amount has an effect on the clarity of bands in a gel.

2 Overview

We ran a single gel with serial dilutions of oligos and ladders, looking for clarity and single base-pair resolution.

3 Safety Information

1. **SYBR Gold** is a mutagen and can penetrate laboratory gloves in a relatively short period of time, please change your gloves in the event of contamination.
2. Working in a communal lab space is dangerous. Do not assume your fellow workers cleaned up sufficiently

4 Materials

1. 1 15% PAGE Urea gel
2. Loading Buffer (dye)
3. TBE Buffer
4. 40 ng/uL 15 bp oligo
5. 40 ng/uL 16 bp oligo
6. Custom ladder mix
7. UltraPure water

5 Procedure

1. Rinse wells with 15 µL TBE buffer. Pre-run for 20 minutes. Get the DNA samples out of the freezer.

5.1 Dilutions

(a) 15 and 16 bp

- For 1 in 5, add 10 µL original sample to 40 µL water.
- For 1 in 10, add 10 µL original sample to 90 µL water.
- For 1 in 100, add 10 µL 10x dilution to 90 µL water.

(b) Ladder

- For 1 in 10, add 1 µL original sample to 9 µL water.
- For 1 in 100, add 1 µL original sample to 99 µL water.
- For 1 in 1000, add 1 µL original sample to 999 µL water.
- For 1 in 10000, add 1 µL 10x dilution to 999 µL water.

2. Obtain a sizable piece of parafilm. Pipette 5 µL of Gel Loading Buffer in a row of 14 droplets.
3. For the 10 bp ladder column, add 0.5 µL ladder stock and 4.5 µL TBE Buffer.
4. For the remaining droplets, add 5 µL of the appropriate sample.
5. As you go, pipette up and down to mix thoroughly.
6. Load the gels (with 10 µL sample in each well) when they are finished pre-running. Ensure pipette tip is fully in the well, and depress slowly and carefully. Work quickly to minimize diffusion.

Well number	Sample
1	10 bp Ladder (control)
2	Undiluted 15 bp (200 ng)
3	1:5 15 bp (40 ng)
4	1:10 15 bp (20 ng)
5	1:100 15 bp (2 ng)
6	Undiluted 16 bp (200 ng)
7	1:5 16 bp (40 ng)
8	1:10 16 bp (20 ng)
9	1:100 16 bp (2 ng)
10	1:10 ladder mix
11	1:100 ladder mix
12	1:1000 ladder mix
13	1:10000 ladder mix
14	Blank

0mm

Figure 1: Wells

7. Run the gel at 150 V until dark blue dye is about 1 cm from the bottom. This should take about 1 hour.
8. Meanwhile, prepare 50 mL 1x SYBR Gold solution in an appropriate container.
9. After gel finishes, soak in SYBR for 30 minutes (try and be exact, but can probably vary within 5 min).
10. Proceed to imaging. Carefully position the gel, ensuring correct orientation, and optimize for intense bands.
11. Post resulting pictures and interpretations to Slack.