Perfect PAGE I

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1 Procedure Purpose

Determine whether pre-cast 15% TBE-Urea gels are capable of single nucleotide resolution, and which reaction conditions enable such detection.

2 Overview

Goal is to decisively answer whether pre-cast gels are adequate for our purposes of single base-pair resolution ideally in no more than two experiments, if not one. This will allow us to either know precisely how to run a gel to allow such narrow detection or otherwise shift focus to other methods.

3 Safety Information

1. Sybr Gold is a carcinogen. Handle with caution.
2. Working in a communal lab space is dangerous. Do not assume your fellow workers cleaned up sufficiently

4 Materials

1. Three pre-cast 15% PAGE Urea gels.
2. 15-Mer DNA Oligo
3. 16-Mer DNA Oligo
4. 25-Mer DNA Oligo
5. 26-Mer DNA Oligo
6. 35-Mer DNA Oligo
7. 36-Mer DNA Oligo
8. Invitrogen Gel Loading Buffer II
9. Nuclease-free Water
10. .5X TBE Buffer

5 Dilutions

1. Basic DNA Oligo dilution - suspend each oligo in an appropriate amount of water to achieve 100uM concentration. Vortex and mix.
2. 15-Mer 2000ng- Stock solution should have 457 nanograms of DNA per microliter. Pipette 8.8 uL of stock primer into 91.2 uL of water. Vortex and mix. Concentration is 40ng/uL - label appropriately.
3. 16-Mer 2000ng- Stock solution should have 505 nanograms of DNA per microliter. Pipette 8 uL of stock primer into 92 uL of water. Vortex and mix. Concentration is 40ng/uL - label appropriately.
4. 25-Mer 2000ng - Stock solution should have 761 nanograms of DNA per microliter. Pipette 5.2 uL of stock primer into 94.8 uL of water. Vortex and mix. Concentration is 40ng/uL - label appropriately.
5. 26-Mer 2000ng - Stock solution should have 786 nanograms of DNA per microliter. Pipette 5.2 uL of stock primer into 94.8 uL of water. Vortex and mix. Concentration is 40ng/uL - label appropriately.
6. 35-Mer 2000ng - Stock solution should have 1070 nanograms of DNA per microliter. Pipette 3.8 uL of stock primer into 96.2 uL of water. Vortex and mix. Concentration is 40ng/uL - label appropriately.
7. 36-Mer 2000ng - Stock solution should have 1103 nanograms of DNA per microliter. Pipette 3.6 uL of stock primer into 96.4 uL of water. Vortex and mix. Concentration is 40ng/uL - label appropriately.
8. Custom DNA Ladder - Add 8.8 uL of 15-Mer stock, 8 uL of 16-Mer stock, 5.2 uL of 25-Mer stock, 5.2 uL of 26-Mer stock, 3.8 uL of 35-Mer stock, and 3.6 uL of 36-Mer stock to 65.4 uL of water. Concentration is 240ng/uL - label appropriately.

6 Procedure

1. Prepare all above dilutions and the custom DNA ladder.

OPTIONAL STOP POINT

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2. Prepare three PAGE gels. Rinse each well with 15 uL of .5X TBE buffer. Allow to pre-run for 20 minutes prior to starting the protocol.
3. On to a piece of parafilm, pipette 5 uL of Gel Loading Buffer II in 24 different locations, in a grid with three rows and eight columns.
4. In the first column, pipette .5 uL of 10 bp ladder onto the loading buffer. Pipette .5 uL of TBE buffer onto those mixtures. Mix thoroughly.
5. In the remaining columns, pipette 5 uL ladder and each oligo in order of increasing size. Mix thoroughly with the loading buffer.
6. Once the gels have finished pre-running, load each 10 uL from each mix in each row onto each gel correspondingly. Run the first gel on 200V for 20 minutes, the second for 35 minutes, and the third for 50 minutes.
7. Post-stain each gel for 30 minutes in 1X Sybr Gold, and use the gel imager to interpret results.

7 Interpretation

1. The results should provide a range of 15 minute increments for how long to run the gel. Further experimentation may be required to provide a more narrow 5-minute time interval. It may also be the case that the amount of DNA loaded is either inadequate or excessive, which should be altered in the subsequent experiment.