Chemical Sequencing of DNA

This is a rapid method for chemical DNA sequencing which is commonly used as ladder for footprinting reactions or for sequencing of short DNA oligonucleotides.

Reference: Bencini et al. (1984) Biotechniques 2: 4-5.

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The method below works well for Sequencing of DNA of greater than ~40 bp. Typically, about 150 bases of sequence can be read from analysis on a 6-8% urea acrylamide gel. For sequencing of short oligonucleotides, the reaction times should be increased as noted below and the reactions should be loaded to 15%-20% acrylamide urea gels.

If making a sequencing ladder for footprinting, use several hundred thousand cpm per reaction if possible. This will give enough sequenced DNA for many gels (typically 5,000 cpm of the sequencing reaction are run on a lane of a sequencing gel for an overnight film exposure). The final yield of this method is typically 30%-50% of the starting cpm.

The DNA to be sequenced needs to be in water. TE or salt will interfere with some of the sequencing reactions. However, 1 microliter or less of TE in a 10 microliter reaction will be O.K.

1. Aliquot DNA in H20 to three 1.5 ml microfuge tubes labeled: G+A, A+C, and C+T. Add water so that the final volume in each tube is 9 microliters. Add one microliter of 1 microgram/microliter carrier DNA. This will aid in the DNA precipitation steps.

2. To the T+C tubes, add 15 microliters of hydrazine, mix well and incubate at room temperature for 10 min. Fill the tube with butanol, vortex very well (make sure there is not a separate water and butanol phase) and spin in the microfuge for 5 min. Remove butanol, briefly spin again and remove any remaining butanol. Dry in speedvac. Resuspend in 150 microliters 1/10 diluted piperidine. Store these reactions on ice while completing the other sequencing reactions. Use piperidine in the fume hood.

3. To the G+A tubes, add 1.0 microliter 1M Na/H Formate pH 2 (use NaOH to adjust the pH of 1M formic acid). Mix well and incubate 30 min at 37 degrees. Add 150 microliters 1/10 diluted piperidine and store on ice.

4. To the A+C tube, add 1.0 microliter 30% NaOH. Incubate 90 degrees for 15 min. This step works best if performed in a PCR tube and incubated in a PCR instrument with a heated lid to prevent evaporation during the reaction. After the reaction is complete, transfer to a 1.5 ml microfuge tube. Add 150 microliters 1/10 diluted piperidine and store on ice.

5. After all the above reactions are complete, incubate tubes at 90 degrees for 30 min. Put a weight on top of the tubes to prevent opening. This step cleaves the DNA at the point of modification. Cool tubes and spin before opening.

6. Add 150 microliters 70% ethanol to the A+C reactions. Fill all tubes with butanol and vortex very well to mix phases. Spin 5 minutes in the microfuge and remove the supernatants. Briefly re spin to remove all remaining butanol.

7. Resuspend pellets in 150 microliters of 1% SDS. Fill with butanol and precipitate again. After removing supernatant, briefly spin and remove any remaining butanol. Dry in speedvac and quantitate radioactivity. Resuspend in a convenient volume of formamide sequencing dye.

For sequencing of short DNAs (less than 50 bp) increase the time of hydrazine reaction to 12-15 min, increase the Formate reaction to 50 min, and increase the NaOH reaction to 25 min.