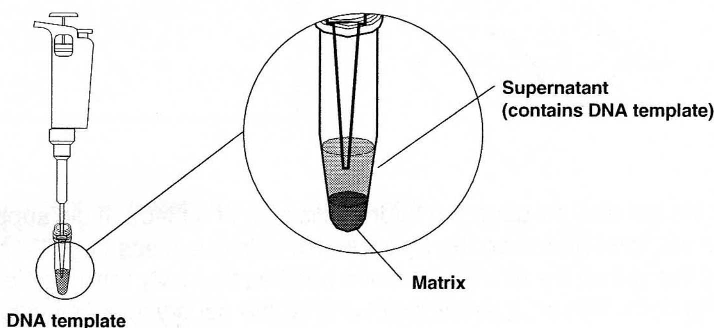
Interpretation of Results and Troubleshooting Guide

Explanations for "Empty Lanes" or Unamplified Samples

Multiple explanations can account for student samples not being amplified in the PCR reactions.

1. **Inadequate collection of cheek cells.** A visible cell pellet about the size of a match head should be obtained following centrifugation of the saline mouthwash. If no cell pellet is visible, or the pellet is too small, additional swished saline may be centrifuged until a pellet of the desired size is obtained. However, it is not recommended to collect more than 3 ml of cells (see point below).
2. **Excessive number of cells.** Just as too few cells will yield insufficient genomic DNA, an excessive number of cells will saturate the capacity of the InstaGene, resulting in little or no amplification.
3. **InstaGene matrix not transferred.** Each workstation is supplied with tubes of InstaGene matrix that were aliquotted by the instructor and placed on ice. These tubes of matrix must be mixed prior to each pipetting to bring the beads up into suspension. If no beads were transferred into the student's tube, the divalent cations will not be removed from the genomic DNA preparation, and the PCR reaction will be inhibited.
4. **Carryover of InstaGene into PCR reaction.** Although the beads in the InstaGene matrix are required for the DNA template preparation, it is critical that none of the InstaGene matrix be carried over into the PCR reaction. If beads are transferred into the PCR tube, the magnesium ions needed by the *Taq* polymerase will be removed, and the PCR reaction will be inhibited.



Interpretation of Heterozygous Samples

1. **Competition during amplification.** Amplification of heterozygous samples is more difficult than both homozygous amplifications because of competition between the reactions that produce the smaller (641 bp) and larger (941 bp) bands. Because the smaller 641 bp band is amplified more efficiently than the 941 bp band, heterozygous samples on agarose gels will show the smaller band being more intense than the larger band (see the band indicated by an asterisk in the gel below). For this reason, heterozygous samples can often be interpreted as homozygous (–/–) because of a faint upper band. **Careful examination of the gels is required to distinguish between heterozygous (+/–) and homozygous (–/–) individuals.** Alternatively, the use of ethidium bromide and photodocumentation equipment (the Bio-Rad gel documentation system) will increase the sensitivity and allow easier visualization of faint heterozygous samples.

2. **Larger band in (+/–) samples.** The heterozygous samples will often contain larger bands which migrate at ~1,100 bp and 1,700 bp in the gel (see the bands indicated by arrows in the gel below). These bands are heteroduplexes that form between the 641- and 941-nucleotide strands and contain secondary structure which results in the DNA bands migrating at a slower rate in the gel (Figure 8).

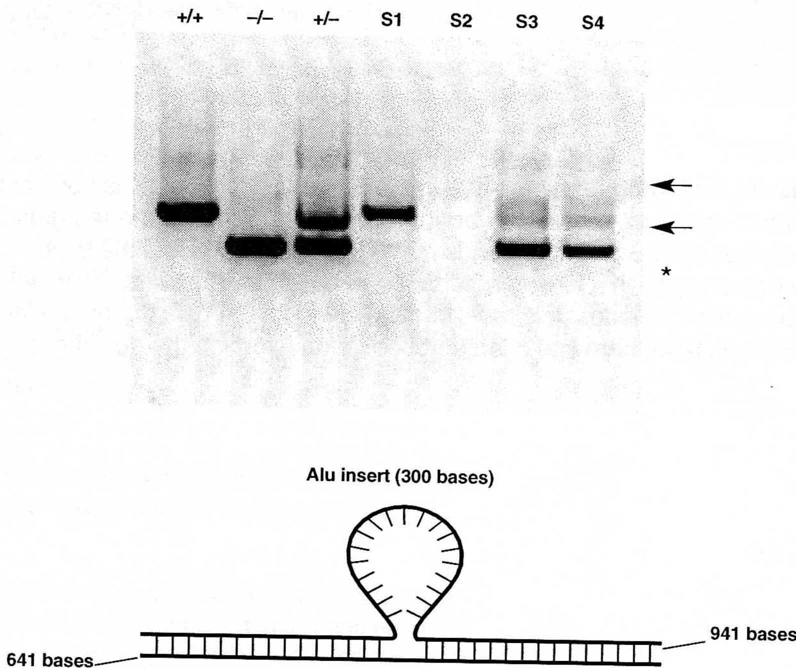
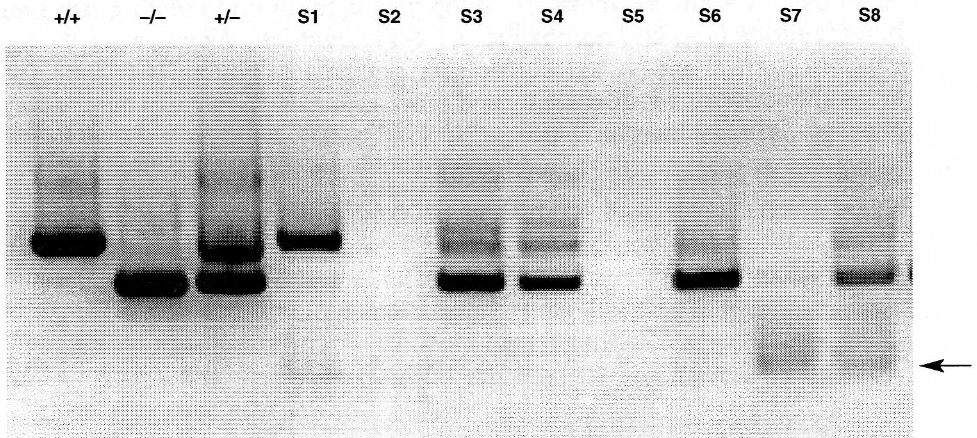


Fig. 8. Heteroduplex formed between 941- and 641-nucleotide strands.

3. **Primer-dimer formation**. Some PCR reactions may show primer-dimer formation. Primer dimers are bands that are seen at the bottom of the gels and which correspond to complexes of both primers. Primer-dimer formation is more intense in reactions that show little or no amplified product. Thus, primer-dimer formation is more likely to occur in reaction tubes with InstaGene contamination, little or no template, or in samples that were prepared well in advance of placing into the thermal cycler. The arrow in the figure below shows primer-dimers.



4. **Bands appear to be fading**. The blue dye in the Fast Blast DNA stain is subject to reversible bleaching when exposed to bright room lights. When the dried gels are examined 3–5 days after drying, the bands may appear faint. Placing the gels in a dark location (in a box or taped in a closed notebook) and examining several hours later will provide the most intense bands. It is most convenient to let the gels dry on the lab bench for 3–5 days, tape them into a lab notebook, and examine the gels the following day.