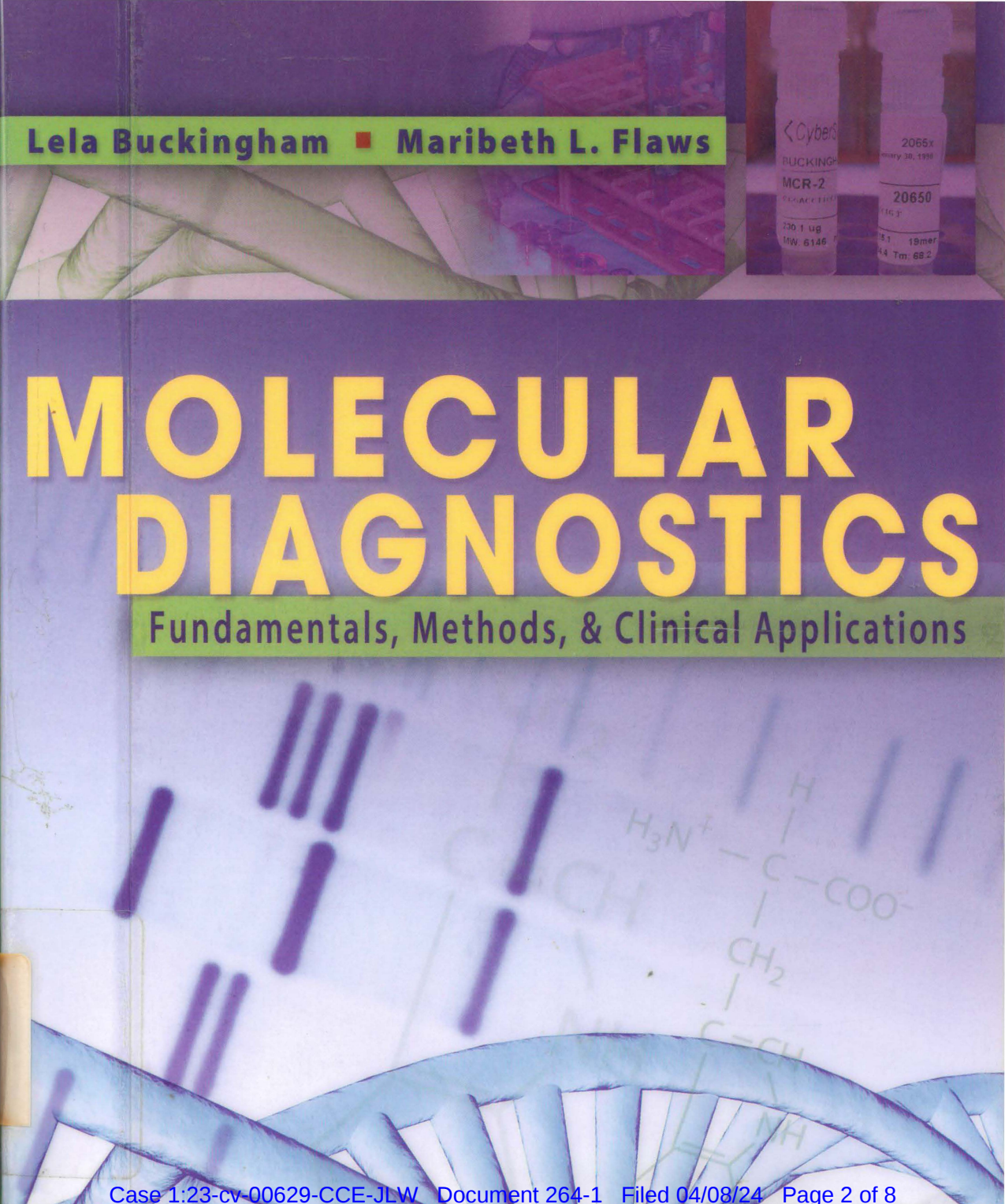


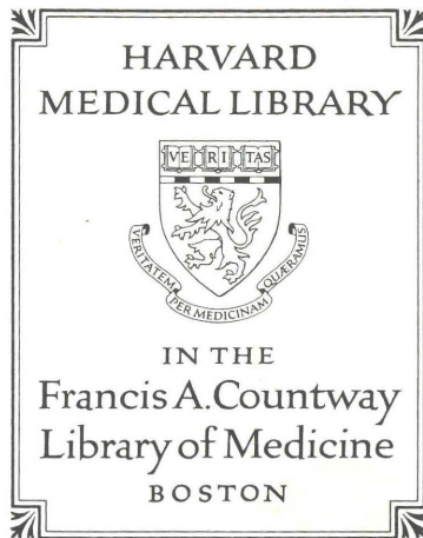
Exhibit A



Lela Buckingham ■ Maribeth L. Flaws

MOLECULAR DIAGNOSTICS

Fundamentals, Methods, & Clinical Applications



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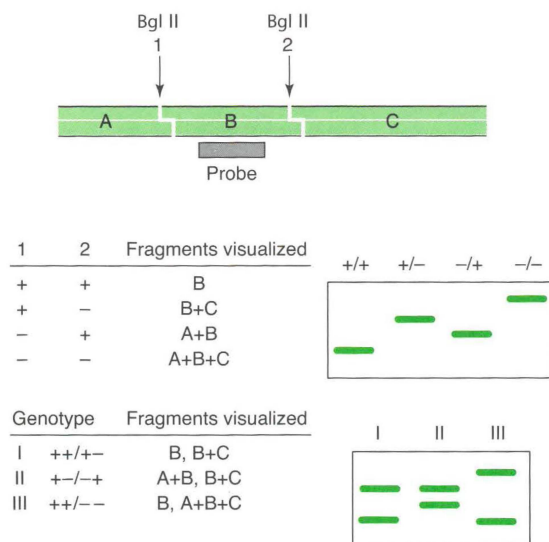
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■ **Figure 11-3** Using a Southern blot to probe for RFLP. With the same region shown in Figure 11-2, only the fragments with complementary sequences to a probe to the B region (top) can be visualized.

phisms so that the offspring inherits a combination of the parental polymorphisms. When visualized as fragments that hybridize to a probe of a polymorphic region, the band patterns represent the combination of RFLPs inherited from each parent. Due to recombination and random assortment, each person has a unique set of RFLPs, half inherited maternally and half paternally. Every genotype will yield a descriptive band pattern as shown in Figure 11-3.

Over many generations, mutations, intra- and inter-chromosomal recombination, gene conversion, and other genetic events have increased the diversity of DNA sequences. One consequence of this genetic diversity is that a single **locus**, that is, a gene or region of DNA, will have several versions, or **alleles**. Human beings are diploid; that is, people have two copies of every locus. In other words, each person has two alleles of each locus. If these alleles are the same, the locus is **homozygous**; if the two alleles are different, the locus is **heterozygous**.

Depending on the extent of diversity or polymorphism of a locus, any two people can share the same alleles or have different alleles. More closely related individuals are likely to share more alleles than unrelated persons. In the

examples shown in Figure 11-3, (+ +), (+ -), (- +), and (- -) describe the presence (+) or absence (-) of *Bgl*II sites making up four alleles of the locus detectable by Southern blot. In the illustration, genotypes I and II both have the (+ -) allele on one chromosome, but genotype I has (+ +), and genotype II has (- +) on the other chromosome. This appears in the Southern blot results as one band of equal size between the two genotypes and one band that is a different size. Two individuals can share both alleles at a single locus, but the chances of two individuals, except for identical twins, sharing the same alleles decrease 10-fold with each additional locus tested.¹

More than 2000 RFLP loci have been described in human DNA. The uniqueness of the collection of polymorphisms in each individual is the basis for human identification at the molecular level. Detection of RFLPs by Southern blot made positive paternity testing and human identification possible for the first time. RFLP protocols for human identification in most North American laboratories used the restriction enzyme *Hae*III for fragmentation of genomic DNA. Many European laboratories used the *Hinf*I enzyme. These enzymes cut DNA frequently enough to reveal polymorphisms in multiple locations throughout the genome. To regulate results from independent laboratories, the Standard Reference Material (SRM) DNA Profiling Standard for RFLP analysis was released in 1992. The SRM supplies cell pellets, genomic DNA, gel standards, precut DNA, electrophoresis materials, molecular weight markers, and certified values for final analysis. These materials were designed to maintain reproducibility of the RFLP process across laboratories.

Genetic Mapping With RFLPs

Polymorphisms are inherited in a mendelian fashion, and locations of many polymorphisms in the genome are known. Therefore, polymorphisms can be used as landmarks, or **markers**, in the genome to determine the location of other genes. In addition to showing clear family history or direct identification of a genetic factor, one can confirm that a disease has a genetic component by demonstrating a close genetic association or linkage to a known marker. Formal statistical methods are used to determine the probability that an unknown gene is located close to a known marker in the genome. The more frequently a particular polymorphism is present in persons with a disease phenotype, the more likely the affected gene is located