Abstract. Microarrays can quantitatively measure the expression of thousands of genes simultaneously but they are also powerful tools to examine the expression of specific genes in different tissues and cell types. They complement other methods used to detect gene expression at the transcript level, including Northern blotting and RT-PCR. This study uses oligonucleotide microarray expression profiling to compare maize β-tubulin (tub) gene expression at the transcript level in different tissues and during various stages of development and adds to what is known about maize β-tubulin expression. In maize, at least nine β-tubulin genes are expressed. The presence of multiple tub genes suggests that some could have evolved to provide fine control of expression or even have distinct biological or physiological properties in the plant. This is supported by the differential expression at the transcript level of the maize β-tubulins in different tissues during development. The following work has added evidence of maize β-tubulin gene differential expression and regulation. Based on microarray data, some β-tubulins exhibit statistically significant differences in transcript abundance in 11-day old seedling shoots, immature ears, 19-DAP embryos, 13-DAP endosperm and 19-DAP endosperm. There are also different expression patterns for many of the tubulin genes within the tissues studied. As the plant develops it may use β-tubulins in different amounts in specific tissues, and these tubulins may even interact differently with other proteins present at these developmental stages. In immature ears, tub1 and tub2 have higher transcript abundances. Comparisons of expression patterns indicate that tub3 and tub4 have very similar expression patterns, which may be a result of similar transcriptional regulation. The highest transcript abundance of these two genes occurs in developing seedling shoots. tub3 and tub4 transcripts have also been detected for the first time in maize endosperm. This chapter describes a variety of methods.
for array analysis of tubulin gene expression as well as important considerations before taking on this type of work.

**Keywords:** GCRMA, GeneChip, hybridize, maize, MAS 5.0 (Affymetrix Microsuite 5.0), microarray, RMA, Significance Analysis of Microarrays (SAM), *tub* (tubulin gene), cDNA

1. Introduction

The major components of microtubules are heterodimers of α- and β-tubulins. In maize (*Zea mays* (L.)), there are at least eight α-tubulin genes but the expression of only six has been detected.¹ There are at least nine expressed β-tubulin (*tub*) genes.²,³ While some or all may be functionally redundant, some could have slightly different biochemical properties or distinct roles in the plant, for example, during development or in response to different stimuli. Determining if there are significant differences in the properties and physiological roles of the maize α- and β-tubulins is an ultimate goal, but the focus of this research is the β-tubulins. Studies using Northern blots, dot blot hybridizations, RNase protection assays, 2D electrophoresis, and antibodies to the β-tubulin family provide evidence of differentially expressed β-tubulins within maize tissues.²⁻⁷ Additional evidence is provided here through the use of oligonucleotide microarray expression profiling of maize β-tubulins.

A DNA microarray is also known as a DNA chip, gene array, genome chip, or GeneChip®, a registered trademark of Affymetrix, Inc. (Santa Clara, CA), referring to its high density, oligonucleotide-based DNA arrays. Affymetrix GeneChips® have become the industry standard but other companies do manufacture oligonucleotide based chips.

Briefly, on a DNA microarray, DNA is covalently attached to suitable, solid matrices as microscopic spots at known, fixed locations. These probes are high density; each spot contains a large number of identical DNA molecules or fragments. These probes range from 20 to 100s of nucleotides long. Targets are hybridized under highly stringent conditions and hybridization is usually determined by fluorophore-based detection.

Microarrays are used as a means for expression profiling at the mRNA level. If a gene is active, many mRNA molecules are produced and the RNA levels are used to extrapolate gene expression. Some benefits of working with RNA rather than proteins are that RNA is more easily produced, fewer precautions are needed with its handling, and RNA will