

Many different tissue types on a convenient single-slide format

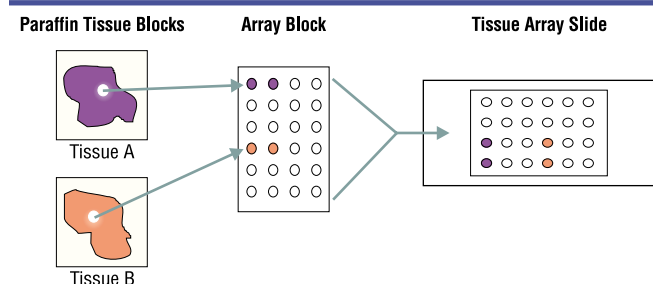
# Innovative Tissue Array Technology for High-Throughput Screening of Gene Expression

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Stratagene's Complete View<sup>®</sup> tissue array slides offer further refinement of tissue array technology through optimized tissue fixation and processing procedures that preserve tissue morphology, antigenicity of proteins, and nucleic acid reactivity. This innovative product line consists of murine (male and female) survey and validation array slides. The Complete View survey array slides represent major organ systems, consisting of approximately 104 cell types mounted in duplicate. The Complete View validation array slides include 12 representative tissues from the survey arrays and are useful to test staining procedures and reagents. We report our results using Complete View array slides for hematoxylin and eosin staining of murine tissue, immunostaining of murine tropomyosin, and labeling RNA of murine salivary gland. These tests confirm that Complete View tissue array slides ensure reliable screening results in a convenient, high-throughput format.

To achieve an understanding of the cellular function of gene products requires localizing gene expression to specific cells and tissues. This task can be accomplished through the use of tissue arrays, a collection of diverse tissue samples mounted on a single slide. Proven successful in examining both normal<sup>1,2</sup> and cancerous tissues,<sup>3,4</sup> tissue arrays can be treated as a single histological slide during staining, immunohistochemistry, or in situ hybridization. The single-slide format affords a high-throughput method for precisely identifying the specific tissues and cell types that express a gene of interest.

**Figure 1**  
**Tissue Arrays Procedure**



The paraffin tissue blocks act as tissue donors for the array block. The array block is then sectioned, and the tissue array slides are generated.

## Single-Slide Whole-Animal Surveys

One particularly useful application of tissue arrays is the whole-animal survey, in which all tissues and cell types in an animal species are screened. Traditional sections for whole-animal surveys require approximately 25 to 40 histological slides for complete screening. With each slide requiring preparation and processing that includes staining, labeling, and analysis, such surveys are so demanding of time, labor, and reagents that they are impractical in most research environments. In contrast, a single Complete View tissue array can contain all the major tissues and cell types of an animal. Hence, tissue array technology makes it possible to screen numerous tissues in a single experiment (Figure 1).

Stratagene has improved tissue array technology by creating the Complete View survey and validation array slides. In this product line, optimized tissue fixation

**Table 1**  
**Tissue Types Represented On Arrays**

Slides	Tissue specimens
Murine Validation Array, Male C57BL/6J or Female C57BL/6J	12 unique tissue types represented from the following groups: <ul style="list-style-type: none"> <li>• connective tissue</li> <li>• skeletal tissue</li> <li>• nervous tissue</li> <li>• lymphatic tissue</li> <li>• integument</li> <li>• alimentary system</li> <li>• respiratory system</li> <li>• urinary system</li> <li>• glandular tissue</li> </ul> Each representative tissue has dual representation per slide. (24 tissue spots per slide + 1 positive and 1 negative control spot)
Murine Survey Array, Male C57BL/6J or Female C57BL/6J	In excess of 40 tissue types representing greater than 100 cell types from the following groups: <ul style="list-style-type: none"> <li>• connective tissue</li> <li>• skeletal tissue</li> <li>• nervous tissue</li> <li>• lymphatic tissue</li> <li>• integument</li> <li>• alimentary system</li> <li>• respiratory system</li> <li>• urinary system</li> <li>• glandular tissue</li> <li>• vascular system</li> <li>• reproductive system</li> </ul>

**Figure 2**  
**Morphological Integrity of Tissue**



A portion of the Complete View™ male mouse survey array slide was stained with hematoxylin and eosin.

**Figure 3**  
**Antigenicity of Proteins**



Muscle section from the Complete View male mouse validation array slide was reacted with antitropomyosin as the primary antibody and probed with the goat anti-rabbit FITC as the secondary antibody.

**Figure 4**  
**Nucleic Acid Reactivity**



Two consecutive sections from the Complete View male mouse validation array slide were prepared for in situ hybridization (Methods). Figure 4A demonstrates the presence of mRNA in salivary gland. Figure 4B shows the result of prelabeled RNase digestion.

preserves morphological integrity, antigenicity of proteins, and nucleic acid reactivity. On each Complete View array slide, tissues are conveniently configured for easy identification with the Complete View target locator, which positions each tissue within the array by column and row. The availability of two categories of array slides provides further convenience. The Complete View survey array slides represent major organ systems that consist of approximately 10<sup>4</sup> cell types mounted in duplicate (Table 1). The Complete View validation array slides include representative tissues from the survey arrays and are useful to test staining procedures and reagents.

**Preserved Tissue Integrity**

We assessed the morphological integrity of tissues and the antigenicity of proteins by assaying the Complete View array slides in hematoxylin and eosin staining and immunohistochemical analysis, respectively. Figure 2 shows multiple tissues stained within the Complete View male mouse survey array slide. To test antigenicity, we used the Complete View male mouse validation array slide as described (Methods) with antitropomyosin as the

primary antibody and goat anti-rabbit FITC as the secondary antibody. The result of this immunological screening assay demonstrates the actin-binding protein within the muscle tissue (Figure 3).

Since many applications of tissue array technology rely on nucleic acid assays, we used the Complete View male mouse validation array slides for an in situ hybridization experiment (Figure 4). One of two slides was treated with RNase prior to hybridization of both slides with a fluorescently labeled poly(dT) probe. Salivary gland tissue on the slide without RNase digestion clearly shows the presence of mRNA (Figure 4A). We used standard conditions for slide preparation, prehybridization, and hybridization reactions (Methods).

**Conclusions**

The Complete View tissue array slide kits permit widespread use of tissue array technology. With the single-slide format and convenient configuration of tissues, these innovative array slides simplify screening procedures so specialized expertise in histology is not required. We demonstrated their use to localize expression to specific tissues and cells, and our data confirm that these optimized array slides maintain the morphological integrity of tissues. By immunostaining of the tropomyosin protein and fluorescent labeling of RNA, we verified that the tissues affixed to the Complete View slides retain antigenicity of proteins and reactivity of nucleic acids, respectively. We are expanding this product line to include human fetal validation and survey arrays.

**Methods**

**In Situ Hybridization:** Paraffin was removed from the array tissue by treating the slides as described in the product instructions. The two consecutive slides were rehydrated and washed in PBS, immersed in 0.1 M glycine, and washed three times in PBS. Then, the slides were incubated with proteinase K and one was subsequently treated with RNase. To stop the reactions, slides were immersed in 4% paraformaldehyde in PBS followed by a PBS rinse. The slides were then incubated with acetic anhydride in triethanolamine and prehybridized in formamide (50% v/v) in 4X SSC at 37°C for 30 minutes, then hybridized with 20 µl of poly d(T)-FITC incubated at 42°C for 12 to 18 hours. This incubation was followed by a series of washes with 4X SSC. Each slide was examined with an Olympus BX60 fluorescent microscope.

**Immunohistochemistry:** Paraffin was removed from the array tissue by treating the slide as described in the product instructions. The slide was rehydrated, then incubated in PBS for 5 minutes and placed in a humidity chamber. The diluted primary antibody was added to the slide and incubated for 30 to 60 minutes at 37°C. After washing with PBS, the diluted secondary antibody was added to the slide and incubated for 30 to 60 minutes at 37°C. Following two additional PBS washes, a coverglass was placed over the slide, and the slide was examined using an Olympus® BX60 fluorescent microscope.

**REFERENCES**

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2. Battifora, H. and Mehta, P. (1990) *Lab. Invest.* 63(5): 722-724.
3. Kononen, J., et al. (1998). *Nature Med.* 4(7): 844-847.
4. Bubendorf, L., et al. (1999) *Cancer Res.* 59: 803-806.

Complete View™ Tissue Array Slides		
Murine Validation Array, Male C57BL/6J	2 slides <sup>a</sup>	#340000
Murine Validation Array, Female C57BL/6J	2 slides <sup>a</sup>	#340001
Murine Survey Array, Male C57BL/6J	2 slides <sup>a</sup>	Coming soon!
Murine Survey Array, Female C57BL/6J	2 slides <sup>a</sup>	Coming soon!

<sup>a</sup> A target locator is supplied with each set of slides.

<sup>a</sup> The two slides contain the same tissues. The two slides are consecutive, serial sections.

