

## Tissue Microarrays: Construction and Use

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### Abstract

Tissue microarrays (TMAs) enable high-throughput tissue analysis by selecting a large number of paraffin-embedded donor tissue block cores and transferring these tissue cores into a positionally encoded array in the recipient TMA block. Once TMAs are constructed, a variety of analysis may be performed on the arrays including histochemical, immunohistochemical, or immunofluorescent staining, and in situ hybridization for DNA or RNA. TMAs offer a cost-effective method for performing parallel analysis of a large number of tissue samples. In this chapter we outline the method of TMA construction with an emphasis on providing useful information in the analysis of a variety of pancreatic neoplasms, including pancreatic adenocarcinomas and pre-invasive lesions. The technique of TMA construction in this chapter is restricted to the use of formalin-fixed paraffin-embedded tissue.

**Key words:** Tissue microarrays, Image analysis, Bioinformatics, Formalin-fixed paraffin-embedded tissue, High-throughput tissue array protocol, Immunohistochemistry, In situ hybridization

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### 1. Introduction

The introduction of the multitumor (sausage) block as a novel method for immunohistochemical antibody testing was initially described by Battifora in 1986 with subsequent modification creating the (checkerboard) block in 1990 ([1](#), [2](#)). Kononen and colleagues refined the construction of the multicore tissue microarray (TMA) and extended analysis to enable the parallel in situ detection of DNA, RNA, and protein targets in a large number of tumors ([3](#), [4](#)).

TMAs offer a cost-effective efficient resource for rapid biomarker analysis for prognostic or therapeutic purposes. TMAs facilitate high-throughput molecular profiling of cancer specimens with minimal tissue requirements. There have been outstanding reviews detailing methods used in constructing TMAs ([5–12](#)).

This review focuses on TMA construction-related issues that are of particular relevance in the analysis of pancreatic neoplasms.

### **1.1. Benefits of TMAs**

1. TMAs allow rapid and high-throughput discovery and validation of biomarkers. Hundreds of molecular targets can be analyzed “in parallel” from consecutive TMA sections.
2. TMAs allow biomarker analysis in the context of tissue morphology. TMAs allow localization of biomarkers to specific cells within the tumor tissue including evaluation of the tumor cells and interacting cells and stroma comprising the microenvironment of the tumor cells. TMAs also permit intracellular compartmental localization of these biomarkers (i.e., cytoplasmic, membranous, and nuclear localization).
3. TMAs are cost effective and provide efficient use of reagents and lab personnel. (Instead of performing immunohistochemical (IHC) staining on 100 slides, one can perform IHC on a single TMA slide created with 100 tissue samples).
4. TMAs are useful for quality control standardization in IHC and in situ hybridization (ISH). The same TMA can be utilized for cross-validation or comparison studies between different techniques.
5. TMAs can be used to evaluate sensitivity and specificity of antibodies or ISH probes with respect to a large variety of tissue types, and a wide spectrum of pathologic conditions and neoplasms.
6. TMAs utilize only a portion of archival tissue, so limited tissue is utilized efficiently.
7. TMAs constructed from formalin-fixed paraffin-embedded (FFPE) tissue samples allow study of archival tissue samples that cannot be analyzed using other high-throughput genomic or proteomic methods.

### **1.2. Limitations of TMAs**

1. One limitation of TMAs involves the issue of tissue heterogeneity, with a key concern of whether the TMA cores sampled are representative of the tumor. In pathology, tumor sampling is always an issue and the initial concern of TMAs was whether they were representative of whole sections since they sample far less tissue. Numerous studies have addressed concordance of IHC staining in TMAs and whole sections. In general, for many immunostains with relatively homogeneous staining, it was observed that two to four tissue cores are representative of whole sections with 95–97% concordance rates; furthermore, five to six cores do not improve concordance rates (13). A second concern involves biomarkers that show significant heterogeneity of expression within the tissue. Some biomarkers also may show relative heterogeneity with regard to the topographic regions of the tumor (central vs. peripheral regions). These

topographic variations may reflect varying degrees of hypoxia within the tumor, or differences in other components of the microenvironment of the tumor cells.

Several studies have reported non-concordance in analysis of TMA core samples compared to whole sections with respect to IHC staining of markers that show heterogeneity of expression in the tumor including proliferation markers (Ki-67), apoptosis markers (bcl-2, p53), and neoangiogenesis markers (CD31, CD105) (14–16). Evaluation of biomarkers that show heterogeneity in the tumor may require increased number of cores sampling different areas of the tumor, as well as a larger core diameter size to be representative of the tumor.

2. Early TMA validation studies demonstrated consistent and representative protein expression by immunohistochemistry (IHC) and DNA copy numbers by fluorescence in situ hybridization (FISH) assays (17–19). It has become increasingly clear that nonuniformity of tissue fixation parameters has a considerable effect on the technical performance of a variety of in situ assays of IHC, ISH, and FISH. Standardization of preanalytic fixation parameters has become an area of intense focus on the technical performance of these assays, particularly if the biomarker is utilized clinically as a predictive marker (20–26). We have found immunohistochemical detection of labile phosphorylated proteins (e.g., pERK, pRB) may show discordant results when comparing TMA studies derived from resection specimens to studies performed on biopsies. This is largely the result that labile proteins are more likely to be detected in core biopsies immediately fixed in 10% neutral buffered formalin, as compared to resection specimens in which time to fixation may vary greatly. In addition for RNA-ISH studies, since degradation of RNA occurs rapidly in pancreatic tissue before fixation, it is imperative to develop a protocol in which representative tissue to be used in the TMA is immediately fixed to optimally preserve proteins and avoid RNA degradation.

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## 2. Materials

Manual tissue arrayer-MTA-1 (Beecher instruments, available through Estigen Tissue Science, <http://www.estigen.com>).

Tissue array punches (varying sizes: 0.6 mm, 1.0 mm, 1.5 mm, or 2.0 mm, available through Estigen Tissue Science, <http://www.estigen.com>).

Paraplast PlusTissue Embedding Media (McCormick Scientific). Note: Paraffin kept at 60°C prior to use.

Oven (Fisher Scientific).

Magnifier on stand with attached light (Fisher Scientific).

Stainless steel molds, extra-large (Labtek).  
Tissue cassettes (Surgipath, Leica).  
Flotation water bath.  
Accu Edge blades.  
Automated rotary microtome (Leica, Deerfield, IL).  
Slide warmer (Fisher Scientific).  
Pilot Pen ultrafine point (Register Office Supply, Baltimore, MD).

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### 3. Methods

Although semiautomated and automated tissue microarrayers for constructing TMAs are available, the manual microarrayer is used in most laboratories. The first and largest commercial supplier of arrayers was Beecher Instruments Inc. that manufactured a variety of manual and robotic arrayers. The manual tissue arrayer MTA-I and the automated tissue arrayer ATA-27 are available through Estigen Tissue Science.

#### **3.1. Collection and Selection of Tissue Blocks**

The first and most time-consuming step of constructing a TMA is collecting and reviewing the appropriate starting material, consisting of FFPE tissue blocks that accurately sample the disease state to be analyzed. The material selected is dependent on the goals of the study. An H&E section is cut from the donor paraffin blocks by standard protocol to assess morphologic features of the tissue (see Note 1). The H&E slide is annotated by the pathologist to indicate the areas of interest for sampling (e.g., tumor and normal areas). Each separate tissue diagnostic region can be given its own number or letter so that it can be uniquely identified with case number, block designation, and tissue diagnosis.

#### **3.2. Design and Organization of TMA**

##### *3.2.1. Determining Type of Array*

Construction of a TMA is primarily based on what particular research question one is trying to answer.

*Multi-tissue and multitumor arrays:* These arrays contain tissues from a variety of anatomic locations, sampling tumor and non-tumor from these different sites. Small arrays can be used for quality control for evaluating reagents/antibodies or documenting the specificity of biomarkers in a spectrum of different tissues and tumors.

*Specific tumor type arrays:* These arrays may be constructed using representative cases of a specific tumor type occurring in a single tissue site (e.g., pancreatic adenocarcinoma). It is recommended that control normal tissue of the primary tumor site also be sampled. These arrays are helpful in studying the prevalence of a biomarker in a given tumor type and comparing different biomarkers in different patients and comparing with their normal tissue.

*Early progression arrays:* These arrays analyze normal tissue, preinvasive lesions (e.g., pancreatic intraepithelial neoplasia (PanIN), or intraductal papillary mucinous neoplasms (IPMN) that may include a spectrum of low- and high-grade dysplastic lesions), in addition to adenocarcinomas. IHC analysis of PanIN TMAs has been useful in confirming the multistep model for pancreatic adenocarcinoma with detection of “early, intermediate, and late” changes occurring in pancreatic neoplasia (27).

*Late progression arrays:* These arrays may identify biomarkers differentially expressed in the primary tumor, regional lymph node metastases, or systemic metastases.

*Tumor and microenvironment arrays:* When studying the interactions between tumor and stromal components, larger diameter cores (1.5 or 2 mm) are recommended. Additional sampling of stroma present at a distance from the tumor may also be useful.

*Outcome-based arrays:* One example of an outcome-based array includes patients with pancreatic adenocarcinoma that received similar therapy and have been clinically followed with long-term outcome data. These arrays may help identify predictive biomarkers that identify specific tumor subtypes responsive to a particular therapy.

### 3.2.2. Determining Size and Number of Cores

All tissue arrayers use two thin-walled needles with slightly different core diameters, one to punch a hole in the recipient (composite TMA) block and one to punch and transfer the core from the donor block. The needles range in diameter from 0.6 to 2.0 mm. When constructing a TMA with 1.5 mm cores, the recipient needle (e.g., outer diameter 1.5 mm) punches a slightly larger hole than the donor needle (e.g., inner diameter 1.5 mm), so the donor core fits tightly into the recipient hole. Selection of the core size is based on the (1) original tissue size in the donor block, (2) scope of the study, and (3) number of blocks to be arrayed.

Usually four to five cores (0.6 or 1.0 mm) or alternatively two to three cores (1.5–2.0 mm) are taken from two to three discrete but representative regions. The increase in the number of cores ensures minimal study case loss due to tissue core dropout, or technical difficulties. Larger sized cores also improve the chance of sampling the entire lesion, or region of interest and adequate surrounding tissue.

The number of cores required for representative sampling may also depend on the degree of heterogeneity of a tumor. In addition, if a tumor such as pancreatic ductal carcinoma is associated with a desmoplastic stroma, a large component of the tumor may consist of stroma and larger cores are recommended. For the majority of TMA studies with pancreatic adenocarcinoma, we have preferred the 1.5 or 2.0 mm core low-density composite TMAs. With more homogeneous tumors (e.g., pancreatic neuroendocrine tumors), sampling of smaller cores (0.6 or 1.0 mm) may be representative.

**Table 1**  
**The number of cores on each tissue array block depends on the size of the needle**

Size of the needle	Layout (L × H)	Max # of cases <sup>a</sup>	Max # of cores (case triplicate)	Space between each core	Setup
0.6 mm	19 × 9	54 <sup>b</sup>	162	1.0 mm	1.5 mm
1.0 mm	15 × 9	42 <sup>c</sup>	126	1.0 mm	2 mm
1.5 mm	12 × 6	22 <sup>d</sup>	66	1.0 mm	2.5 mm
2.0 mm	10 × 6	18 <sup>e</sup>	54	1.0 mm	3 mm

<sup>a</sup> Divide these numbers by two for cases that are comparing tumor/normal

<sup>b</sup> 54 cases plus 1 marker, 2 reference

<sup>c</sup> 42 cases plus 1 marker, 2 reference

<sup>d</sup> 22 cases plus 1 marker, 1 reference

<sup>e</sup> 18 cases plus 1 marker, 1 reference

**3.2.3. Creation of TMA Map**

1. The technologist matches the donor tissue blocks with the corresponding H&E slides in which areas of interest have been previously marked by the pathologist with a marker (xylene-free pen or Pilot pen).
2. The technologist creates a detailed map with core designations for the tumor tissue (2–3 per case in general) and normal control tissue from the same case. The TMA layout is determined and the corresponding TMA block summary is prepared to record information about each tissue core in the array (Position *x*, *y*: case #: Code: Tumor type, Organ tissue, Diagnosis/type, etc.). For creation of pancreatic tumor TMAs in our laboratory, the number of sample cores per array ranges from 54 to 162 (see Table 1). If samples are performed in triplicate the number of unique cases per TMA block ranges from 18 to 54 (see Figs. 1, 2, 3).

**3.3. Technical Construction/  
Punching of TMA**

This protocol is tailored for the use of a manual arrayer MTA-1 (Beecher Instruments/Estigen Tissue Science).

1. The technologist matches the blocks to the annotated slides. Blocks and slides are organized so that they match. Donor blocks should be at least 1 mm thick. If the donor block is thin, cores can be stacked (see Note 2). Slides, blocks, and TMA map should be kept together.  
TMA-coded samples should correspond to matching blocks and slides (see Table 2). Prepare corresponding TMA block summary to record all the information about each tissue core in the array (Position *x*, *y*: case #: Code: Tumor type, Organ tissue, Diagnosis/type, etc.). It may be helpful to prepare a color-coded graphic map of the TMA (see Table 3).

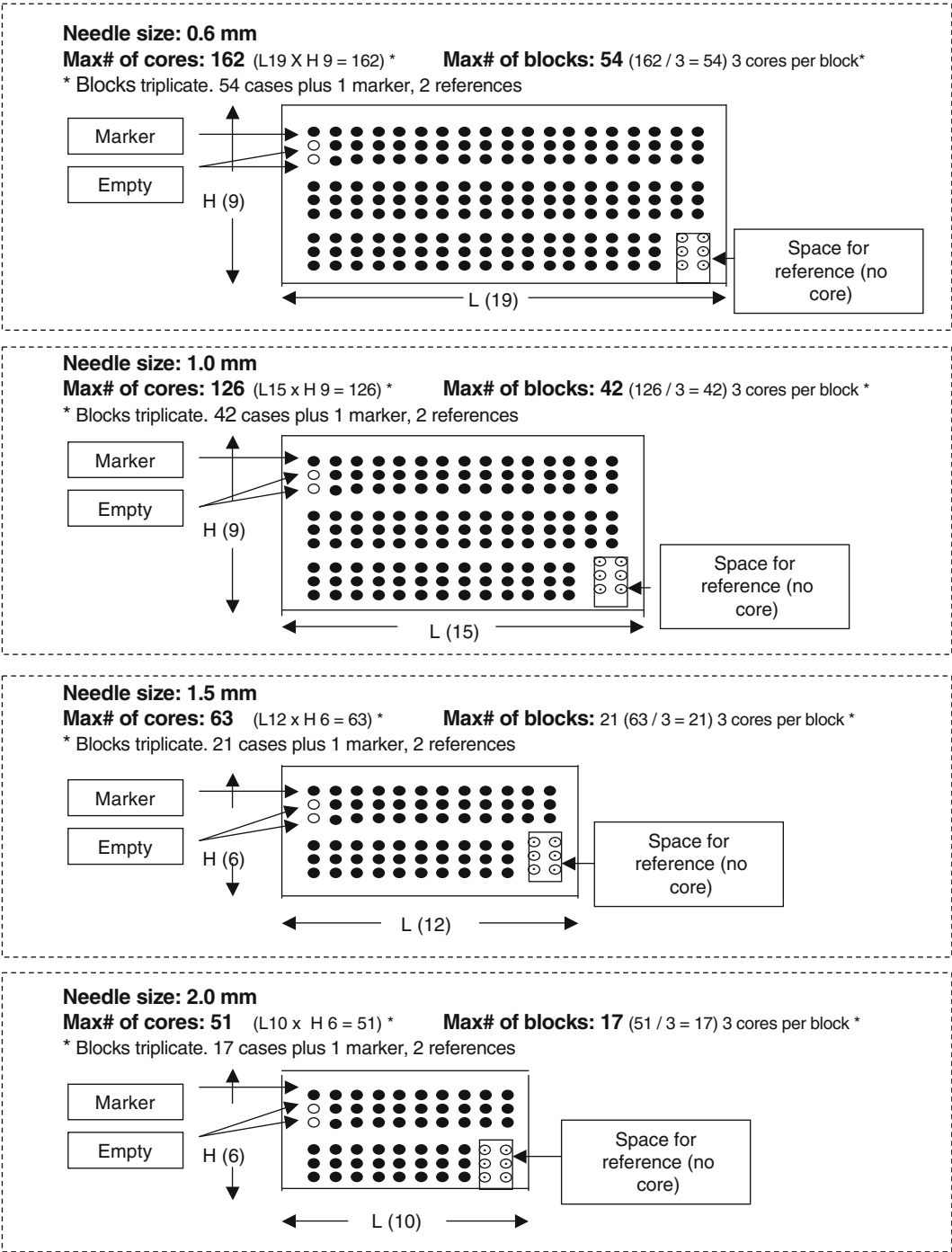


Fig. 1. Pictorial representation of TMAs with differing sized needle cores.



## THE MANUAL ARRAYER

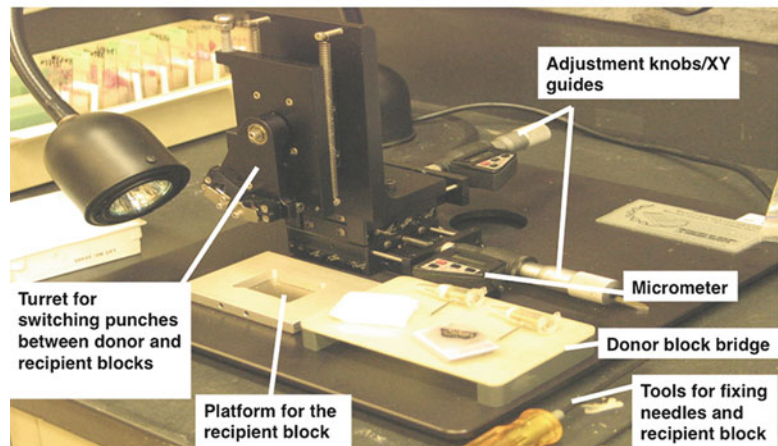


Fig. 2. Workstation set up with manual tissue arrayer.

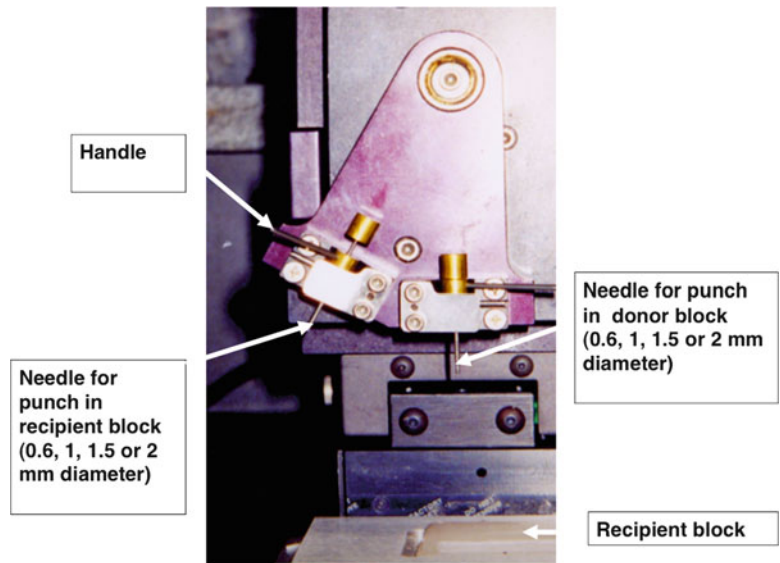


Fig. 3. Closer view of needle punches for recipient and donor blocks in the manual tissue arrayer.

2. Prepare the recipient paraffin block by pouring liquid paraffin into a stainless steel base mold. A variety of paraffin can be used. We use high-temperature Paraplast X-tra and extra large molds. Cover with a slotted tissue cassette and allow to cool. Remove the recipient block from the mold and check for any bubbles or holes. Leave a margin of 3 mm around the array (see Note 3).



**Table 2**  
**Initial spreadsheet with essential necessary for creating a**  
**TMA array including ID number for donor block and tissue**  
**diagnosis**

Code	Accession #	Block #	Diagnosis
IPMN5-001	SPXX-218608	A2	IPMN
IPMN5-002	SPXX-206635	A2	IPMN
IPMN5-003	SPXX-248434	A3	IPMN
IPMN5-004	SPXX-936324	A6	IPMN
IPMN5-005	SPXX-678733	A3	IPMN
IPMN5-006	SPXX-952667	B2	IPMN
IPMN5-007	SPXX-167946	A4	IPMN
IPMN5-008	SPXX-204091	A1	IPMN
IPMN5-009	SPXX-232040	A8	IPMN
IPMN5-010	SPXX-246582	A28	IPMN
IPMN5-011	SPXX-179234	A26	IPMN
MCN5-012	SPXX-270681	B14	MCN
MCN5-013	SPXX-157892	A1	MCN
IPMN5-014	SPXX-182621	A5	IPMN
IPMN5-015	SPXX-230490	A5	IPMN
IPMN5-016	SPXX-861556	A3	IPMN
IPMN5-017	SPXX-255629	A1	IPMN
PANCA5-018	SPXX-861523	A10	Adenocarcinoma
SA5-019	SPXX-451334	B8	Serous cystadenoma
AA5-020	SPXX-499721	B5	Acinar cystadenoma
AA5-021	SPXX-178690	B26	Acinar cystadenoma

- Place the recipient block in the block holder. Adjust the depth stop by rotating the adjustment nut until the punch stops at the desired depth within the paraffin block (typically 0.5–1 mm above the base of the plastic tissue cassette).
- Check the alignment of the donor (larger) and recipient (smaller) punches of the MTA I arrayer. The circular imprints of the punches on the paraffin block surface should be identically centered if they are correctly aligned. Adjust alignment of punches if necessary.

5. With the small punch mark a hole in the first position of the array (intersection of the upper and left margins, position A1). All other array positions will be in reference to this first spot. Accordingly, set the  $X$  and  $Y$  micrometers of the MTA-1 to zero. When the depth stop blocks the downward motion, slowly release the tissue punch and eject the paraffin core.
6. Place the donor block bridge over the array block holder and move the larger punch into the sampling position. Manually hold the donor block in position on top of the donor block bridge while positioning the area to be sampled directly underneath the sample punch. Note: Superimposing the corresponding marked H&E slide over the tissue block will assist in positioning the area to be sampled underneath the tissue punch. For orientation purposes, we use a control lung tissue as a standard marker for tissue in position A1 of all TMA blocks, to facilitate orientation during microscopic evaluation (see Note 4).
7. To retrieve the tissue core push downward on the sample punch. Note: The depth stop will not block the punch motion at the proper position for the donor block, so be careful to prevent the punch from entering too deeply into the block (see Note 5).
8. Remove the donor tissue block and bridge and push the punch downward until its tip reaches the top of the hole in first hole of the recipient array block. Use the large punch stylet to inject the tissue core into the hole created by the smaller punch.
9. Adjust the micrometers to move the tissue punch to the next  $x$ -,  $y$ -position. We use spacing of 1.5 and 2.0 mm between sample centers when using 0.6 and 1.0 mm needles. For 1.5 and 2.0 mm needles, a spacing of 2.5 mm and 3.0 mm is used.
10. Align the marked H&E slide and the corresponding block (repeat steps 6–9, until TMA is completed).
11. The cores are gently pressed down with a spatula, to insure that they are flushed with the surface of the block (see Note 6).
12. The recipient block is dusted clean or wiped with a kimwipe after the placement of every punch. After several punches, the stylus/punch complex may retain some paraffin. Move the stylus up and down to dislodge the paraffin and wipe with a kimwipe.
13. The TMA block is placed in an adjusted metal mold with a 2 lb weight on it to prevent displacement of the cores when the paraffin warms up. To allow annealing of cores with the paraffin, the TMA block is placed in a slide warmer and gradually heated over 1 h (40°C for 15 min; 45°C for 15 min; 50°C for 15 min; and 55°C for 15 min), followed at 60°C for 1 min (see Note 7).

**Table 3**  
**Pictorial map of TMA core data for easy visualization**

**Block label: IPMN-5**

**Needle size: 1.5 mm/number of cases (cores): 21(63)**

1	2	3	4	5	6	7	8	9	10	11	12	
1	Marker	IPMN5-002	IPMN5-004	IPMN5-006	IPMN5-008	IPMN5-010	MCN5-012	IPMN5-014	IPMN5-016	PANCA5-018	AA5-020	AA5-021
2		IPMN5-002	IPMN5-004	IPMN5-006	IPMN5-008	IPMN5-010	MCN5-012	IPMN5-014	IPMN5-016	PANCA5-018	AA5-020	AA5-021
3		IPMN5-002	IPMN5-004	IPMN5-006	IPMN5-008	IPMN5-010	MCN5-012	IPMN5-014	IPMN5-016	PANCA5-018	AA5-020	AA5-021
4	IPMN5-001	IPMN5-003	IPMN5-005	IPMN5-007	IPMN5-009	IPMN5-011	MCN5-013	IPMN5-015	IPMN5-017	SA5-019		
5	IPMN5-001	IPMN5-003	IPMN5-005	IPMN5-007	IPMN5-009	IPMN5-011	MCN5-013	IPMN5-015	IPMN5-017	SA5-019		
6	IPMN5-001	IPMN5-003	IPMN5-005	IPMN5-007	IPMN5-009	IPMN5-011	MCN5-013	IPMN5-015	IPMN5-017	SA5-019		

14. Carefully transfer block in adjusted metal mold on cool plate ( $-5^{\circ}\text{C}$ ) for 30 min. Weight should be kept on mold during cooling.

### **3.4. Technical Cutting of TMA**

1. The TMA block is trimmed on standard microtome (using new blade). Sections should be no more than  $5\ \mu$  (see Note 8).
2. The TMA is placed on cool plate/ice water for standard microtome sectioning.
3. In our laboratory we use standard tissue sectioning techniques with a  $34^{\circ}\text{C}$  water bath to float off the sections onto positive-charged sections or polylysine-coated slides. It is important to maintain orientation of tissue on glass slides (see Note 9).

In the past, the tape transfer method (Instrumedics, Inc.) was used due to the ease of transferring of tissue sections in the desired orientation (3, 28). One disadvantage of the tape transfer method involved increased background staining from the adhesive residue that interfered with molecular assays, such as ISH, FISH, and phosphorylation-specific IHC, particularly if using automated methods.

4. In sectioning a TMA block, one H&E is stained for every 20 sections. For most studies we cut 40 sections at a time. Slides are allowed to dry in vertical position in an open slide box for 48 h. Slides for IHC studies are optimally used within 1 or 2 weeks. For longer storage, unstained sections are stored at  $-20^{\circ}\text{C}$ , without baking the slides.

### **3.5. Biomarker Analysis of TMA**

Histochemical, IHC, and ISH studies performed on TMAs can follow similar protocols as conventional slides prepared with whole tissue sections.

### **3.6. TMA Construction from Frozen Tissue and Cell Line TMAs**

Although most TMAs are created from FFPE tissues, TMA methods may be modified to include sampling of frozen tissue or cell lines.

Frozen TMAs are technically more difficult to construct and require special handling. When constructing frozen TMAs specialized equipment is needed. Special donor tissue and recipient block requirements with a common cutting media such as OCT and special adhesives for section transfer for retained TMA core orientation are used (29).

One of the principal advantages of frozen TMAs includes better RNA quality for applications in ISH assays. Frozen tissue microarrays appear to provide excellent target material for the study of DNA, RNA, and proteins by fixing each array slide in a manner specific to the corresponding technique used (30). Another advantage is that those procedures requiring fixation can be conducted in samples fixed in an identical manner, since fixation is performed for a limited time on the TMA slide.

The disadvantages include altered morphology with significant loss of fine detail in frozen tissue compared to formalin-fixed tissue. Newer commercially available methodologies for performing molecular analysis including RNA-ISH on FFPE tissues provide alternatives to frozen tissue TMAs (31).

Several protocols have been described for TMA preparation from cell lines (32–34). One method involves growing cells, creating a cell pellet with subsequent formalin fixation to create an FFPE block (5).

### **3.7. Digital Image Scanning and Analysis**

Analysis of TMA data can be assessed manually using an ordinal grading system. If the number of markers and number of tissues are relatively small, this method can be used; however it is time consuming, semiquantitative, and requires an experienced pathologist.

In order to optimally handle large-scale IHC analysis or to objectively quantify IHC, ISH results, a variety of scanners are available that will automate the acquisition of data generated with the TMA technology. A variety of TMA analysis software programs are available to standardize the analysis of biomarker data. These programs may be able to analyze both chromogenic or immunofluorescent markers to quantify biomarkers. The automated systems can use morphometric parameters to distinguish tumor cells from background normal cells.

Data management and data analysis are therefore a major concern to ensure both documentation and archiving data of the experiment in addition to facilitating analysis in an efficient and quantitative manner. Conventional spreadsheet-based data organization is feasible for relatively small projects. For larger projects, analyzing thousands of samples with hundreds of biomarkers, robust and scalable applications have to be utilized, which allow data access and manipulation for researchers from different laboratories in a secure way.

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## **4. Notes**

1. A new recut H&E should be cut from the donor block, instead of evaluating the H&E slide in the file. This will ensure that the tissue has not been cut through and diagnostic tissue is still present in the block.
2. If the thickness of the selected area of the donor block is too thin (<1 mm), if possible another sample area or block should be used, since the donor core will be exhausted and absent in deeper sections of the TMA. Although it is recommended to use paraffin blocks of similar thickness in order to maintain

uniform sections with sampling of all cores throughout the TMA, there is another alternative for utilizing thin tissue blocks. This technique involves “stacking” or “packing” of the core with two punches from the original donor block, one on top of each other occupying the same hole in the recipient block.

3. We use a margin of 3.0 mm of paraffin around the entire TMA. This prevents the recipient block from cracking during construction.
4. The use of control tissue is recommended. We use a “marker” lung tissue for the first core in the array. Other standard tissues including normal pancreas and adenocarcinoma may be references that can be utilized as a quality control for assessing uniformity of staining of multiple TMAs.
5. Although the depth of the recipient core can be set by the depth stop, there is no depth stop that will set the depth of the donor core. This depth varies when using different donor blocks, since the tissue blocks are of varying thickness, which determines the limit of the depth at which the cores are taken. For an experienced technologist the depth of the donor core is modified based on the thickness of the donor block. A separate “Depth Stop Kit” can be purchased from Beecher that can be used to obtain uniform lengths of donor cores; this kit may be useful if all donor blocks are of similar thickness. If the size of the donor core punched is too long, eject the core from the stylus and place on a clean flat surface and trim with a clean razor blade to the desired length. The core can then be placed in the recipient hole with a small forceps.
6. Do not push the donor punch all the way into the receiver hole; let it slightly protrude. It can be gently pressed with a spatula to ensure that its position is level with the recipient block surface.
7. It is important that the TMA block be remelted at a temperature slightly higher than the melting point of the wax used in the recipient block for a short time (1 min) and then cooled. This step is critical if sections of the TMA block are cut with a standard microtome using water floatation.
8. It is important to face off the recipient block using a dedicated rotary microtome prior to use. This ensures that the block face is smooth and that all the arrays will be made in the identical sectioning plane. This will minimize the amount of block realignment that is necessary during sections and will help

optimize the number of complete sections that a TMA block will provide.

9. It is important to maintain correct orientation of the slides in both vertical and horizontal direction so that the sections are oriented similarly on the slide and are easier to be read and analyzed.

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