## 1.0 PURPOSE

The purpose of this document is to establish the procedure to create a tissue microarray (TMA).

## 2.0 SCOPE

This standard operating procedure (SOP) describes how TMAs should be constructed from FFPE tissue blocks. This SOP applies to all personnel from ACSR Regional Biospecimen Repositories (RBRs) and affiliates that are responsible for constructing TMAs. The ACSR RBRs and affiliates process and bank biospecimens under site specific approved Human Subjects Protocols. Biospecimens, Samples, Aliquots and Derivatives are entered into the ACSR database when consent for banking and research use have been verified by the Protocol PI or ACSR designee. Each RBR and affiliate site is responsible for Human Subjects compliance as per their institutional guidelines and their local approved Human Subjects Protocol. The SOP does not cover detailed safety procedures for handling biohazardous material and it is recommended that personnel follow institutional safety guidelines

## 3.0 REFERENCE TO OTHER ACSR SOPS OR POLICIES

ACSR SOP Tech009 Biospecimen Handling

ACSR SOP Tech010 Biospecimen Labeling

## 4.0 DEFINITIONS

| Term/Acronym | Definition |
|---|---|
| ACSR | AIDS and Cancer Specimen Resource |
| Aliquot | The sample has the original characteristics of the original or parent specimen but in smaller quantities (FFPE block vs unstained sections from the FFPE block) |
| ATLAS | Annotation of a Tissue Library and Searching Platform |
| | |
| Biospecimen | Human material such as urine, blood, tissue stored in a biorepository for use in laboratory research. For the ACSR this is considered the original or parent biospecimen. |
| Derivative | The original characteristics of the specimen are changed (FFPE |

| | |
|---|---|
| | vs DNA derived from FFPE). |
| Form | A paper or electronic document on which information or results are captured; **NOTE:** Once completed, a form becomes a record. |
| PI | Principal Investigator |
| PPE | Personal Protective Equipment such as gloves, lab coat, face shield. |
| Procedure | Specified way to carry out an activity of a process. A procedure is a set of instructions that describes the stepwise actions taken to complete activities identified in processes. |
| Process | Set of interrelated or interacting activities that transform input into outputs. |
| RBR | Regional Biorepository |
| Record | Document stating results achieved or providing evidence of activities performed. Some examples include freezer logs, incident reports, the master equipment file, etc. |
| Sample | This is an aliquot, derivative or if the Parent Specimen received is stored as a whole specimen, it is referred to as a sample, as per ACSR database definition. |
| SOP | Standard Operating Procedure. A set of written instructions that document a routine or repetitive activity followed by an organization. |
| Specimen | The same as Biospecimen. Human material such as urine, blood, tissue stored in a biorepository for use in laboratory research. For the ACSR this is considered the original or parent biospecimen/ specimen. |
| TMA | Tissue Microarray |
| Universal Precautions | This is an approach to infection control to treat all human blood and certain human body fluids as if they were known to be infectious for HIV, HBV and other bloodborne pathogens, |

## 5.0 ROLES AND RESPONSIBILITIES

This SOP applies to all personnel from ACSR RBRs and affiliate sites that are responsible for creating TMAs.

| ACSR Personnel | Responsibility/Role |
|---|---|

| | |
|---|---|
| ACSR Staff Member/ Laboratory Technician/Technologist or Histology Laboratory Technician/ Technologist | Responsible for preliminary case selection to present to pathologist for validation, organizing selected blocks, creating TMA map, constructing TMA, making unstained cuts and H&E for |
| Pathologist | Validate slides and path reports of cases, selects cases to be utilized for TMA and circles area on H&E to be cored and review of completed cores in TMA |

## 6.0 MATERIALS, EQUIPMENT AND FORMS

The materials, equipment and forms listed in the following list are recommendations only and may be substituted by alternative/equivalent products more suitable for the site-specific task or procedure.

| **Materials and Equipment** | **Materials and Equipment (Site Specific or equivalent)** |
|---|---|
| Donor tissue blocks with tissue specific control blocks | |
| Formula R or equivalent Tissue Embedding Infiltration Medium | Leica Microsystems, Item No.3801450 |
| UNI-CASSETTE | Tissue-Tek, Item No.4157 |
| Incubator set at 65°C | VWR, Fisher, or equivalent |
| Quick-Ray Mold 1 – 1mm x 170 holes (other core sizes available) | IHC World #IW-UMO1-1 |
| UNITMA Quik-Ray Manual Tissue Microarrayer kit with 1 mm tip (other tip sizes available) | IHC World # IW-UT06 |
| Blue or black pen | |
| Magnifying lamp with light | ProVue Magnifying Lamp #26501-SIV |
| Razor blades or disposable scalpel | blades- VWR # 55411-050 or Fisher #12-640 or disposable scalpels- Fisher #31- |

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|---|---|
| | 200-32 or VWR # 21909-654 |
| Sharps disposal container | Fisher #14-827-63 or VWR#19001-005 |
| Can of air – moisture-free, residue-free, with extension tube | Office Depot # 911280 or Staples # IM1GE4780 |
| Flat end Tweezers | |
| Double sided tape 0.75inch wide | |
| Superfrost Plus Microscope glass slides *or other charged slides* | Fisher # 67-762-14 or VWR#48311-703 |
| Cold pack or ice block | |
| Timer | VWR #62344-641 or Fisher #06-662-5 |
| Tissue Tek Base stainless Mold | #4165 *(*37 x 24 x 5mm*)* |
| TMA template/ Map that is to be constructed | |
| Ice block to rehydrate blocks if necessary or to cool created TMA | |
| Tray to hold blocks for workflow – 1) blocks to be cored; 2) blocks that have been cored | |

## 7.0 PROCEDURES

### 7.1 SPECIAL SAFETY PRECAUTIONS

**7.1.1** Comply with "Universal Precautions" when handling all specimens.

**7.1.2** Use PPE (personal protective equipment) in accordance with the institution's guidelines.

**7.1.3** Standard best-practice working procedures include careful manipulation of the patient biospecimens, disinfection of countertops and equipment used during testing, and disposal of biohazard waste into appropriate receptacles.

**7.2** Verification of Identification Information on Biospecimens

As applicable, verify the accuracy of patient information (in keeping with privacy and ethical policies) and ensure that it corresponds with the information on the biospecimen labels or on the biospecimen vessel. Ensure that all personnel are trained in the use of the ACSR database and local electronic filing system(s).

**7.3 SELECTION OF BLOCKS**

**7.3.1** Donor blocks are selected by the research staff and pathologist based upon study criteria. Research staff will assemble H&Es, pathology reports and potential donor blocks for pathologist review.

**7.3.2** The pathologist examines the H&E stained slides, pathology report and tissue blocks.

**7.3.3** H&E slides are marked with a felt-tipped waterproof pen by the pathologist in order to identify the appropriate location to obtain the core(s) for the TMA.

**7.3.4** The marked areas are matched to the corresponding paraffin blocks when cores are taken for TMA construction.

**7.4 CREATION OF THE TMA TEMPLATE**

**7.4.1** After all usable donor tissue is identified; a spreadsheet is constructed listing all the donor blocks to be used in the TMA construction.

**7.4.2** A map for the TMA is constructed using the spreadsheet to map each patient to a specific x,y coordinate. The map should be designed to best accommodate the variety of cases, number of biospecimens, matching normal tissue, and the purpose of the array.

**7.4.3** It is good practice to avoid using the outside rows and columns, and to insert recognizable/orientation cores at indicator positions to define the TMA orientation and ensure correct case identification when the TMA is scored.

**7.5 MAKING A RECIPIENT BLOCK**

**7.5.1** Melt Formula R Tissue Embedding Infiltration Medium at 65°C.

**7.5.2** Make sure the block mold is free of debris by using canned air to blow out the mold.

**7.5.3** Pre-warm the mold to 65$^{o}$C for 30 minutes.

**7.5.4** Place a UNI CASSETTE on top of the TMA mold and Dispense melted paraffin into the TMA mold taking care to not introduce air bubbles.

**7.5.5** Set the TMA mold with Cassette on top on a cold surface for 15-20 minutes or at room temperature for 1 hour.

**7.5.6** Prepare a "blank" block using a stainlessTissueTek base mold. Place a UNI CASSETTE on top of the stainless base mold, fill the mold with melted paraffin and then place on a cold surface.

**7.5.7** Allow the blocks to cool on a flat surface. When the blocks are cooled, carefully remove the mold.

**7.5.8** Check the blocks for any holes or cracks that may have arisen during the block preparation. Also ensure that the block surface is flat and parallel to the underside of the cassette.

**7.5.9** Wrap the blocks in foil, date and initial, and store the blocks at 4°C until they are ready to be used.

## 7.6 CONSTRUCTION OF THE TISSUE MICROARRAY – Recipient block method

**7.6.1** Gather all donor blocks to be cored and place them in ordered rows in a tray. The order of the blocks in the tray should represent the order of the cores in the TMA.

**7.6.2** For each block, check the block ID against the TMA map before taking a core. The number must be taken directly from the FFPE block and compared to the TMA map to ensure that the map is an accurate representation of the actual block.

**7.6.3** Inspect the recipient block by placing it under the magnifying lamp. Make sure there is not any residual paraffin/medium in the wells. If there is, use an air can to gently remove.

**7.6.4** Place the donor tissue block under the magnifying lamp and using the UNITMA Manual Tissue Microarrayer take a 1mm core of the previously marked area on the tissue block.

**7.6.5** Slowly release the core from the microarrayer into the pre-identified well. Stop releasing when the last end of the tissue reaches the tip of the microarrayer, use your finger to gently push the core the rest of the way down into the well. Use a sharp blade (razor blade or disposable scalpel) to cut off any excess paraffin that may be sticking up above the paraffin block.

**7.6.6** Clean the microarrayer between samples using a Kim Wipe.

**7.6.7** If the donor block is thin and the core does not fill the well, a second core from the same tissue block should be taken and steps 7.6.4-7.6.6 should be repeated. The second sample is stacked on top of the first core, until the well is filled. Stacking as many cores as determined to fill the well. NOTE: Stacking cores will create an issue when sectioning the TMA, as stacked cores tend to pull out of the well instead of being cut. The likelihood of core pull-out when sectioning increases as the core length diminishes.

**7.6.8** As each core is placed into the recipient block, the block identification number should be noted on the array map – checking off the cases as the wells are filled.

**7.6.9** After the donor FFPE block is used, return the blocks to a different box in the same order as used to generate the recipient block. This system will avoid confusion as the number of the block and the order of the block in the storage box can be used to verify the position in the TMA.

**7.6.10** After all the tissue cores have been placed in the TMA, fill the empty wells with cores taken from the “blank” block (step 7.5.6).

**7.6.11** After all the wells have been filled, make sure cores are level with top of block by taping with flat side of flat end tweezers. Invert the TMA on a glass slide and incubate at 65°C for 15 minutes.

**7.6.12** Apply gentle pressure to flatten the surface and "set" the cores within the paraffin block.

**7.6.13** Repeat if necessary only heating for 5 minutes each additional time.

**7.6.14** The block should be labeled with the TMA name and date.

**7.6.15** The block will then be cut – 30 cuts with H&E stains made of slides 10 and 30.

**7.6.16** The H&E slides are reviewed by the pathologist for quality control, presence of tumor and presence of core.

**7.6.17** Immunohistochemistry staining for Pancytokeratin and In situ hybridization for U6 probe are done for quality assessment of the tissues in the block.

**7.6.18** This information is noted and filed with the electronic map and case list for the TMA.

## 7.7 CONSTRUCTION OF THE TISSUE MICROARRAY – Tape method

**7.7.1** NOTE: No recipient block is required. This method should be used for blocks that are at least 5mm in depth. No stacking of cores is performed with this method and dexterity is required.

**7.7.2** Melt Formula R Tissue Embedding Infiltration Medium at 65°C for use once the cores have been placed.

**7.7.3** Gather all donor blocks to be cored and place them in ordered rows in a tray. The order of the blocks in the tray should represent the order of the cores in the TMA.

**7.7.4** For each block, check the block ID against the TMA map before taking a core. (See Appendix 9.3 for how to make a TMA map when using the Tape Method. Note the grid in the mold is a mirror image of the resulting

product.) The number must be taken directly from the FFPE block and compared to the TMA map to ensure that the map is an accurate representation of the actual block.

**7.7.5** Make sure the stainless mold is free of debris by using canned air to blow out the mold.

**7.7.6** Place a grid in the stainless mold. Place double stick tape across the entire floor of the mold.

**7.7.7** Place the donor tissue block under the magnifying lamp and using the UNITMA Manual Tissue Microarrayer take a 1mm core of the previously marked area on the tissue block.

**7.7.8** Slowly release the sample from the microarrayer. Stop releasing when the tissue reaches the tip of the microarrayer, and use a sharp blade (razor blade or disposable scalpel) to cut off the excess paraffin, so that the tissue is now at the very end.

**7.7.9** Place the tissue in the pre-identified spot on the tape by slowly releasing the tissue from the microarrayer. Clean the microarrayer between samples using a Kim Wipe.

**7.7.10** As each core is placed onto the stainless mold the block identification number should be noted on the array map – checking off the cases as the wells are filled.

**7.7.11** After the donor FFPE block is used, return the blocks to a different box in the same order as used to generate the recipient block. This system will avoid confusion as the number of the block and the order of the block in the storage box can be used to verify the position in the TMA.

**7.7.12** Add tissue control and orientation cores.

**7.7.13** Place a tissue cassette upon the stainless mold. Slowly and steadily pour melted paraffin through the cassette grid. Allow to sit undisturbed for 30 minutes at RT. The TMA which is still attached to the metal tray is then placed in 4$^{o}$C for an additional 30 minutes, after which the TMA is removed from the metal tray and the double stick tape discarded.

**7.7.14** The block should be labeled with the TMA name and date.

**7.7.15** The block will then be sectioned – 30 sections with H&E stain made of slides 10 and 30.

**7.7.16** The H&E slides are reviewed by the pathologist for quality control.

**7.7.17** Immunohistochemistry staining for Pancytokeratin and In situ hybridization for U6 probe are done for quality assessment of the tissues in the block.

**7.7.18** This information is noted and filed with the electronic map and case list for the TMA.

## 8.0 APPLICABLE REFERENCES, REGULATIONS AND GUIDELINES

**8.1** Quick-Ray Manual Tissue Microarrayer - Short Version – PLEASE REFER TO PROTOCOL FOR ALTERATIONS – THIS IS HELPFUL FOR VISUALIZATION http://www.youtube.com/watch?v=0lSngf-eG-4

**8.2** NCI Best Practices for Biospecimen Resources http://biospecimens.cancer.gov/practices/default.asp

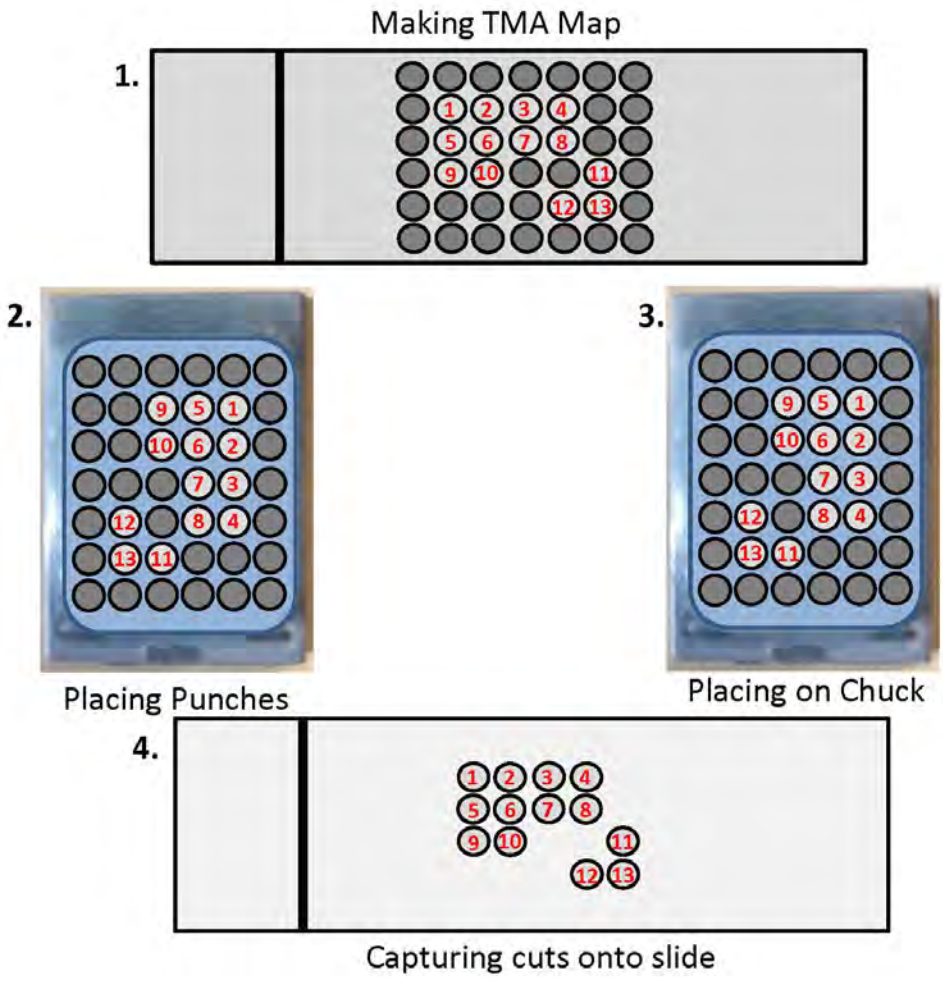
**8.3** Best Practices for Repositories I. Collection, Storage and Retrieval of Human Biological Materials for Research. International Society for Biological and Environmental Repositories (ISBER). http://www.isber.org/Search/search.asp?zoom_query=best+practices+for+repositories

**8.4** US National Biospecimen Network Blueprint http://biospecimens.cancer.gov/resources/publications/reports/nbn.asp

**8.5** National Bioethics Advisory Commission: Research involving human biological materials: Ethical issues and policy guidance, Vol. I: Report and recommendations of the National Bioethics Advisory Committee. August 1999. http://bioethics.georgetown.edu/nbac/hbm.pdf

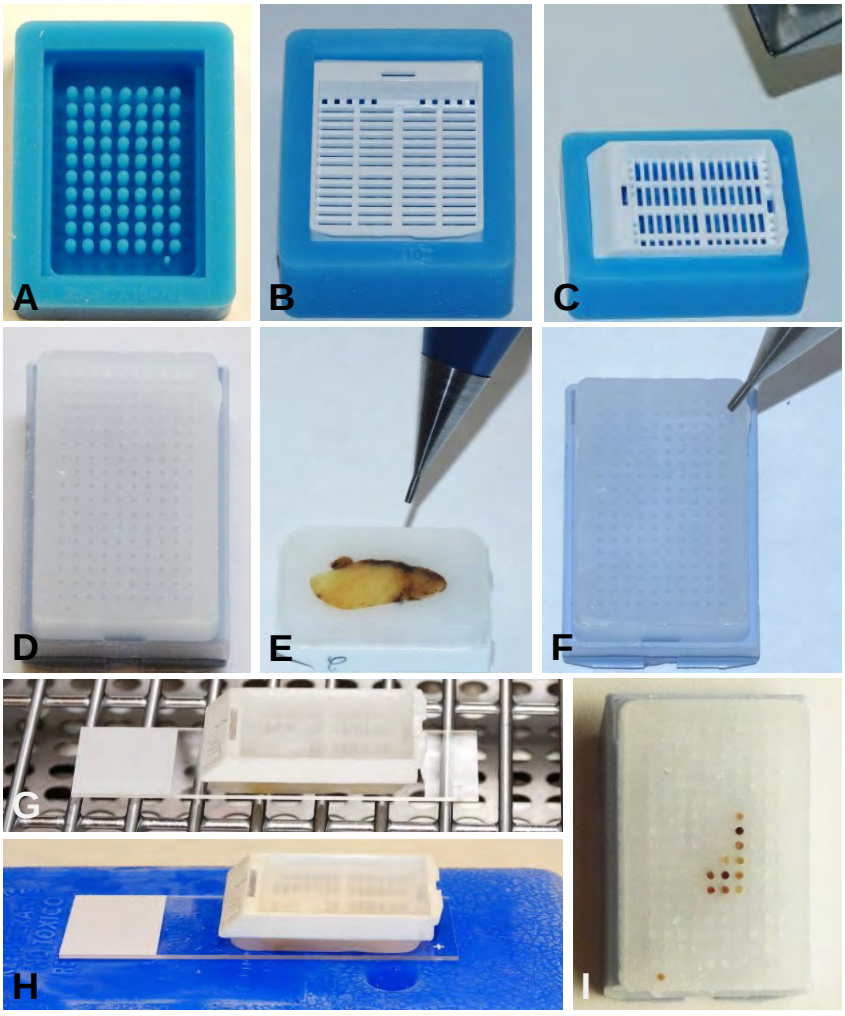
## 9.0 APPENDICES

### 9.1 RECIPIENT BLOCK METHOD – TMA MAP AND RESULTING ALIGNMENT OF CORES ON SECTIONS



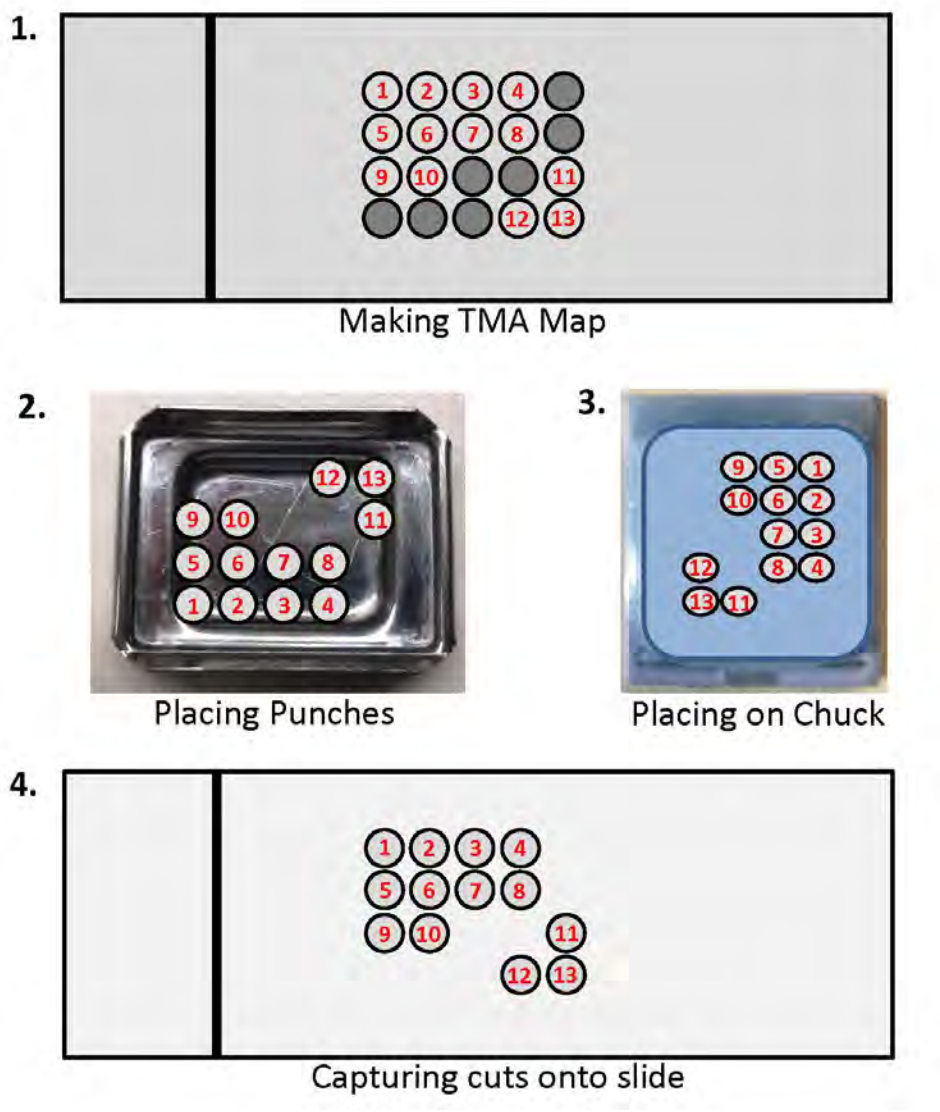
## 9.2 RECIPIENT BLOCK METHOD – TMA CONSTRUCTION VISUAL AIDES

A) Silicon mold used to make paraffin recipient block. B) Placement of cassette on mold prior to addition of paraffin. C) Pour melted paraffin into mold, allow mold to cool to RT for 30 minutes and gently pull to separate mold from block. D) Resulting recipient block. E) Donor block about to have a core removed. F) Core being placed in recipient block. G) Heat completed TMA at 60$^{o}$C for 15 min and then gently press the block against slide to align cores. H) Cool slide 30 minutes on an ice pack, then remove slide from block. I) Completed TMA



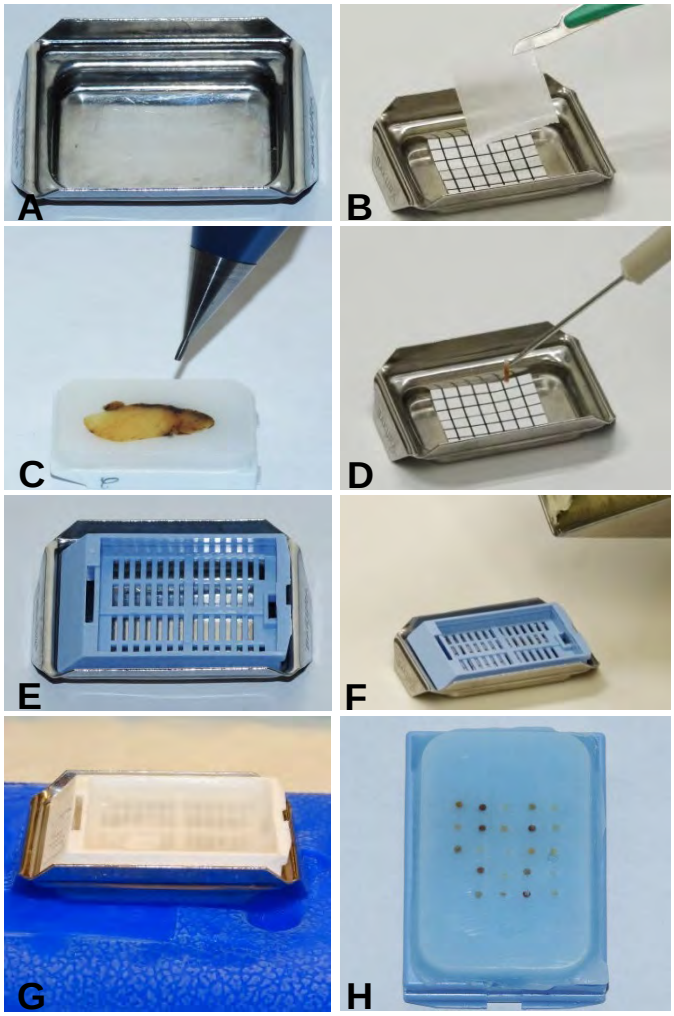
### 9.3 TAPE METHOD – TMA MAP AND RESULTING ALIGNMENT OF CORES ON SECTIONS

NOTE: Alignment in Mold is a mirror image of the resulting TMA when looking at the face of the block.



### 9.4 TAPE METHOD – TMA CONSTRUCTION VISUAL AIDES

A) Stainless block mold. B) TMA core placement grid in the bottom of the mold, and placing double stick tape on top of grid. C) Donor block to be cored for TMA. D) Expel core and pick up with dissection stick and place on grid. E) Cassette on completed TMA. F) Gently pour melted paraffin through cassette to form TMA. G) Cool TMA on ice pack for 30 minutes and then remove from mold. H) Completed Tape TMA



## 10.0 REVISION HISTORY

| SOP Number | Date revised | Author | Summary of Revisions |
|---|---|---|---|
| Tech011 | 6-6-2018 | BGG/TY | Add Tape Method, replace sample with biospecimen, format and add definitions. |
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