Agena MassARRAY DICER1 Assay

Purpose	This procedure provides instructions for preparing samples and reagents, setting up multiplex, polymerase chain reaction (PCR) and Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF) reactions using the Agena Bioscience MassARRAY DICER1 assay on Formalin-Fixed, Paraffin-Embedded (FFPE) tumor tissue.
Policy Statements	This procedure applies to all technical staff performing testing on the Agena MassARRAY instrument.
Principle and Clinical Significance	The *DICER1* gene provides instructions for making the Dicer protein which plays a role in regulating the expression of other genes. The Dicer protein aids in the production of a molecule called microRNA (miRNA). MicroRNAs are short lengths of RNA. Dicer cleaves precursor RNA molecules to produce miRNA. MicroRNAs control gene expression by blocking the process of protein production. In the first step of making a protein from a gene, messenger RNA (mRNA) is formed and acts as the blueprint for protein production. MicroRNAs attach to specific mRNA molecules and stop the process by which protein is made. Sometimes, miRNAs break down the mRNA, which also blocks protein production. Through this role in regulating the expression of genes, Dicer is involved in many processes, including cell growth and proliferation and the maturation of cells to take on differentiation.[3] Mutations in the *DICER1* gene can cause *DICER1* syndrome. People with this condition have an increased risk of developing many types of tumors, particularly certain tumors of the lungs (pleuropulmonary blastoma), kidneys (cystic nephroma), ovaries (Sertoli-Leydig tumors), and thyroid (multinodular goiter). Most of these mutations lead to an abnormally short Dicer protein that is likely unable to produce miRNA. Without regulation by miRNA, genes are expressed abnormally, which could cause cells to grow and divide uncontrollably and lead to tumor formation.[3] The DICER1 Panel for use on the MassARRAY system, is a multiplex Polymerase Chain Reaction, Matrix-Assisted Laser Desorption Ionization – Time of Flight (PCR/MALDI-TOF) test intended for single nucleotide variant (SNV) and small indel genotyping and somatic mutation analysis of genomic DNA extracted from tumor tissue. Formalin-Fixed, Paraffin-Embedded tissue (FFPE) is the validated clinical sample type. A spin column based nucleic acid extraction on the QIACube Connect instrument is utilized. Following DNA extraction, PCR with iPLEX Pro chemistry then amplifies custom target regions of interest; after the inactivation of unincorporated dNTPs, a sequence-specific primer extension step is performed using the supplied custom set of Extend primers and iPLEX Pro reagents. Sample amplification is carried out on the Applied Biosystems 2720 and Veriti Thermalcyclers following the parameters listed in the iPlex Pro IFU. The extension products (analyte) are desalted, transferred to a SpectroCHIP Array (a chip with pre-spotted matrix crystals) and then loaded into the MassARRAY Analyzer (a MALDI-TOF mass spectrometer). The analyte/matrix co-crystals are irradiated by a laser, inducing desorption and ionization. The positively charged molecules accelerate into a flight tube towards a detector. Separation occurs by time-of-flight, which is proportional to the mass of the individual molecules. After data processing, a spectrum is produced with relative intensity on the y-axis and mass/charge on the x-axis. Data acquired by the MassARRAY analyzer is processed by MassARRAY Typer software, and results then are interpreted.[1] The Agena Bioscience DICER1 panel consists of 68 variants in *DICER1*, *DROSHA*, *FOXL2*, *TERT*, and *TP53* genes interrogated by 37 assays divided into 6 primer sets. See **Tables 1** and **2** for details.

Table 1: Panel Variants

Gene	p.	c.	Gene	p.	c.	Gene	p.	c.	Gene	p.	c.
DICER1	p.A234V	c.701C>T	DICER1	p.D1709N	c.5125G>A	DICER1	p.G1809W	c.5425G>T	DICER1	p.E1813S	c.5437_5438delinsTC
DICER1	p.N477S	c.1430A>G	DICER1	p.D1709Y	c.5125G>T	DICER1	p.G1809A	c.5426G>C	DICER1	p.E1813A	c.5438A>C
DICER1	p.R656*	c.1966C>T	DICER1	p.D1709A	c.5126A>C	DICER1	p.G1809E	c.5426G>A	DICER1	p.E1813G	c.5438A>G
DICER1	p.R688*	c.2062C>T	DICER1	p.D1709G	c.5126A>G	DICER1	p.G1809V	c.5426G>T	DICER1	p.E1813V	c.5438A>T
DICER1	p.R937C	c.2809C>T	DICER1	p.D1709V	c.5126A>T	DICER1	p.D1810Y	c.5427_5428delinsTT	DICER1	p.E1813D	c.5439G>C
DICER1	p.S1344L	c.4031C>T	DICER1	p.D1709E	c.5127T>A	DICER1	p.D1810H	c.5428G>C	DICER1	p.E1813D	c.5439G>T
DICER1	p.G162D	c.485G>A	DICER1	p.D1709E	c.5127T>G	DICER1	p.D1810N	c.5428G>A	DICER1	p.Y1874*	c.5622C>G
DICER1	p.E1705*	c.5113G>T	DICER1	p.D1713H	c.5137G>C	DICER1	p.D1810S	c.5428-5429delinsAG	DROSHA	p.E1147K	c.3439G>A
DICER1	p.E1705K	c.5113G>A	DICER1	p.D1713N	c.5137G>A	DICER1	p.D1810Y	c.5428G>T	FOXL2	p.C134W	c.402C>G
DICER1	p.E1705Q	c.5113G>C	DICER1	p.D1713Y	c.5137G>T	DICER1	p.D1810A	c.5429A>C	TERT	n/a	c.-124C>T (C228T)
DICER1	p.E1705A	c.5114A>C	DICER1	p.D1713A	c.5138A>C	DICER1	p.D1810G	c.5429A>G	TERT	n/a	c.-146C>T (C250T)
DICER1	p.E1705G	c.5114A>G	DICER1	p.D1713G	c.5138A>G	DICER1	p.D1810V	c.5429A>T	TP53	p.R175H	c.524G>A
DICER1	p.E1705V	c.5114A>T	DICER1	p.D1713V	c.5138A>T	DICER1	p.D1810E	c.5430T>A	TP53	p.R248W	c.742C>T
DICER1	p.E1705D	c.5115A>C	DICER1	p.D1713E	c.5139C>A	DICER1	p.D1810E	c.5430T>G	TP53	p.R248Q	c.743G>A
DICER1	p.E1705D	c.5115A>T	DICER1	p.D1713E	c.5139C>G	DICER1	p.E1813*	c.5437G>T	TP53	p.R273C	c.817C>T
DICER1	p.D1709C	c.5125_5126delinsTG	DICER1	p.G1809A	c.5425G>C	DICER1	p.E1813K	c.5437G>A	TP53	p.R273H	c.818G>A
DICER1	p.D1709H	c.5125G>C	DICER1	p.G1809R	c.5425G>A	DICER1	p.E1813Q	c.5437G>C	TP53	p.R282W	c.844C>T

Table 2: Panel Assay IDs and Primer Sets

Primer Set 1	Primer Set 2	Primer Set 3
DICER1_c5127TtoAG	DICER1_c5622CtoG	DICER1_c2809CtoT
TP53_c743GtoA	TP53_c742CtoT	TP53_c817CtoT
TP53_c844CtoT	DICER1_c5114AtoCGT	TP53_c524GtoA
DICER1_c1966CtoT	DICER1_c5113GtoACT	DICER1_c5428GtoACT_r
DROSHA_c3439GtoA	DICER1_c485GtoA	FOXL2_c402CtoG
DICER1_c1430AtoG	DICER1_c2062CtoT	DICER1_c5427GtoT
DICER1_c5428GtoACT_f	DICER1_c5430TtoAG	DICER1_c5115AtoCT
DICER1_c5429AtoCGT		DICER1_c701CtoT
Primer Set 4	**Primer Set 5**	**Primer Set 6**
DICER1_c5438AtoCGT	DICER1_c5125GtoACT	TERT_C228T
DICER1_c5137GtoACT	DICER1_c5425GtoACT	DICER1_c5439GtoCT
DICER1_c5138AtoCGT	DICER1_c5126AtoCGT	DICER1_c5139CtoAG
DICER1_c4031CtoT	DICER1_c5426GtoACT	TERT_C250T
TP53_c818GtoA		
DICER1_c5437GtoACT		

Test Code

DICER1 – Formalin-Fixed, Paraffin-Embedded (FFPE) tissue.

Sample

A. Acceptable specimens:

- Formalin-Fixed, Paraffin-Embedded (FFPE) tissue as scrolls or unstained sections.

B. Unacceptable specimens:

- Improperly labeled or unlabeled samples.

- <40% tumor cellularity, with or without macrodissection (unless approved by molecular pathologist on case-by-case basis).
- Decalcified specimens.

C. Specimen Collection and Transport:

- FFPE scrolls or slides received from histology department; room temperature.
- Refer to *Lab Test Directory* on StarNet

D. Specimen assessment:

- Refer to the policy *MB1.02v7 Specimen Rejection Criteria for Molecular Biology*
- Do NOT discard rejected samples unless approved by molecular pathologist.

Special Safety Precautions

- Standard precautions. Refer to MB 2.02 Biohazard Containment
- Use of engineering controls: Refer to MB 3.01 Engineering Controls to Prevent Nucleic Acid Contamination

Laboratory staff are subject to occupational risks associated with specimen handling. Refer to the safety policies located in the safety section of the *Molecular Biology Policy Manual*:

- *Safe Work Practices in Molecular*
- *Biohazardous Spill in Molecular*
- *Biohazard Containment*

1. Wear appropriate personal protective equipment (PPE) including disposable gloves and lab coats.
2. Handle all samples and waste materials as if they were capable of transmitting infectious agents.
3. Change gloves often when handling reagents or samples.
4. Dispose of materials used in this assay, including reagents and used buffer vials, in biohazardous waste.

Materials

Reagents	Supplies	Equipment
-Sani-Cloth Bleach wipes or 1:10 diluted bleach solution -70% ethanol -100% ethanol -5% Extran -DNA Away -Deionized water (DI) -Nuclease Free Water (NFW) -Clean Resin -Ultrapure HPLC-grade water -Positive Amplification Control material -QIAamp DNA FFPE kit (REF 56404) Room 1: **MassARRAY DICER1 Panel Kit** -Agena DICER1 Panel (REF 06205) – store at -25 °C to -10 °C: • DICER1 PCR Primers	-Gloves (powder-free) -Filtered pipette tips, various sizes including 200 uL, extended tips -Sharps disposal container -Microtubes -Clear adhesive plate seals (ThermoFisher, Cat #: AB0558) -96 well semi-skirted, color microtiter plates -0.2 mL PCR strip tubes (8) -Reservoirs -QIAcube kit concumables • 1000 uL tips • 200 uL tips • RB Tubes • CB Tubes • Rotor adaptors -Eppendorf epMotion 50 uL tips -Eppendorf 1.5 mL Snap-cap tubes	-Plate centrifuge -Vortex -Mini tube centrifuge -Tube centrifuge -Thermocycler -PCR work station with UV irradiation -Multichannel pipettes -Single channel Pipettes -Tube racks -Sealing paddle -SpectroCHIP arrays -10 to -30° C freezers -Laminar flow Hood -Refrigerators 2 – 8° C -BSC BSL-2 -70^0 C freezer -QIAcube Connect -Nanodrop -Agena MassARRAY with Chip Prep Module (CPM) 96, including:

	• DICER1 Panel Extend Primers • -PCR Reagent Set (REF 21327M) - store at -25 °C to -10 °C: • $MgCl_2$, 25mM • 10x PCR Buffer • dNTP Mix • PCR Enzyme -iPLEX Pro Reagent Set (REF 10212)- store at -25 °C to -10 °C: • 3 Point Calibrant • iPLEX Termination Mix • iPLEX Buffer Plus, GPR • iPLEX Pro Enzyme • SAP Buffer • Shrimp Alkaline Phosphatase (SAP) **NOTE:** The MassARRAY DICER1 Panel Kit can be used up to ten times (freeze/thaw), as long as components are maintained on ice or a cold block during access and returned to storage conditions after use. **NOTE:** Keep all reagents in cold blocks once thawed and only take enzymes out of freezer immediately prior to use. Mark white board for each freeze-thaw cycle.		• Typer software v5.01 or greater • RT-Workstation v4.1 or greater • Chip Prep Controller v2.2 or greater -Eppendorf epMotion 5073 -Eppendorf epMotion 5075 -Eppendorf Thermorack TMX -Eppendorf Thermoadaptor
	Record shipment receipts on log: MP 4.02.F1 Agena DICER1 Kit and QC Inventory Log		
Calibration	Annual instrument function check and maintenance performed by Agena BioScience.		
Quality Control	**See SOP:** MP 4.02 Agena MassARRAY DICER1 Assay Quality Control **Daily Quality Control:** For extraction quality control, the Nanodrop will be used to assess DNA concentration and purity. **External Quality Control:** • Perform QC using an external positive control on a rotating basis based on primer set and a negative control with **every run**. • Perform QC using a previously tested positive sample (historical patient sample or positive synthetic control if patient sample is not available) for each primer set (positive TERT assay required for primer set 6), a previously tested negative sample for each primer set, positive QC (PC), and a negative control with **new lot/shipments**.		

- Record and file results in the appropriate binder.
- Positive Amplification QC – store at – 70 °C
- Negative/Contamination QC (NTC) – Nuclease-free water

QC Monitors:

Control	Control Monitor
Historical Positive and Negative Patient (or synthetic control) Samples	Reagent, instrument, equipment, or supply failure.
Positive Amplification Control (PC)	Reagent, instrument, equipment, or supply failure.
Negative/Contamination Control (NTC)	Reagent, instrument, equipment, or supply failure. Environmental contamination monitoring.

Record QC results on worksheet and log:
MP 4.02.F1 Agena DICER1 Kit and QC Inventory Log
MP 4.02.F2 Agena DICER1 New reagent QC worksheet
MP 4.02.F3 Agena DICER1 QC Log

- Before reporting patient results, all controls must yield valid results
- If results are invalid, repeat testing

Negative Control (NTC)

1. Lot number (L/N), expiration date
2. Record lot information in appropriate binder

Positive Controls (POSC) – Rotating targets

1. Previous positive patient
2. Synthetic controls

Test controls as you would patient samples.

Record and file results in QC binder

Control Type	QC Status	Expected Sample Result
Historical Positive and Negative Patient (or synthetic control) Samples	Pass	Match to historical interpreted results
POSC: Positive Amplification Control	Pass	Match to documented target

	<table><tr><td>**NTC: Nuclease-free water**</td><td>Pass</td><td>No Alleles</td></tr></table> **NOTE:** When reagents are in use, update the information on the reagent run prep worksheet. **NOTE:** Additional external quality control may be performed on an as needed basis if certain circumstances arise. Examples include: • Drift in results (e.g., increasing/decreasing positivity rates) • Potential contamination (negative control) • After dramatic instrument maintenance or movement
Assay Procedure: Sample Preparation and Extraction on QIAcube (Room 2)	**NOTE:** Clean hood and supplies with a 1:10 bleach dilution or wipes followed by DI water and alcohol before and after any sample processing **Testing Preparation – Sample prep and DNA extraction: Room 2** 1. Open the DICER1 extraction worksheet template; SharePoint – Lab – Documents – Molecular – Molecular Pathology – Agena-Dicer1 – Forms and Worksheets – DICER1 QIAcube and Nanodrop Worksheet. 2. Click File-Save As-Save a Copy → change name as described in Step 3 3. Delete "- Copy" from the name and add the date (mm.dd.yy) in front of the File name followed by a space and save to DICER1 Run Worksheets. 4. Fill out Date, Initial, Lot#, Exp, and Run name fields. **NOTE:** Format Run name as follows: "mmddyy DICER1" a. e.g. March 3, 2020 = 0**30320 DICER1** **NOTE:** Base runs off available chip spots in room 3 if applicable. **Note:** Store the QIAamp UCP MinElute columns at 2-8°C. They can be stored up to 4 weeks at room temperature if necessary. The rest of the kit contents should be stored at room temperature. Expiration is 1 year after receipt. **Note:** Prior to first time use, prepare Buffer AW1 by adding 25 mL ethanol and Buffer AW2 by adding 30 mL ethanol, dating both bottles and checking the box to indicate they have been prepared. **Note:** Ensure the "2" shaker adapter is in place on the QIAcube for the FFPE protocol (not the S2). 1. On the QIAcube, log in with username: Admin and password: Admin. Go to the Tools tab and then the Run Modules tab. Select Heat Shaker and set the temperature to 56°C at 8 rpm for 30 minutes to get the Heat Shaker up to temperature prior to loading the samples. 2. Labeled Sample Tubes RB (990381) with the correlating FFPE sample in it will be delivered to Molecular from Histology along with the paper requisitions. 3. Fill in the Sample ID fields on the DICER1 QIACube and Nanodrop worksheet and label the caps of the tubes with the corresponding QIACube Number from the worksheet. 4. Centrifuge briefly. Add 300 uL Deparaffinization solution and vortex for 10 seconds. Centrifuge briefly. 5. On the QIAcube, select Stop to terminate the preheat step. Load sample tubes. Set the temperature back to 56 °C at 8 rpm for 3 minutes and 30 seconds. Select Start. 6. Cool to room temperature (around 7 minutes). If the solution becomes solid or waxy at room temperature, add another 300 uL deparaffiniazation solution and repeat the incubation. The blue solution should remain liquid at room temperature if it has completely melted the paraffin.

NOTE: A master mix that comprises the components in Step 8 may be prepared in advance during sample cool down.

Samples	Buffer FTB (uL)	Ultrapure Rnase-free Water (uL)	Proteinase K (uL)
1	25.0	55.0	20.0
2	52.5	115.5	42.0
3	78.8	173.3	63.0
4	105.0	231.0	84.0
5	131.3	288.8	105.0
6	157.5	346.5	126.0
7	183.8	404.3	147.0
8	210.0	462.0	168.0
9	236.3	519.8	189.0
10	262.5	577.5	210.0
11	288.8	635.3	231.0
12	315.0	693.0	252.0

7. On the QIAcube, go to the Tools tab and then the Run Modules tab. Select Heat Shaker and set the temperature to 56°C at 8 rpm for 30 minutes to get the Heat Shaker up to temperature prior to loading the samples.
8. If a master mix was prepared in advance during Step 6, add 100 uL of master mix to each sample. Otherwise, add 25 uL Buffer FTB, 55 uL ultrapure RNase-free Water, and 20 uL Proteinase K to each sample. Vortex and briefly centrifuge.
9. On the QIAcube, select Stop to terminate the preheat step. Incubate for 1 hour and 3 minutes at 56°C and 1000 rpm onboard the QIAcube.
10. Set the Eppendorf ThermoMixer F1.5 to 90°C by using the arrows to start heating. Insert the thermometer into the glycerin on top of the ThermoMixer.
11. Once the QIAcube incubation has finished, take tubes out and place into the ThermoMixer at 90°C without shaking for 1 hour.
12. After incubation, briefly centrifuge.
13. There should be a complete phase separation. Remove and discard the upper blue phase using a fine-tip disposable pipet. Keep the lower ~100 uL aqueous lysate in the Sample Tube RB. This is the 2 mL Eppendorf Safe-Lock Tube.
14. On the QIAcube, go to DNA tab and select QIAamp DNA FFPE Advanced.
15. Select material: FFPE Tissue and click Next.
16. Select Protocol: DNA from FFPE and click Next.

	17. Elution volume: 40 and click Next. 18. Select the correct number of samples and click Next. 19. Load Buffer Bottles with enough volume of each respective reagent as indicated on the screen. **NOTE**: Elution bottle should be filled with Ultrapure HPLC-grade water instead of Buffer ATE. **NOTE:** If opening a new QIAamp FFPE Extraction kit, prepare AW1 and AW2 by adding absolute ethanol. Follow bottle instructions for volumes. 20. Click Next. 21. Load Tip Racks. 22. Prepare RNase A and Proteinase K in 1.5 mL elution tubes; volumes are shown on QIAcube screen. 23. Label 1.5 mL elution tubes with Sample ID on the side and QIAcube number on the cap. 24. Assemble Centrifuge Rotor Adaptors with the spin columns and elution tubes in the correct positions according to the screen diagram. Ensure lids are also in the correct location and pushed all the way down and at a 90° angle. Place assembly in numbered holding rack according to QIAcube Number on worksheet. 25. Load Centrifuge Rotor Adaptors into the correct location in the instrument. 26. Load the heat shaker with the Sample Tubes RB containing the specimens in the correct locations. 27. Close lid and hit Start. Approximate run time is 80 minutes. 28. Once the program is complete, open instrument and take out rotor apparatus, take out the spin column and dispose in a dual waste container, close elution tube and set in rack to be quantified on the Nanodrop. Dispose all reagents and consumables in a dual waster container.
Assay Procedure: Nanodrop (Room 2)	1. Turn on instrument. 2. Lift the instrument arm and clean the upper and lower pedestals with DI water and a clean, lint free laboratory wipe. 3. From the instrument home screen, select the Nucleic Acids application tab. 4. Select the dsDNA application. 5. Pipette 1 – 2 µl Ultrapure HPLC-grade water onto the lower pedestal and lower the arm. 6. Tap Blank. 7. Lift the arm and clear both pedestals with a new laboratory wipe. 8. Pipette 1 – 2 µl of the sample onto the pedestal and lower the arm. 9. Tap Measure. When complete, the spectrum and reported values are displayed. 10. Record the concentration and the A260/280 values on the DICER1 QIACube and Nanodrop worksheet. 11. After each measurement, lift the arm and clean both pedestals with a new wipe to prevent carryover.

	NOTE: Wiping the sample from both the upper and lower pedestals upon completion of **each sample** is usually sufficient to prevent sample carryover and avoid residue buildup. 2 µl of DI water can be used to clean the measurement surfaces after particularly high concentration samples are measured. 12. Repeat steps 7-11 for all samples. 13. Once all samples have been measured, tap End Experiment. Optional: Name and save your experiment along with Sample names. 14. Clean measurement surfaces with de-ionized water after the last measurement: a. Apply 5 µl of dH_2O onto bottom pedestal. Lower the upper pedestal arm to form a column and let sit for 2 – 3 minutes. Wipe away the water from both the upper and lower pedestals with a clean lab wipe. **NOTE**: Do **NOT** use a squirt bottle to apply de-ionized water 15. Turn off instrument. For additional information, maintenance and troubleshooting, consult the Nanodrop Procedure.
Assay Procedure: Sample Dilution (Room 2)	**NOTE:** Clean hood and supplies with a 1:10 bleach dilution or wipes followed by DI water and alcohol before and after any sample processing 1. Enter the hand-written concentrations and the A260/280 values into the digital version of the worksheet, SharePoint – Lab – Documents – Molecular – Molecular Pathology – Agena-Dicer1 – Forms and Worksheets – DICER1 QIAcube and Nanodrop Worksheet. Dilutions will be automatically calculated once the concentrations are entered into the DICER1 QIACube and Nanodrop worksheet. 2. If the concentration is greater than 100 ng/uL then a 1:10 dilution will be calculated. The word “yes” or “no” will populate to indicate the necessity of a 1:10 dilution. 3. Label 1.5 mL tubes with Specimen ID on the side and the QIACube number and “1:10” on the cap for all samples that generated a 1:10 dilution and place in rack. 4. Add 18 uL of Ultrapure HPLC-grade water into each tube. 5. Add 2 uL of each extract into the corresponding tube. 6. Vortex and centrifuge briefly. 7. In a rack, organize all samples in order that will be used to make the final 5 ng/uL dilution, either the original extract (< 100 ng/uL) or the prepared 1:10 dilution (> 100ng/uL). 8. Label 1.5 mL tubes with Specimen ID on the side and the QIACube number and “5 ng/uL” on the cap for all samples and place in rack. 9. Add Ultrapure HPLC-grade water to the “5 ng/uL” tubes according to volumes on worksheet. 10. Add sample (either original extract or 1:10 dilution) to the “5ng/uL” tube according to the “Extract volume” field on worksheet. **NOTE:** Only open one set of corresponding tubes at a time. 11. Vortex and centrifuge briefly. 12. Discard “1:10” dilution tubes in biohazard waste. 13. Store original extract in -70° C freezer.

	14. If moving on to Sample Addition the same day, store the "5ng/uL" dilutions in the refrigerator, otherwise, store in -70° C freezer.
Assay Procedure: Option 1 - Automated Sample and Master Mix Addition Performed by epMotion 5073	**IMPORTANT:** This section is for automated sample and master mix addition performed by the epMotion 5073. Skip to Option 2 for the manual procedure. **NOTE:** Place the Thermorack TMX in the fridge until epMotion 5073 setup 1. Prepare Plate Map and Master Mix Calculator. a. SharePoint – Lab – Documents – Molecular – Molecular Pathology – Agena-Dicer 1 – Forms and Worksheets – DICER1 Plate Map and Calculator 2. Type in sample names and controls in the "Samples" tab at the bottom. 3. Click the "1Plate" tab and change the run name in the upper left corner, e.g. 030320DICER1 4. Click on the "Run Prep Sheet" tab and fill in the Date and Run Name. **NOTE**: Fill in Lot# and Exp at each step. 5. Print the "1Plate", "Run Prep Sheet" and "Singlet DICER1 Calculator" tabs **NOTE**: Fill in Date, Tech and Thermocycler fields on the Singlet DICER1 Calculator at each step 6. Click File-Create a Copy-Create a Copy Online → change name, e.g. 030320 DICER1 Plate Map and Calculator and then delete "Copy" from the name 7. Click the "Choose Location" dropdown box and click "More save locations" 8. Click the path as follows: Lab-Molecular-Molecular Pathology-Agena-DICER1-Forms and Worksheets-DICER1 Run Worksheets-DICER1 Runs (current year) 9. Click "Save Here" 10. Take all printed worksheets to Room 1. 11. Clean the Room 1 hood with 5% Extran followed by 70% ethanol. 12. Pull the PCR reagents listed in the Table below to thaw. Leave Enzyme in freezer until needed. 13. Once other reagents have thawed, take enzyme out of freezer and place in cold block. 14. Vortex all reagents except the enzyme (flick), quick spin and place in cold block. 15. Label seven 1.5 mL tubes, one MMX and the remaining 1-6, place in cold block. 16. Prepare the PCR master mix (cocktail) in the MMX labeled 1.5 mL tube by adding reagents in order, as listed in the table below. Write how many tests are being removed on the white board in room 1. When opening a new box, write the numbers 1 through 5 on top of each vial as corresponds to the table below. 17. Once the PCR (MMX) cocktail has been prepared, evenly distribute into Tubes 1-6 according to the calculator. 18. Add each PCR Primer Set to the corresponding labeled tube. 19. Vortex and spin tubes briefly.

Reagent	Per Reaction (uL)
1. Ultrapure HPLC-grade Water	0.80
2. 10x PCR Buffer	0.50
3. $MgCl_2$	0.40
4. dNTP mix	0.10
5. PCR Enzyme	0.20
PCR (MMX) Cocktail Final Volume	2.00
Split MMX across Tubes 1-6	PCR (MMX) Cocktail Final Volume/6
6. PCR Primers (1-6 accordingly)	1.00
PCR Cocktail (MMX + Primer) Final Volume	3.00
Sample DNA/Positive Control/Negative Control	2.00
PCR Total Reaction Volume	5.00

NOTE: reagent volumes on the worksheet allow for 25% overage
NOTE: The worksheet calculates volumes accordingly

20. Bring the Master Mix in the cold block into Room 2 for dispersal.
    NOTE: If for any reason transfer of the master mix is delayed, place the entire block in the fridge

NOTE: Leave the Master Mix Calculator worksheet in Room 1 for SAP and iPLEX Pro Extension Cocktail prep below.

21. If frozen, thaw samples.

22. Turn on epMotion computer if necessary.

23. Turn on epMotion 5073.

24. Double click epBlue 50.0 sofware
    Note: Username: bioapps
    Password: Bioapps123!

25. Click Application Runner under Application Library

26. Highlight BioApps

27. From the middle pane, highlight “DICER set up- from bottom tolerance 3/10/2025”

28. Click Next

29. Make sure 5073MK710035 is highlighted

30. Click Next

31. The Air Filter System pane will appear, click Start

32. Click Next

33. Click Next

34. Enter the number of samples, including controls

35. Click OK

36. Click Next

37. Load the deck according to the layout on the screen

	38. Take the Thermorack TMX out of the fridge and place it in position C2 on the deck 39. Click on each master mix or sample listed in the Volume Input table and a position will be highlighted in the Vessels pane. Add master mixes and samples to the Thermorack TMX as indicated. 40. Label a 96-well PCR plate with the Run name: "date, DICER1" 41. Place the 96-well PCR plate on the Thermoadapter in position C1 42. Place 50 uL tips in positions B1 and B2 Note: partial boxes can be used; the software will indicate if more tips are needed. 43. Ensure there is enough room in the waste container 44. Click Run 45. Instrument will scan all consumables and reagents. It will alert if there is a missing requirement. Respond to the instrument message and rescan. If nothing is wrong after repeated scan, click ignore. 46. Clean hood and supplies with a 1:10 bleach dilution or wipes followed by DI water and alcohol 47. Once the run is complete, place 96-well plate in a plate holder in the hood 48. Discard master mix tubes in the waste container 49. Freeze samples in designated box 50. Put lids on the tip boxes 51. Wipe down deck with alcohol, turn on UV light and change gloves 52. Manually add 2.0 uL of the controls to designated wells of the plate according to plate map 53. Seal the PCR reaction plate, briefly pulse vortex 1-2 times, then do a quick spin in the centrifuge by bringing up to 1200 rpm. **NOTE:** Crease all four sides with the paddle. **NOTE:** Visually inspect the individual wells from the bottom of the reaction plate to confirm uniformity before continuing. 54. Place the plate in the cold block and move to Room 3 to start thermal cycling. **NOTE: Skip to PCR Amplification (Room 3)**
Assay Procedure: Option 2 - Manual Sample Addition (Room 2)	**IMPORTANT:** This section is for manual sample and master mix addition. See to Option 1 for the automated procedure. **NOTE:** Clean hood and supplies with a 1:10 bleach dilution or wipes followed by DI water and alcohol before and after any sample processing 55. Prepare Plate Map and Master Mix Calculator. a. SharePoint – Lab – Documents – Molecular – Molecular Pathology – Agena-Dicer 1 – Forms and Worksheets – DICER1 Plate Map and Calculator 56. Type in sample names and controls in the "Samples" tab at the bottom. 57. Click the "1Plate" tab and change the run name in the upper left corner, e.g. 030320DICER1

	58. Click on the "Run Prep Sheet" tab and fill in the Date and Run Name. **NOTE**: Fill in Lot# and Exp at each step. 59. Print the "1Plate", "Run Prep Sheet" and "Singlet DICER1 Calculator" tabs **NOTE**: Fill in Date, Tech and Thermocycler fields on the Singlet DICER1 Calculator at each step 60. Click File-Create a Copy-Create a Copy Online → change name, e.g. 030320 DICER1 Plate Map and Calculator and then delete "Copy" from the name 61. Click the "Choose Location" dropdown box and click "More save locations" 62. Click the path as follows: Lab-Molecular-Molecular Pathology-Agena-DICER1-Forms and Worksheets-DICER1 Run Worksheets-DICER1 Runs (current year) 63. Click "Save Here" 64. Take the "Run Prep Sheet" and "Singlet DICER1 Calculator" to Room 1 and "1Plate" to Room 2. Save "File for MassARRAY" to a flash drive for instrument set up at the end 65. Label a 96-well PCR plate with the Run name: "date, DICER1" a. e.g. March 3, 2020 = 0**3.03.20 DICER1.** Place in cold block. 66. Divide and mark plate based on Primer Set layout according to plate map. 67. Add 2.0 uL of either sample or control to designated well of the plate according to plate map. **NOTE:** The NTC and each sample will be pipetted six times, once for each primer set. **NOTE:** Controls are primer set based thus pipetted only once and are routinely rotated. Do not vortex synthetic positive QC. Thaw and quick spin only. **NOTE:** A previously tested positive sample needs to be run for each primer set for new lots/shipments. 68. Seal the PCR plate and place in fridge until master mix is prepared. **NOTE:** Visually inspect the individual wells from the bottom of the reaction plate to confirm uniformity before continuing.
Assay Procedure: Option 2 cont. - PCR Master Mix Preparation (Room 1):	1. Clean hood with 5% Extran followed by 70% ethanol. 2. Pull the PCR reagents listed in the Table below to thaw. Leave Enzyme in freezer until needed. 3. Take enzyme out of freezer and place in cold block. 4. Vortex all reagents except the enzyme (flick), quick spin and place in cold block. 5. Label seven 1.5 mL tubes, one MMX and the remaining 1-6, place in cold block. 6. Prepare the PCR master mix (cocktail) in the MMX labeled 1.5 mL tube by adding reagents in order, as listed in the table below. Write how many tests are being removed on the white board in room 1. When opening a new box, write the numbers 1 through 5 on top of each vial as corresponds to the table below. 7. Once the PCR (MMX) cocktail has been prepared, evenly distribute into Tubes 1-6 according to the calculator. 8. Add each PCR Primer Set to the corresponding labeled tube. 9. Vortex and spin tubes briefly. 10. Label 0.2 mL PCR strip tubes with designated primer set and place in cold block.

11. Evenly aliquot cocktail from Tubes 1-6 into corresponding labeled strip tube according to the calculator worksheet.

12. Cap tubes and spin briefly.

Reagent	Per Reaction (uL)
1. Ultrapure HPLC-grade Water	0.80
2. 10x PCR Buffer	0.50
3. $MgCl_2$	0.40
4. dNTP mix	0.10
5. PCR Enzyme	0.20
PCR (MMX) Cocktail Final Volume	2.00
Split MMX across Tubes 1-6	PCR (MMX) Cocktail Final Volume/6
6. PCR Primers (1-6 accordingly)	1.00
PCR Cocktail (MMX + Primer) Final Volume	3.00
Sample DNA/Positive Control/Negative Control	2.00
PCR Total Reaction Volume	5.00

NOTE: reagent volumes on the worksheet allow for 25% overage
NOTE: The worksheet calculates volumes accordingly

13. Bring the Master Mix in the cold block into Room 2 for dispersal.
    NOTE: If for any reason transfer of the master mix is delayed, place the entire block in the fridge.

NOTE: Leave the Master Mix Calculator worksheet in Room 1 for SAP and iPLEX Pro Extension Cocktail prep below.

Assay Procedure: Option 2 cont. - Master Mix Dispersal (Room 2)

1. Take the sample plate out of the fridge, carefully remove seal and change gloves.

2. Using a multi-channel pipet, add 3.0 uL of master mix to the sample plate according to the map, changing tips each time.

3. Seal the PCR reaction plate, briefly pulse vortex 1-2 times, then do a quick spin in the centrifuge by bringing up to 1200 rpm.
   NOTE: Crease all four sides with the paddle.
   NOTE: Visually inspect the individual wells from the bottom of the reaction plate to confirm uniformity before continuing.

4. Place the plate in the cold block and move to Room 3 to start thermal cycling.

Assay Procedure: PCR Amplification (Room 3)

NOTE: Turn on the thermocycler so that the heated cover can come to the correct operating temperature and there is no time delay between the PCR reaction plate being prepped and cycling being started.

1. Place plate in the thermocycler (rotate usage). Run program **DICER-**PCR. See the Table below for program details.
   NOTE: Total volume: 5.0 uL
   NOTE: Cycle program takes approximately 2 hour and 20 minutes in Veriti

Step		Temperature	Time	Number of Cycles
1	Initialization	95°C	5 minutes	1 cycle
2	Denaturation	95°C	30 seconds	45 cycles of steps 2-4
3	Annealing	56°C	30 seconds	
4	Extension	72°C	1 minute	

5	Final extension	72°C	5 minutes	1 cycle
6	Sample preservation	4°C	--	Hold

NOTE: If not proceeding directly to the next step, the reaction plate should be sealed and stored at 4 °C – 10 °C (if storing for less than 24 hours), or at -20 °C (if storing for more than 24 hours). Do not store for more than 2 weeks.

Assay Procedure: Shrimp Alkaline Phosphatase (SAP) Cocktail Preparation (Room 1)

1. Pull the reagents listed in the Table below to thaw to room temp. Vortex all reagents except enzyme (flick) and quick spin.
   NOTE: Leave enzymes in the freezer until use, and keep in a cold block when pipetting.

2. In a cold block, prepare the SAP cocktail in a labeled 1.5 mL tube by adding reagents in order, as listed in the table below.

Reagent	**Per reaction (uL)**
1. Ultrapure HPLC-grade water	1.53
2. SAP Buffer	0.17
3. Shrimp Alkaline Phosphatase (SAP)	0.30
SAP Cocktail Final Volume	2.00

NOTE: reagent volumes on the worksheet allow for 25% overage
NOTE: The worksheet calculates volumes accordingly

3. Pulse vortex the tube briefly 3 times and quick spin.

4. Label 0.2 mL PCR strip tubes and place in cold block.

5. Evenly aliquot SAP master mix into strip tubes according to calculator.

6. Immediately bring to Room 3 for dispersal.

NOTE: Leave the Master Mix Calculator worksheet in Room 1 for iPLEX Pro Extension Cocktail prep below.

Assay Procedure: SAP Cocktail Addition to Samples (Room 3)

NOTE: If plates were stored frozen prior to this step, make sure they are thawed completely, gently homogenized, spun down, and kept in a cold block

NOTE: Clean hood and supplies with a 1:10 bleach dilution or wipes followed by DI water and alcohol before and after adding the cocktail

1. Perform a quick spin of the PCR reaction plate in the centrifuge by bringing up to 1200 rpm.

2. Remove and discard seal. Change gloves.
   NOTE: Take care when removing seal: do not let it curl back onto the plate or fold into itself.

3. Using a multi-channel pipet, dispense 2.0 uL of SAP cocktail into each well of the reaction plate, changing tips each time.

4. Seal the reaction plate, briefly pulse vortex 1-2 times, then perform a quick spin in the centrifuge by bringing up to 1200 rpm.
   NOTE: Crease all four sides with the paddle.

NOTE: Visually inspect the individual wells from the bottom of the reaction plate to confirm uniformity before continuing.

5. Place the plate with compression pad in the thermocycler and run program **DICER-SAP**. See the table below for program details.
   NOTE: Total volume: 7.0 uL
   NOTE: Cycle program takes approximately 45 minutes

Step		Temperature	Time	Number of Cycles
1	Dephosphorylation	37°C	40 minutes	1 cycle
2	Enzyme inactivation	85°C	5 minutes	1 cycle
3	Sample preservation	4°C	--	Hold

NOTE: If not proceeding directly to the next step, the reaction plate should be sealed and stored at 4 °C – 10 °C (if storing for less than 24 hours), or at -20 °C (if storing for more than 24 hours). Do not store for more than 2 weeks.

Assay Procedure: iPLEX Pro Extension Cocktail Prep (Room 1)

1. Pull the reagents listed in the Table below to thaw. Vortex all reagents except enzyme (flick) and quick spin.
   NOTE: Leave enzymes in the freezer until use, and keep in a cold block when pipetting.
2. Label 7 1.5 mL tubes, one MMX and the remaining 1-6, place in cold block
3. In a cold block, prepare the iPLEX Pro Extension master mix (cocktail) in the MMX labeled 1.5 mL tube by adding the reagents in order, as listed in the table below. When opening a new box, write the number 1 through 4 on the top of each vial as corresponds to the table below.
4. Once the Extension (MMX) cocktail has been prepared, evenly distribute into Tubes 1-6 according to the calculator.
5. Add each Extend Primer Set to corresponding labeled tube.
6. Vortex and spin tubes briefly.
7. Label 0.2 mL PCR strip tubes with designated primer set and place in cold block.
8. Evenly aliquot cocktail from Tubes 1-6 into appropriately labeled strip tube according to the calculator worksheet.
9. Cap tubes and spin briefly.

Reagent	Per reaction (uL)
1. Ultrapure HPLC-grade water	0.62
2. iPLEX Buffer Plus, GPR	0.20
3. iPLEX Termination Mix	0.20
4. iPLEX Pro Enzyme	0.04
Extension (MMX) Cocktail Final Volume	1.06
Split MMX across Tubes 1-6	Extension Cocktail Final Volume/6
EXTEND Primers (1-6 accordingly)	0.94
Extension (MMX + Primers) Final Volume	2.00

NOTE: reagent volumes on the worksheet allow for 25% overage

NOTE: The worksheet calculates volumes accordingly

10. Bring the Master Mix in the cold block into Room 3 for dispersal.
    NOTE: If for any reason transfer of the master mix is delayed, place the entire block in the fridge.

Assay Procedure: iPLEX Pro Extension Cocktail Addition to Samples (Room 3)

NOTE: If plates were stored frozen prior to this step, make sure they are thawed completely, gently homogenized, spun down, and kept in a cold block

NOTE: Clean hood and supplies with a 1:10 bleach dilution or wipes followed by DI water and alcohol before and after adding the cocktail

1. Perform a quick spin of the PCR reaction plate on the centrifuge by bringing up to 1200 rpm.

2. Remove and discard seal. Change gloves.
   NOTE: Take care when removing seal: do not let it curl back onto the plate or fold into itself.

3. Using a multi-channel pipet, dispense 2.0 uL of extension cocktail into designated wells of the reaction plate according to plate map, changing tips each time.

4. Seal the reaction plate, briefly pulse vortex 1-2 times, then perform a quick spin on the centrifuge by bringing up to 1200 rpm.
   NOTE: Crease all sides, corners and between wells with the paddle.
   NOTE: Visually inspect the individual wells from the bottom of the reaction plate to confirm uniformity before continuing.

5. Place the plate along with compression pad in the thermocycler and run program **DICER-Extension**. See the table below for program details.
   NOTE: Total volume: 9.0 uL
   NOTE: Cycle program take approximately 2 hours and 30 minutes

Step		Temperature	Time	Number of Cycles
1	Initialization	95°C	30 seconds	1 cycle
2	Denaturation	95°C	5 seconds	40 cycles
3	Annealing	52°C	5 seconds	5 cycles
4	Extension	80°C	5 seconds	
5	Final extension	72°C	3 minutes	1 cycle
6	Sample preservation	4°C	--	Hold

NOTE: If not proceeding directly to the next step, the reaction plate should be sealed and stored at 4 °C – 10 °C (if storing for less than 24 hours), or at -20 °C (if storing for more than 24 hours). Do not store for more than 2 weeks.

Assay Procedure: Water Addition (Room 3)

NOTE: If plates were stored frozen prior to this step, make sure they are thawed completely, gently homogenized, spun down, and kept in a cold block

NOTE: Clean hood and supplies with a 1:10 bleach dilution or wipes followed by DI water and alcohol before and after adding the cocktail

1. Perform a quick spin of the reaction plate on the centrifuge by bringing up to 1200 rpm.

	2. Discard and remove seal. Change gloves. **NOTE:** Take care when removing seal: do not let it curl back onto the plate or fold into itself. 3. Using a multi-channel pipet, add 41.0 uL Ultrapure HPLC-grade water to each well of the reaction plate, changing tip each time. 4. Seal the plate and perform a quick spin of the reaction plate on the centrifuge by bringing up to 1200 rpm. **NOTE:** Crease all four sides with the paddle. **NOTE:** If not proceeding directly to the next step, the reaction plate should be sealed and stored at 4 °C – 10 °C (if storing for less than 24 hours), or at -20 °C (if storing for more than 24 hours). Do not store for more than 2 weeks.
Creating a plate in the MassARRAY Software	**Create a Sample Group** 1. Open the MassARRAY Typer Plate Editor software, log in. a. User: charles b. Password: darwin 2. Click on the **Sample view** tab. 3. Expand the customer and project (DICER1->Registry Samples). Right-click on the sample project that you want to add the sample group to and select **Add New Sample Group**. 4. Enter the Experiment/Run name and click the folder button in the toolbar to browse to the location of your sample group text file, and click **Open**, then click **OK**. Check sample IDs and QC IDs against plate set up sheet for accuracy. **NOTE:** properties – select **"Horizontal"** and **"Keep Selected Region True"** **Create a Project & Plate** 1. In the MassARRAY Typer Plate Editor software, click on the **Plate tab**. 2. Create a plate. a. Right-click on the project (DICER1->Registry Samples) that you want to add the plate to and select **New Plate**. b. Enter the Experiment/Run name and select the plate type (96- or 384-well) and click **OK**. c. The new plate will appear in the Plate tab and a plate layout will be created automatically, based on the plate type specified. **Apply Assays to the Plate** 1. In the **Plate tab**, select the plate that was just created. 2. Select the **Assay View** tab at the bottom and in the plate layout. 3. Select wells for Primer set 1 based on the plate map worksheet. 4. Expand the assay design file. 5. Right click on the test tube "1" icon and select add plex 1. 6. Repeat steps 3-5 for each of the 6 Primer sets. **Apply Samples to the Plate** 1. Click on the **Sample View** tab. 2. In the plate layout, select all the wells in which a "plex" number has been added. 3. Right-click the plate, right click "Apply Samples from Group"…, choose file from the flash drive.

	4. Check plate locations with sample IDs and QC IDs against plate set up sheet for accuracy. 5. Select **File** > **Save** from the toolbar.
Assay Procedure: Creating an Input File (Room 3)	1. Double-click the **Chip Linker** icon on the desktop. 2. In the dialog box that appears, enter your username, password, and server. a. User: charles b. Password: darwin 3. Click **Connect**. The Chip Linker window appears. 4. Select a plate in the Chip Linker directory tree (DICER1->Registry Samples). 5. Select **iPLEX** as the terminator chemistry. 6. Select **Allelotype** for the process method. 7. Select **Nanodispenser 96 to 96** as the dispenser method. 8. Enter the Experiment/Run name. 9. Enter the SpectroCHIP Array barcode or other SpectroCHIP Array identifier. Record chip ID on the Run Worksheet. 10. Click **Add**. The input information appears in the Chip Linker table. 11. If a second SpectroCHIP Array will be processed, repeat step 4 to step 10 for the second SpectroCHIP Array. 12. Click **Create** to create an input XML file. This file will be selected for use when you set up the automatic run.
Assay Procedure: Preparing the Instrument (Room 3)	**NOTE:** Ensure the daily maintenance has been performed prior to advancing. 1. In the Status section in the **Run Setup** tab of SpectroACQUIRE check the Waste Tank, System Fluid, and Resin buttons; they should be green/Okay. If any are red (Waste Tank Full, System Fluid Empty, Resin Low or Empty) perform the necessary maintenance. 2. Click **Chip prep module Deck In/Out** at the top of the SpectroACQUIRE window. The deck will extend. 3. If there are SpectroCHIP Arrays in the completed chips position on the deck remove them. **NOTE:** the completed position is the deck that is closer to you **NOTE:** a used chip is always needed on the right side of the chip holder for balance 4. Allow the calibrant to equilibrate to room temperature for 5 minutes (if it has been refrigerated) or 10 minutes (if it has been frozen). Quick spin and pipette 75 µL of calibrant into the calibrant vial and place in the calibrant vial holder on the deck. 5. Load the SpectroCHIP Arrays. a. Orient the Chip holder so that the beveled corner is at the top right. b. Open a new SpectroCHIP Array pouch and insert the new SpectroCHIP Array into the chip holder in position 1 (on the left); orient the SpectroCHIP Array such that the Agena logo and barcode are at the **bottom**. Make sure that the SpectroCHIP Array is properly seated so that the chip holder and SpectroCHIP Array surface are flush.

NOTE: Use flat tweezer for transfer and pointed tweezer for any necessary adjustments
NOTE: Care must be taken to avoid touching the surface
NOTE: Chips may be sent on counter on top of a kim wipe
NOTE: Save the SpectroCHIP Array pouch and holder if chip will not be completely used. Write the open date on the pouch.

c. If you are processing two plates, insert another new SpectroCHIP Array into the chip holder in position 2 (on the right). If you are only processing one plate, place a previously completed SpectroCHIP Array in position 2, as the MassARRAY Analyzer requires both SpectroCHIP Array positions to be filled to function properly.
d. Place the full chip holder in the chip holder tray, new chips position, on the deck.

6. Load microtiter plates (MTPs). Up to two 96-well microtiter plates of analyte may be loaded.
   a. Quick spin the microtiter plate(s) at ~1200 – 1500g
   b. Remove the seal, change gloves, and place the first MTP on MTP holder 1 (on the left). This corresponds to chip position
      i. Orient the plate such that well A1 is in the front left corner (match to label on instrument loading deck)
   c. If a second MTP is being processed, place it on MTP holder 2 (on the right). This corresponds to chip position 2.

7. Click **Chip prep module Deck In/Out** again to retract the deck.

Assay Procedure: Setting up and Starting the Run (Room 3)

1. Select the **Run Setup** tab in SpectroACQUIRE.

2. In the Analyzer Setup section:
   a. In the Experiment Setup section:
      i. Under MTP 1, click on the browse button next to Experiment Name and select the XML input file created earlier.
      ii. In the Wells to Process field, select **Automatic**.
      iii. Repeat steps 1 and 2 for MTP 2 if running two plates. Otherwise, select **None** in the Experiment Name and Wells to Process fields for MTP 2.
   b. Make sure all settings are as shown in the Table below.

	SpectroCHIP CPM-96
Use Autotune	NOT selected
Start Dispense Condition	600
Resin Volume	13
Sample Volume	n/a
Shots (n)	30
Maximum acquisitions	9
Minimum good spectra	5
Maximum good spectra	5
Turn off HV After Analysis	Selected
Analyze Calibrant Pads	Selected
Filter Saturated Shots	NOT selected
Chip Type	SpectroCHIP CPM-96

3. In the Chip Prep Module Setup section:
   a. Check the **Normal Operation** box.
      i. If NOT running 2 plates: unselect “Transfer Resin to MTP2”
      NOTE: if rerunning a plate that has already been spotted unselect “Transfer Resin to MTP1 and/or MTP2”
   b. Select **iPLEX** in the Chemistry drop-down menu.

	4. In the Temperature Control section: a. If the run will sit on the instrument overnight, select the **MTP Cool** box, and check the **Auto** box. b. If using a SpectroCHIP CPM-96, check the **Chip Heat** box and enter **30** in the Setpoint field. 5. Click **Start Chip prep module** to start the run. Approximate run time is 1 hour and 40 minutes.
Assay Procedure: Removing plates, calibrant and SpectroCHIP Array when run is complete	1. Once the run is completed, click **Remove Old Chips from MA4** in the Run Setup tab. The instrument will move the completed SpectroCHIP Arrays from the MassARRAY Analyzer to the completed chips position on the Chip Prep Module deck, and then extend the deck so you may remove the SpectroCHIP Arrays from the completed chips position. **NOTE:** save incompletely used chips in saved holder and pouch with the desiccant. Write the open date and which wells have been used on the outside of the pouch. Seal with tape. 2. Remove the MTPs and calibrant vial. a. Pipette remaining calibrant into a 2 mL cryovial labeled with the calibrant lot number, expiration date, and date the vial was created b. Freeze calibrant for future use c. Rinse calibrant tube with DI water: i. Pipette 100 uL DI water and mix 5-8 times d. Store MTP in freezer for two weeks and then discard in red biohazard waste 3. Click **Chip prep module Deck In/Out** to move the deck in.
Assay Procedure: Recalling Chip to Create a Genotype+Area Report	1. Create a folder with the run's name (mm.dd.yy DICER1) in Documents 2. Open Chip Linker (log in if necessary): a. Select the plate from the folder tree b. Settings: iPlex, Genotype+Area, Nanodispenser 96 to 96 c. Name the experiment exactly as the Allelotype run d. Enter Chip ID 3. Click Recall and select the correct chip from the pop-up window 4. Open Typer to display recalled chip 5. Find run according to date
Analyzing Data	6. In the Project Explorer pane double click on the Allelotype (blue font) and Genotype+Area (magenta font) SpectroCHIP Arrays. The SpectroCHIP Arrays will be added to the Chip List. 7. Check the box next to Genotype + Area in the Chip List. 8. Save a picture (Snip tool) of the GenotypeArea "traffic light" diagram in the newly created dated folder in Documents. a. Note: If any well is NOT dark green (except for wells with known synthetic controls or NTC) notify the molecular pathologist. Ensure optimal threshold (dark green) is set at 100. 9. Ensure positive, negative, and no template controls gave expected results. a. Note: If any control is not as expected, notify the molecular pathologist and save original plate and chip for potential troubleshooting and/or repeat run. 10. While still on the GenotypeArea chip, select **File > Reports > Typer Reports > GenotypeArea Report-v1** in the MassARRAY Typer Analyzer menu bar. **NOTE:** When the report is complete, it will automatically open in OpenOffice. Save this file as a .xls in an appropriately dated folder in the same folder as the GenotypeArea traffic light picture above, keeping the generated name.

	11. Load the Allelotype chip and select **File > OncoPanel Reports > CHMN_DICER1_v1.0-v1** in the MassARRAY Typer Analyzer menu bar. Save these files in the same folder as the GenotypeArea traffic light picture and report as above. 12. Save (drag and drop) the dated folder containing the GenotypeArea traffic light picture and report and OncoPanel reports to a USB thumb drive. Remove USB. 13. Insert USB into a networked computer. save files located here: 14. Go to SharePoint\Lab\Documents\Molecular\Molecular Pathology\Agena - Dicer 1\Results – folder by year 15. . Create new folder (mm.dd.yy DICER1) 16. Save (drag and drop) files from the USB to the new folder Files to be included: a. GenotypeArea.xls b. Traffic light c. (Allelotype) OncoReport.tsv d. (Allelotype) OncoReport mutation list e. (Allelotype) OncoReport uncertain mutation list f. Plate map and calculator (with lot numbers and expiration dates) g. QiaCube and Nanodrop (with lot numbers and expiration dates) i. To save the Plate map and calculator and QiaCube and Nanodrop worksheets to the new folder: 1. Open worksheet (Sharepoint-Lab-Documents-Molecular-Molecular Pathology-Agena-Dicer 1-Forms and Worksheets) 2. File 3. Save As 4. Save a Copy 5. Expand “Choose Location” drop down box 6. Click “More save Locations” 7. Click Lab-Molecular-Molecular Pathology-Agena-Dicer 1-Results-(folder by year) 8. Save here 9. Rename so the date is in front of the file name and the “copy” is removed at the end 10. Make sure all information (including lot numbers and expiration dates) has been filled in on the Run Prep Sheet tab, Singlet DICER1 Calculator tab, and DICER1 QIAcube and Nanodrop worksheet 17. Notify pathologist of results available for review.
Result Interpretation	Interpretation of the results is performed by the molecular pathologist on service. **Quality control and validity of results** 1. One no template control and one positive patient sample (or synthetic positive sample if patient sample is not available; rotating through a positive for each assay within a primer set set each run) for each primer set are processed with each run. **NOTE:** If any control fails, notify the molecular pathologist and store plates and chips while waiting for further instructions. **NOTES on well colors:**

	There are 6 assays in each well. The color shown in the Typer software relates to the number of targets that were called (AKA: performed). Green = 100% called Light Green = 99% – 50% called Yellow = 49% - 16% called Red = 15% or less called
Sample Storage	**Storage and Retention of Test Specimens** 1. Store eluates in positive or negative box (based on results) in the -80 °C freezer in Room 2. 2. Store completed and sealed 96-well plates in the -20 °C freezer in Room 3. 3. Discard samples in red biohazard container after molecular pathologist approval.
Equipment and Room Decontamination	**Refer to:** MB 3.03 Cleaning and Decontamination of Equipment and Work Areas
Limitations	1. The performance characteristics of the MassARRAY DICER1 panel have been evaluated by Children's MN Laboratory. This is a RESEARCH USE ONLY assay. 2. All results from this and other tests must be considered in conjunction with the clinical history, epidemiological data, and other data available to the clinician evaluating the patient. 3. Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of samples may hinder the ability of the assay to detect the target sequences. 4. The performance of the DICER1 Agena MassARRAY Research Panel was established using extracted DNA from formalin fixed, paraffin-embedded tissue samples. 5. This test is a qualitative test and does not provide the quantitative value of detected alleles present. 6. There is a risk of false positive values resulting from: a. Non-specific signals in the assay. b. Cross-contamination during sample handling or preparation. c. Cross-contamination between patient samples. d. Sample mix up. e. DNA contamination during product handling. 7. There is a risk of false negative values due to: a. The presence of sequence variants in the regions of interest of the assay, procedural errors, amplification inhibitors in samples, or inadequate tumor cellularity for amplification. b. Improper sample collection/preservation. c. Sample mix up. d. Degradation of sample DNA during shipping/storage. e. The presence of PCR inhibitors. 8. This test cannot rule out potentially clinically relevant variants in parts of the genome not specifically targeted by the assay. 9. Negative results do not preclude low-level clinically relevant variants and should not be the sole basis of a patient management decision. 10. This panel has been evaluated for use with human sample material only.

Method Performance Specifications

In-house performance (per the assay validation studies): 2x2 Table, FFPE Samples – overall arbitrated results

Agena	Expected result	
	Positive	**Negative**
Positive	37	1
Negative	0	42

Results (95% CI):
Positive Percent Agreement: 100% (90.51% - 100.00%)
Negative Percent Agreement: 97.67% (87.71% - 99.94%)
Overall Percent agreement: 98.75% (93.23% - 99.97%)

LoD: 0.02 $TCID_{50}$/mL

References

1. Agena iPLEX Pro and Gold Reagents User Guide, USG-CUS-071 Rev03, San Diego, CA: Agena Bioscience; 2019.
2. QIAamp DNA FFPE Tissue Handbook, HB-0353-004, Germantown, Maryland: Qiagen; 2020
3. MedlinePlus [Internet]. Bethesda (MD): National Library of Medicine (US); [updated 2022 Feb 9]. DICER1 syndrome; [updated 2020 Aug 18; reviewed 2016 May 01; cited 2022 Feb 9]; [about 5 p.]. Available from: https://medlineplus.gov/genetics/condition/dicer1-syndrome/.

Alternate Methods

1. Send out test

Proficiency Testing

Not applicable. Research Use Only assay.

Training Plan/ Competency Assessment

Training Plan	**Initial Competency Assessment**
1. Employee must read the procedure. 2. Employee will demonstrate the ability to perform procedure, record results, and document corrective action after instruction by the trainer.	1. Direct observation

Historical Record

Version	**Written/Revised by:**	**Effective Date:**	**Summary of Revisions**
1	Kristi Prokop /Damon Olson	06/09/2023	Initial Version
2	Kristi Prokop	07/25/2024	Updated instrument set up and Analysis
3	Kristi Prokop	04/28/2025	Added automated sample and master mix addition by epMotion
Archived by:		**Archived date:**	