Cell-free methylated DNA immunoprecipitation (cfMeDIP) & ctDNA Targeted Sequencing Assay

Purpose

To define the cell-free methylated DNA immunoprecipitation (cfMeDIP) library preparation procedure, using KAPA Hyper Prep Kit, IDT unique molecular indexes (UMIs), Diagenode washes, and 5-mC antibodies.

Scope

This standard operating procedure (SOP) describes two library prep arms, both requiring **10ng** (in <50ul) as input for immunoprecipitation (IP) of cell-free DNA (cfDNA):

1. **Standard Arm (10ng)**
    - **IP:** Uses ~9.12ng for immunoprecipitation (IP) of methylated cfDNA using 5-mC antibodies
    - **IC**: Remaining ~0.88ng of library is NOT subjected to immunoprecipitation, it is used as an input control (IC) to measure successful methylation enrichment using sWGS (shallow Whole genome sequencing).

2. **Targeted Enrichment Arm (50ng):** Use if ≥50ng of cfDNA available
    - **IP:** Uses ~10.00 ng for immunoprecipitation (IP) of methylated cfDNA using 5-mC antibodies
    - **IC:** Remaining ~40ng of library is NOT subjected to immunoprecipitation, it is used as an input control (IC) to measure successful methylation enrichment using sWGS. As a higher input was used (~40ng vs 0.88ng), this IC can be hybridized to custom targeted sequencing panels

Both assay arms are offered as part of a Research Use Only (RUO) sequencing service by the OICR Genome Research Platform.

Responsibilities

Management:
Review and update procedure, as required

Laboratory Staff:
Follow the SOP, document all required metrics, and report any non-conformances that may occur

Reagents and Consumables

Company	Product	Catalog #
ThermoFisher	Dynamag-96 (96 well magnetic rack)	123331D
	Dynamag-2 Magnet	12321D
	MicroAmp® Optical Adhesive Film	4311971
	MicroAmp Adhesive Film Applicator	4333183
BioRad	Sealing Roller	MSR0001
FisherScientific	Fisherbrand™ Mini Tube Rotator (rotating wheel)	88861051
Medstore	PCR STRIP(8), 0.2ml NEUTRAL, ATT FLAT CAP,120/pk	72.991.002
VWR	TUBE MICRO CLR 1500UL PK250	22234-044
Covaris (DMARK)	Covaris M220 (for shearing genomic DNA input only)	N/Ap
Covaris	Microtube-50 AFAF Fiber screw cap (25 or 250 tubes)	PN520166 or PN520167 (case of 250)
Beckman Coulter/Cedarlane	AMPure XP beads	A36881 (60 ml)
Medstore	Phosphate Buffered Saline (PBS); pH=7.4, 500mL	10010023
EpigenDx	High Methylation Control (Human Genomic DNA); 5ug, 100ng/µl	80-8061-HGHM5

IDT	xGen Duplex Seq Adapter-Tech Access; 2nmol at 15uM, dual 3bp UMI; approximately 133.3µl (stock solution)	1080799
	xGen Duplex Seq Primers (duplex I5/I7 Indicies for NextSeq); 4nmole Ultramer DNA Plate Oligo at 40uM or 100µl; Pre-mixed Amplification Primers	IDT_cfMeDIP_xgen-duplex-seq-oligo-ordering-form.xlsx
	Nuclease-Free Duplex Buffer; 10X2mL	11-01-03-01
ThermoFisher	Lambda (λ) DNA (dam-,dcm-), 500ug, 0.3ug/µl (Phage genomic DNA)	SD0021
ThermoFisher	1M Tris, pH=8.0, 100mls	AM9855G
Diagenode	MagMeDIP kit (48 rnxs)	Cat# C02010021
	IPURE Kit V2 (100 purifications)	Cat# C03010015
	DNA Methylation Control Package (40 rxns)	Cat# C02040012
	5-methylcytosine (5-mC) monoclonal antibody 33D3-Premium (100 ug)** lot controlled.	Cat# C15200081-100
Roche	KAPA HiFi HS RM (6.25 mls, final library PCR amplification)	7958935001 (KK2602)
	KAPA Hyper Prep (96rxn kit, no PCR module)	07962371001
MedStore/Greenfield Specialty Alcohols	Ethanol anhydrous 100% (brown bottle), case of 12X 500ml	P006EAAN
	Ethanol anhydrous 100%, 4X4L white jugs (cleaning only)	P016EAAN
	Nuclease-free water (Sigma-Aldrich)	W4502-1L
	2-Propanol (for molecular biology, >=99%), 500mL(Sigma-Aldrich)	I9516-500ML

Variables and Observations to Record

Samples

If the SOP has branching paths, optional steps, or variable conditions that are not explicitly specified, these **MUST** be recorded during the performance of the experiment. Observations may include:

No.	Library Type	Sample Name	Library Index	Insert Size (bp)	Qubit(ng/μl)	Matrix Barcode
1)	**IC**					
2)	**IP**					

Procedure

1. Component Preparation

1. Dilute the antibody as follows:

Reagent	**Reaction, 1X**
PBS	14 µL
5-mC antibody	1 µL
TOTAL REACTION	**15 µL**

    1. Pipette to mix.
    2. ***New antibody lots may require titration.
    3. It is recommended to make a large batch, and then aliquot into smaller volumes in 0.2-mL PCR tubes. Store the aliquots at −80 °C until needed
        i. Limit freeze thaws to a **maximum of 3X** (Dot tube to indicate thaws)
2. Prepare IPURE buffers
    1. Add 9 mLs of 100% isopropanol to **9** mLs of IPURE Wash Buffer 1, as indicated on the bottle.
    2. Add 9 mLs of 100% isopropanol to **9** mLs of IPURE Wash Buffer 2, as indicated on the bottle.

2. Shear DNA by Covaris

For detailed instructions please refer to SOP **TM. Covaris M220 & E220 Use and Maintenance (CE220M v2)**

2.1. Shear the following samples (Final volume must be **50 µL** for shearing):

1. **Genomic control sample** (Human High Methylated DNA or NA12878): Aliquot 10ng or 50ng depending on assay arm used.
2. ***A. thaliana spike-in*:** Combine and aliquot methylated & unmethylated *A. thaliana* controls.
    I. Qubit DNA controls to verify concentration before aliquoting.
    II. Combine equal ng of methylated & unmethylated DNA controls.
    III. Depending on the concentration of the stocks, shear sufficient DNA for the expected sample batch size.
        1. 0.1ng are required per sample (to be spiked-in at Step 3.2)

2.2. Use the Covaris **DNA_0150_bp_microTUBE-50_HolderXTU** protocol (verify the following parameters are met):

Target Shearing Size: ~150p
Temperature Range: Min: 18C/ Set Point: 20.0C / Max: 22.0C

Running Time	Peak Power	Duty Factor	Cycles/Burst
360 seconds	75.0	10.0	200

Safe Stop: Samples can be sealed and stored at -20°C after shearing.

2.3. Briefly spin micro tube containing sheared DNA, twist off cap and transfer sheared DNA to new tube/plate.
2.4. Qubit sheared samples and store at -20°C.

3. Methylated λ Filler DNA

Please see the Methylated Lambda Filler DNA SOP.

4. Kapa Hyper Prep + MeDIP Preparation of Libraries: Day 1

4.1. Aliquot **10ng** (Standard Arm) or **50ng** (Targeted Sequencing Arm) of cfDNA and Genomic Control Sample into a PCR plate.
4.2. Add **0.1ng** of sheared *A. thaliana* control mix from Step 2.3.
4.3. **If working with PBCM samples:** Add 0.01ng synthetic spike-in DNA.
4.4. Bring the volume up to 50µL with pre-chilled H_2O.

5. Kapa Hyper Prep: Day 1 (ER & A-T)

Notes:

- **The buffer and enzyme of the ER & AT Mix should be pre-mixed and added in a single pipetting step.**

5.1. Prepare the following, accounting for extra volume to compensate for pipetting error, and keep on ice:

ER & AT Mix	**1X**
End Repair & A-Tailing Buffer	7 µL
End Repair & A-Tailing Enzyme	3 µL
Total ER & AT Mix	**10 µL**
cfDNA	50 µL
TOTAL REACTION	**60 µL**

5.2. Add 10µL of ER & A-T Mix to the 50 µL of cfDNA. Pipette to mix.

5.3. Incubate in thermal cycler as follows:

File name: **COMP_ER_A**
Reaction Volume: **60 µL**
Cover Temperature: **85°C**

ER/AT Conditions		
Step	**Temperature (C)**	**Time**
1	20	30 min
2	65	30 min
3	4	Hold

6. Kapa Hyper Prep: Day 1 (Adapter Ligation)

Notes:

- **Water, buffer, and ligase should be pre-mixed and added in a single pipetting step.**
- **Dilute the** xGen Duplex Seq Adapter to 2µM using the **Nuclease-free Duplex Buffer (4°C).**

6.1. Prepare the following, accounting for extra volume to compensate for pipetting error, and keep on ice:

Adapter Ligation Mix	**1X**
Ligation Buffer	30 µL
DNA Ligase	10 µL
xGen Duplex Seq Adapter (diluted to 2µM)	5 µL
Pre-chilled PCR-grade water	5µL
Total Mix	**50 µL**
ER & AT Product	60 µL
TOTAL REACTION	**110 µL**

6.2. Add 50µL of Adapter Ligation Mix to the 60µl of ER & AT product. Pipette to mix.
6.3. Incubate in thermal cycler at **20°C** for 2 hours. Followed by **4°C hold.**

Reaction Volume: 110µL
Heated lid: **OFF**

7. Kapa Hyper Prep: Day 1 (Post-Ligation Cleanup)

- **Obtain the following reagents:**
  - **a. Ampure XP beads (stored at 4°C).**
    - i. Let equilibrate at room temperature at least 30 minutes before use.
  - **b. 5X MagBuffer** A, MagBuffer B, **MagBeads, and pre-chilled nuclease-free water from the MagMeDIP kit @ 4°C (Cat# C02010021, Diagenode)**
    - i. Place Magbeads at RT until use.
    - ii. If MagBuffer A is cloudy or crystallized, gently warm in hands before returning to ice.
    - iii. Place MagBuffer B on ice.
  - **c. MagBuffer** C **from the MagMeDIP kit @-20°C (Cat# C02010021, Diagenode)**
    - i. Place on ice
  - **d. Methylated** λ DNA (stored at -20**°C).**
    - i. Place on ice.
  - **e. Diluted 5-methylcytosine antibody.**
    - i. Place on ice.

7.1. Add **88µL** of Ampure XP beads to each 110 µL ligation reaction product for a 0.8X SPRI cleanup (final volume is 198µL)
7.2. Pipette to mix and incubate at RT for 15 min
7.3. Place tubes on magnet and allow solution to clear for 5 min
7.4. Remove supernatant without disturbing beads
7.5. Remove residual supernatant with 10µL pipette
7.6. Add 200 µL of fresh 80% EtOH while still on magnet and let sit for 30s
7.7. Remove EtOH
7.8. Repeat for a total of 2 washes
7.9. Remove residual supernatant with 10µL pipette
7.10. Dry beads at RT for ~4 min or until all residual ethanol evaporates

***Over drying beads may reduce yield**

7.11. Re-suspend beads in **27µL** of nuclease-free water, and visually confirm re-suspension.
7.12. Incubate at RT for 2 min to elute DNA.
7.13. Place on magnet until clear (~5 min) and transfer **25 µL** of supernatant to new PCR well.
7.14. Confirm no beads were carried over by placing new PCR well in magnet
7.15. If beads present incubate for 5 min, and transfer supernatant to new PCR well
7.16. Depending assay arm, proceed as follows:

Standard Arm (10ng)	**Targeted Sequencing Arm (50ng)**
7.17. Add **90ng** of methylated lambda-DNA Filler (see step 3) to entire **25µL** of library from Step 6.9: 7.17.1. Top up volume to 42.8µL with nuclease-free water. This is the **IP.** 7.17.2. Remove 2.2µL and save as **IC.** 7.18. Store **IC** at -20°C until library amplification in **Step 14.** 7.19. Keep **IP** on ice until **Step 5.1.**	1. <u>Divide</u> **25µL** of library as follows: a. **IC**: 20µL for input control. b. **IP**: 5µL for cfMeDIP. i. **ONLY** add 90 ng of methylated lamda-DNA filler (see step 3) to each IP library. ii. Top up volume to **23µL** with nuclease free water. 2. Store **IC** at -20°C until library amplification in **Step 14.** 3. Keep **IP** on ice until **Step 5.1.**

8. MeDIP Day-1

8.1. Prepare Bead Wash Buffer:
8.1.1. Prepare an ice-slush bath
8.1.2. Make a 1:5 dilution of 5X MagBuffer A in nuclease-free water as follows:

8.1.3. This dilution is now known as **Bead Wash Buffer** (77 µL required per reaction)

Bead Wash Buffer	**1X**
5X MagBuffer A	20 µL
PCR-grade water	80 µL
Total Mix	**100 µL**

8.2. Prepare Magbeads:

⚠ **Avoid vortexing the Magbeads. Pipette up and down to mix.**

8.1.1. Aliquot 11 µL of **Magbeads** (per reaction).
8.1.2. Wash by adding 27.5 µL of **Bead Wash Buffer** (per reaction).
8.1.3. Pipette to mix. **DO NOT VORTEX.**
8.1.4. Place on a magnetic rack until supernatant is clear, then discard supernatant.
8.1.5. Repeat Steps 8.1.2 – 8.1.4 for a total of two washes.
8.1.6. Re-suspend beads in 22 µL of Bead Wash Buffer.
8.1.7. **Keep on ice** (for use in Step 9.13).

9. Immunoprecipitation

9.1. Prepare IP Incubation Mix, according to assay arm used:

a. Keep on ice.

Standard Arm (10ng)

IP Incubation Mix	**1X**
5X MagBuffer A	24 µL
MagBuffer B	6 µL
Cold PCR-grade water	19.4 µL
Total IP Mix	**49.4 µL**
cfDNA	40.6 µL
TOTAL REACTION	**90 µL**

Targeted Sequencing Arm (50ng)

IP Incubation Mix	**1X**
5X MagBuffer A	24 µL
MagBuffer B	6 µL
Cold PCR-grade water	37 µL
Total IP Mix	**67 µL**
cfDNA	23 µL
TOTAL REACTION	**90 µL**

• Add **49.4 µL** of IP Mix to cfDNA (pipette to mix).	• Add **67 µL** of IP Mix to cfDNA (pipette to mix).

9.2. Incubate in thermal cycler as follows:

File name: **IP_**
Reaction volume: **90 µL**
Cover temperature: **95°C**

IP Incubation			
Step	**Temperature (C)**	**Time (HH:MM:SS)**	**Ramp Rate**
1	4	00:00:02	
2	95	00:10:00	1°C/s
3	4	HOLD*	

9.3. **Immediately perform the following once HOLD stage is reached**:
9.4. Transfer to ice-slush bath.
9.5. Let rest for 1s, then immediately transfer to new ice.
9.6. Repeat at least 3x, to ensure ssDNA remains cold.

⚠ The initial position where the tubes are placed in the ice water slush mixture will heat up instantly; therefore, it is important to transfer the tubes to a new location in the ice water slush mixture to prevent the DNA strands from re-annealing.

9.7. Incubate 10min on ice.
9.8. After ice incubation, quick spin samples and return to ice.
9.9. Thaw an aliquot (-80°C) of diluted anti-5mC and place on ice.
9.10. Pipette to mix (**DO NOT VORTEX**).
9.11. Prepare antibody master mix, accounting for extra volume to compensate for pipetting error. **Order of addition is critical**!
9.11.1. Add diluted Antibody to an empty tube (e.g. 1.5ml).
9.11.2. Add MagBuffer A, **pipette to mix**.
9.11.3. Add MagBuffer C, **pipette to mix.**
9.11.4. **Keep on ice.**

REQUIRED ORDER	Antibody Master Mix	1X
1	Diluted Antibody (anit-5mC)	2.4 µL
2	5X MagBuffer A	0.6 µL
3	***Pipette to Mix***	
4	MagBuffer C	2 µL
5	***Pipette to Mix***	
N/Ap	**Total Mix**	**5µL**

9.12. Pipette to mix antibody Master Mix prior to use, then add 5 µL to each sample, pipette to mix.
9.13. Add 20 µL of the washed Magbeads to each sample, pipette to mix.
9.14. Place the samples on a rotating wheel at 4°C for 17h-20h at 40 RPM.

10. MeDIP Washes-Day 2

- Obtain the following reagents from the 4°C IPure Kit (Cat: C03010015):
    1. **IPure Wash Buffer 1**- Place at RT
    2. **IPure Wash Buffer 2**- Place at RT
        1. Ensure both have isopropanol added in 1:1 ratio
    3. **IPure Buffer A**- Warm to RT 30min before use
    4. **IPure Buffer B**- Place on ice
    5. **IPure Buffer C**- Place on ice
    6. **IPURE Magnetic Beads v2**- Place at RT

- Obtain the following reagents from the 4°C MagMeDIP qPCR Kit (Cat:C02010021)
    1. **Magwash** Buffer 1- Place on ice
    2. **Magwash** Buffer 2- Place on ice

- Thaw -20°C Carrier Buffer (Labelled **'Carrier'**) at RT, then place on ice

- Obtain the following:
    1. **Magnetic Rack 1**: Place on **ICE**
    2. **Magnetic Rack 2**: Place at RT

10.1. Gently Prepare the following (each sample needs 100 µL):

IPure Elution Buffer Mix (IEB)	**1X**
IPure Buffer A	115.4 µL
IPure Buffer B	4.6 µL
Total Mix	**120 µL**

10.2. Keep **IEB** at RT until needed
10.3. Remove samples from rotating wheel and quick spin.
10.4. Use Magnetic Rack 1 (**ICE**) for all of the following steps:
10.5. Bind the DNA-anti-5mC-Magbeads on a magnetic rack (keep rack on ice) for 1 min.
10.6. Discard supernatant.
10.7. Add **100 µL** of **cold** **MagWash Buffer 1** to each sample and re-suspend by inverting/flicking.
10.8. Place samples on the rotating wheel and incubate for 4 min at 40 rpm at RT.
10.9. Quick spin tubes to collect droplets, place on magnetic rack and incubate for 1 min.
10.10. Discard supernatant.
10.11. Repeat Steps 10.7 – 10.10 for a total of **3** washes with **MagWash Buffer 1.**
10.12. Add **100 µL** of **cold** **MagWash Buffer 2** to each sample and re-suspend by inverting/flicking.
10.13. Incubate for 4 min at 40 rpm at RT.
10.14. Quick spin tubes to collect droplets, place on magnetic rack and incubate for 1 min.
10.15. Discard supernatant (use p10 to remove remaining liquid).

11. Methylated DNA Elution-Day-2

Note: From this point forward, use Magnetic Rack 2(RT) for all steps.

11.1. Gently pipette to mix the IEB Mix.
11.2. Re-suspend beads in 50 µL IEB Mix by pipetting.
11.3. Incubate samples on rotating wheel, **40 rpm for 15 min at RT.**
11.4. Remove samples from rotating wheel and place on magnetic rack at **RT** for 1 min.

11.5. **DO NOT DISCARD**!
11.6. Transfer **48 µL** of supernatant to a new strip tube.
11.7. Re-suspend beads in **50 µL** IEB Mix by pipetting.
11.8. Incubate samples on a rotating wheel, **40 rpm for 15 min at RT.**
11.9. Remove samples from rotating wheel and place on magnetic rack at RT for 1 minute.
11.10. Transfer **48 µL** of supernatant into tube containing supernatant (Step 14.4a).
11.11. Samples should now have total volume of 96µL (48µL+48µL)

12. Methylated DNA Washing and Purification-Day 2

12.1. Add 2µL of carrier buffer to the samples.
12.2. **Pipette to mix** then quick spin.
12.3. Add 100 µL of 100% isopropanol to each tube.
12.4. **Vortex to mix** and then quick spin (samples may become cloudy).
12.5. Vortex to re-suspend the **IPure Beads v2** (black magnetic beads) and add **10µL** of beads to each sample.
12.6. Pipette to mix very well.
12.7. Ensure beads are suspended between additions when working with many samples.
12.8. Incubate samples on a rotating wheel, 40 rpm for 10 min at RT.
12.9. Quick spin tubes and place on the magnetic rack for 1 min.
12.10. Discard supernatant.
12.11. Add **100 µL** of **IPure Wash Buffer 1**, inverting to mix.
12.12. Incubate on the rotating wheel, 40 rpm for 5 min at RT.
12.13. Quick spin tubes and place on the magnetic rack for 1 min.
12.14. Discard supernatant.
12.15. Add **100 µL** of **IPure Wash Buffer 2**, inverting to mix.
12.16. Incubate on the rotating wheel, 40 rpm for 5 min at RT.
12.17. Quick spin tubes and place on the magnetic rack for 1 minute.
12.18. Discard supernatant (use p10 to remove remaining liquid).
12.19. Air dry the beads for 2 min.
12.20. Re-suspend beads in 25 µL of **IPure Buffer C** by pipetting.
12.21. Incubate on rotating wheel at 40 rpm for 15 min at RT.
12.22. Quick spin tubes and place on the magnetic rack for 1 min.
12.23. **Keep and transfer** 23 µL of supernatant to a new tube (**library elution**).
12.24. Re-suspend beads in 25 µl of **IPure Buffer C** by pipetting.
12.25. Incubate on rotating wheel at 40 rpm for 15 min at RT.
12.26. Quick spin tubes and place on the magnetic rack for 1 min.
12.27. **Keep and transfer** 25 µL of supernatant and add to **library elution.**
12.28. Total volume of eluted IP library is 48 µL (verify beads have **NOT** carried over).

13. Library Amplification and Indexing-Day 2

13.1. Remove **IC** from the -20°C storage (**Step 7.17.2**).
13.2. Prepare PCR Mixes for IP and IC, according to the assay arm used:

Standard Arm (10ng)

IP PCR Mix	1X
2X KAPA HiFi HotStart ReadyMix	50uL
PCR-grade water	0.5ul
Total PCR Mix	50.5ul
XGEN Duplex Primers	1.5ul
cfMeDIP IP Lib	48ul
Total Reaction	100ul

IC PCR Mix	1X
2X KAPA HiFi HotStart ReadyMix	50 ul
PCR-grade water	46.3ul
Total PCR Mix	96.3ul
XGEN Duplex Primers	1.5 ul
IC Lib	2.2 ul
Total Reaction	100 ul

Targeted Sequencing Arm (50ng)

IP PCR Mix	1X
2X KAPA HiFi HotStart ReadyMix	50ul
PCR-grade water	0.5ul
Total PCR Mix	50.5ul
XGEN Duplex Primers	1.5ul
cfMeDIP IP Lib	48ul
Total Reaction	100ul

IC PCR Mix	1X
2X KAPA HiFi HotStart ReadyMix	50 ul
PCR-grade water	28.5ul
Total PCR Mix	78.5
XGEN Duplex Primers	1.5 ul
IC Lib	20 ul
Total Reaction	100 ul

13.3. Incubate in a thermal cycler as follows:

File name: **cfMeDIP**
Reaction Volume: **100 µL**
Cover Temperature: **105°C**

Step	Temperature (°C)	Time	Cycles
1	95	5 min	1
2	98	20 seconds	15
3	65	15 seconds	
4	72	30 seconds	
5	72	1 min	1
6	4	Hold	1

14. Post-Amplification Cleanup-Day 2

14.1. Add **180µL** of AMPureXP beads to each 100 µL PCR reaction product for a 1.8X SPRI cleanup (final volume is now 280µL).
14.2. Pipette to mix and incubate at RT for 15 min.
14.3. Place on magnet and allow solution to clear for 5 min.
14.4. Remove supernatant without disturbing beads.
14.5. Remove residual supernatant with 10µL pipette.
14.6. Add 200 µL of fresh 80% EtOH while still on magnet and let sit for 30s.
14.7. Remove EtOH.
14.8. Repeat for a total of 2 washes.
14.9. Remove residual EtOH with 10µL pipette.
14.10. Dry beads at RT for ~4min or until all residual ethanol evaporates*
14.11. ***Over drying beads may reduce yield.**
14.12. Re-suspend beads in **34µL** of nuclease-free water, and visually confirm re-suspension.
14.13. Incubate at RT for 2 min to elute DNA.
14.14. Place on magnet until clear (~5 min) and transfer 32 µL of supernatant to new PCR well/matrix tube.
14.15. **Safe Stop:** Store at -20°C if not proceeding directly to next step (Quality Assessment).

15. Library Quality Assessment:

15.1. Assess the library quality by running **1 µL** of each library on Qubit dsDNA HS Assay (refer to appropriate protocol)
15.2. Dilute library based on Qubit quantification to be run on the TapeStation 2200 using the High Sensitivity Tape or the Fragment Analyzer

References

- TM-015 Initial Sample QC – Qubit
- TM-009 Fragment Analyzer Assays