



# ASTM Standard Guide for

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Helping our world work better

## Application of Molecular Biological Tools to Assess Biological Processes at Contaminated Sites

[www.astm.org](http://www.astm.org)

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on Bioremediation and Sustainable  
Environmental Technologies  
Austin, Texas, May 11, 2023**

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12,900 ASTM standards operate globally

Applied to just about everything from **steel** to **sustainability**

They improve the lives of millions every day

# Over a Century of Openness



## How ASTM Works

- Principles of openness and **voluntary consensus**
- Experts, individuals, organizations, academia, governments, trade associations, consultants and consumers
- **Over 30,000 members from 160 countries** exchange expertise and knowledge
- **Transparent process – open to anyone, anywhere**
- Timely and relevant. Fully representative of sectors. An aid to innovation



# Committee E50

## Environmental Assessment, Risk Management, and Corrective Action

- One of ASTM's 148 Main Committees
- **Home of RBCA** – Risk Based Corrective Action and **ESA** – Environmental Site Assessment
- Has 7 subcommittees:
  - Storage Tanks
  - Real Estate Assessment and Management
  - Beneficial Use
  - **Corrective Action**
  - Risk Management
  - Climate and Community
  - Biological Effects and Environmental Fate



# ASTM Molecular Biological Tools Team



## Co-Leads

<b>Trent Key</b>	<b>Andrew Madison</b>	<b>Stephanie Fiorenza</b>
MBT Overview, Application, Selection	Sampling Methods	Overall ASTM process



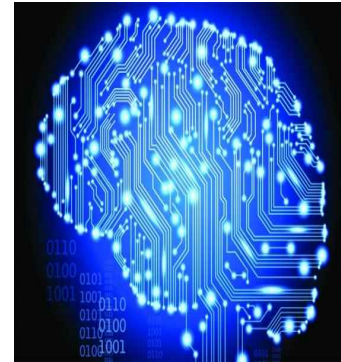
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# Required Sections of an ASTM Standard



- **Scope** – what the standard covers
- **Referenced Documents**
- **Terminology**
- **Significance and Use** – why the standard is important; how the standard is to be used



**These first four sections are publicly available on [www.astm.org](http://www.astm.org)**

# Molecular Biological Tools (MBT) Standard Guide



## Scope:

- Presents a framework for applying molecular biological tools to in situ remediation.
- Develops a consistent way to apply MBTs.
- Emphasizes nucleic acid-based tools.

**E3354 – 22**

# MBT Standard Guide



## Referenced Documents:

- ASTM Methods
- EPA Methods
- Other published papers and sources



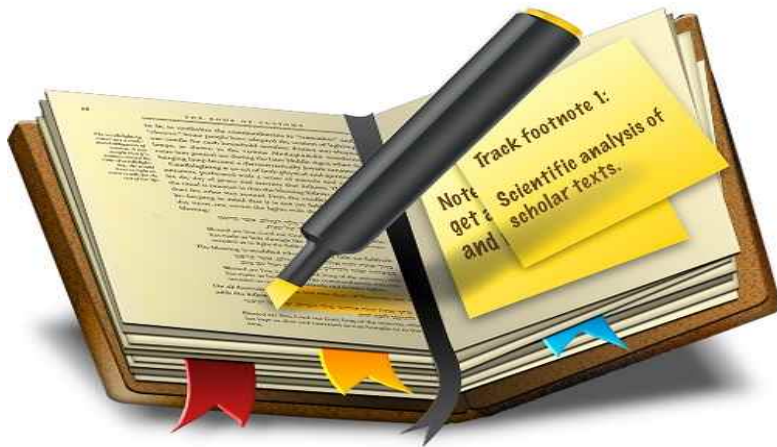


# MBT Standard Guide



## Terminology:

- Thirty-eight terms specific to the application of MBTs are defined and discussed
- Includes Next Generation Sequencing, qPCR, RT-qPCR
- Provides an overview of key terms in this rapidly developing field



3.2.19 molecular biological tools (MBTs), n—a suite of molecular genetic analyses that can be used to characterize and evaluate microorganisms and their related activity.

3.2.19.1 Discussion—MBTs may also be referred to as or included in Environmental Molecular Diagnostics (EMDs).

## Significance and Use:



- Aids managers of contaminated sites in the selection and application of MBTs
- Presents the limitations of methods
- Describes quantitative techniques to determine biological processes, including a summary comparison table

# Significance is What MBTs Tell Us

What is the concentration of contaminant degraders?

qPCR

qPCR Arrays

Is biodegradation occurring?

Stable Isotope Probing (SIP)

Compound Specific Isotope Analysis (CSIA)

What is the community structure?

Next Generation Sequencing

What treatment strategy should be selected?

Microcosms and In Situ Microcosms (ISMs)

# Molecular Biological Tools (MBTs) Standard Guide



**Table 1. Overview of Molecular Biological Tools**

MBT	Type of Data	Advantages	Disadvantages
Quantitative Polymerase Chain Reaction	Presence/absence of a gene	Culture independent	Positive results depend upon sequence similarity to a known gene
		Sensitive	Does not give information on activity
		Results easy to understand	Does not differentiate between live and dead cell DNA
		Commercially available	

# Molecular Biological Tools (MBTs) Standard Guide

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## Table 1. Overview of Molecular Biological Tools

**Additional tools covered:**

**RT-PCR**

**16S rRNA Amplicon Sequencing (Targeted method)**

**Metagenomics (Non-targeted method)**

**Transcriptomics**

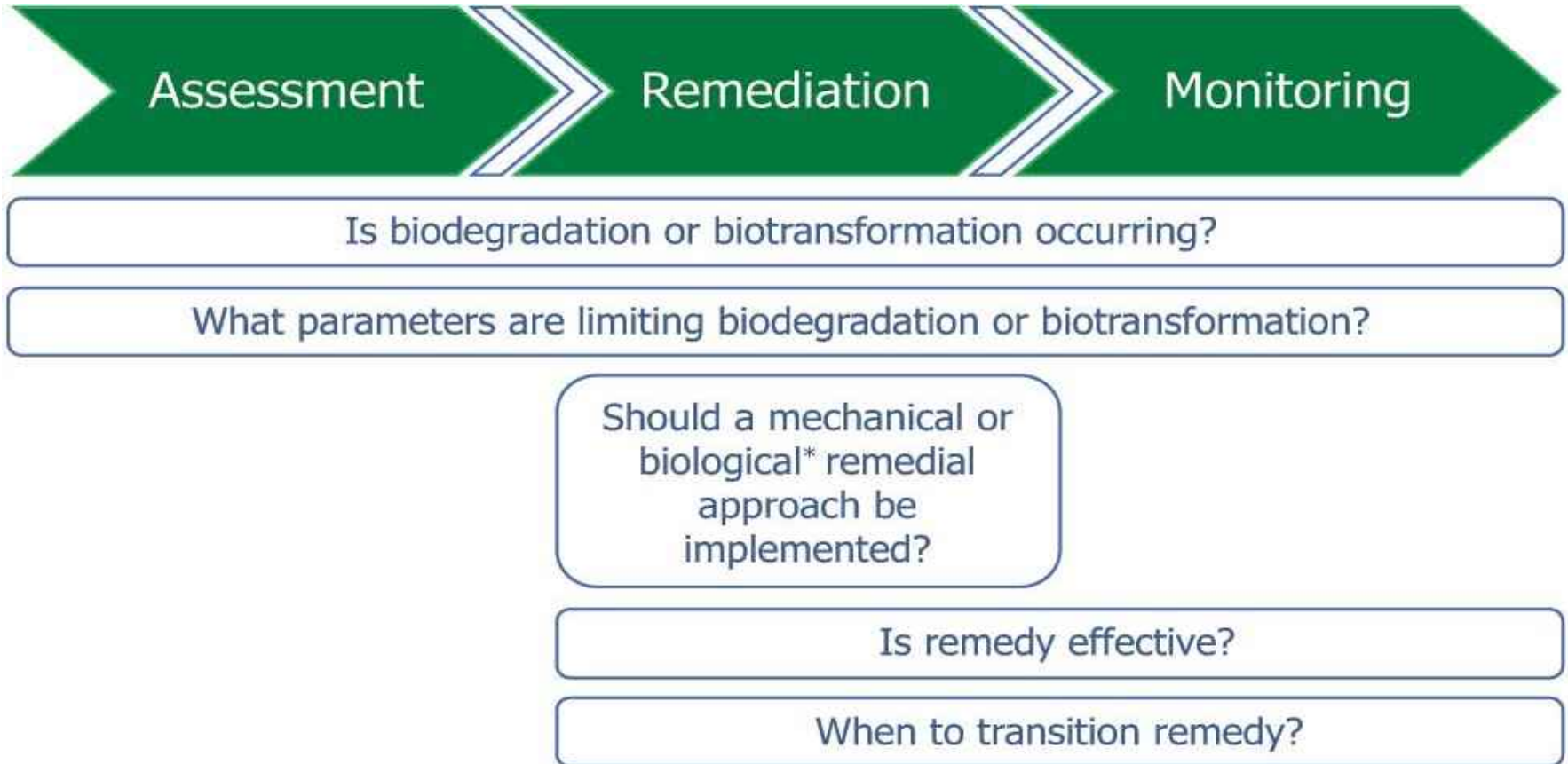
**Proteomics**

**Stable Isotope Probing**

**CSIA**

**Metabolomics**

# Application of MBTs Throughout Project Lifecycle



\*"biological" = monitored natural attenuation (MNA), natural source zone depletion (NSZD), enhanced bioremediation

Image Excerpt from Figure 3 Guidance Document E3354-22

# Molecular Biological Tools (MBTs) Standard Guide



**Table 2. Selection of Commercially Available Genetic Targets  
Chlorinated Ethenes, Anaerobic**

Acronym	Target	Relevance
Dhc	<i>Dehalococcoides</i>	Reductively dechlorinates PCE, TCE, all DCE isomers, VC
bvca	BAV 1 Vinyl chloride reductase (bvca)	Dechlorination of cDCE and VC to ethene
tceA	Trichloroethene reductase (vcrA)	Dechlorination of PCE and TCE to cDCE and VC

# Molecular Biological Tools (MBTs) Standard Guide



## Table 2. Selection of Commercially Available Genetic Targets

### Additional Targets Covered:

**Chlorinated Ethenes, Aerobic**

**Chlorinated Benzenes, Biphenyls, Phenols**

**BTEX, Anaerobic**

**BTEX, Aerobic**

**PAHs**

**N- compounds**

**Prokaryotic Groups**



# Molecular Biological Tools (MBTs) Standard Guide

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## Molecular Biological Tool Selection:

- **Targeted Methods**

- Requires prior knowledge of organism or gene of interest
- Methods designed to target specific bit of DNA/RNA of organism or gene (i.e., primers)
- Example of targeted methods
  - PCR
  - qPCR
  - 16S rRNA amplicon sequencing

- **Non-targeted Methods**

- Large data generated
- Challenge associated with data interpretation
- Example of non-targeted methods
  - Metagenomics

# Molecular Biological Tools (MBTs) Standard Guide



## Molecular Biological Tool Selection:

- Considered commercial availability
  - qPCR
  - NGS of 16S rRNA gene
- **Align tool selection with project goals**
  - Misuse of MBTs can result in waste time, effort, money
  - Identify question to be answered
  - Awareness of tool advantages/disadvantages
  - Always employ a **multiple lines of evidence** approach:
    - COCs
    - Geochemistry
    - MBTs



# Molecular Biological Tools (MBTs) Standard Guide



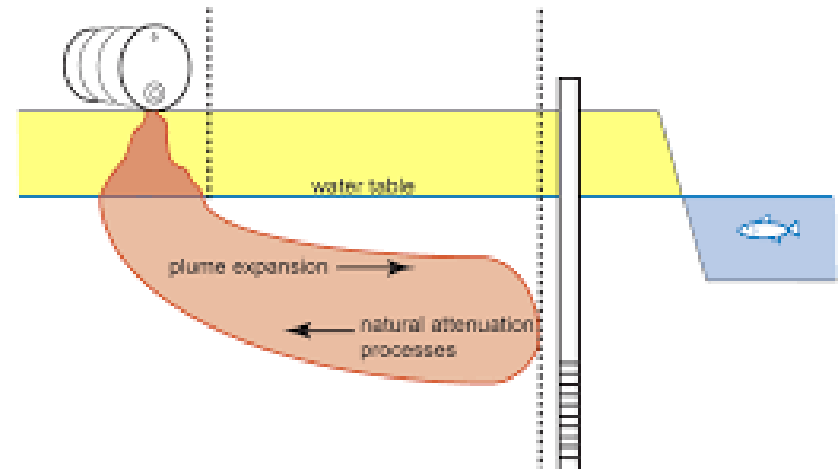
## Sampling Plan Development:

- **Sample locations**

- Align with CSM, site goals, e.g., plume vs. source area
- General locations: background, source, mid-plume, and plume edge

- **Sampling for Multiple Lines of Evidence**

- Site COCs
- Geochemical Parameters
- MBTs



# MBT Standard Guide



## Section 9

### Sample Collection, Preservation and Shipping

#### AQUEOUS SAMPLES

Groundwater

Surface water

Porewater

#### Consider Purpose

Single Time Point

Time-weighted Average



# MBT Standard Guide



**Grab Samples are Discrete or Single Time Point**

**Passive Samples are Time-weighted Average**

# MBT Standard Guide



Aqueous Sampling Method	Advantages	Disadvantages	Comments
Grab – field filtration	Larger volumes, ease of preservation	Increased field time, filter and equipment costs	Well purging recommended; high pressure pumps can ruin filter
Grab – lab filtration	Less time in field	Potential for low biomass	Method consistency
Bio-Trap <sup>R</sup>	Easy to deploy	Only a subsample is extracted	Time-average

# Solid Medium Sample Collection



## Important variables:

- Soil moisture
- Exposure to air
- Geochemical conditions
- Heterogeneity of soil
- Time

**Soil homogenization should be done by lab**

**Characteristics of Microorganisms in Soils:**

Interface dwellers

Proliferate in organic-rich soils

Contaminant-degrading potential in contaminated and uncontaminated soil



# MBT Standard Guide



Soil Sampling Method	Advantages	Disadvantages	Comments
Drilling  Rotary Hollow Stem Auger Sonic	Soil Core obtained	Sonic disrupts soil texture Cooling fluids added can affect core representativeness	Soil Core is obtained Geologist or soil scientist needed on site
Probing  Direct push	Less time in field Minimal disturbance of soil	Depth / soil type limitations	Easy to subsample for MBT test

**Discrete – one aliquot per sampling interval**  
**Composite – aggregation of aliquots from a larger area**



# Sample Collection and Handling

**Volume:** about 50 grams

**Containers:** polymer preferred to glass, Whirl-Pak bags<sup>®</sup>, conical serum vials

**Preservation:** consult with shipping expert at lab. For example:

MBT	Condition
qPCR DNA	≤ 4 degrees C
NGS, transcriptomics	-80 degrees C

## Shipping:

- Overnight at -80°C (dry ice) best
- If cold storage not possible, use nucleic acid preservatives (e.g., DNAgard<sup>®</sup>)
- Use a temperature logger
- Chain of Custody with all MBT analyses specified



## Section 10 DATA INTERPRETATION

- **Data quality depends on sample collection and preservation**
- **Data value depends on planning**
- **Considered a tertiary line of evidence for biodegradation**
- **“Not Detected” doesn’t necessarily mean “No”**
- **Need to connect the MBT result (gene or process) with a process**



# Standard Guide for Application of Molecular Biological Tools to Assess Biological Processes at Contaminated Sites

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## E3354 – 22

- **Identifies tools that can be used to understand biological processes at contaminated sites**
- **Provides guidance for the selection of methods, sample collection and data interpretation**
- **Summarizes important factors for selecting tools**

# MBT Standard Guide

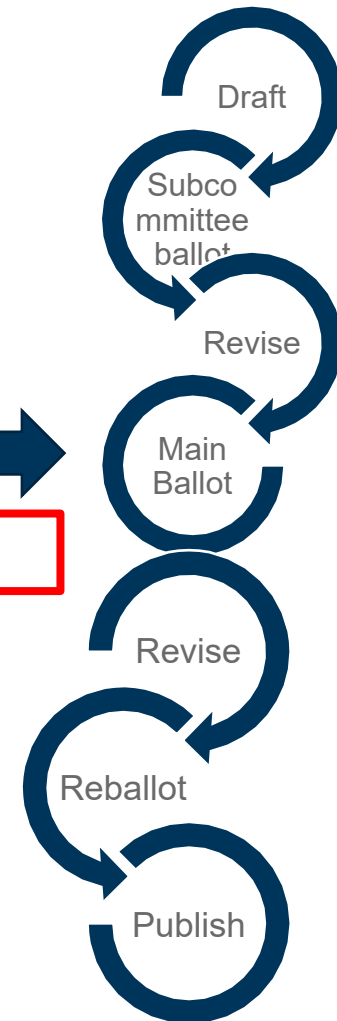


## Status

**E3354 – 22**

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**Passed on first main committee ballot**



# Molecular Biological Tools

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## Acknowledgments

### All Task Group Members

**ASTM** Molly Lynyak, E50 Staff Manager



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# Thank you

For more information on the Standard Guide for Application of Molecular Biological Tools to Assess Biological Processes at Contaminated Sites, contact Andrew.Madison at [andrew.madison@wsp.com](mailto:andrew.madison@wsp.com) or Stephanie Fiorenza at [sxfiorenza@gmail.com](mailto:sxfiorenza@gmail.com)

[www.astm.org](http://www.astm.org)



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