Introduction to Nucleic Acid Purification:

Purification Basics and Their Application to Different Sample Types

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welcome

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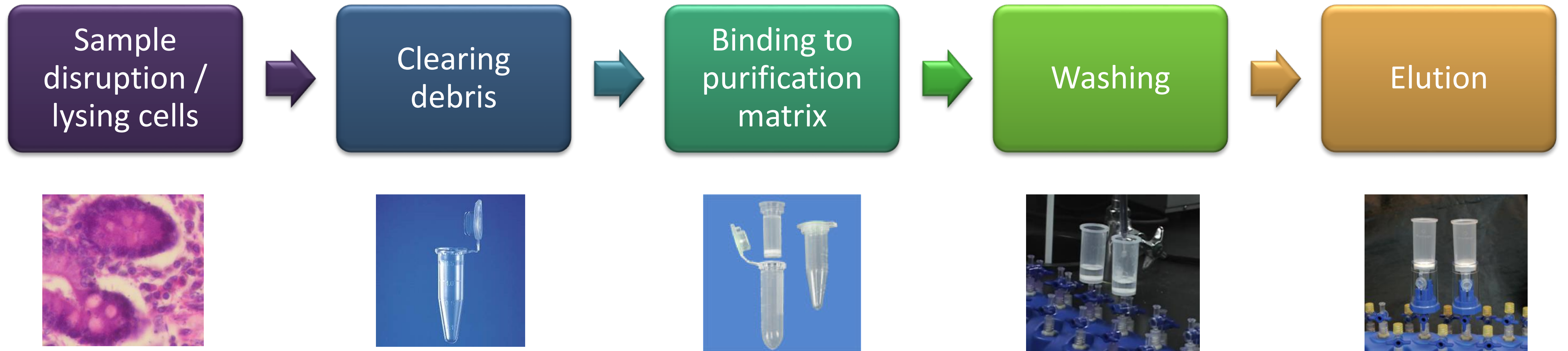
Agenda

- Basics of Nucleic Acid Purification
- Purification of Plasmid DNA
- Purification of Nucleic Acid from FFPE
- Purifying RNA or DNA from Blood
- Adapting Purification Methods to Sample Type & Throughput Needs

Basics of Nucleic Acid Purification



Breaking the Problem into Distinct Parts

Sample Extraction

Nucleic Acid Isolation

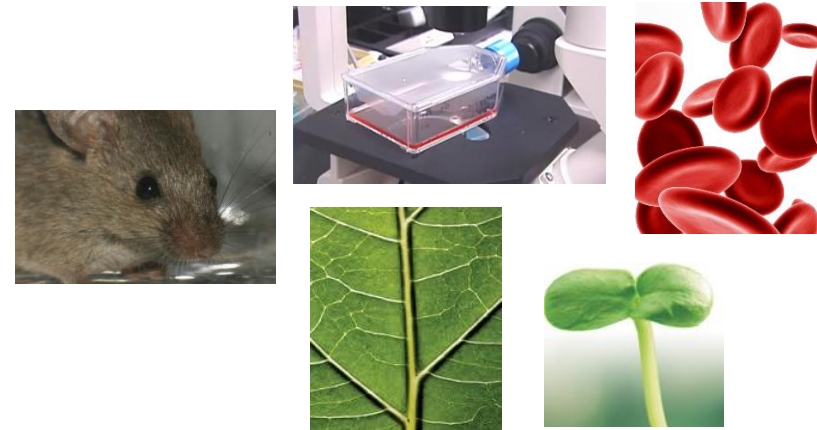
Sample Disruption Considerations

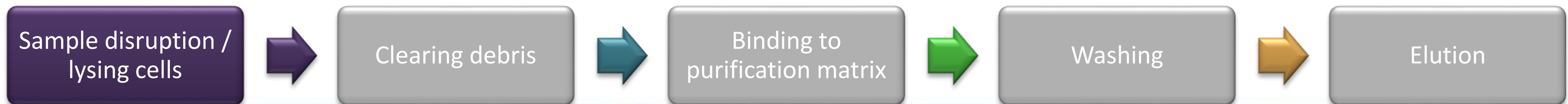
Tip: there is usually a visible physical change to a sample when the cells lyse

Goal: rapid and complete disruption of cells in sample releasing nucleic acid into lysate

Starting material and target (DNA or RNA) may influence your choice of lysis method:

- Chemical
- Physical
- Enzymatic
- Combination

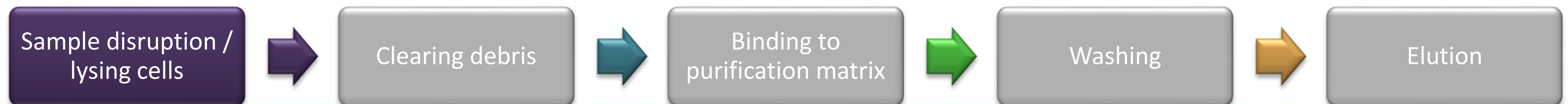




Chemical Disruption

Cellular disruption is accomplished with a variety of agents which disrupt membranes and denature proteins:

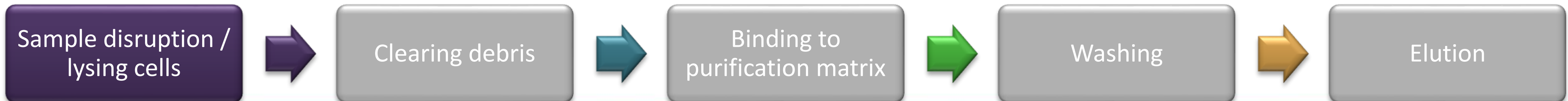
- Detergents (SDS)
- Chaotropes (guanidine salts)
- Alkaline solutions



Physical Disruption

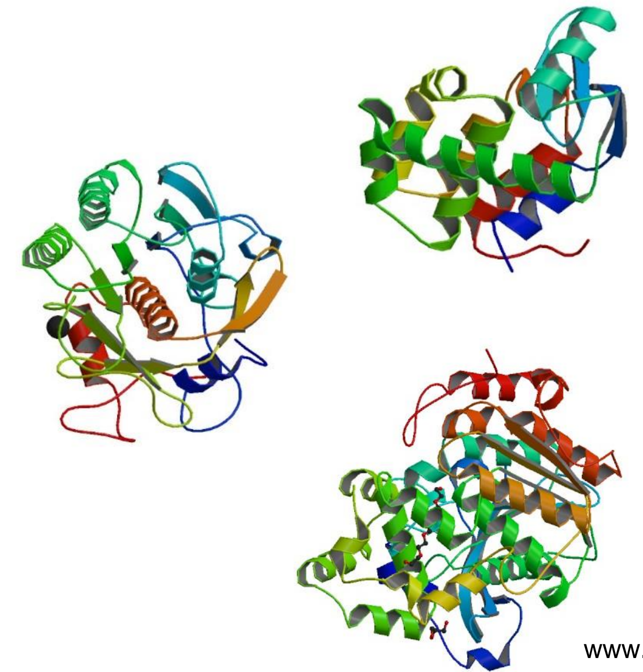
- Usually in combination with chemical or enzymatic
- Used with more structured starting material
  - Tissue pieces
  - Plant materials (cell wall disruption)
- Manual processes (grinding under liquid nitrogen)
- Automated single to multi sample processing
  - Bead beating
  - Sonication
  - Tissue grinders
- Heat can have negative consequences

Tip: for RNA, cellular material need to be rapidly exposed to lysis buffer to avoid degradation



Enzymatic Disruption

- Used with more structured starting material in combination with other methods:
  - Tissue pieces
  - Plant materials
  - Bacteria
  - Yeast
- Common treatments include (depending on starting material):
  - Lysozyme, Zymolase and lyticase
  - Proteinase K
  - Collagenase
  - Lipase



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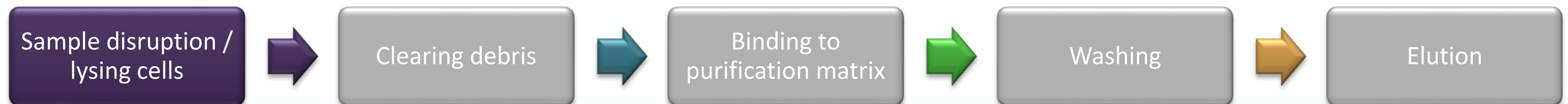
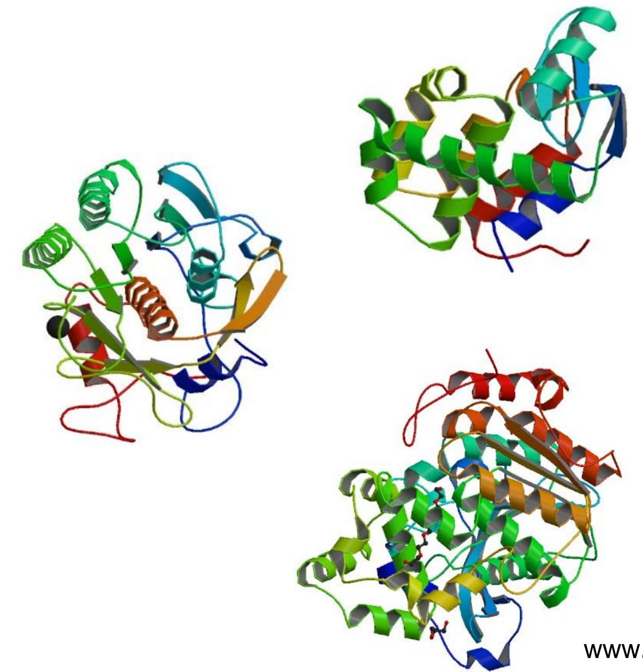
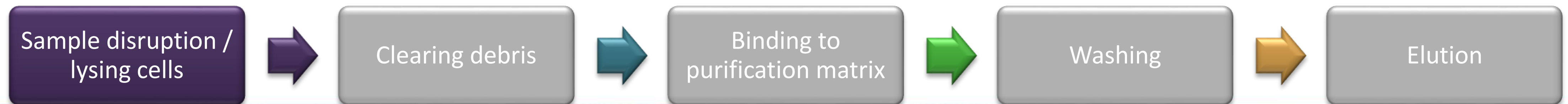


Enzymatic Disruption - continued

- Can be amenable to higher throughput processing
- Increases cost
- Concern of RNA stability with digestion of samples at elevated temperature





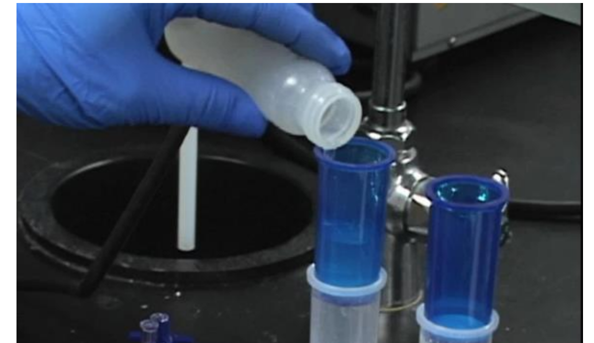
Clearing Debris

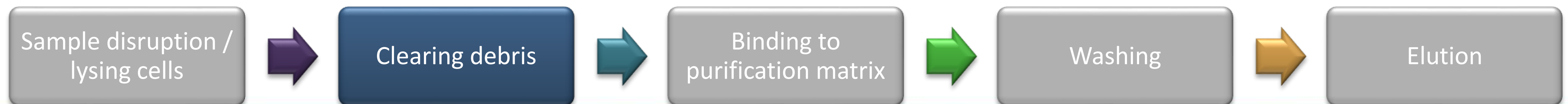
Goal: remove cellular debris

Depending on the starting material and lysis conditions, cellular debris may need to be removed prior to nucleic acid purification to reduce carryover and prevent clogging or aggregation during purifications

Methods:

- Centrifugation – works in most cases; can handle large amounts of debris
- Filtering – can be faster; too much debris can clog
- Bead-based clearing – best for automation; can be overwhelmed with biomass

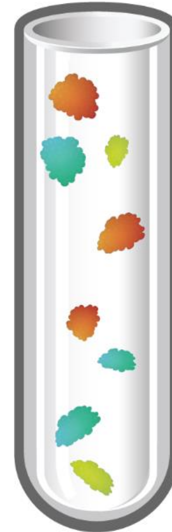




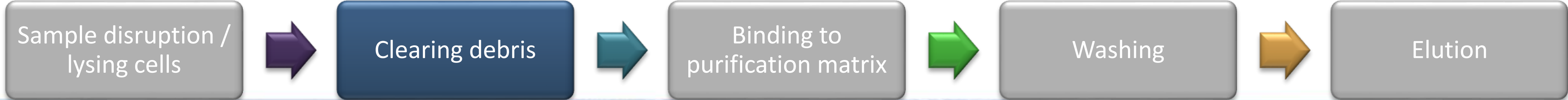
Cell Lysate

Cleared lysate contains:

- Nucleic acids
  - DNA's
  - RNA's
- Proteins
- Smaller molecules (salts and detergents)



Tip: when transferring lysate, avoid any pelleted material which can interfere with the purification

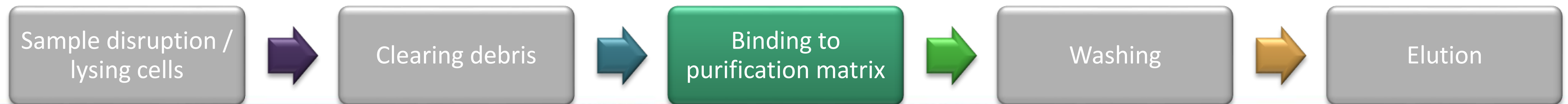


Nucleic Acid Binding

Under appropriate conditions nucleic acid will bind to matrices:

- Silica
- Cellulose
- Ion Exchange

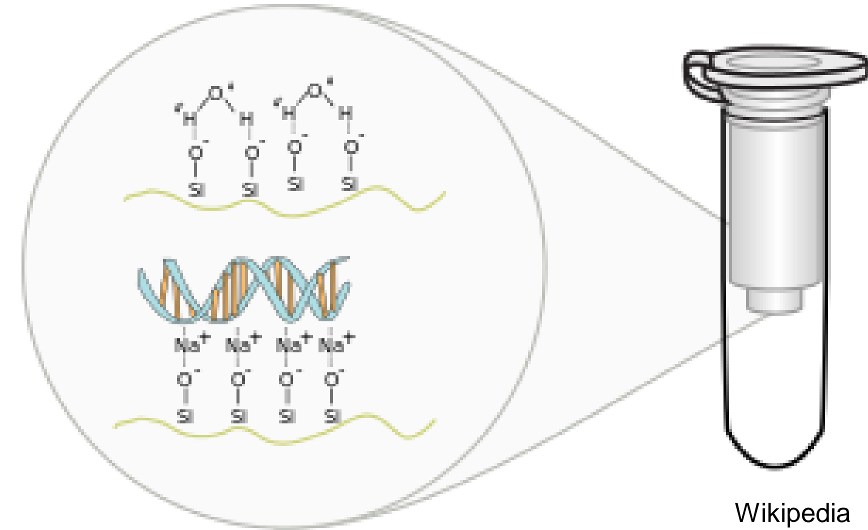
Manipulating binding conditions can affect the efficiency of nucleic acid binding (RNA vs. DNA or large vs. small)



Silica Binding Matrix

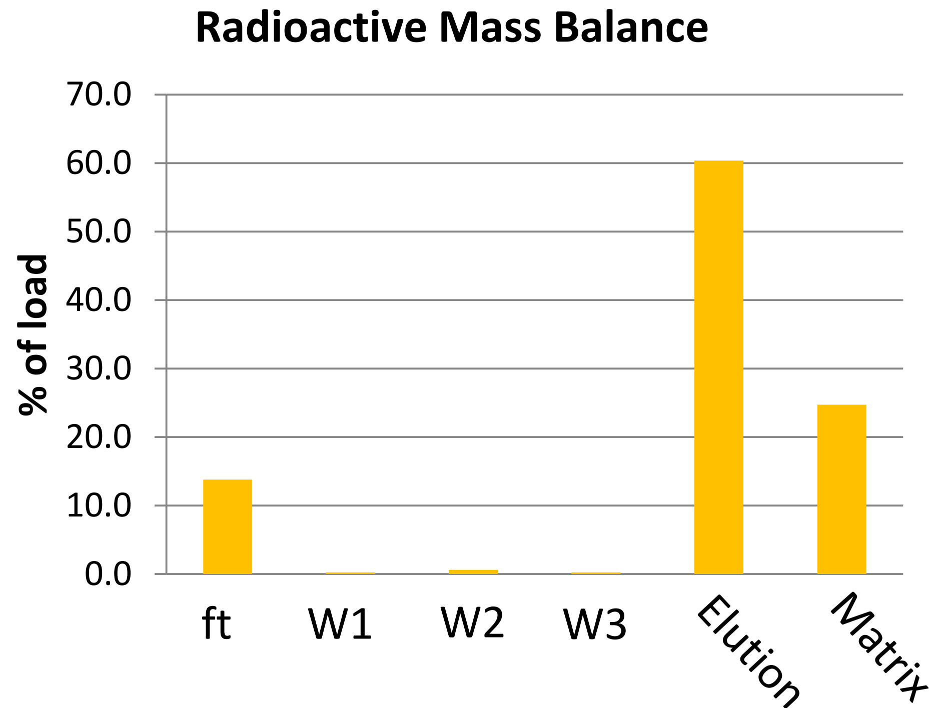
Can be many forms:

- Silica membrane
- Diatomaceous earth
- Slurries
- Particles
- …



Nucleic acid interacts with silica in the presence of chaotrophic salts (NaI, guanidine salts)

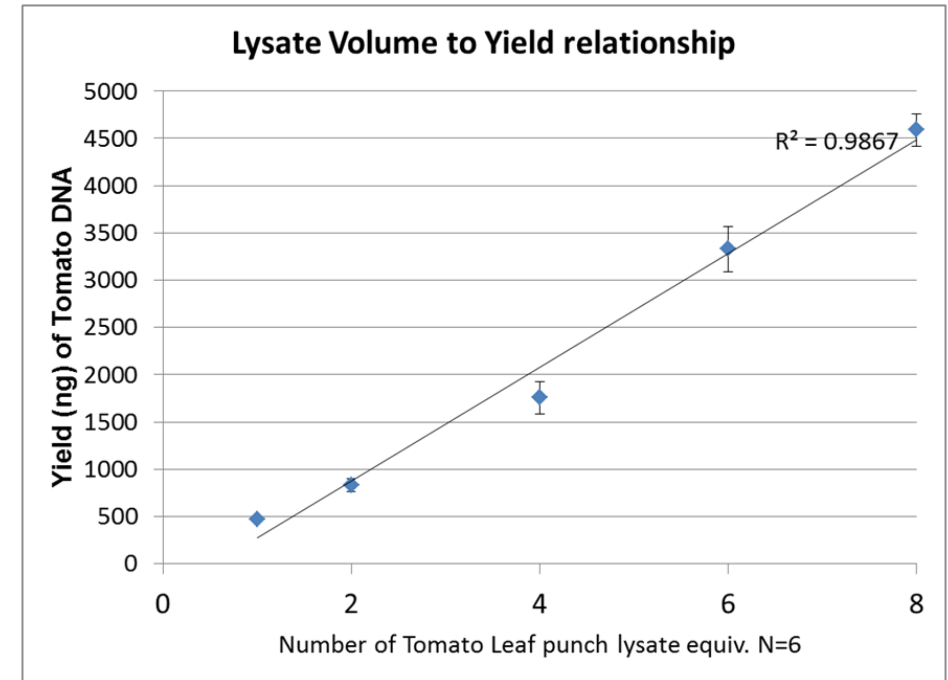
Example of Tracing DNA Through a Purification Chemistry



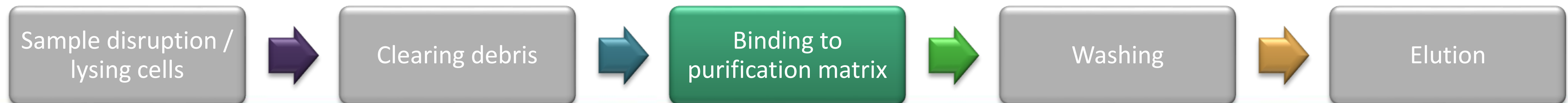
- Labeled DNA with radioactivity
- 80-90% of target binds to resin
- Very little target lost during washes
- 30% of target remains on resin
- Elution efficiency must be increased

Cellulose Binding Matrix

- Nucleic acid binds to cellulose in the presence of high salt and alcohols
- Conditions can be adjusted to preferentially bind different species and sizes of nucleic acid
- Cellulose chemistries have high binding capacities (less matrix material required, able to elute in smaller volumes)

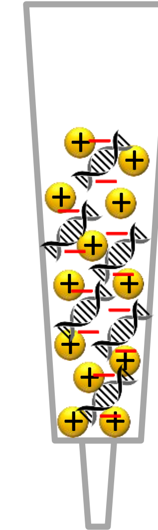


Pooled tomato leaf lysate isolated with cellulose-based paramagnetic particle chemistry



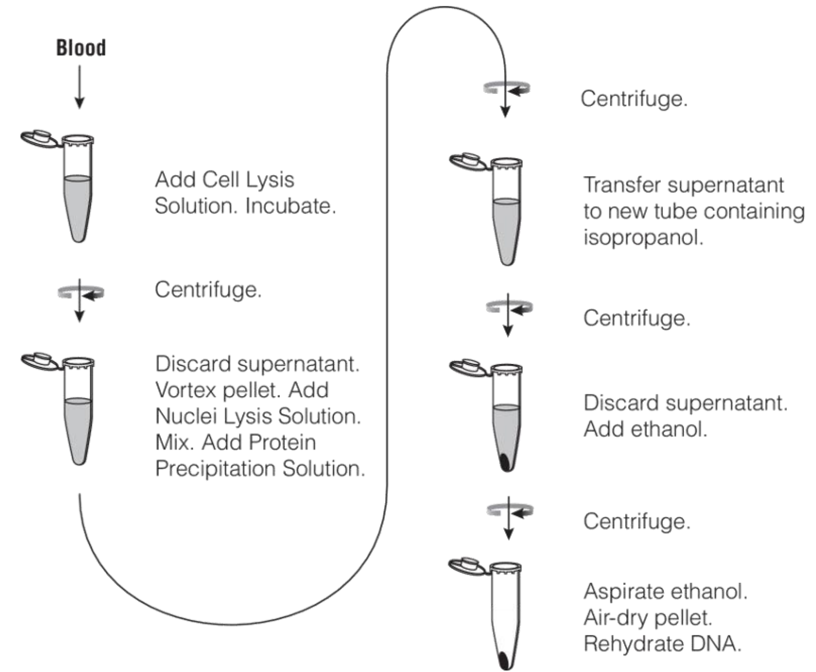
Ion Exchange

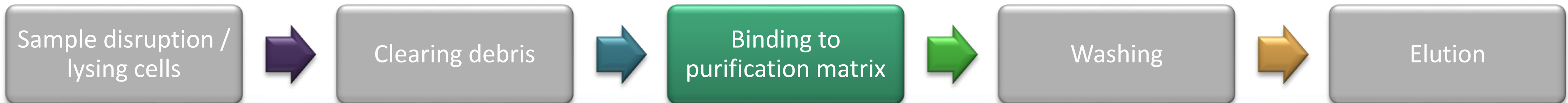
- Based on interaction between negatively charged phosphates in DNA and positively charged particles
- DNA binds under low salt conditions
- Protein and RNA can be washed away with higher salt
- DNA is eluted in high salt and recovered by ethanol precipitation
- Nucleic acid can be bound to some resins based on pH



Solution-Based Purification

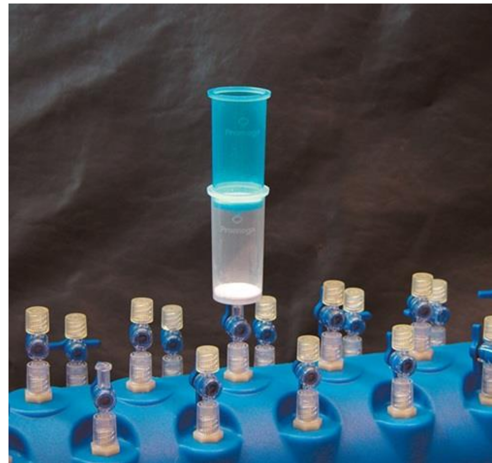
- Based on differential precipitation
- No binding matrix (alcohol precipitation)
- Truly scalable





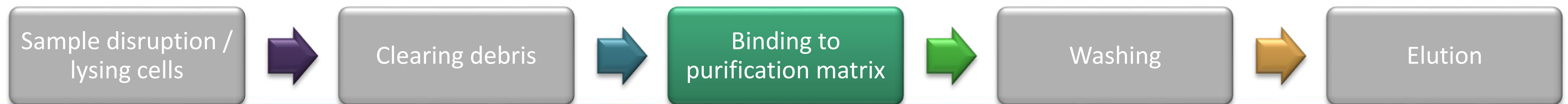
Purification Matrices

Purification surface chemistries can be configured in different formats for purification of nucleic acid



Column chromatography

Paramagnetic particles



Column-Based vs. Magnetic Particle-Based

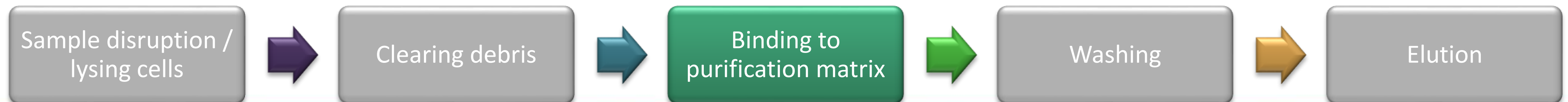
Tip: throughput and laboratory workflow are considerations when choosing column- or particle-based purification

Column-based

- Manual
- Generally lower elution volumes
- More hands on time

Magnetic particle-based

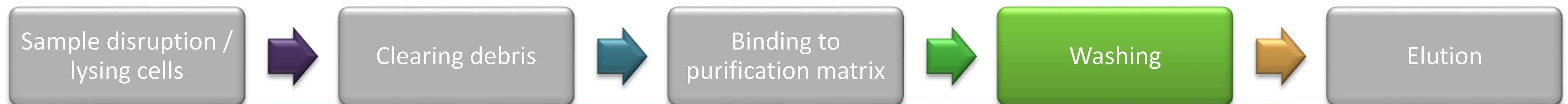
- Low risk of clogging
- More amenable to automation
  - Increased consistency
  - Reduced hands on time
- Scalable



Washing

Wash buffers (usually containing alcohol) are used to remove proteins, salts and other contaminants

- Alcohols associate nucleic acids with the matrix
- Removal of salts allows for elution



Elution Considerations

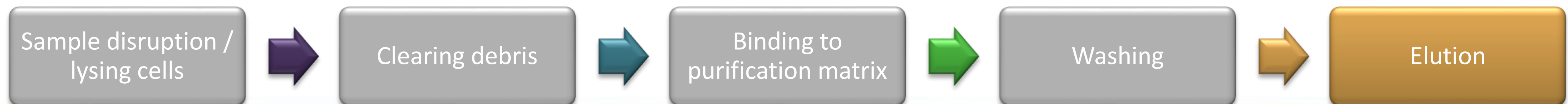
Tip: nucleic acids are typically stored in buffered solutions – the acidic backbone of DNA can autohydrolyze over time

Samples may be eluted in a variety of buffers:

- Nuclease-free water
- Buffered solutions
- TE

Requirements of downstream processes may effect your choice of elution buffer

The higher the elution volume, the higher the yield and the lower the concentration

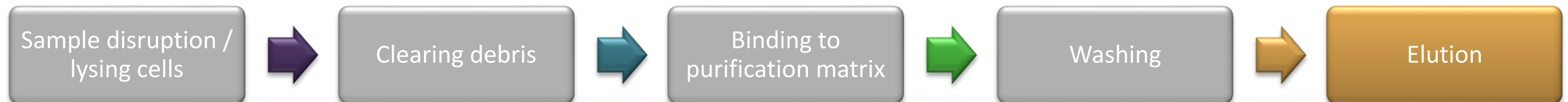


Storage of Eluted DNA or RNA

Nucleic acid is typically recovered in water or elution buffer

For storage there are several considerations:

- Time
- How often will sample be accessed
- Preservation of integrity



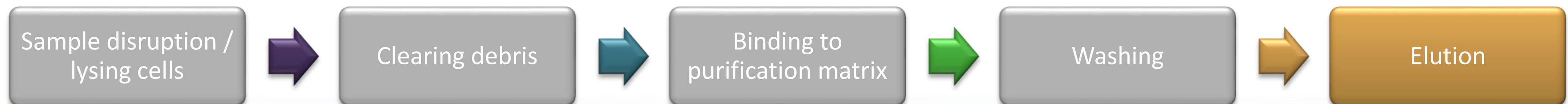
Considerations for DNA and RNA Storage

RNA

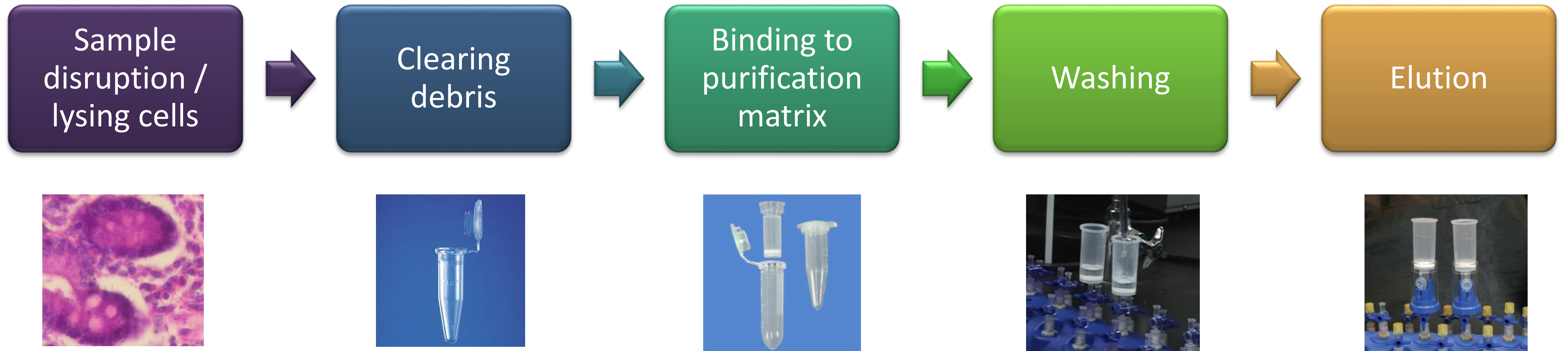
- For best integrity, prepare and use fresh
- If you must store RNA, store at -80°C
- Consider using RNasin® Ribonuclease Inhibitor to protect against RNase contamination

DNA

- If retaining the largest size is your primary consideration, do not freeze DNA
- Store DNA in a buffered solution to reduce auto-hydrolysis



Basics of Nucleic Acid Purification

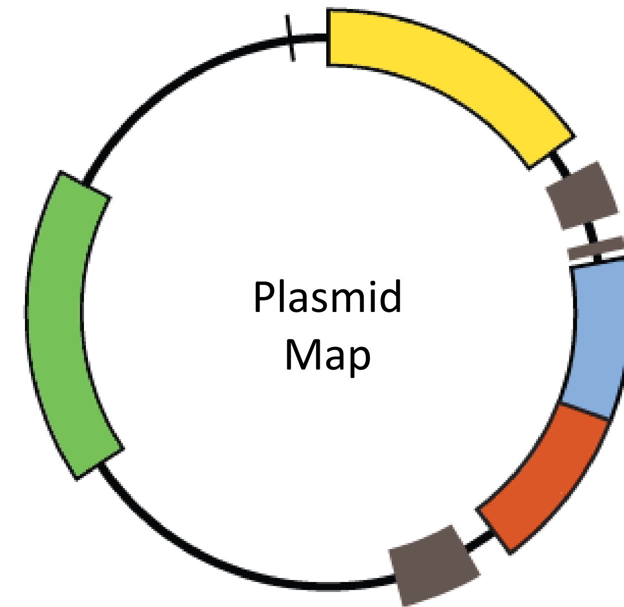


Applications: Purification of Plasmid DNA

Plasmid DNA Purification

Yield and recovery of plasmid from culture is dependent on several parameters:

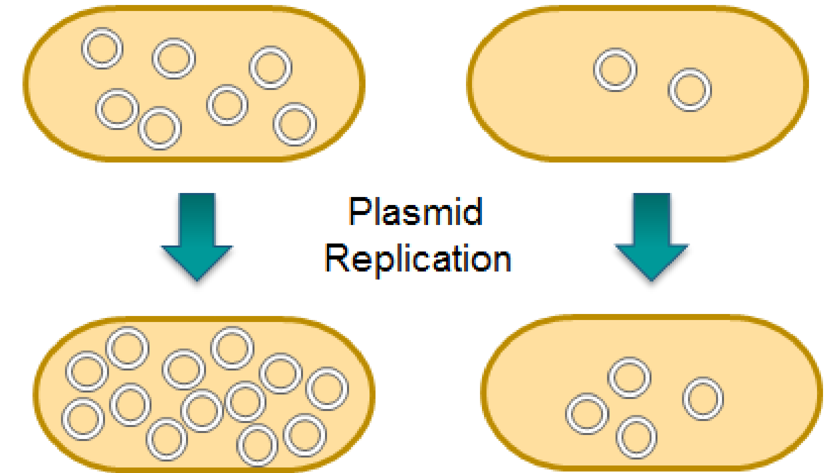
- Plasmid copy number
- Volume of culture
- Culture conditions
- Plasmid prep capacity



Plasmid Copy Number

Origin: where plasmid replication begins

- Different origin sequences give different characteristics
- Origin determines the copy number
  - Origins are classified roughly in classes
  - Low (less than 15) to high (300+) copy
  - Resulting plasmid yields may range from 0.1 to nearly 10µg plasmid per ml of culture



Sample disruption/ Lysis of cells

Lysis of bacterial cells preferentially releasing plasmid

Clearing debris

Removal of cell debris and gDNA by centrifugation

Binding to purification matrix

Binding plasmid to silica matrix

Washing

Removal of salts and debris by washing with ethanol containing buffer

Elution

Elution of plasmid DNA

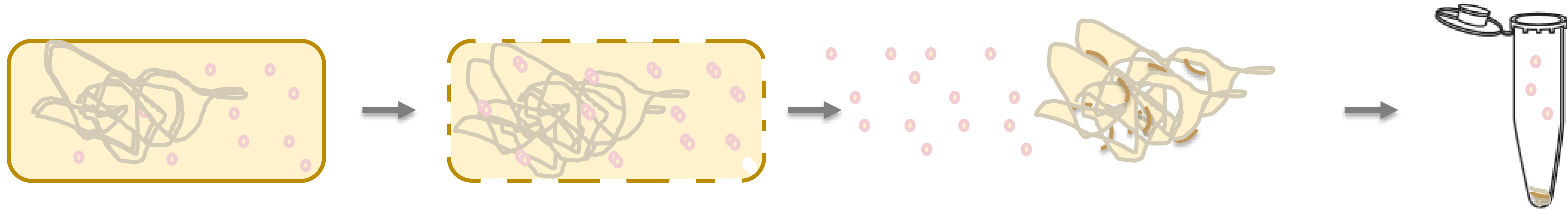
Add Lysis Buffer Directly to Bacterial Culture

Neutralize

Bind & Wash

Elute

Alkaline Lysis – A Key Step for Plasmid Purification



Alkaline (pH>10) Lysis
denatures DNA gDNA
and plasmid

Rapid Neutralization (pH7)
plasmids re-anneal;
gDNA and proteins do not

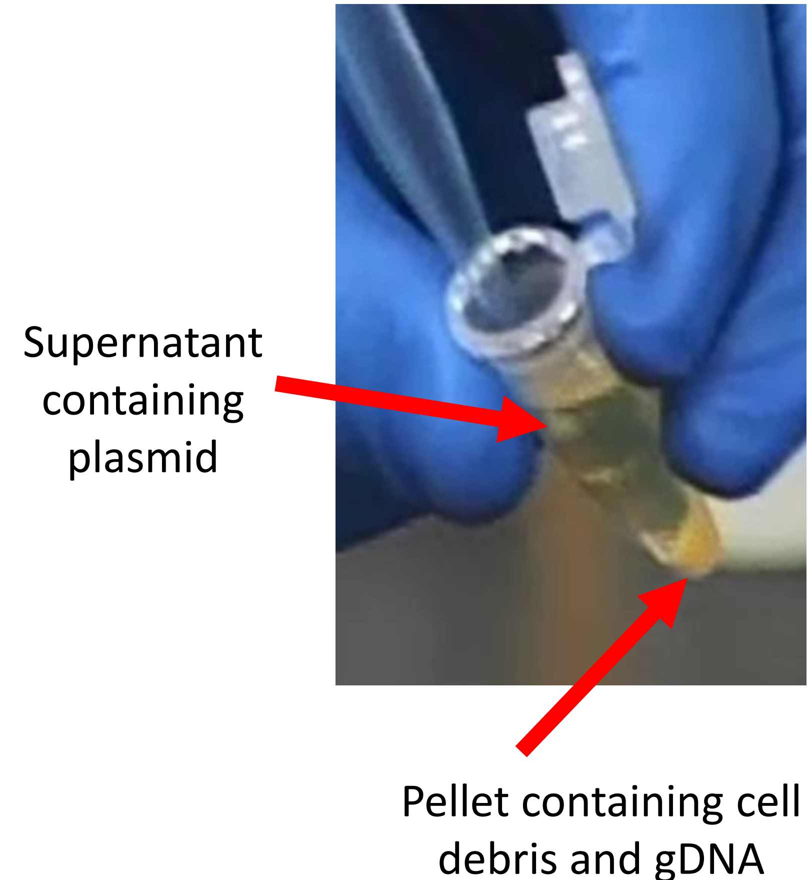
Centrifugation
clears insoluble material
(protein and gDNA) leaving
plasmid in the lysate

Removal of Debris

Alkaline lysis followed by neutralization:

- Releases plasmid into solution
- Captures proteins and gDNA in an insoluable mass
- Insoluable material is removed by centrifugation, filtering or magnetic particles

In this context, the gDNA is part of the debris



Washing

Wash steps utilizing alcohol containing buffers remove unwanted components:

- Proteins
- Unbound nucleic acid
- Salts

Prepares for elution

Elution

Water or low salt buffer are used to elute the plasmid either using a centrifuge or with a vacuum

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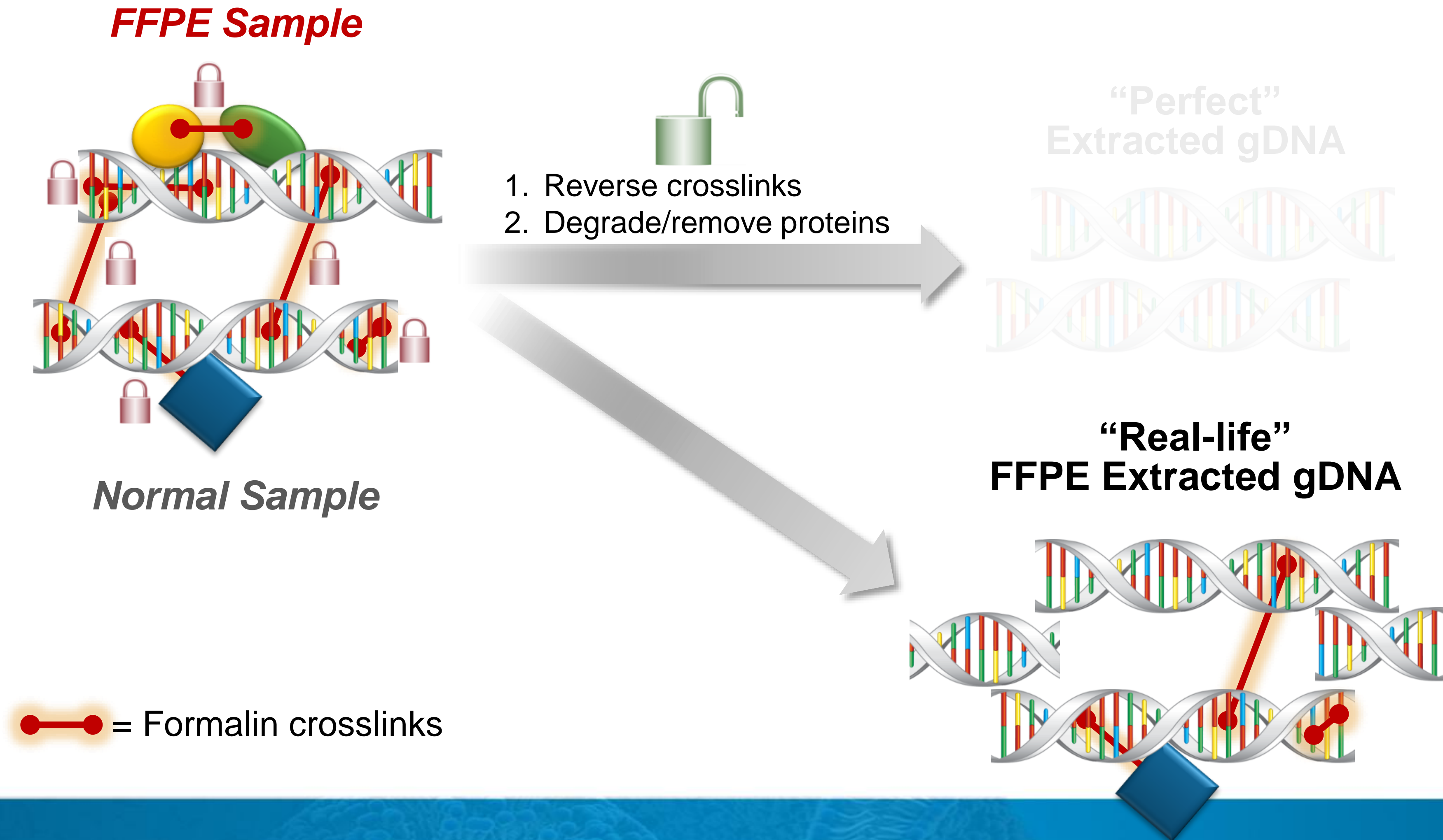
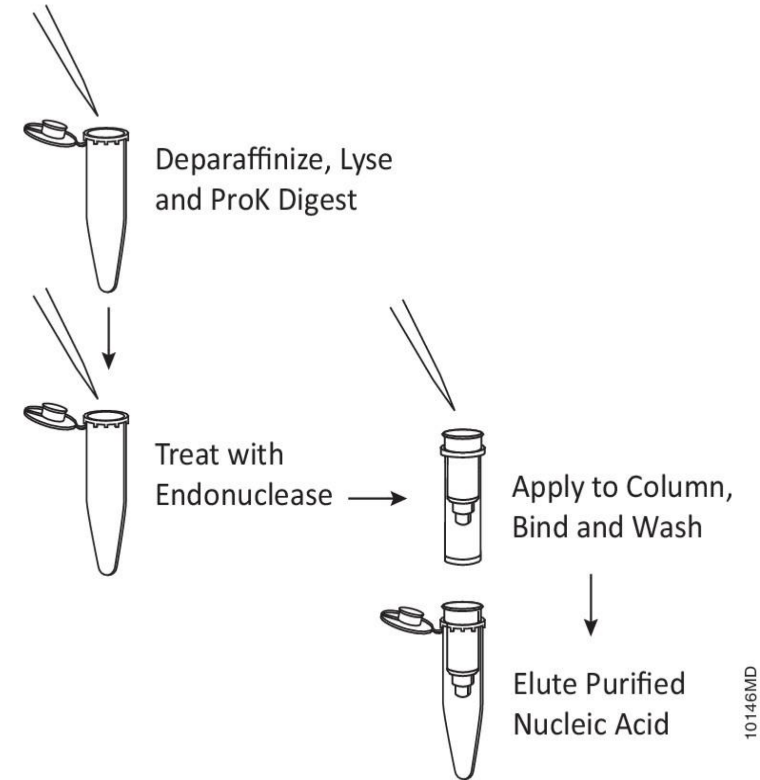
Applications: Purification of Nucleic Acid from FFPE

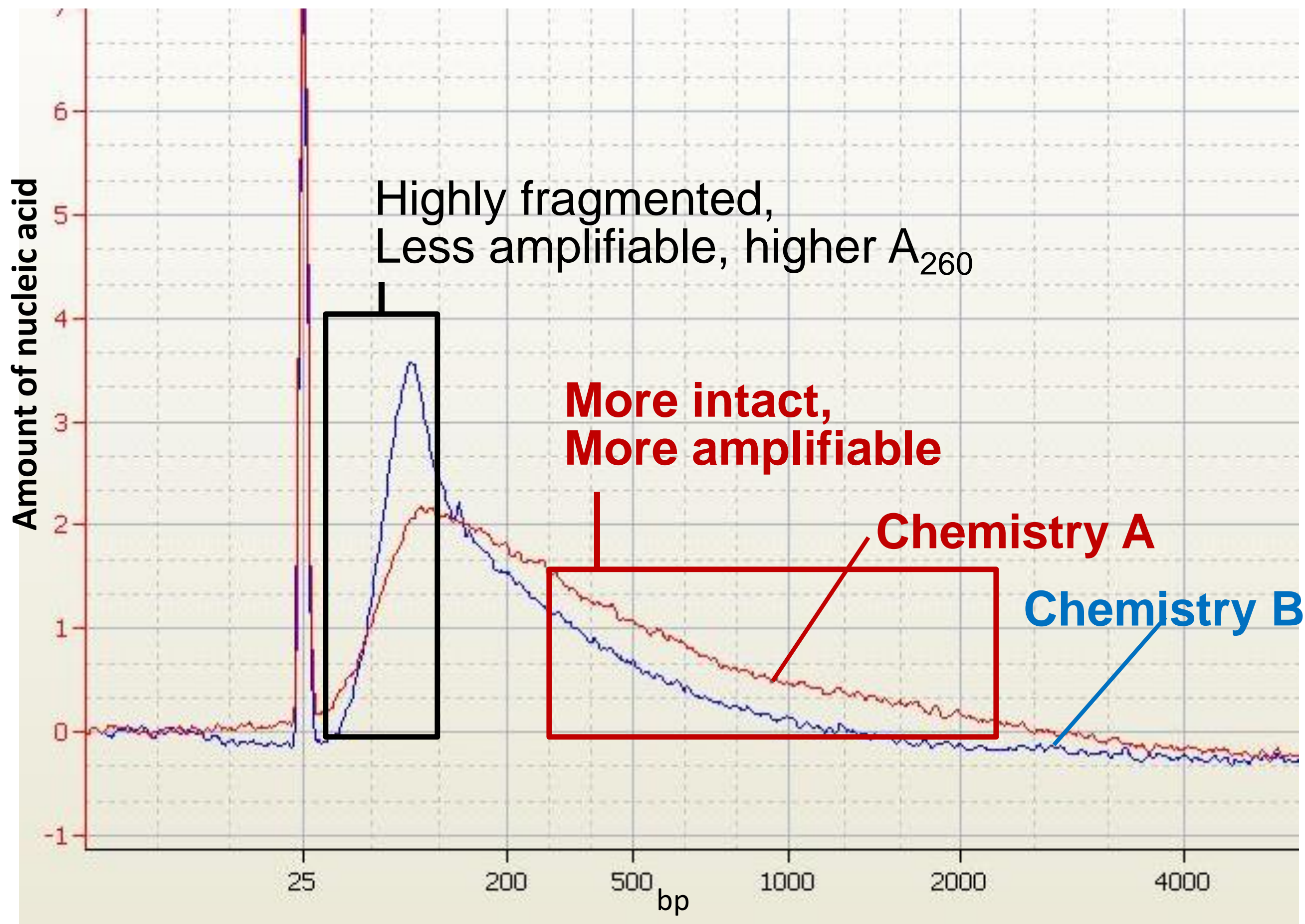
FFPE Samples are Highly Crosslinked and Difficult Starting Samples for Molecular Analyses



Step	Description
Sample disruption/ Lysis of cells	Deparraffinization/Lyse de-crosslink
Clearing debris	Not needed
Binding to purification matrix	Binding of nucleic acid to matrix
Washing	Removal of salts and debris by washing with ethanol containing buffer
Elution	Elution of DNA



Purification Chemistry Affects Average gDNA Fragment Size Recovered



- Fragmentation occurs as part of fixation
- Highly fragmented gDNA is less amplifiable, as the peak is about 100bp
- The difference in area under the curve >300bp is the advantage of Chem A over Chem B

Applications: Purifying RNA or DNA from Blood

Sample disruption/ Lysis of cells

Lysis of blood cells (chemical / enzymatic)

Clearing debris

Not needed

Binding to purification matrix

Binding of nucleic acid to matrix nuclease treatment to remove contaminating nucleic acid

Washing

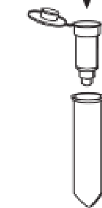
Removal of salts and debris by washing with ethanol containing buffer

Elution

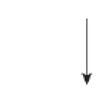
Elution of DNA or RNA

Mix, ProK, Add CLD. Mix well by vortexing.

Incubate at 56°C for 10 minutes.

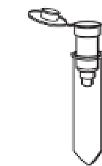


For each sample, place a Minicolumn into a Collection Tube.



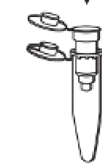
Add BBA. Mix well by vortexing.

Mix, Transfer lysate to Minicolumn.



Centrifuge for 1 minutes. Discard liquid from Collection Tube.

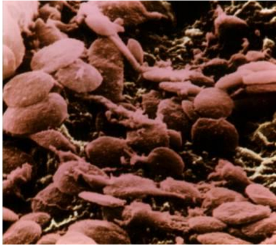
Add CWD to Minicolumn. Centrifuge for 3 minutes. Discard liquid. Repeat for a total of 3 washes.



Transfer the Minicolumn to and Elution Tube. Add Nuclease-Free Water to the Minicolumn membrane. Centrifuge for 1 minute. Discard the Minicolumn.

Special Considerations for Blood…

Three major cell types (~45% of volume):

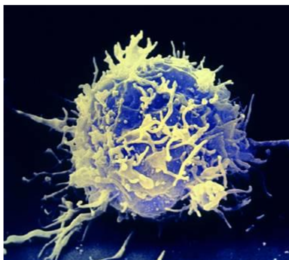


~1% Thrombocytes = Platelets

- Coagulate blood → clots
- Anti-coagulants - Heparin, citrate, EDTA

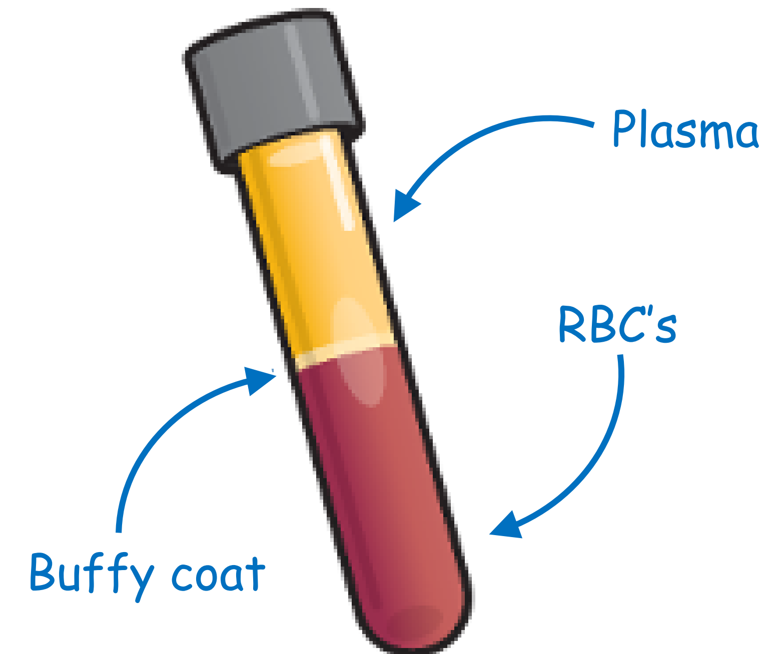
~96% Erythrocytes = Red Blood Cells

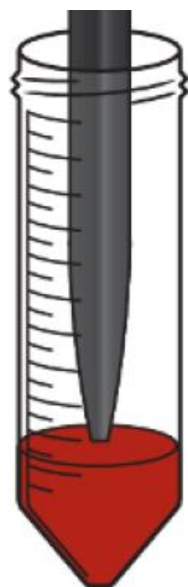
- In mammals, no nuclei = no DNA
- Heme (iron) (Abs 405nm) → may inhibit PCR
- Can be preferentially lysed



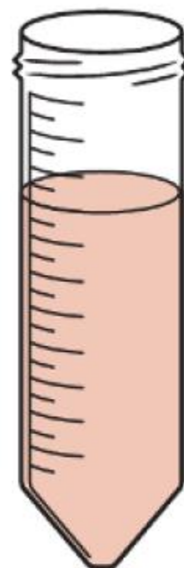
~3% Leukocytes = White Blood Cells

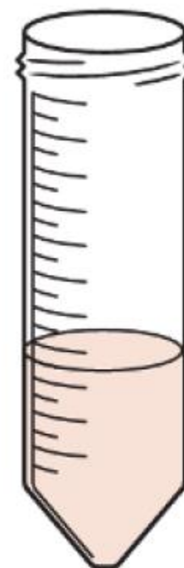
- Lymphocytes, granulocytes, macrophages
- Cell # ∴ gDNA yield, variable
- Can be fractionated as 'Buffy-Coat' -->

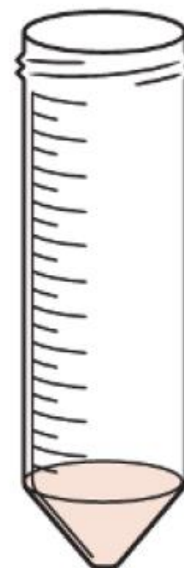



Detect blood volume.

Lyse.


Bind to particles.

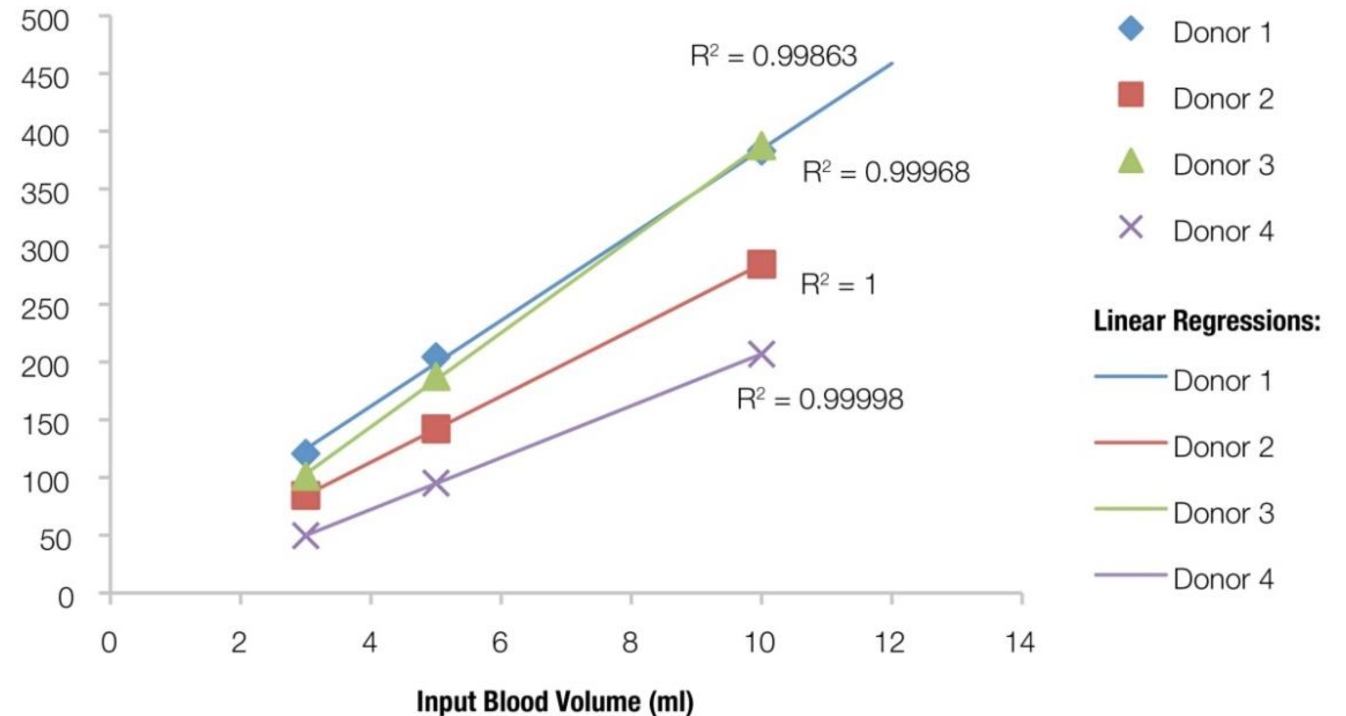

Capture and release washes.


Elute pure DNA.
8525MB

Performance of Automated Purification from Large Volumes of Blood

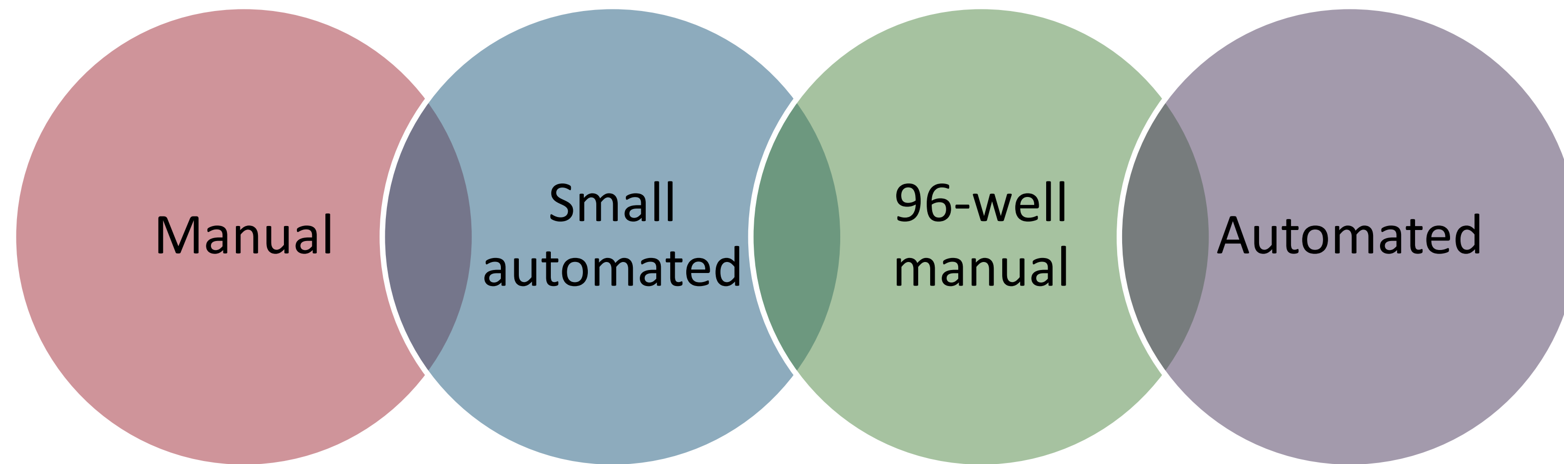
Yield is dependent on:

- Input volume
- Donor (White blood cell count)



- Blood from (4) different donors
- 3, 5 and 10ml aliquots from each donor were extracted and eluted in water
- Yield was measured by UV absorbance

Purification Technologies
All Provide Advantages Depending on Specific Needs



Use our Online Protocol Selector

DNA and RNA Purification

To find the appropriate nucleic acid purification product for your application, filter by the radio button items on the left side. If you have a question about a particular sample type that is not included in this selector, please contact Technical Services to find the solution that is right for you.

clear all

Format

- Manual 22
- Semi Automated 1
- Automated Benchtop 38
- High Throughput Liquid Handler 13

Sample Type

- Whole Blood 18
- Buffy Coat 5
- Plasma 4
- Serum 3
- Body Fluids 4
- Cultured Cells 17
- Bacteria Culture 8
- Tissue 17
- FFPE Tissue 9
- Plant 9
- Food Samples 2
- PCR and Sequencing Reactions 4
- Agarose Gels 2
- Buccal Swabs 5
- Forensic Samples 3
- Total RNA 2

Nucleic Acid

- DNA 35
- RNA 24
- Total Nucleic Acid 4
- Plasmid 7

Customized Automated Solutions

High throughput implementation of DNA and RNA purification of a wide variety of sample types and instrumentation platforms.

Contact HTgenomics@promega.com to begin the discussion to solve your challenges.

Maxwell® CSC Blood DNA Kit
Cat. # AS1321

Extract DNA from up to 16 samples of whole blood using this cGMP-compliant automated method for in vitro diagnostic assays.

View Protocol »

ReliaPrep™ 96 gDNA Miniprep HT System
Cat. # A2670, A2671

Isolate gDNA from up to 96 whole blood samples or Oragene®·Discover sample collection devices using an automated binding resin 96-well plate system.

View Protocol »

Maxwell® CSC RNA Blood Kit
Cat. # AS1410

Extract RNA from from up to 16 samples of whole blood using this cGMP-compliant automated method for in vitro diagnostic assays.

View Protocol »

ReliaPrep™ Blood gDNA Miniprep System
Cat. # A5081, A5082

Isolate gDNA from whole blood using a binding column-based system.

View Protocol »

Maxwell® CSC DNA FFPE Kit
Cat. # AS1350

Extract DNA from from up to 16 samples of FFPE tissue with this cGMP-compliant automated method for in vitro diagnostic assays.

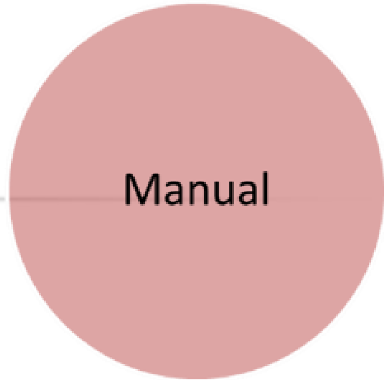
View Protocol »

ReliaPrep™ FFPE gDNA Miniprep System
Cat. # A2351, A2352

Maxwell® CSC RNA FFPE Kit
Cat. # AS1360

http://www.promega.com/products/pm/dna-and-rna-selectors/dna-and-rna-selectors/

Purification, Manual
Low Investment and Scalability are Attractive



Manual columns and scalable solution-based purification are attractive low-throughput options for standard or difficult samples

Advantages	Reasons to Consider Other Options
Low initial investment vs. automation	Greater throughput desired
Flexibility in sample processing	Time constraints
Lower price per prep	Error reduction
Minimal set up time	

Many sample types supported: Blood, Tissue, FFPE, Plant…

Purification, Small Scale Automation

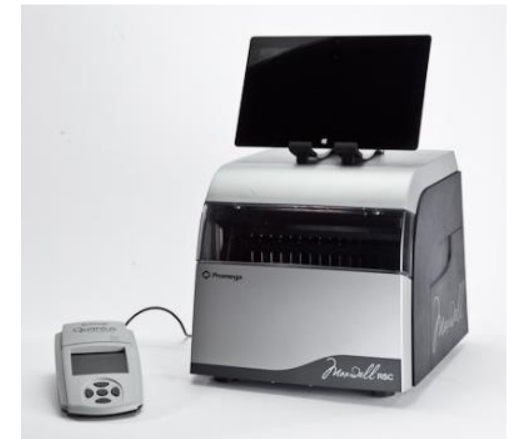
Small Automated Systems Offer Major Benefits

Small automated

Small, dedicated purification instruments allow individuals to automate purification and increase productivity

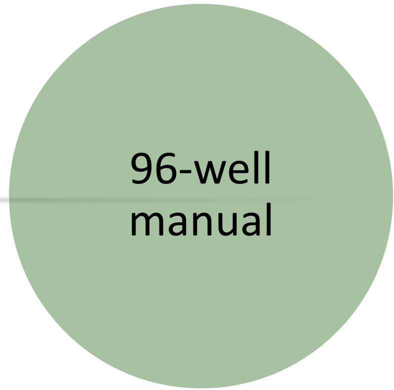
Advantages	Reasons to Consider Other Options
Minimal initial investment	Not enough throughput to justify
Frees time for other activities	Even greater throughput desired
Fewer purification errors	Input sample volume incompatibility
Increases sample throughput	

Maxwell® RSC:
5 minute setup – 30-45 minutes to extract 1-16 samples

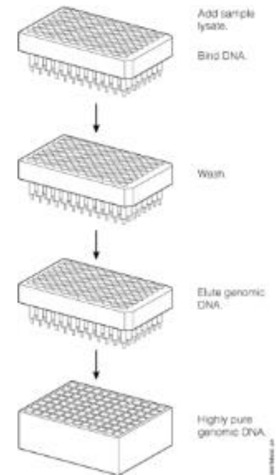


Purification, Manual 96-Well

Vacuum Purification Increases Sample Throughput



Advantages	Reasons to Consider Other Options
Low initial investment	96-well processing can be tedious
High sample throughput	Desire to reduce errors
Offers performance equal to spin columns	Staff time has become rate limiting
	Greater throughput desired
	Input sample volume incompatibility



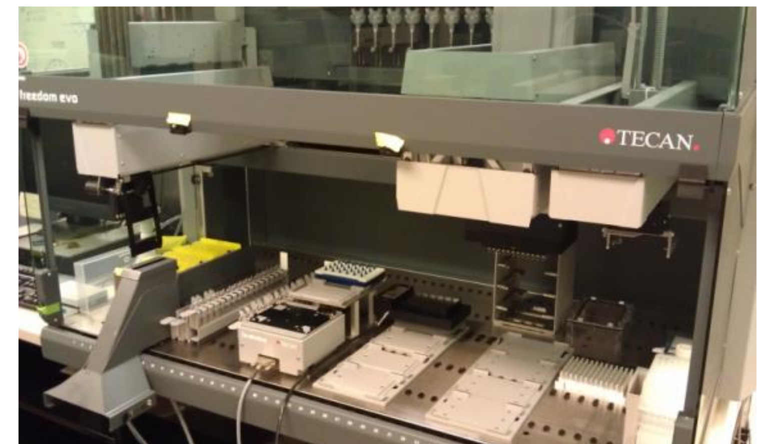
The Wizard® SV 96 Genomic System can isolate gDNA from many sample types in less than 60 minutes

Purification, Automated 96-Well
Increases Laboratory Throughput and Lowers Costs

Automated

Advantages	Reasons to Consider Other Options
Increases laboratory productivity	High initial cost
Aids in sample tracking	Not enough throughput to justify
Increases consistency of results	
Can automate many activities	

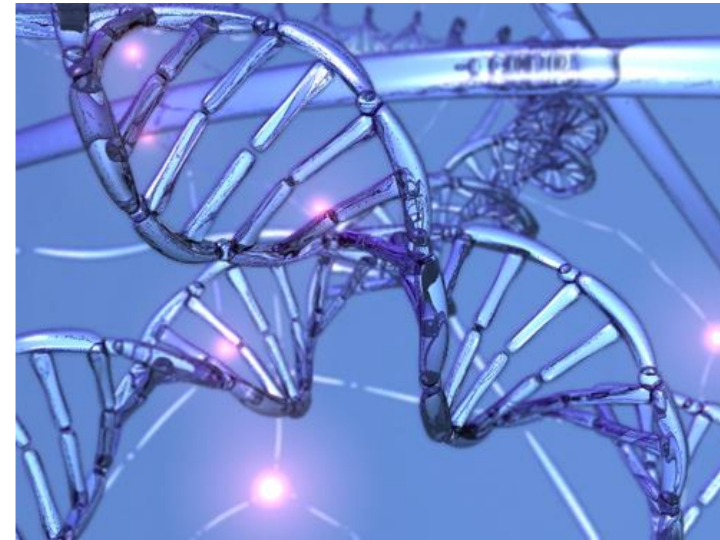
ReliaPrep™ 96 HT DNA Isolation System can isolate DNA from blood and oral samples on automated liquid handlers



Promega Breadth of Product Line

DNA Purification

- Plasmid Purification
- PCR Product/Fragment Cleanup
- Sequencing Product Cleanup
- Genomic DNA Purification



RNA Purification

- Total RNA
- mRNA
- miRNA

Need Assistance?

Contact Promega Technical Services:

Techserv@promega.com

Ask a Scientist

Thank you for your interest in Promega. Your questions and comments are important to us. To receive help please choose an option below.

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7:00am - 6:00pm CST

Thank You for Attending!

Questions?

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