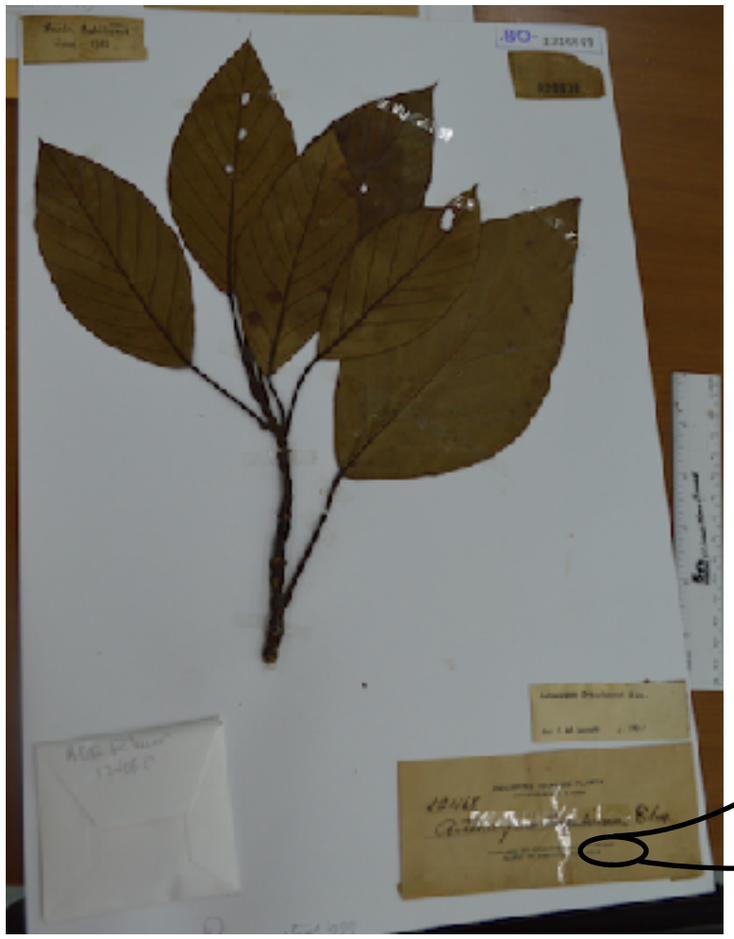
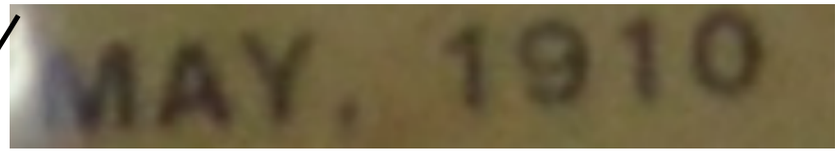
DNA EXTRACTION AND PREPPING YOUR SAMPLES FOR GENOMIC LIBRARY PRODUCTION





Elmer 12468,
>500 ng DNA
recovered

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OBJECTIVES

1. Describe different approaches to DNA extraction and choose the most appropriate for their sample type.
2. List the instruments and assays typically involved in DNA quantification and quality control.
3. Interpret DNA fragment-size data, including agarose gels and BioAnalyzer traces.
4. Evaluate different methods for DNA cleanup, including alcohol, column, and bead-based approaches.
5. Evaluate different approaches for extracting and purifying sufficient quantities of herbarium DNA for library preparation.
6. Develop and apply proper protocol modifications to prevent excessive loss of highly-fragmented herbarium DNA during cleanups.

DNA EXTRACTION

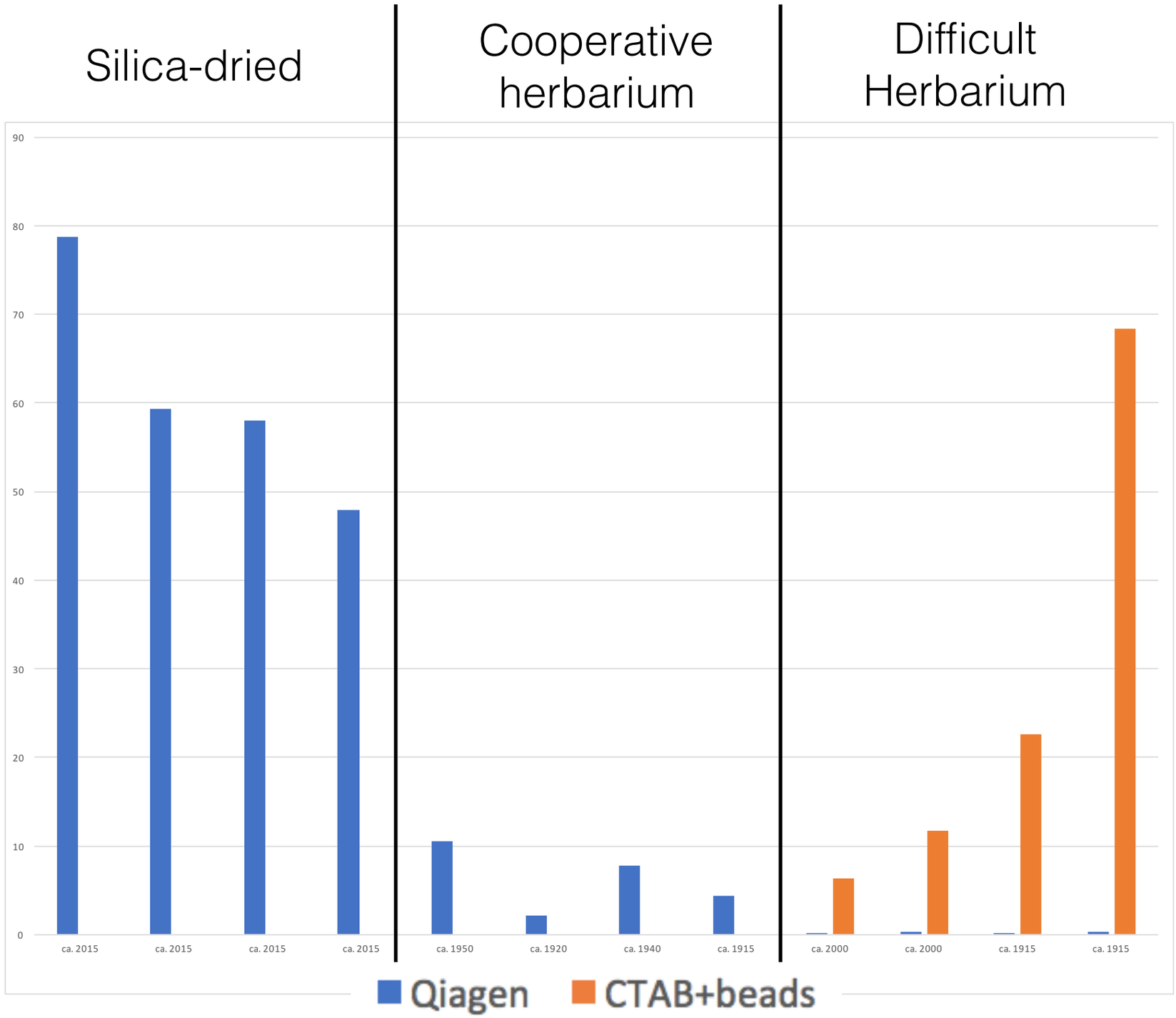
Higher Yield

Kits

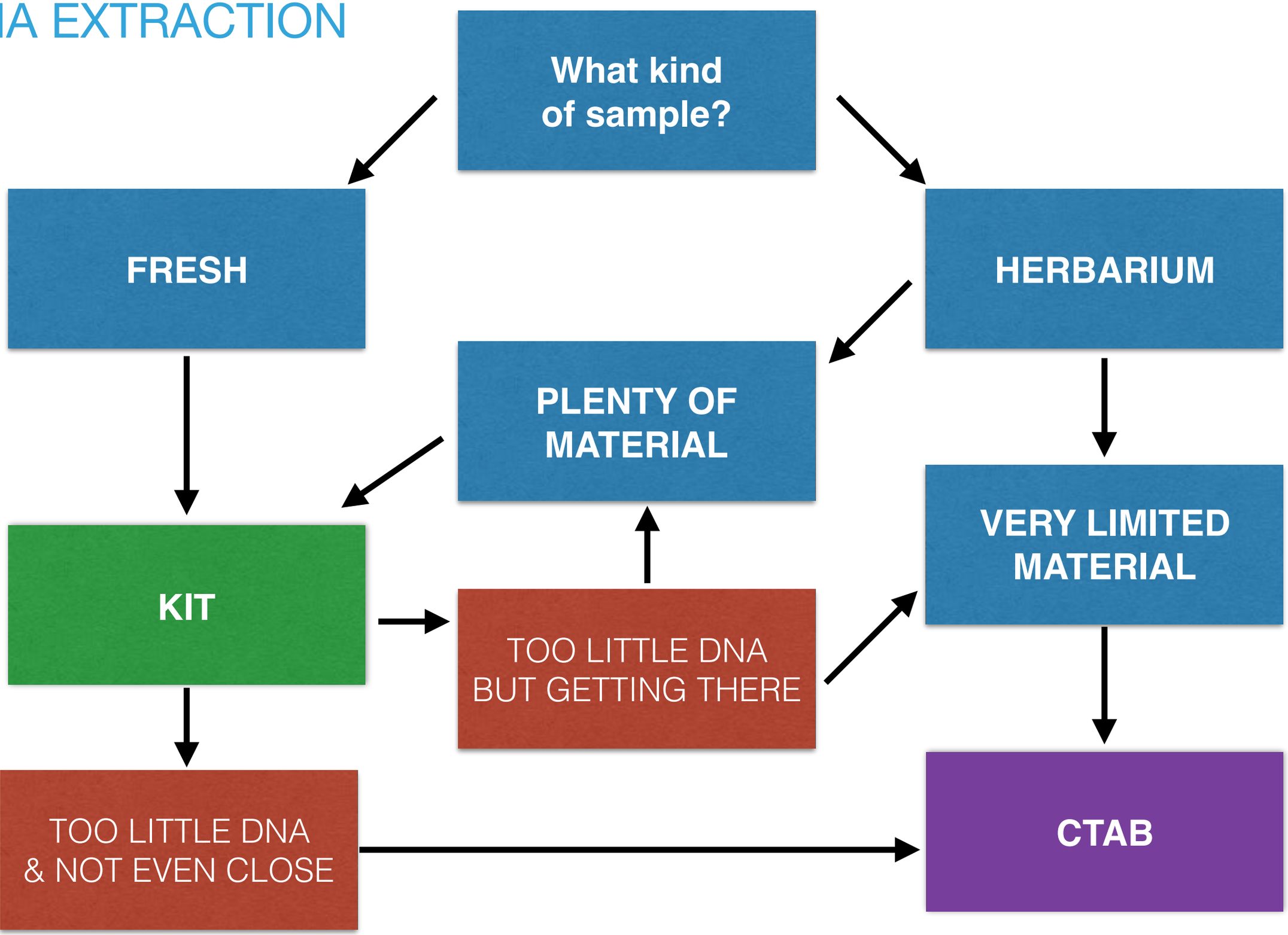
CTAB

Higher Purity
Higher Cost

DNA EXTRACTION



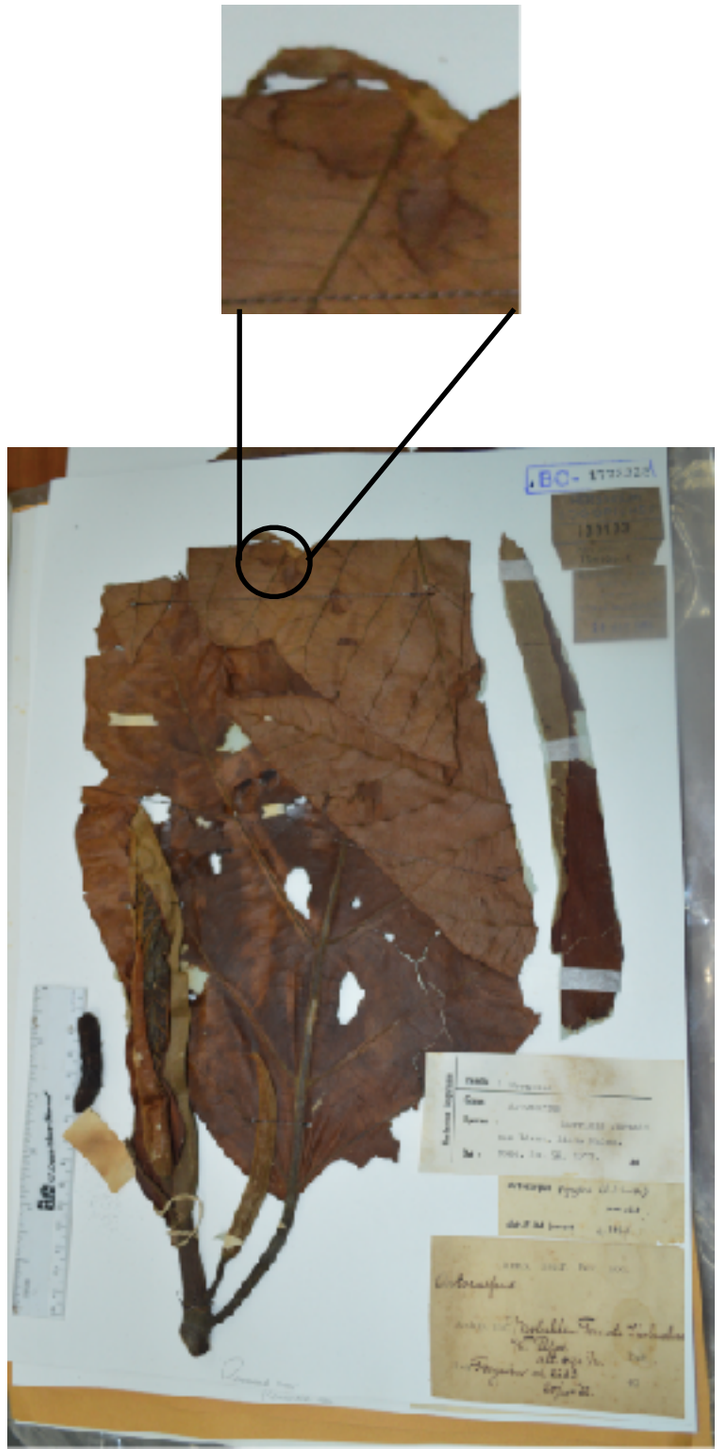
DNA EXTRACTION



DNA EXTRACTION - HERBARIUM SPECIMENS

Best practices for sampling herbarium sheets:

- Cardinal rule: the sheet should look the same before and after sampling.
- Fragment packet: preferred source if you are confident that the fragments come from the sheet.
- Material invisible from the front (e.g. folded behind another leaf): another preferred method.
- If all else fails, sample from already damaged areas.
- Useful tools:
  - extra-fine curved forceps
  - fine dissecting scissors
  - 10% bleach for cleaning tools between samples
  - small paintbrush and water for reactivating gummed tape
- Quantity: typically 0.5–1 cm^2



DNA EXTRACTION - HERBARIUM SPECIMENS

Kit modifications to improve yield and quality for herbarium specimens (tested with Qiagen DNeasy Plant Mini Kit):

- Add 75 uL beta-mercaptoethanol + 35 uL proteinase-K to the lysis step.
- Increase incubation time to at least several hours (or overnight)
- Add 1 volume EtOH to the sample before binding it to the column (precipitates small fragments)
- Use filter tips and work in small batches

DNA EXTRACTION - HERBARIUM SPECIMENS

Further reading:

OPEN ACCESS Freely available online

PLOS ONE

How to Open the Treasure Chest? Optimising DNA Extraction from Herbarium Specimens

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DNA QUANTIFICATION AND QUALITY CONTROL

Quantification: how much is there?

- Qubit fluorometer
- (BioAnalyzer if you are very careful)

Quality control: how good is it?

- How large are the fragments?
  - BioAnalyzer
  - Agarose gel
- How clean is it?
  - NanoDrop
  - Your eyes (i.e. does it look like tea? is it viscous?)

DNA QUANTIFICATION

How much is enough?

- Illumina TruSeq Nano
  - Official recommendation: 100–200 ng
  - In reality: for degraded DNA, as much as possible (up to 1000 ng), but at least 20 ng
  - Non-degraded DNA: even less is possible

- KAPA Hyper Prep (also applicable to NEB kit)
  - Official recommendation: 1–1000 ng
  - In reality: for degraded DNA, as much as possible (up to 1000 ng), but at least 20 ng

DNA QUANTIFICATION

- SpeedVac your DNA to the desired volume for library preparation.
- Buffers TE and AE contain EDTA, which can inhibit end-repair in high concentrations. EB does not contain EDTA.
- If you need to dramatically concentrate DNA in TE or AE, you will need to clean it.
- If you know in advance that you will need to concentrate your DNA, consider suspending it in water and then drying it down and resuspending in the buffer of your choice at the end.

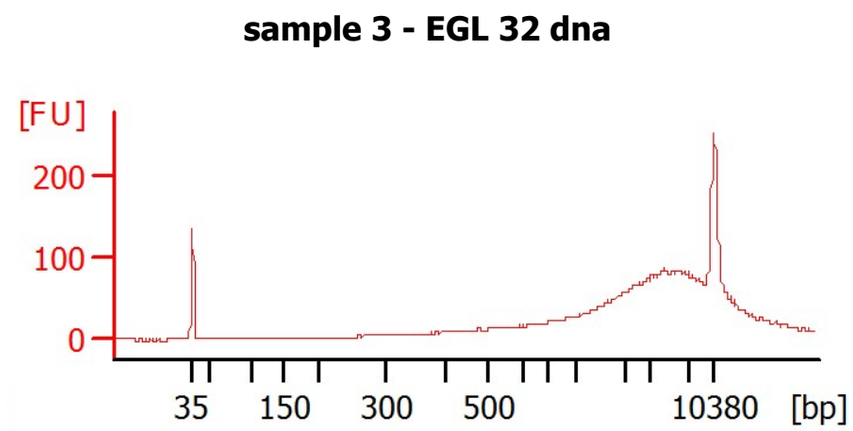
DNA QUALITY CONTROL

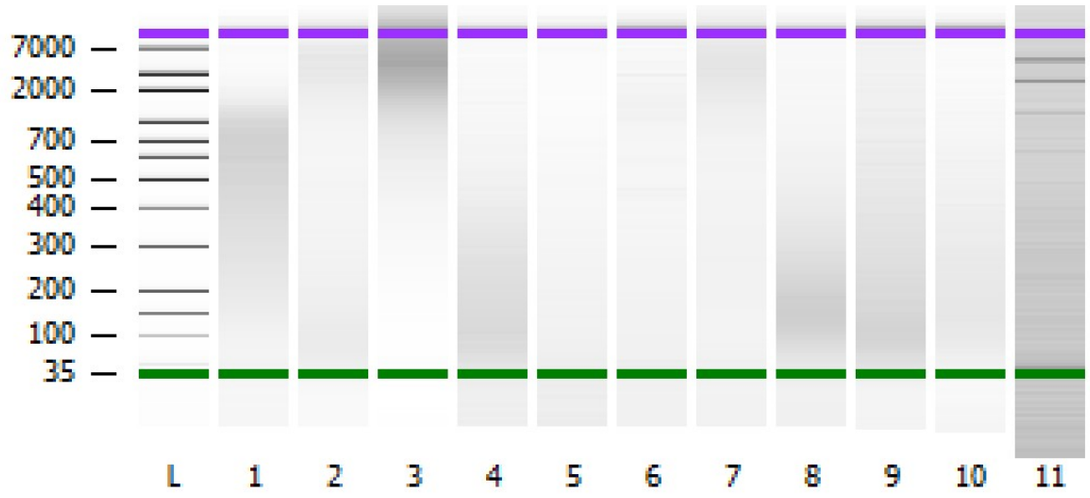
How large should DNA fragments be?

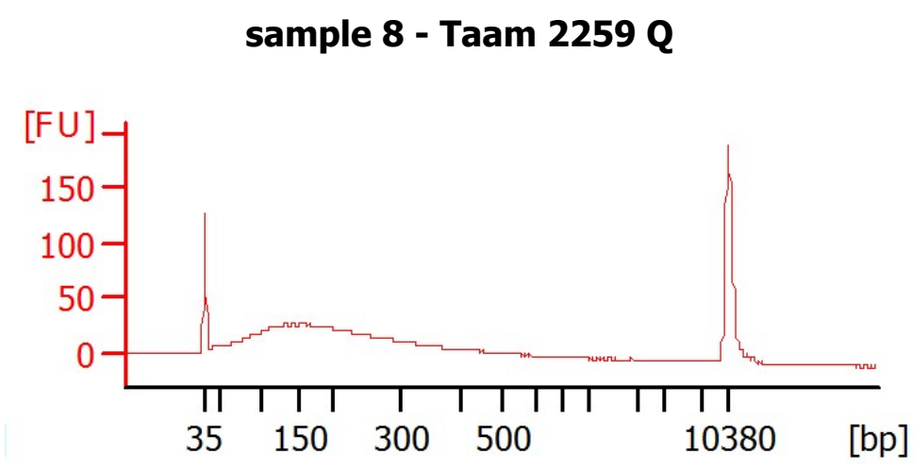
- Ideally, as large as possible. You will then sonicate them to about twice the read length:
  - ~550 bp for 2x300 bp sequencing
  - ~180 bp for 2x100 bp sequencing
- For herbarium specimens, >50 bp is preferable.

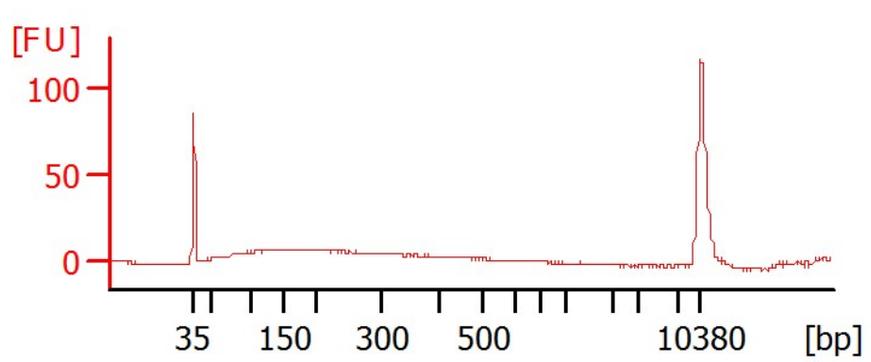
DNA QUALITY CONTROL

Reading BioAnalyzer traces



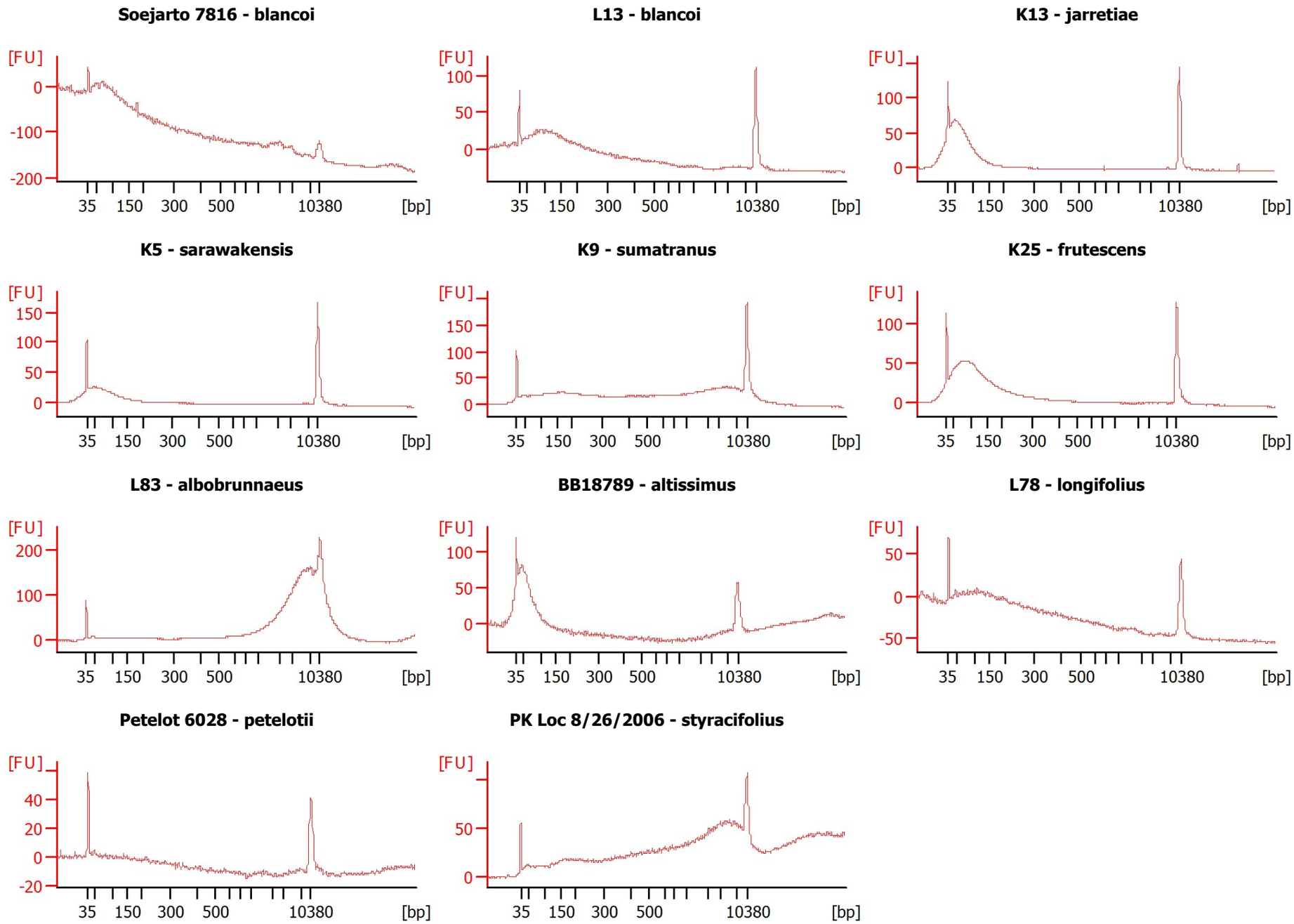






DNA QUALITY CONTROL

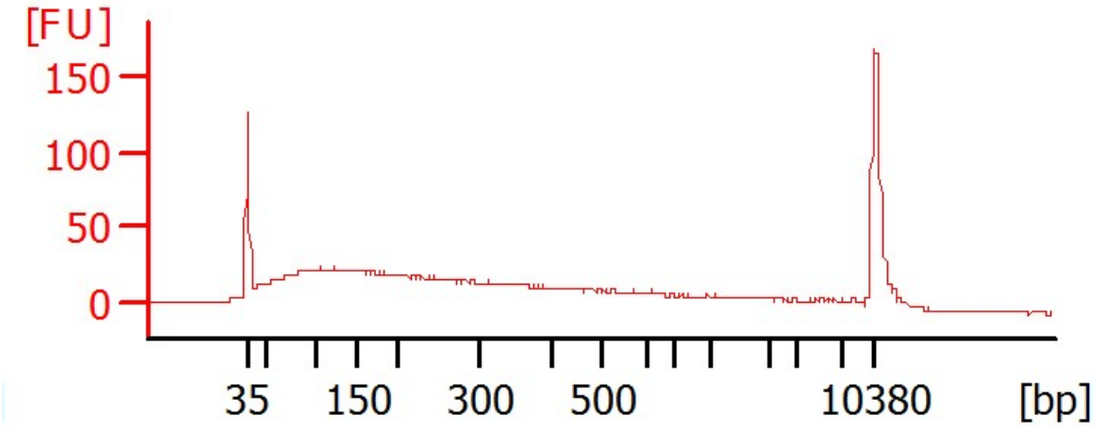
Sonicate or not?



DNA QUALITY CONTROL

When in doubt, sonicate.

Before:



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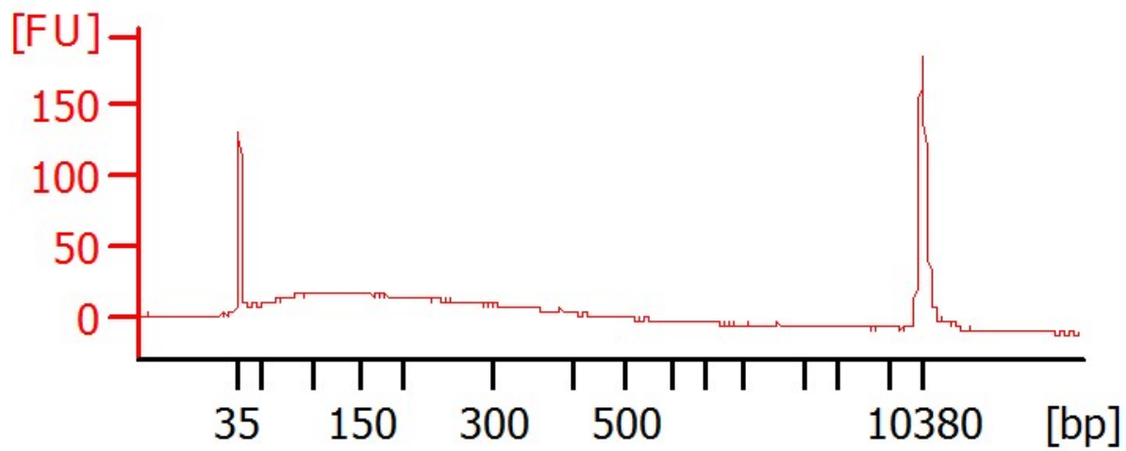


DNA QUALITY CONTROL

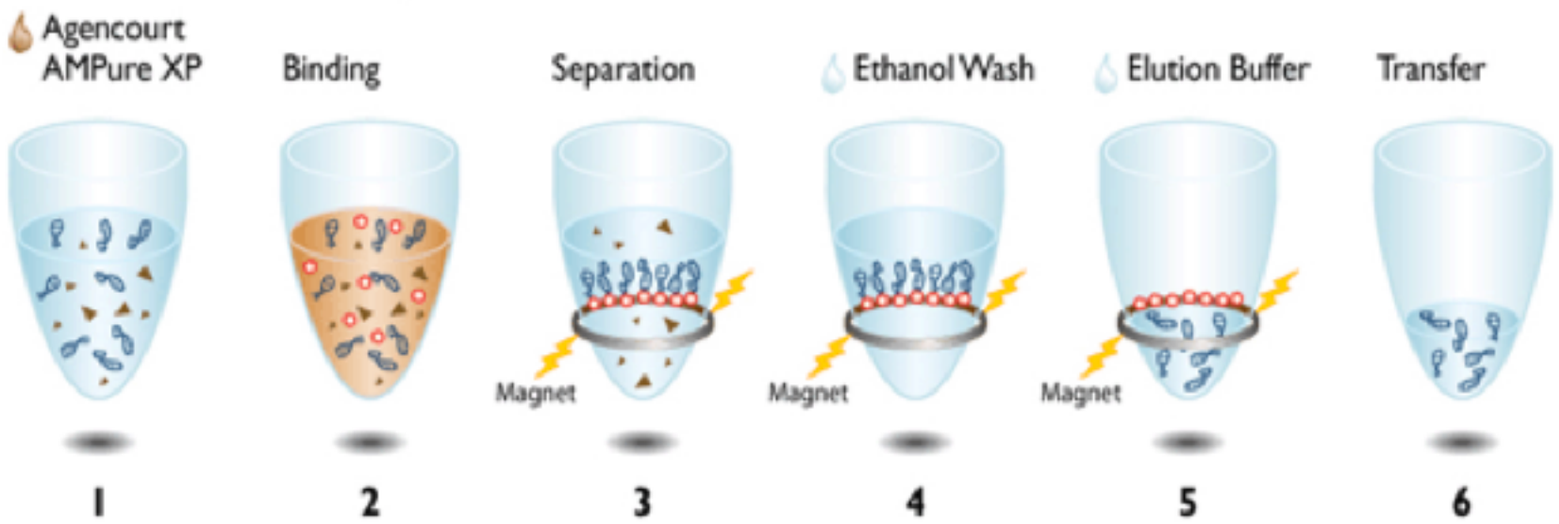
How clean should DNA be?

- Pure DNA has:
  - No color
  - A 280/260nm ratio of ~1.8 on the NanoDrop
- When to clean, generally speaking:
  - If 280/260nm ratio is very different from ~1.8
  - If DNA is darker than a light oolong

DNA QUALITY CONTROL

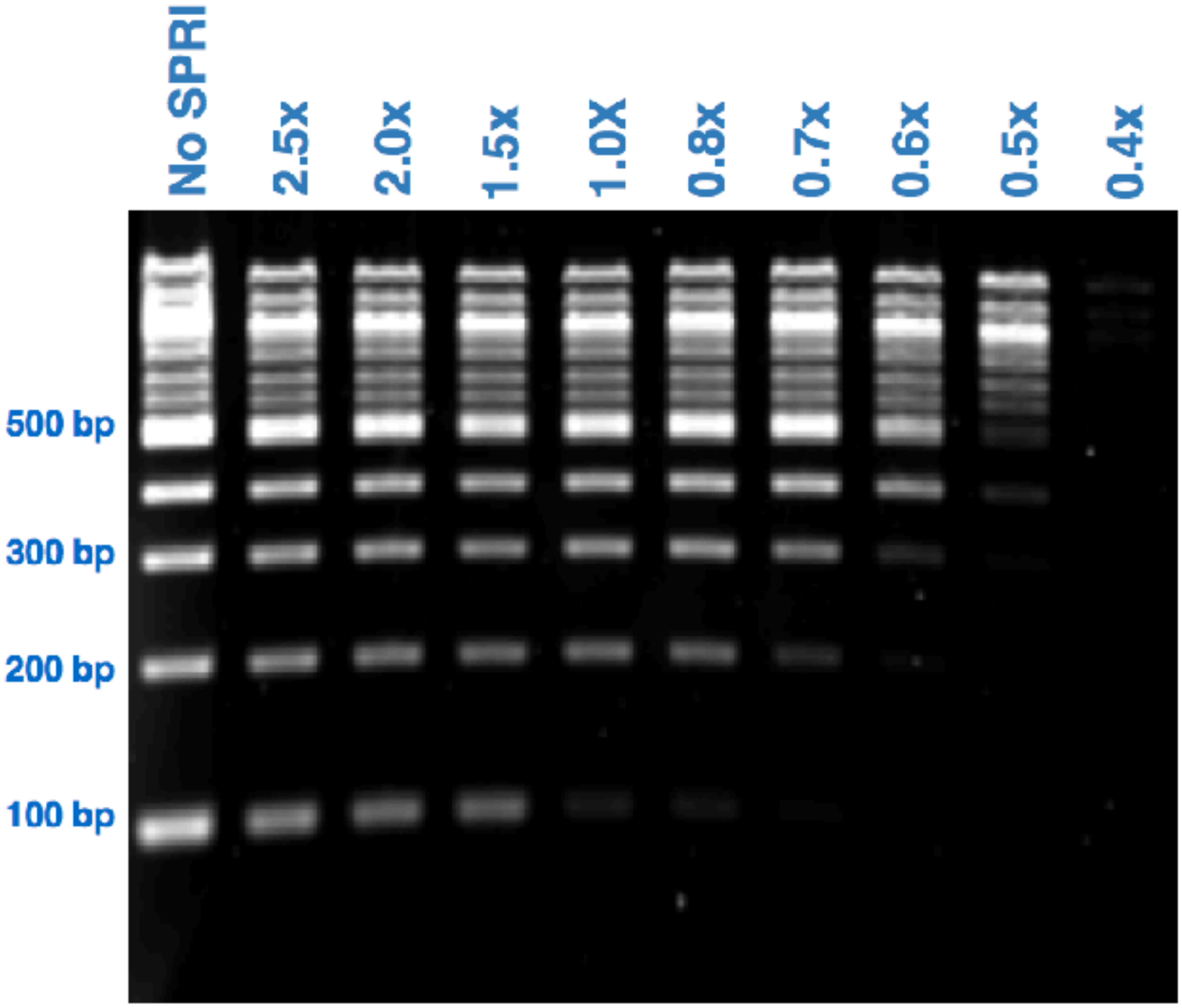
Options for cleaning impure DNA:

- PCR cleanup kits (e.g. QiaQuick)
  - Cleanest DNA but yield may be low
- SPRI beads



DNA QUALITY CONTROL

SPRI beads



http://core-genomics.blogspot.co.uk/2012/04/how-do-spri-beads-work.html

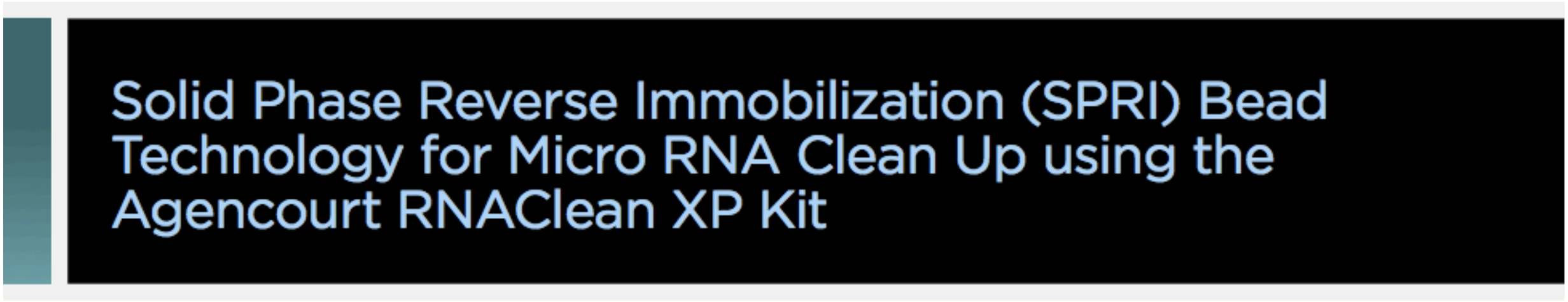
DNA QUALITY CONTROL

SPRI bead cleanup for small DNA fragments

- Micro RNA cleanup protocol preserves small fragments by precipitating them with isopropanol
- Bead-binding step:

  50 : 90 : 270 ul

  sample : beads : isopropanol



Beckman Coulter Protocol AAG-245APP06.14-A

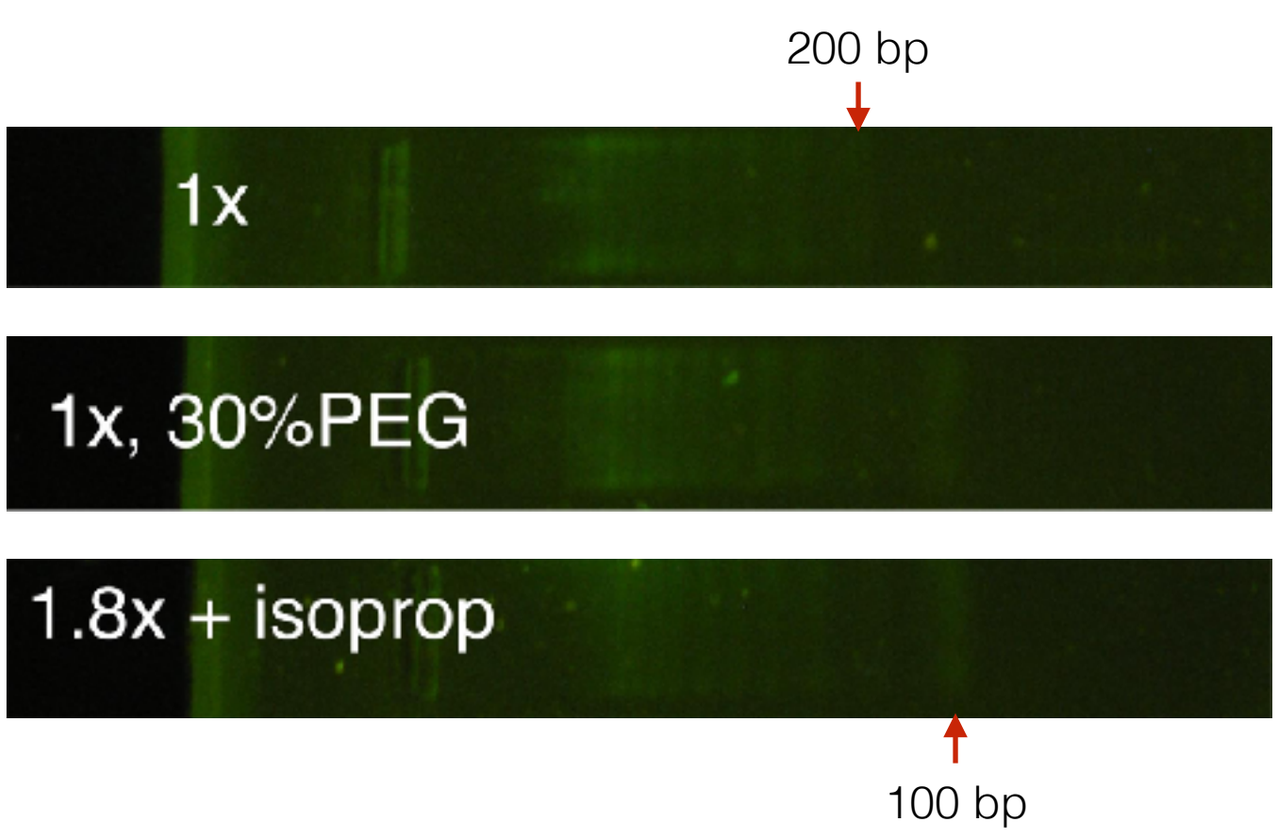
DNA QUALITY CONTROL

Another approach: home-brew your beads and increase PEG concentration.
30% PEG will bind fragments at least down to 50 bp, possibly as low as 20 bp.

Home-brewing beads can reduce costs by roughly 50% at high volumes.

Required reagents (Faircloth & Glenn 2011 https://ethanomics.files.wordpress.com/2012/08/serapure_v2-2.pdf):

- Sera-mag SpeedBeads (Fisher # 09-981-123) <— **one 15 mL bottle @ ~£300 will make 750 mL of SPRI beads**
- PEG-8000 (Amresco 0159)
- 0.5 M EDTA, pH 8.0 (Amresco E177)
- 1.0 M Tris, pH 8.0 (Amresco E199)
- Tween 20 (Amresco 0777)
- 5M NaCL



COSTS

Extractions

CTAB
  Varies, but very low (< £0.25 each)

Qiagen DNeasy kit
  For 250 samples: £2.80 each
  For 50 samples: £3.14 each

Quantification

Qubit
  ~ £1 per assay

Quality control

NanoDrop
  no per-assay cost

Agarose gel
  ~ £1 per gel

BioAnalyzer
  ~ £5.45 per sample (£60 per 11-sample chip)

AmPure XP beads
  £769 for 60 mL

Home-brewed beads
  ~ £350 for 750 mL

ACTIVITY AND DISCUSSION

DNA extraction and preparing samples for genomic library preparation.

1 Outline a plan for extracting DNA, quantifying it, performing quality-control, and preparing it for library preparation. For groups with herbarium specimens, discuss what issues might arise during sample preparation and how you might address them. Consider problems with DNA quantity, purity, and fragment size, along with any other considerations.

2 List the consumables you will need to purchase for no. 1 and estimate the total cost.

3 In preparation for the group discussion, discuss the following items relating to extracting DNA from herbarium specimens:

- What strategies have you used?
- What challenges have you faced?
- Have you dealt with any particularly challenging taxonomic groups (e.g., those with secondary compounds that confound DNA extraction)?
- Any other related issues you would like the group's feedback on.