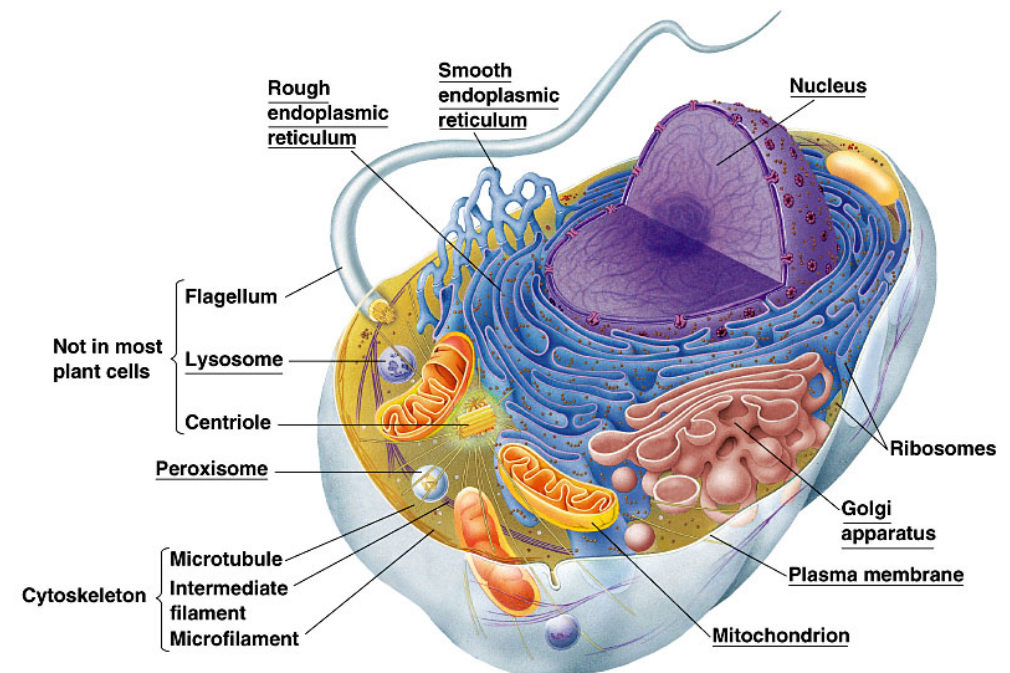
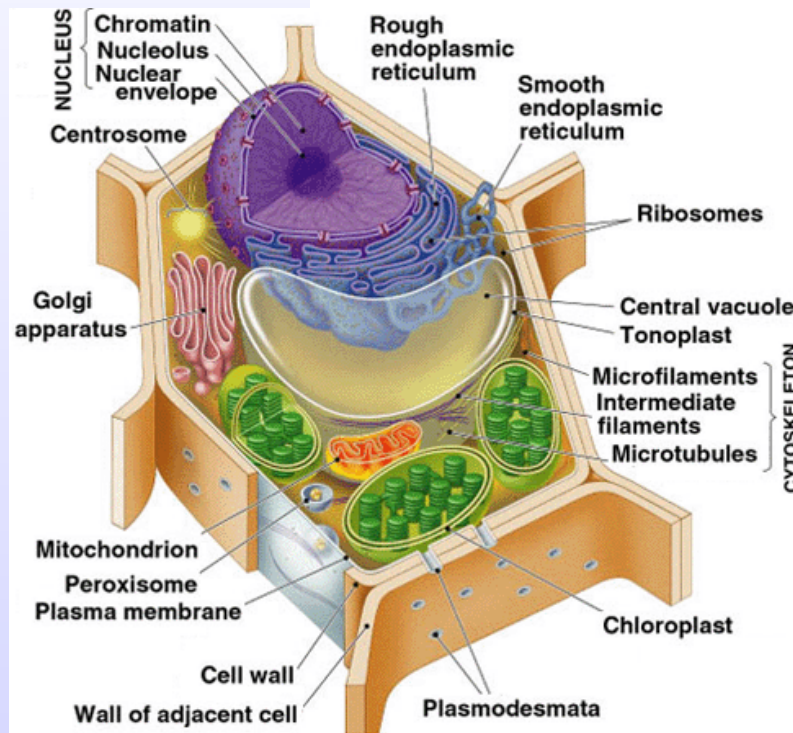


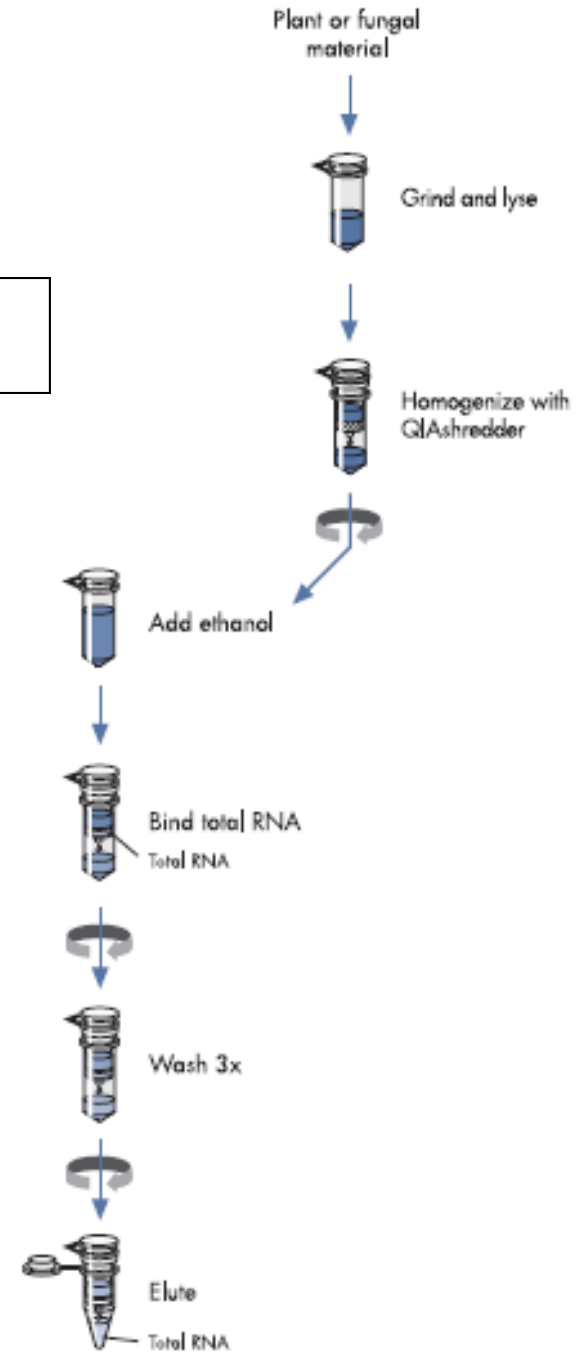
# RNA extraction PLANT tissue

- RNeasy plant mini kit Qiagen
  - Total RNA extraction plant material
- Difference plant cell / animal cell



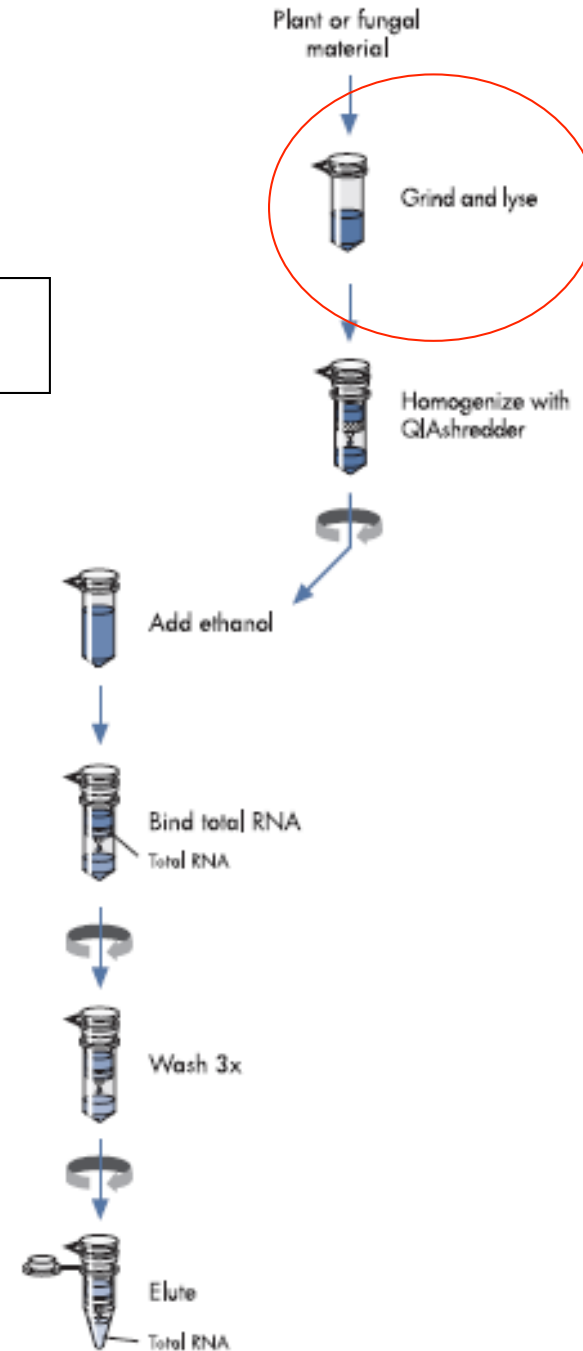
# Overview RNA extraction

## RNeasy Plant Mini Procedure



# Overview RNA extraction

## RNeasy Plant Mini Procedure

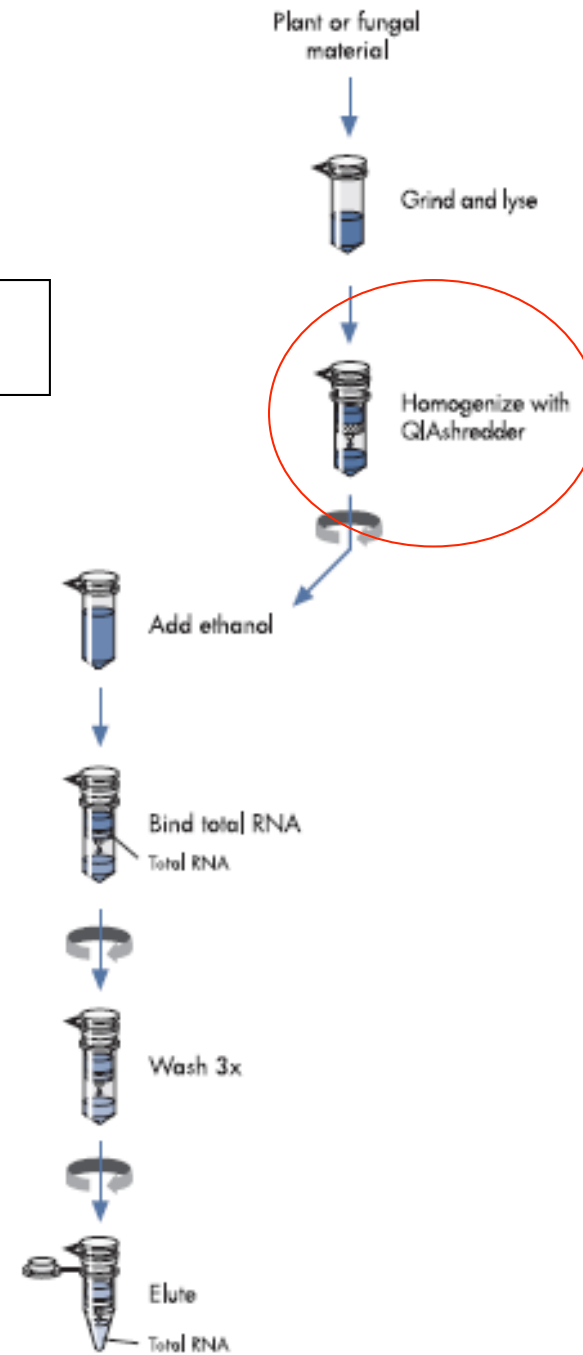


## Disruption + lysis

- 100 mg plant material → 35 µg total RNA
- Complete disruption cell walls + plasma membranes
- Mortar + pestle
  - Freeze tissue in liquid nitrogen
  - Grind to fine powder under liquid nitrogen
  - Transfer tissue powder and liquid nitrogen into tube
  - Allow liquid nitrogen to evaporate without allowing sample to thaw
- Add lysis buffer
  - Highly denaturing, immediate inactivation of RNAses

# Overview RNA extraction

## RNeasy Plant Mini Procedure



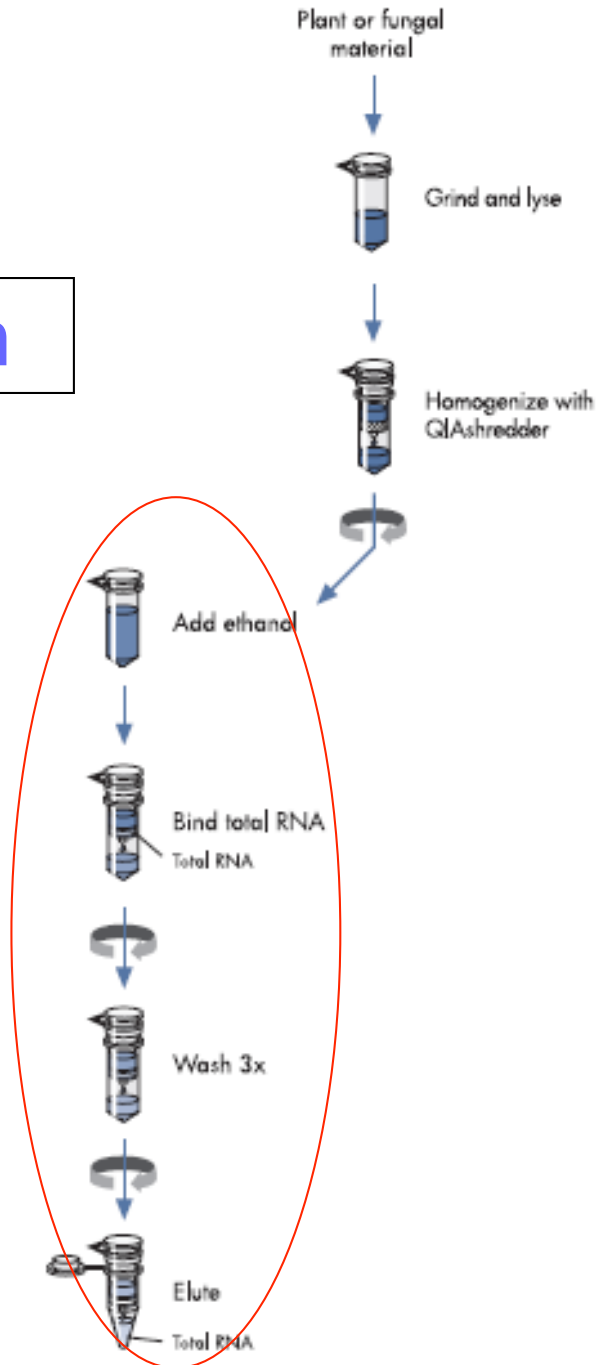
# Homogenization

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- To reduce viscosity of lysate
- Shear high-molecular-weight genomic DNA and other cellular compartments
- Using QIAshredder
- Lysate loaded on QIAshredder spin column placed on 2 ml collection tube, 2 min max rpm
- Lysate is homogenized as it passes through shredder

# Overview RNA extraction

## RNeasy Plant Mini Procedure



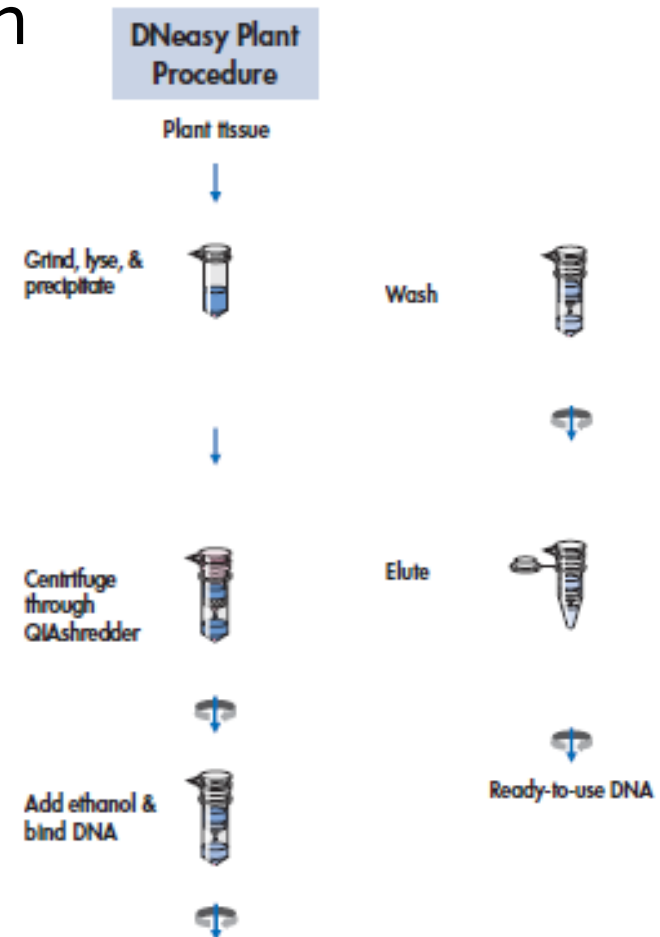
# RNA binding/elution

- Ethanol
  - For appropriate binding conditions
  - Selective binding RNA to RNeasy membrane
- RNA binding to membrane
  - Specific binding nucleotide chains > 200 nucleotides
  - Enrichment mRNA since most RNA's < 200 nucleotides are excluded (rRNA, tRNA)
- Contaminants washed away (3x)
- RNA eluted in water



# DNA extraction PLANT tissue

- DNeasy plant mini kit Qiagen
- Similar to RNA extraction
- 100 mg plant tissue  
→ 3-30 µg DNA



# Overview DNA extraction

- **Disruption of plant tissue**
  - Mortar and pestle (liquid nitrogen)
- **Lysis**
  - Addition lysis buffer + RNase solution + incubation for 10 min at 65 °C
  - Addition of buffer to lysate to precipitate proteins and polysaccharides
- **Removal cell debris and precipitates**
  - QIAshredder column: filtration + homogenization

# Overview DNA extraction

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- Add buffer with ethanol to cleared lysate
  - For appropriate binding conditions
  - Selective binding DNA to DNeasy membrane
- DNA binding to membrane
- Washing away contaminants
- DNA elution