DNA Quality Assessment

Dr John Tembo

We will record these sessions and put them online so you can refer back to them later on

We will also put the slides up online so you can access the notes (links and image credits)

Aims of the session

- Explain the principals of the techniques used for genomic DNA Quality assessment:
  - DNA Quantification
  - DNA Purity
  - DNA integrity
- Apply relevant techniques to QC our DNA extracts
- Explain the Principals of DNA concentration calculations
- Apply this on our DNA extract to calculate the amounts needed per sample to pool the sequencing library at equimolar concentrations

Purity

- Nanodrop™

Quantity

- Qubit ™
- Nanodrop™
- TapeStation™/Bioanalyser™

Integrity

- TapeStation™/Bioanalyser™
- Gel Electrophoresis

Purity

≠

Contaminants

- Proteins
- RNA
- Chemical impurities e.g detergents, denaturants, chelating agents
- high concentration of salts (affect efficiency of enzymatic steps

→

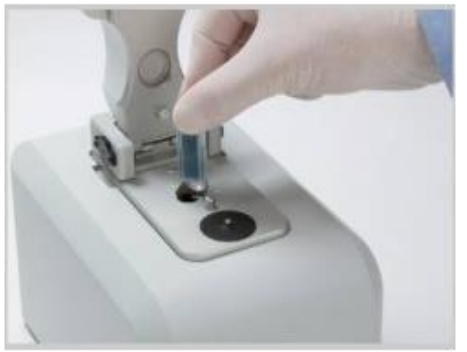
Poor Sequencing Library

Nanodrop™ Microvolume UV-Vis Spectrophotometer

- NanoDrop™ is a spectrophotometer that can be used to quantify the DNA and protein content in a 1-2 µl sample.
- RNA is a common contaminant in genomic DNA extracts
- Nanodrop cannot distinguish between DNA and RNA very well, hence less accurate for quantification of DNA for library preparation purposes
- Detection limit is 2ng/µL

Nanodrop 2000™

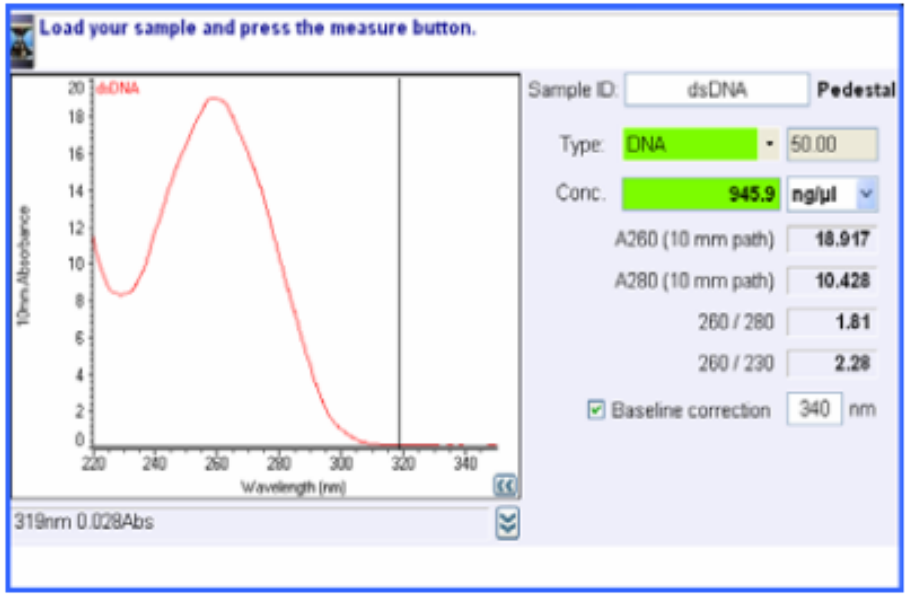
Nanodrop 2000c™



Nanodrop One/One^{c}

Nanodrop™Microvolume UV-Vis Spectrophotometer

- Nucleic acids (such as DNA) absorb light at the 260 nm
- Proteins absorb light at 280 nm
- Phenolic compounds, EDTA, Carbohydrates read at 230 nm



Measure the purity of DNA extracts through measuring the

✓ A260/A280 ratio (recommended values ~1.8)
- <1.8 -> High protein content and other impurities
- (poor library preparation)
- >2 -> High RNA content

✓ A260/A230 recommended value (2.0 – 2.2)
- A260/A230 significantly <2 indicates the presence of contaminants -> purification or PCR amplification (in library prep)

Nanodrop™ Microvolume UV-Vis Spectrophotometer

Purity

- Nanodrop™

Quantity

- Qubit ™
- Nanodrop™
- TapeStation™/Bioanalyser™

Integrity

- TapeStation™/Bioanalyser™
- Gel Electrophoresis

Qubit ™ Fluorometer

- High Specificity of Qubit dsDNA Assay Kits provides accurate quantification of Nucleic acids.
- Target **>53 ng/µl**
- DNA Kits
  - High sensitivity (HS) kit 0.01-100 ng/uL
  - Broad range (BR) kit 0.2-4000 ng/uL
- Components of the kit
  - Qubit® dsDNA HS Reagent (Component A)
  - Qubit® dsDNA HS Buffer (Component B)
  - Qubit® dsDNA HS Standard #1 (Component C)
  - Qubit® dsDNA HS Standard #2 (Component D)
  - Qubit® Assay tubes

Qubit ™ Fluorometer

Prepare the Assay Tubes* according to the table below.

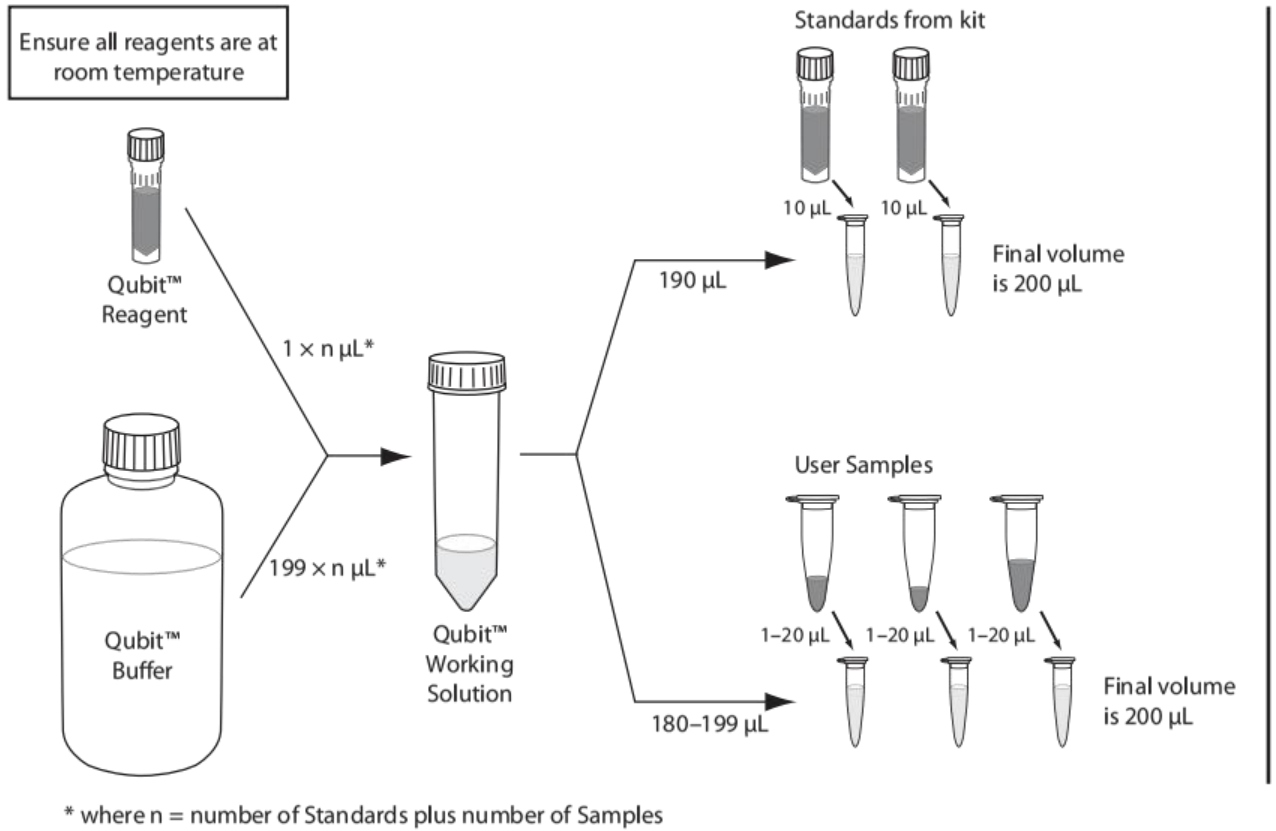
	Standard Assay Tubes	**User Sample** Assay Tubes
Volume of **Working Solution** (from step 2) to add	190 µL	180–199 µL
Volume of **Standard** (from kit) to add	10 µL	—
Volume of **User Sample** to add	—	1–20 µL
Total Volume in each Assay Tube	200 µL	200 µL

Dilution Factor 20x

1uL -> ? 200x

2uL -> ? 100x

Qubit ™ Fluorometer



molecular probes | invitrogen
by life technologies

Vortex all assay tubes for 2–3 seconds

Incubate at room temperature for 2 minutes (15 minutes for Qubit™ protein assay)

Read tubes in Qubit® 2.0 Fluorometer

Purity

- Nanodrop™

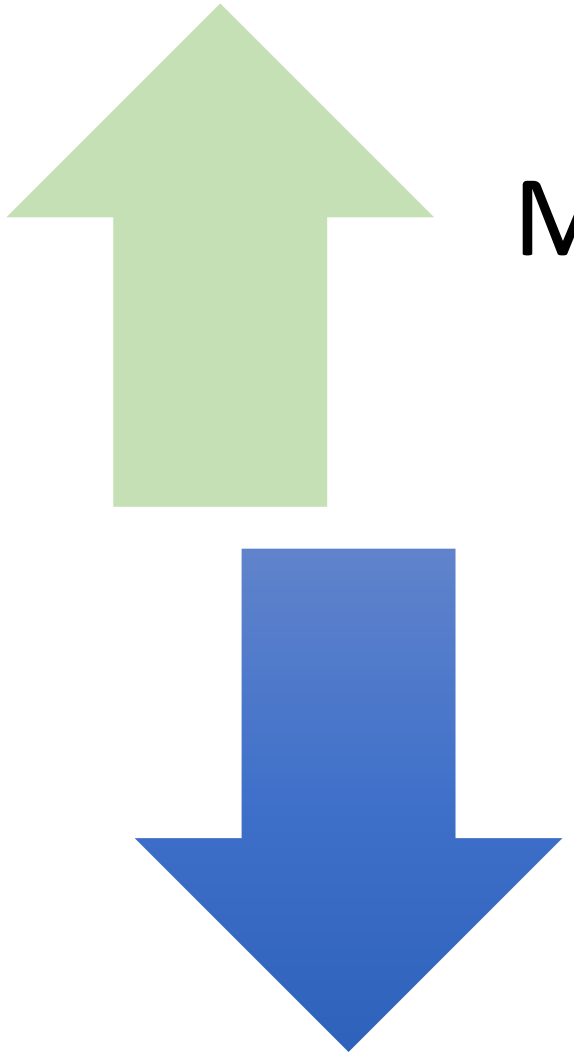
Quantity

- Qubit ™
- Nanodrop™
- TapeStation™/Bioanalyser™

Integrity

- TapeStation™/Bioanalyser™
- Gel Electrophoresis

Integrity



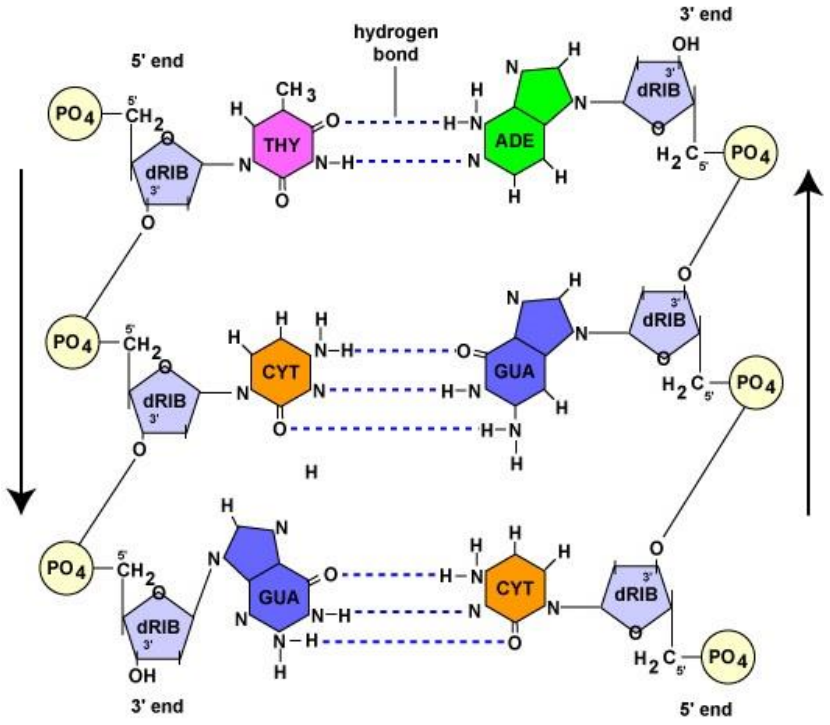
Mwt

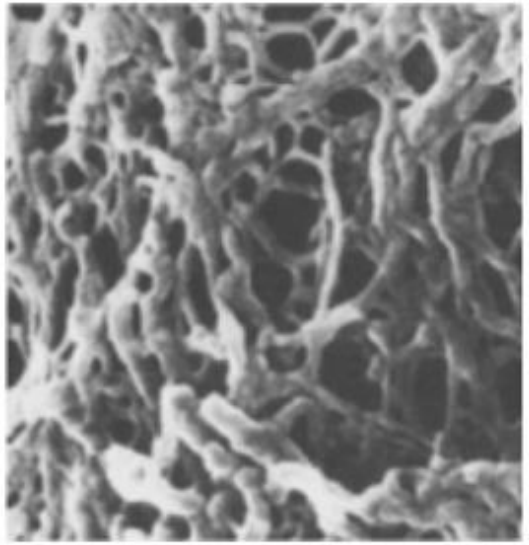
Degradation

Gel Electrophoresis Principal

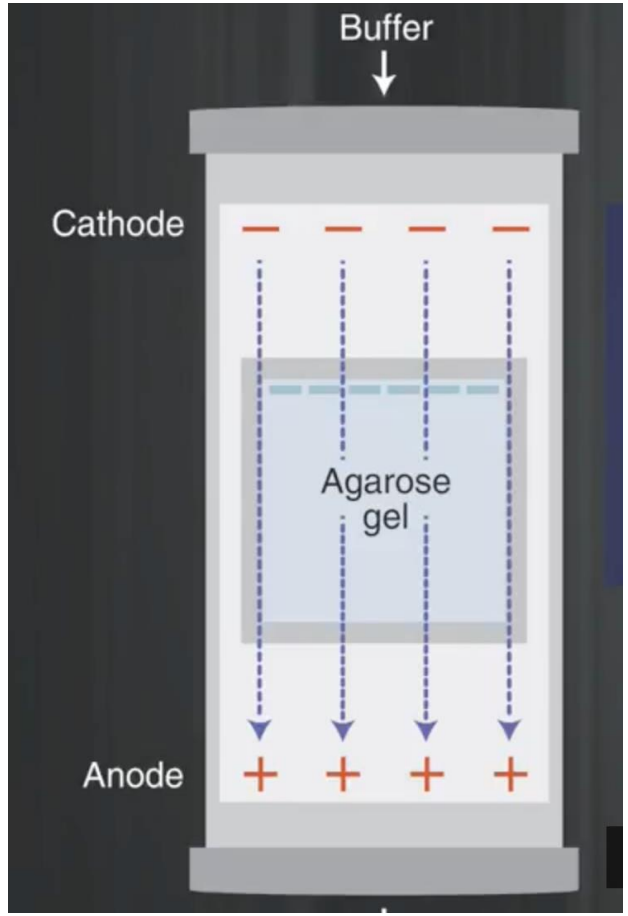
DNA is separated based on their migration rate in a gel matrix (e.g Agarose) under the influence of electric field

DNA carry charge ?

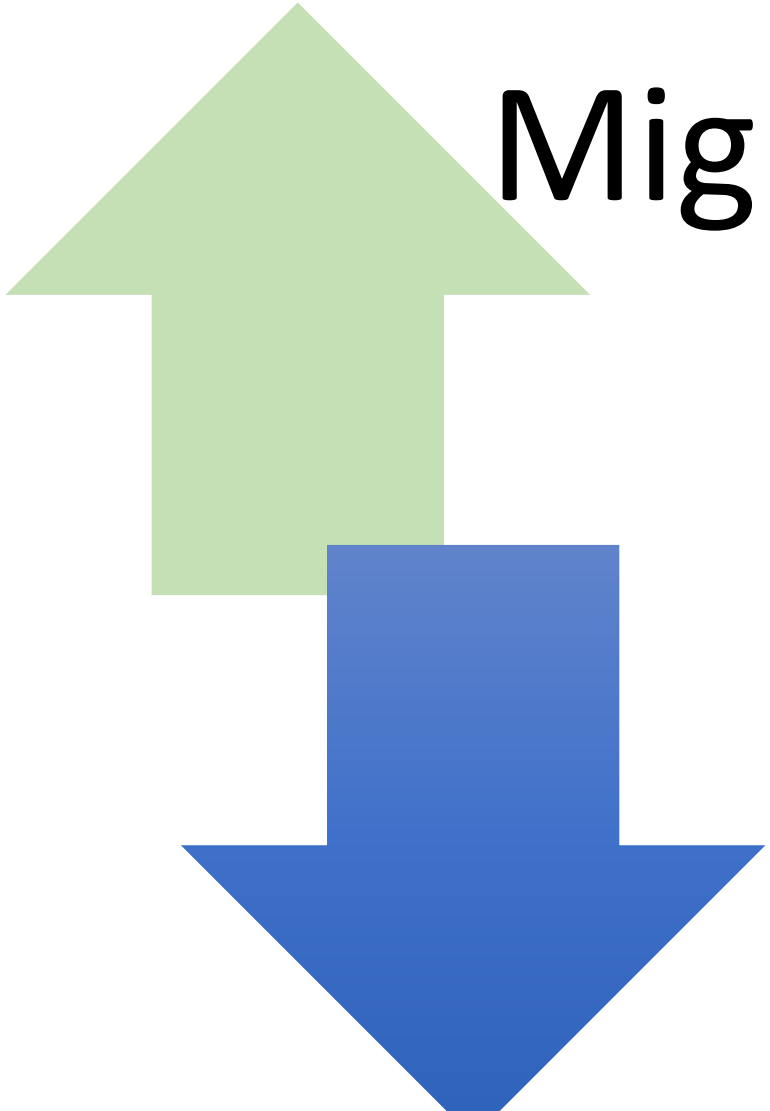




Scanning Electron Micrograph of Agarose Gel



What are Factors?



Migration Rate

- Size of the DNA fragment
- Voltage of Electric field
- Porosity of the gel matrix (Resistance)

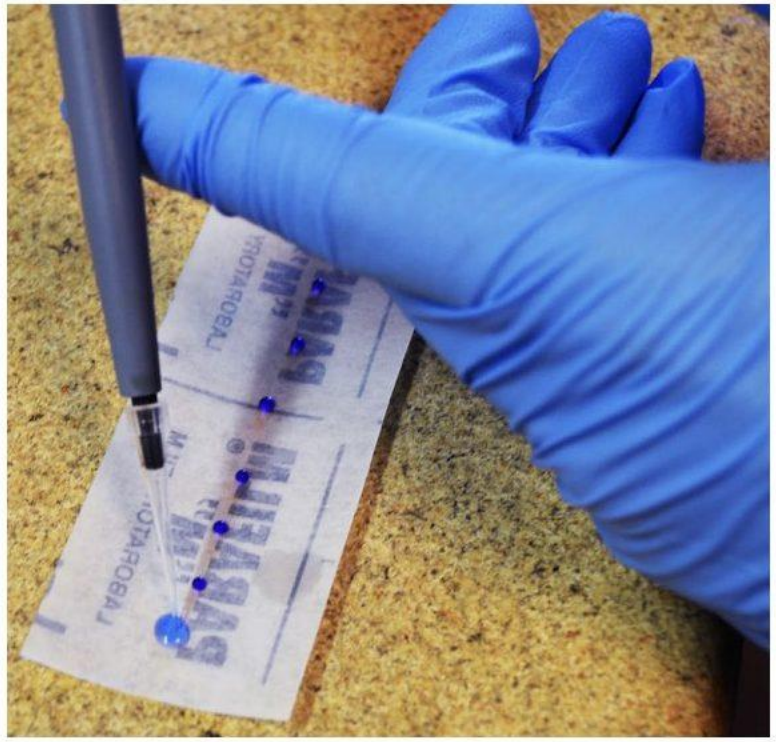
Resolution

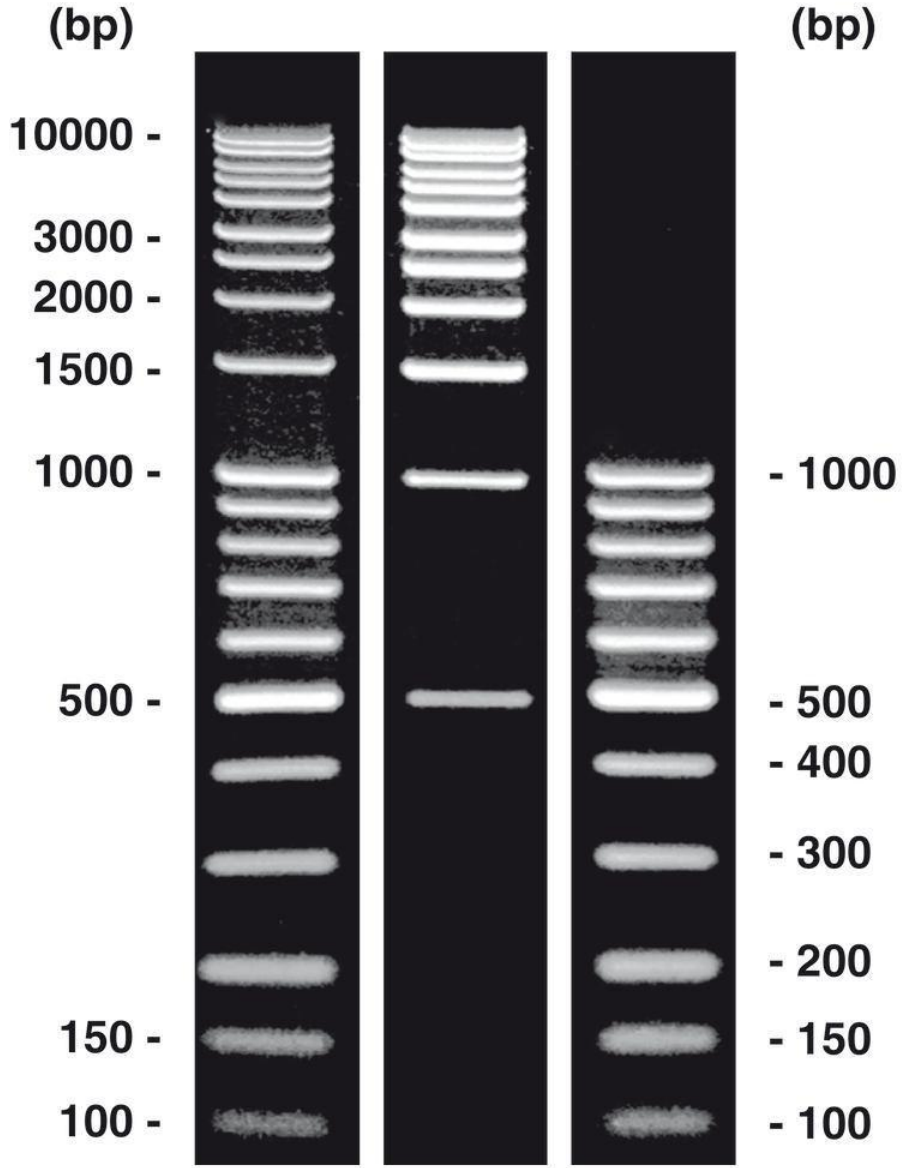
DNA Ladder + Tracking Dye

Loading Dye 6x Dilution 1:5

Tris/Borate/EDTA buffer (TBE buffer) Prepare

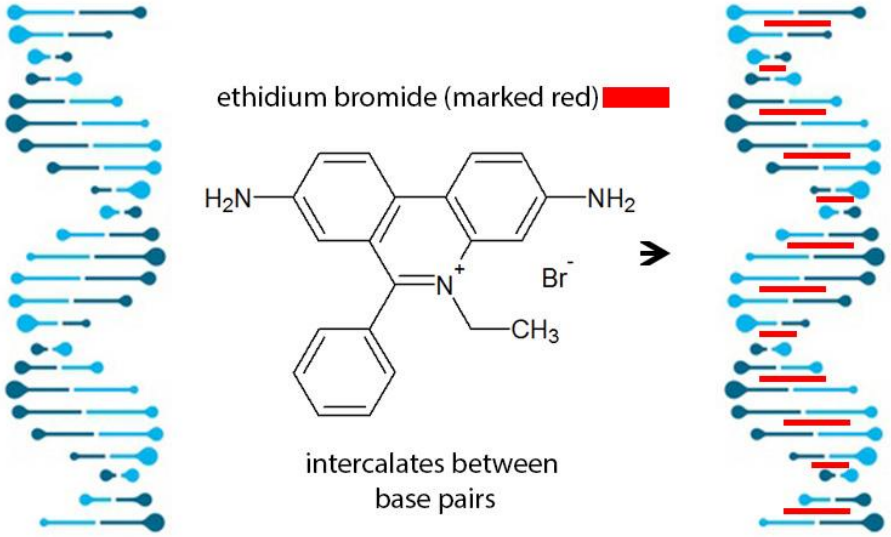
1x or 0.5x TBE buffer



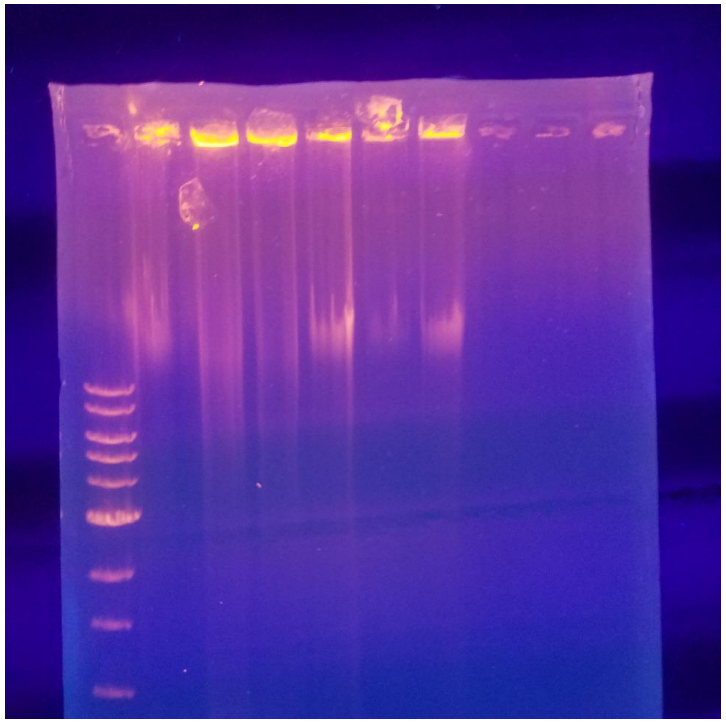


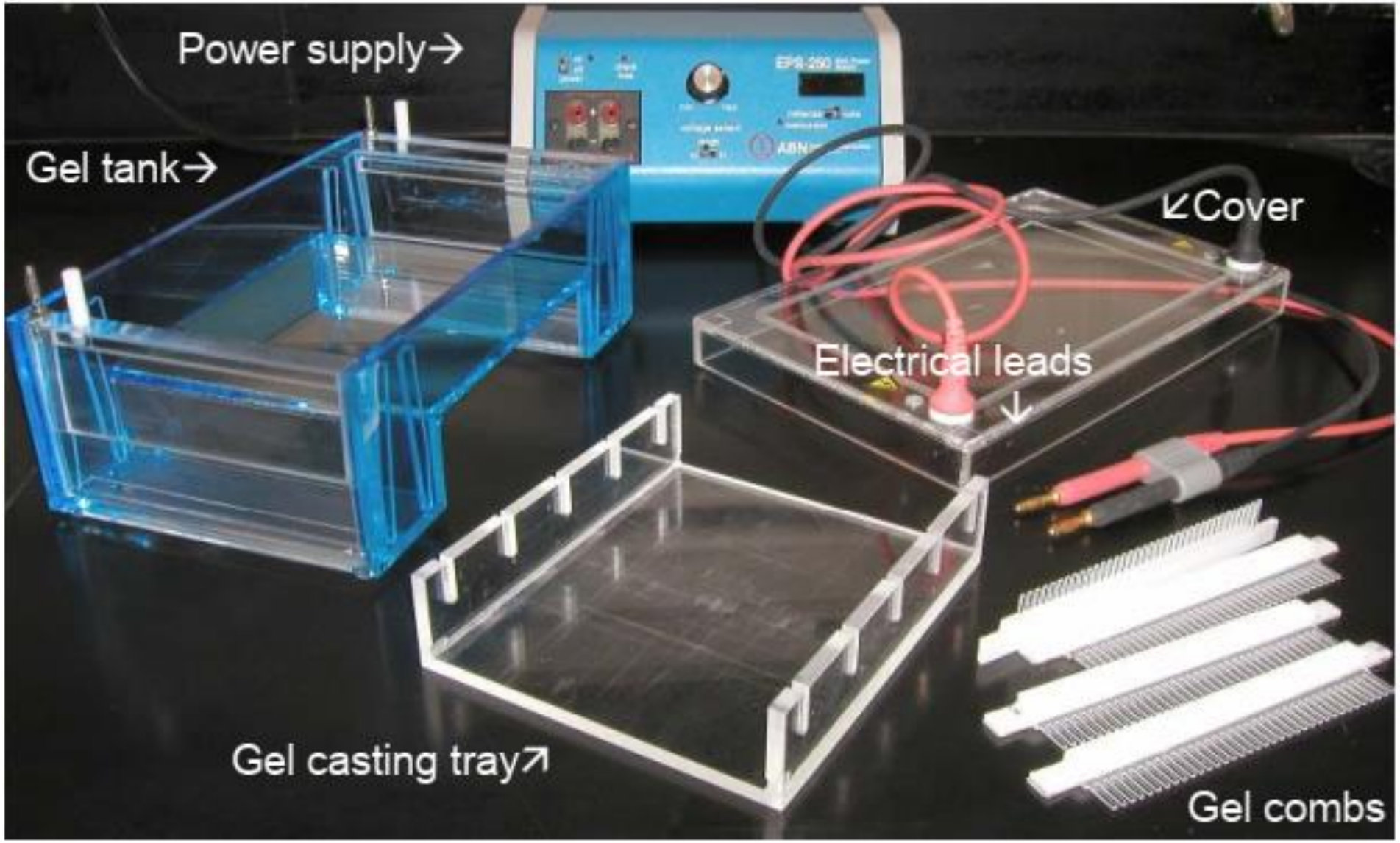
Agarose (0.7%) (? -> 100mL) (?->50 mL) TBE buffer

GelRed® (Biotium) or Ethidium Bromide



UV Lamp

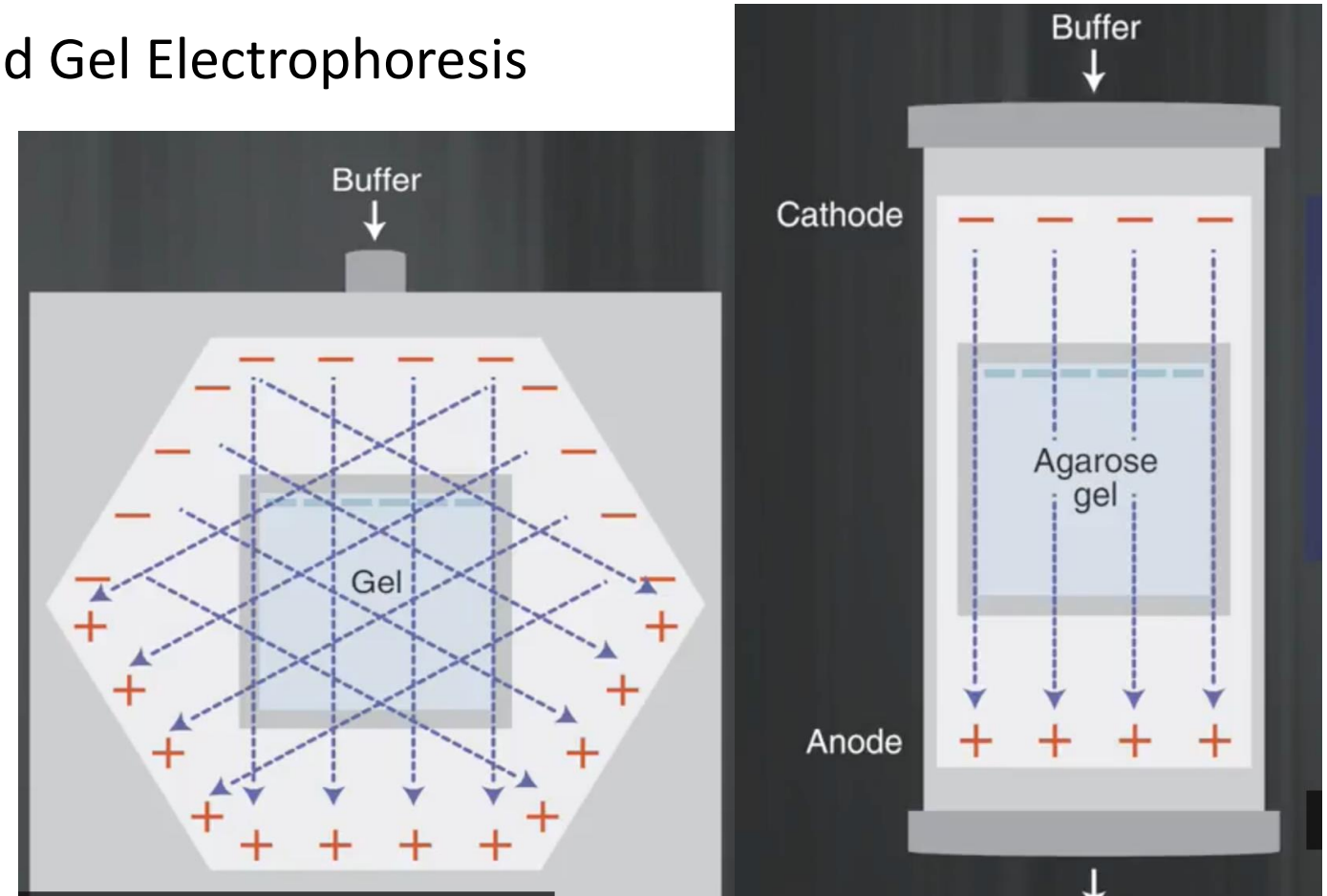



Power supply→
Gel tank→
↙Cover
Electrical leads
↓
Gel casting tray↗
Gel combs

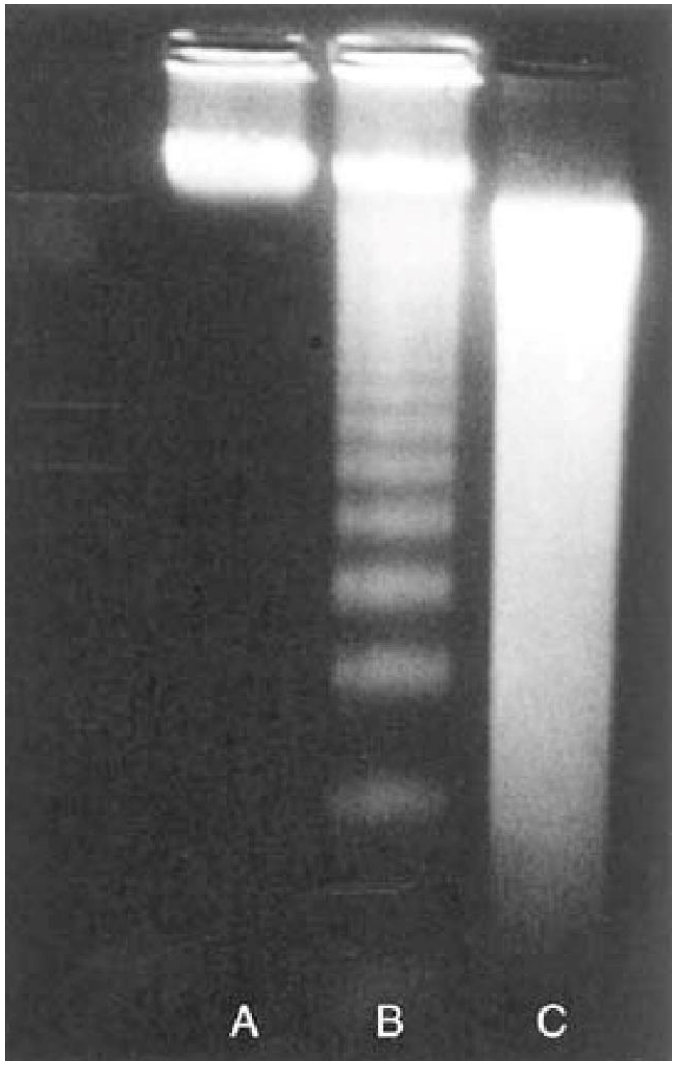
Gel Electrophoresis

- Conventional agarose gel electrophoresis employs a static field and can resolve DNA fragments up to 50 kb
- For Resolution of larger Fragments: Pulsed Gel Electrophoresis (PFGE) is more useful

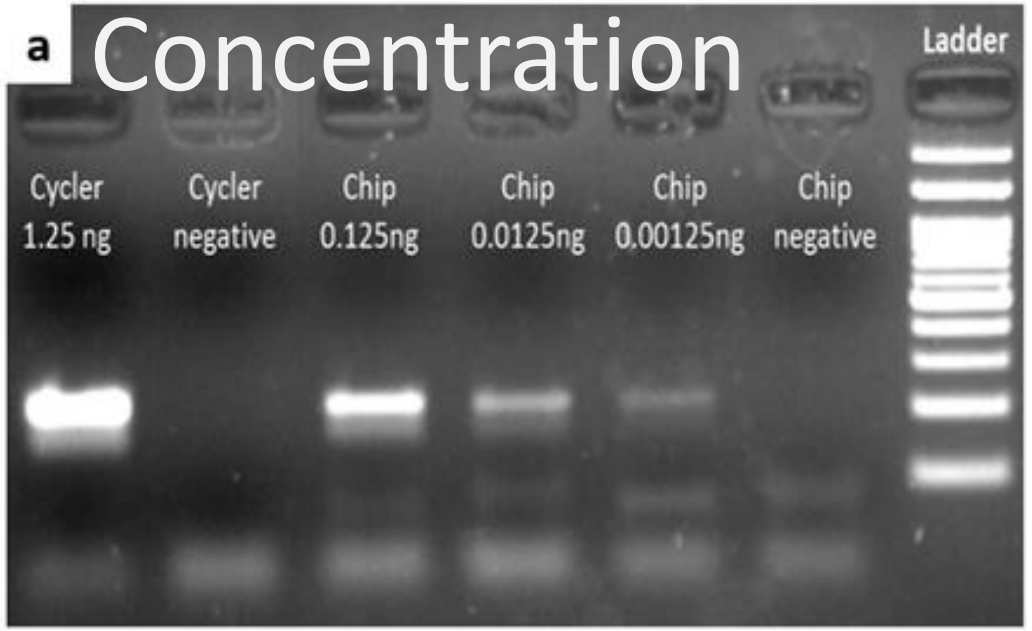
https://www.youtube.com/watch?v=k_QAXLGuQ5w

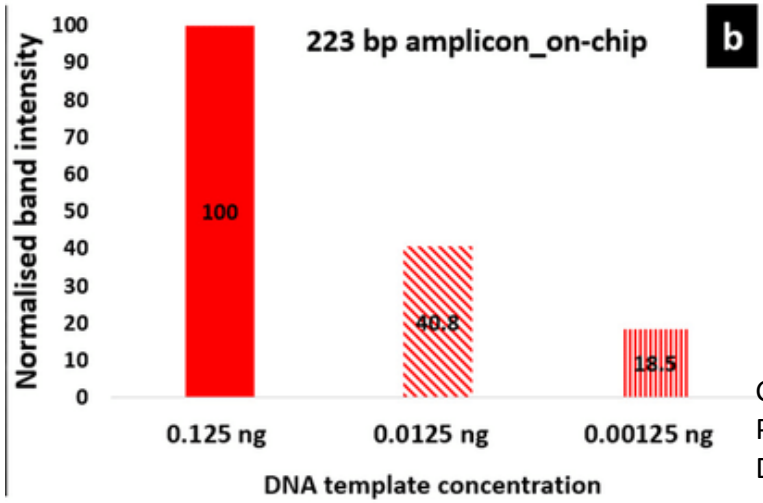


Diffuse Smearing



Concentration





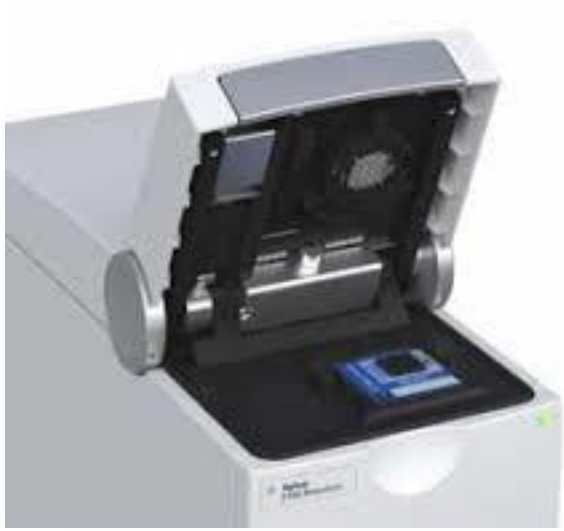
Georgia D. Kaprou et al. 2020. Towards PCB-Based Miniaturized Thermocyclers for DNA Amplification

Agilent Bioanalyzer/ TapeStation 1µL Sample

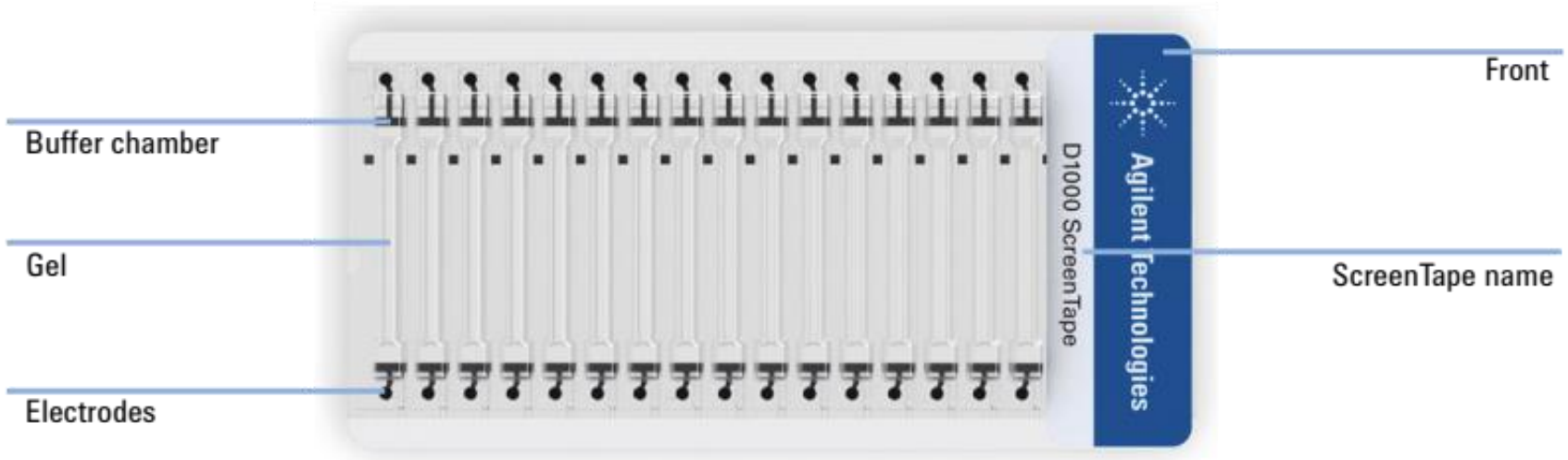
- Agilent 2100 Bioanalyzer instrument
- Chips disposable
- **Kits**
- **Agilent DNA 12000** kits are designed for the sizing and quantitation of double-stranded DNA fragments from 100 to 12000 bp.
- **Agilent DNA 7500** 100 – 7500 bp
- Can analyse 12 samples/chip, chip can not be reused.

Agilent 4200 or 2200 TapeStation
Credit card size Screen Tape 16 Samples/ Card
Can run up to 95 samples in a plate
The unused lanes in Card is reusable -> fixed cost per sample
Kits (Reagents + Screen tape):
Genomic DNA kit :Broad Range 100-60,000 bp
Agilent D1000 Kit/Agilent HS D1000 up to 1000 bp
Agilent D5000 Kit/Agilent HS D5000 up to 5000 bp

Bioanalyzer

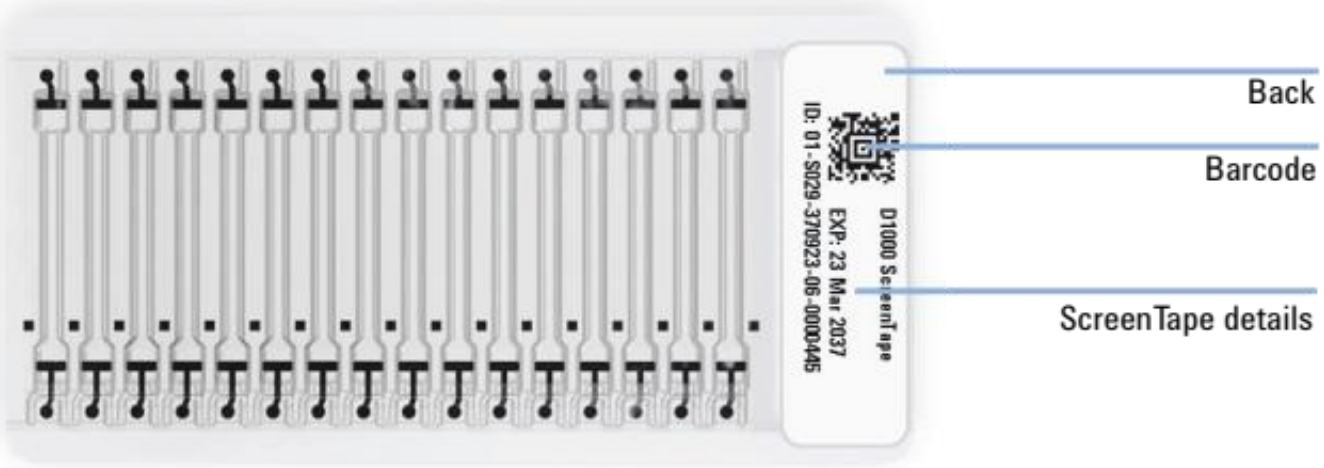


TapeStration Card Size Screen Tape



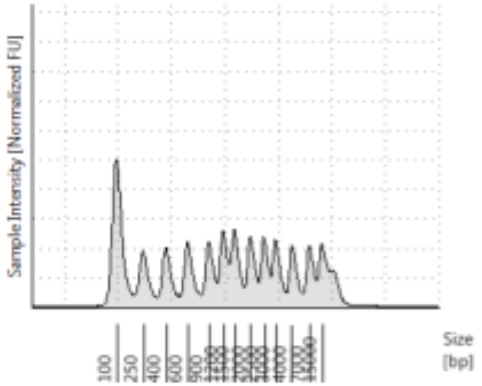
Kit Components (Genomic DNA ScreenTape Assay)

Part Number	Name	Color	Amount
5067-5365	Genomic DNA ScreenTape		7 ScreenTape devices
5067-5366	Genomic DNA Reagents		2 vials
	• Genomic DNA Ladder		25 µL
	• Genomic DNA Sample Buffer		1350 µL

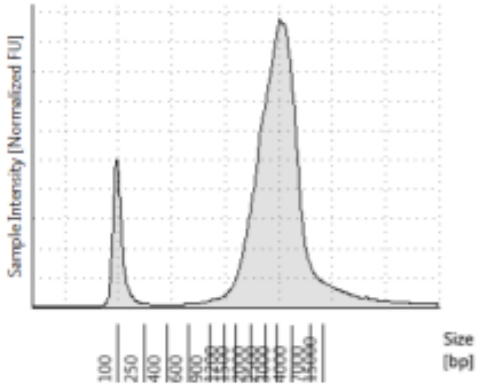


Tapestation results

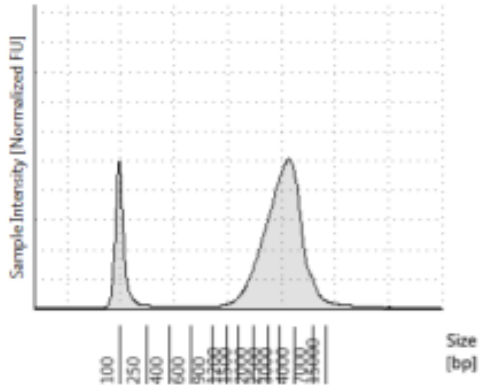
A1: Ladder



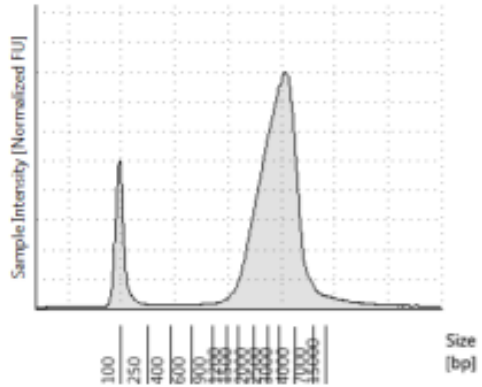
B1: 2

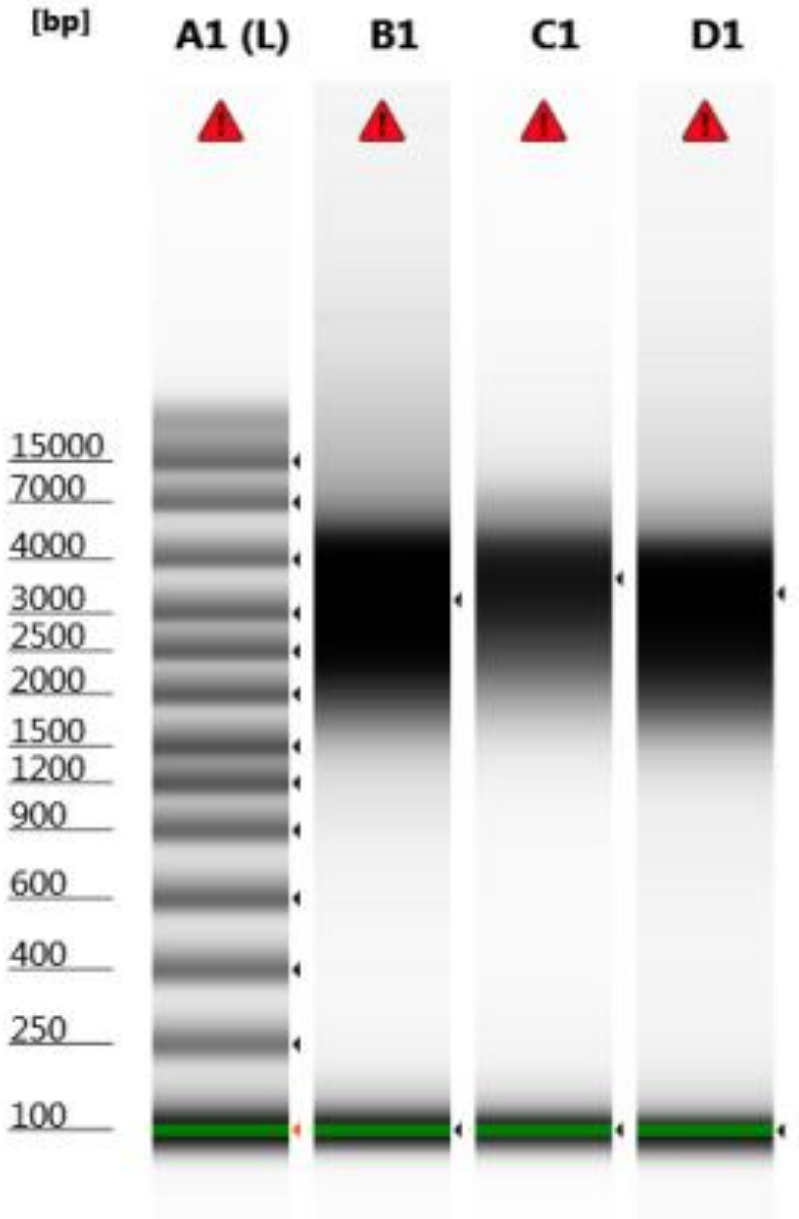


C1: A



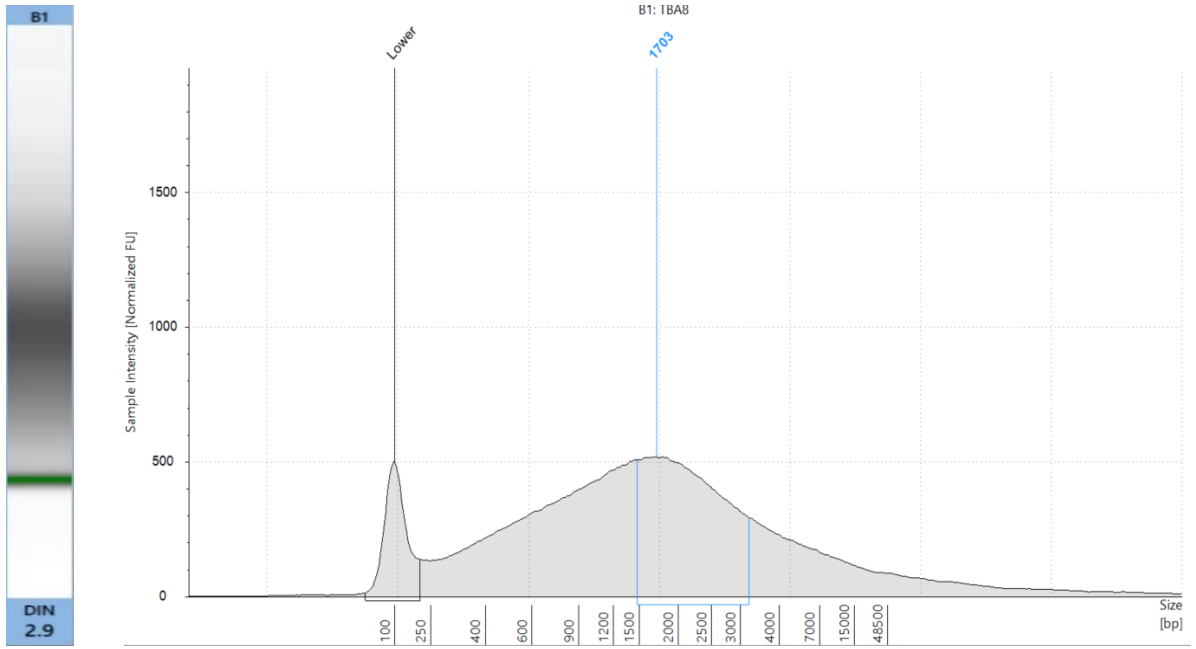
D1: C



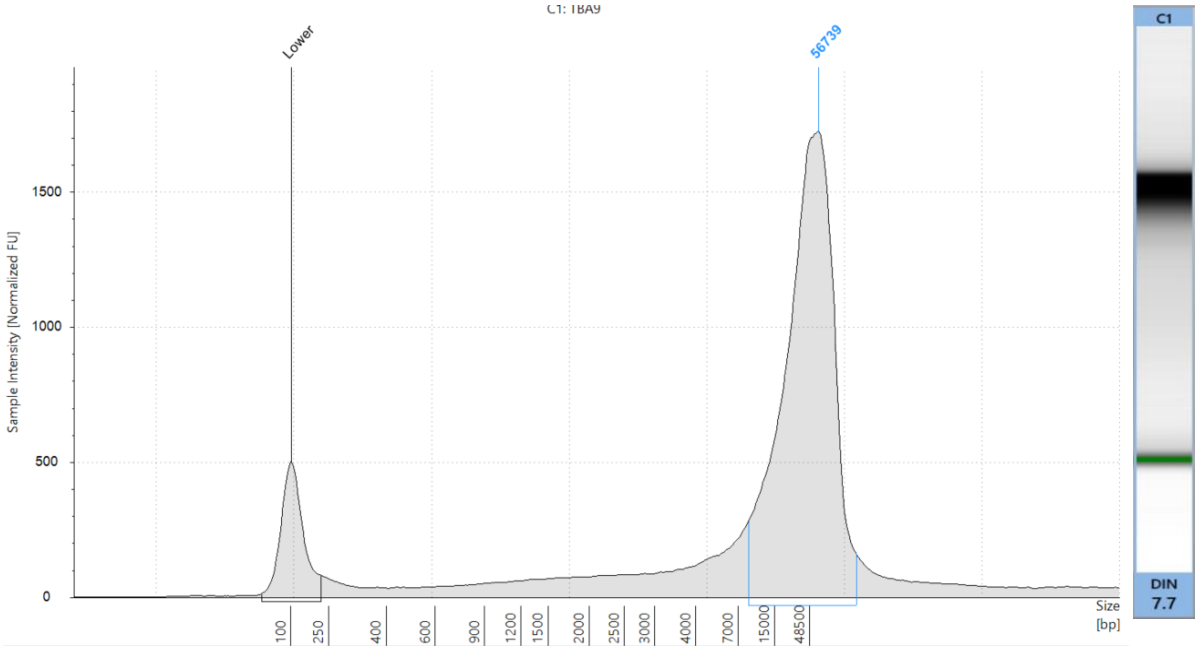


TapeStation™ Results, Which is Better?

A



B



Purity

- Nanodrop™

Quantity

- Qubit™
- Nanodrop™
- TapeStation/Bioanalyser

Integrity

- TapeStation/Bioanalyser
- Gel Electrophoresis

What to aim for?

Purity

- Nanodrop™
  - A260/A280 1.8 - <2.00
  - An Estimate of the concentration

Quantity

- Qubit™
  - Concentration >44 ng/uL

Integrity

- TapeStation/Bioanalyser
- Gel Electrophoresis
  - High Mwt genomic DNA >12 kb
  - No Degradation (Smearing, High sharp peak)

$$\text{Concentration} = \frac{weight}{Volume}$$

moles

$$\text{Molarity} = \frac{weight\ (g)}{Mwt} \times \frac{1}{volume\ (Lt)}$$

10^{-3}	=	m	milli
10^{-6}	=	μ (mu)	micro
10^{-9}	=	n	nano
10^{-12}	=	p	pico
10^{-15}	=	f	femto

Mwt of DNA Fragment =
size of a fragment (TapeStation) x average Mwt of bp (660 Da)

A mole of anything has Avogadro's number **6.022×10^{23}** of this thing

Rule: Pooling the Sequencing library at equal equimolar ratio

Any Questions?