

NUCLEIC ACID QUALITY CONTROL

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predict the problem before seeing the QC data

A rhizosphere sample was bead-beaten strongly, purified on a silica column, washed quickly, and eluted in a very small volume.

Predict two possible downstream problems.

Think about: DNA integrity • residual ethanol • inhibitors • concentration vs total yield

Quality control asks three different questions

1

Quantity

How much nucleic acid?

NanoDrop / Qubit

2

Purity

What contaminants remain?

UV spectrum / ratios

3

Integrity

Is the molecule intact?

Gel / capillary electrophoresis

Quantity ≠ Purity ≠ Integrity

UV spectrophotometry: the Beer–Lambert concept

$$A = \varepsilon \times c \times l$$

Absorbance ∝ concentration × path length

At A_{260} = 1.0 (1-cm equivalent)

dsDNA	**≈ 50 µg/mL**
RNA	**≈ 40 µg/mL**
ssDNA	**≈ 33 µg/mL**

NanoDrop uses a very short optical path and software converts absorbance to concentration.

A_{260}/A_{280}: a clue about protein and phenol contamination

Typical reference values

DNA ≈ 1.8

RNA ≈ 2.0

Low A260/A280 may indicate:

- Protein contamination
- Phenol carryover
- Some other UV-absorbing contaminants

Important

A “good” ratio does not prove intact DNA/RNA.

A_{260}/A_{230}: often the more sensitive warning sign

Typical clean-sample range

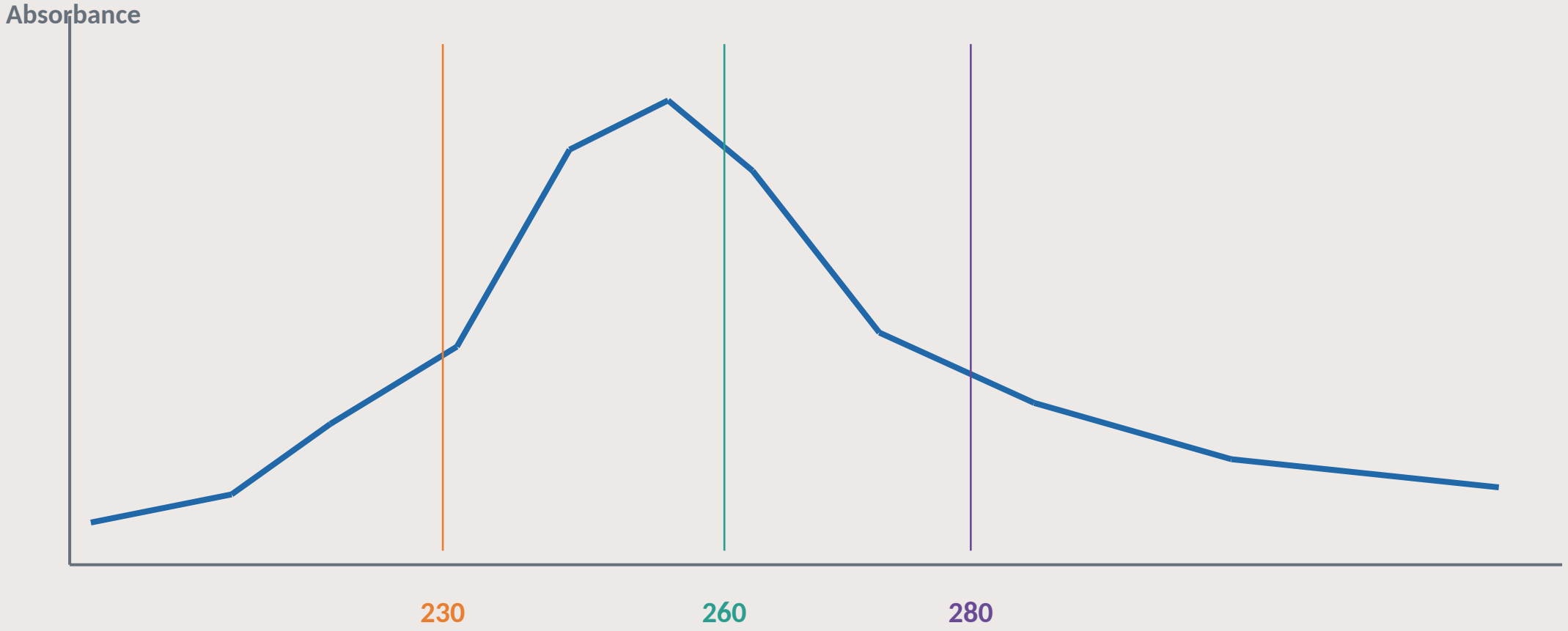
~ 2.0–2.2

Low A260/A230 may indicate

- Guanidinium salts
- Phenol
- Carbohydrates
- EDTA / buffer components
- Residual alcohol
- Humic substances / other extraction contaminants

For soil and plant DNA, a low 260/230 ratio is especially informative because many matrix-derived inhibitors absorb near 230 nm.

Do not read ratios without the full spectrum



Ratios can be unstable at low concentration or with a mismatched blank.

Fluorimetry: Qubit, PicoGreen and RiboGreen

Target-selective fluorescence

Dye signal increases when bound to the target nucleic acid.

Less influenced by proteins, salts and many free UV-absorbing molecules.

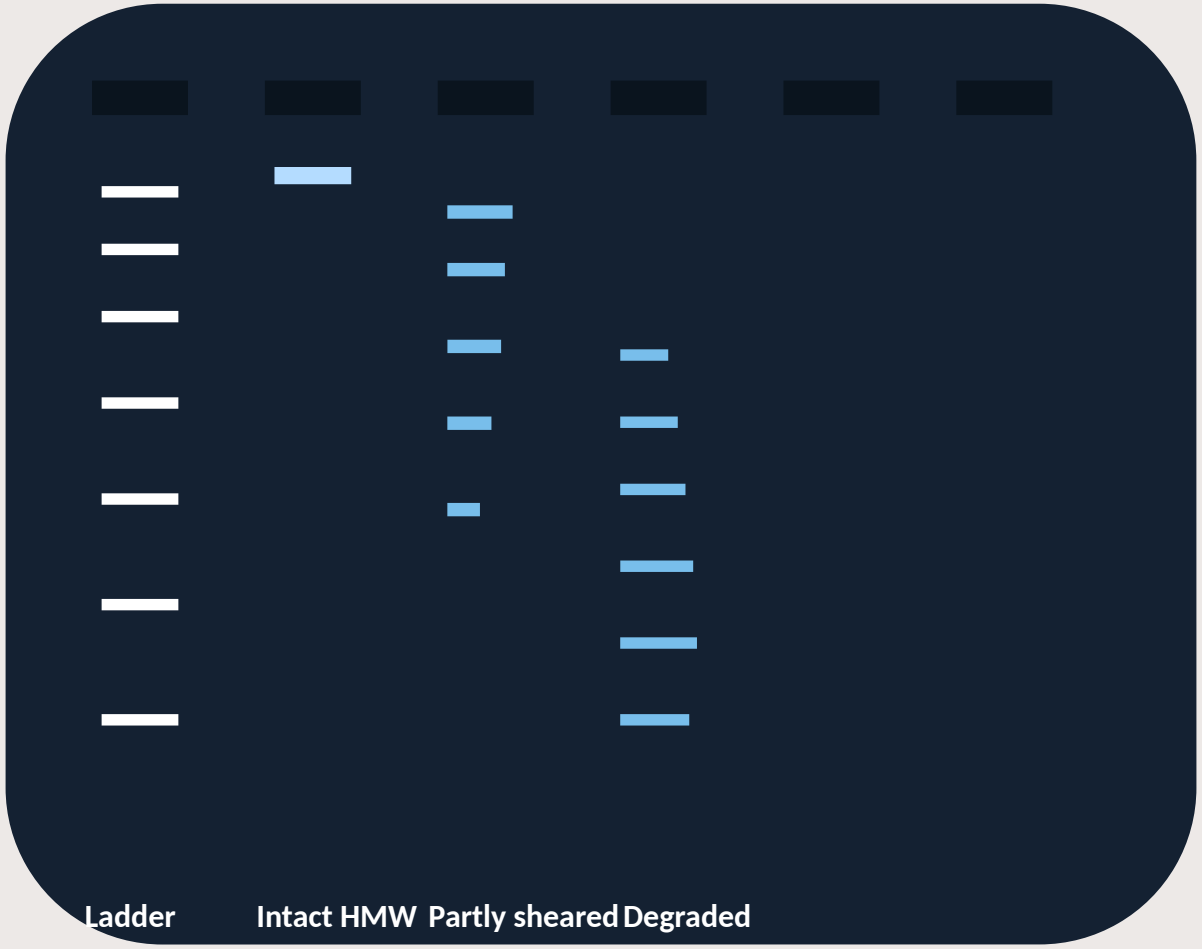
1	**Qubit dsDNA**	Specific DNA quantitation
2	**PicoGreen**	Highly sensitive dsDNA fluorescence
3	**RiboGreen**	Sensitive RNA fluorescence

NanoDrop vs Qubit: different questions, complementary tools

Feature	NanoDrop	Qubit
Principle	UV absorbance	Target-selective fluorescence
Best for	Rapid concentration + purity clues	Accurate target-specific concentration
Low concentration	Less reliable	Generally better
Purity ratios	Yes	No
Contaminant sensitivity	High	Lower for many contaminants
Use together?	Yes	Yes

If NanoDrop = 120 ng/µL but Qubit dsDNA = 45 ng/µL, suspect that UV absorbance is measuring more than dsDNA.

Agarose gel electrophoresis: visualize DNA integrity



Interpretation

- High-molecular-weight DNA stays near the well
- Smearing toward lower sizes indicates fragmentation
- Very small fragments migrate farther
- Gel intensity is only semi-quantitative

RNA integrity: eukaryotic and bacterial patterns differ

Eukaryotic total RNA



Degradation → diffuse lower-molecular-weight smear

Bacterial total RNA



Do not interpret bacterial RNA using only eukaryotic 28S/18S expectations.

Capillary electrophoresis and integrity metrics

1	**Bioanalyzer**	Microfluidic electrophoresis	RNA/DNA sizing + electropherogram
2	**TapeStation**	Automated electrophoresis	High-throughput QC
3	**Femto Pulse**	High-sensitivity / large-fragment analysis	Useful for HMW DNA and low-input samples

Integrity metrics

RIN

RNA Integrity Number
1 → degraded
10 → highly intact

DIN

DNA Integrity Number
Higher values generally indicate greater integrity

RIN/DIN interpretation is application- and sample-dependent; use instrument guidance and assay requirements.

“Good DNA/RNA” is defined by the downstream application

Application	Most important quality features	Why
Routine PCR	Enough amplifiable template; low inhibitors	Only a short target region must remain intact
qPCR	Consistent purity + accurate template input	Inhibitors distort amplification efficiency
RNA-seq	RNA integrity + low contamination	Degradation changes transcript representation
Illumina DNA-seq	Accurate concentration + compatible fragment quality	Library preparation requires controlled input
Long-read sequencing	Very high-molecular-weight, clean DNA	Read length depends on molecule length

There is no universal “good DNA” value. Quality is fit-for-purpose.

Integrated case: high concentration, but no PCR

Rhizosphere DNA sample

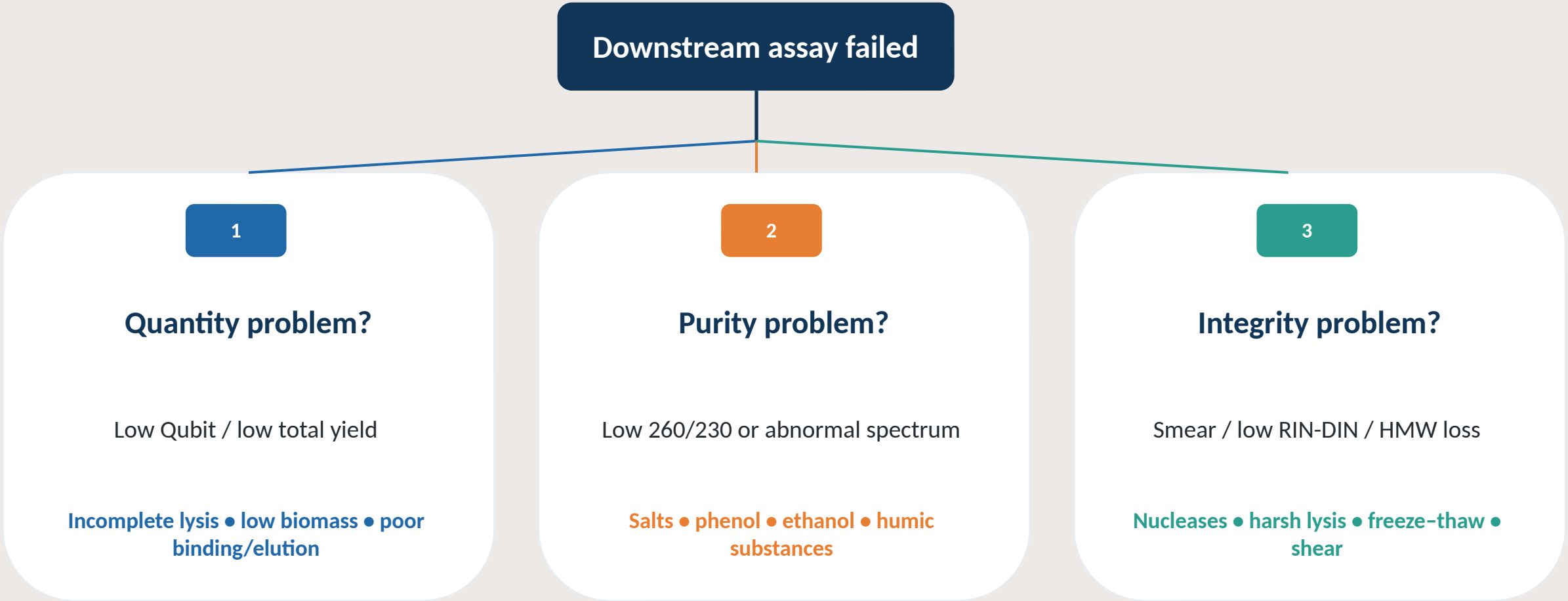
NanoDrop	180 ng/µL
A260/A280	1.82
A260/A230	0.65
Qubit dsDNA	42 ng/µL
PCR	No amplification

1. 260/280 looks acceptable — but that is not enough.

2. 260/230 = 0.65 strongly suggests contamination / inhibitors.

3. NanoDrop ≫ Qubit: UV absorbance is likely measuring more than dsDNA.

Troubleshooting: diagnose the dimension that failed



Do not repeat the experiment blindly. Start with the evidence.

Mini decision challenge: choose the extraction strategy

A

Plant leaf for RT-qPCR

Protect RNA rapidly; remove polysaccharides/polyphenols; DNase control

B

Gram-positive isolate for 16S PCR

Lysozyme + suitable lysis; routine DNA purity

C

Soil microbiome DNA

Strong unbiased lysis + inhibitor removal

D

Long-read bacterial genome

Gentle HMW DNA extraction; avoid shear

Ask: What is the biological barrier? What is the inhibitor risk? What does the downstream assay require?

Take-home messages

1. Sample type determines the lysis strategy.
2. Purification methods exploit phase behavior, adsorption, magnetism or charge.
3. Precipitation concentrates nucleic acids but can co-precipitate contaminants.
4. Quantity, purity and integrity are different QC dimensions.
5. NanoDrop, Qubit and electrophoresis provide complementary information.
6. Nucleic-acid quality must be judged by the downstream application.

The best extraction protocol is the one that produces fit-for-purpose nucleic acid from your specific sample.