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A Quantitative Determination of Dielectric Thresholds for DNA Precipitation by Ethanol

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Protocol status: Working
This protocol provides a quantitative, dielectric-threshold model for ethanol-mediated DNA precipitation, validated experimentally across four DNA concentrations. All steps have been optimized for reproducibility, minimal reagent waste, and compatibility with high-throughput molecular workflows.

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Abstract

Current DNA preservation practices rely on empirically determined volumetric rules rather than quantitative theoretical frameworks. Existing models overestimate ethanol requirements, limiting reproducibility and hindering the development of efficient protocols. A dielectric threshold–based model for predicting ethanol volumes during DNA precipitation establishes a framework that will reduce reagent waste and thus, environmental impact. This quantitative framework predicts the minimum ethanol requirement for human somatic DNA precipitation as a function of DNA molarity, derived from spectrophotometric absorbance data using the Beer-Lambert Law and Coulomb's Law. For four different DNA conditions, 12 replicate samples were prepared across an ethanol concentration gradient from 54% to 90% v/v in 2% increments (n = 12 per concentration). DNA molarity and the dielectric constants of ethanol (ε = 24.5) and water (ε = 80.1) were used to calculate the dielectric constant of each solution, enabling determination of the threshold for DNA precipitation. DNA precipitation begins at 58-60% ethanol and reaches a maximum yield of 95% at 72% ethanol. Across all four DNA concentrations (2, 5, 8, and 10 ng/µL), replicates demonstrated consistent dielectric thresholds and precipitation profiles, generating a reproducible sigmoidal recovery curve. ε = 40.07 is the dielectric threshold at which DNA precipitates optimally, corresponding to the point at which our yield plateaus (varying ± 3% across replicates). These results validate the dielectric-threshold framework, showing a slight shift in the onset relative to theoretical predictions, while demonstrating that ethanol volumes can be optimized without compromising DNA integrity. Adopting the dielectric-threshold framework reduces ethanol requirements by 3% compared to standard DNA precipitation protocols, while ensuring optimal yield and quality. Standardizing this model will improve reproducibility, enable automation, and reduce biohazardous and flammable waste across high-throughput workflows.

Guidelines

Controls

Three controls are utilized in this protocol to verify that DNA precipitation is a function of ethanol dielectric effects:

Negative Control: A tube containing nuclease-free water, sodium acetate, and glycogen, without DNA, in the same concentrations as the other samples, was mixed with all variations in ethanol concentrations across the gradient.

Positive Control: A 20ng/µL sample of DNA with sodium acetate and glycogen was precipitated at 70% to confirm that precipitation will proceed as expected beyond the threshold under standard conditions.

Spectrophotometer Blank: The spectrophotometer was blanked with a solution containing the base amounts of sodium acetate, glycogen, and nuclease-free water for all A260 baseline samples.

Materials

- 96-well plate x 3
- 1mL or 1.5mL microcentrifuge or 15mL centrifuge tubes x 500

- Centrifuge
- Incubator
- Spectrophotometer

- Gloves (nitrile or neoprene) are necessary to resist ethanol corrosion and prevent contamination.
- Lab coat
- Safety glasses
- 70% ethanol spray

- Double-stranded DNA of any accessible form (Lyophilized pellets or flakes of DNA preferred) ~ 5g
- Nuclease-free water
- Sodium Acetate (NaOAc) ~ 500g
- Glycogen (20mg/mL stock) ~ 100µL per X stock

Methodology

1 This derivation applies the Beer-Lambert Law to convert spectrophotometric absorbance (A) into the molarity of double-stranded human somatic DNA. The molar output served as the input for subsequent electrostatic and dielectric modelling of ethanol-mediated precipitation.

Beer-Lambert substitution

2 As per the Beer-Lambert Law (Swinehart, 1962), let $a = \epsilon cl$

a: Absorbance (unitless)	a = Independent Variable
ϵ: Molar absorptivity	ϵ = 50 $mL/\mu g\ cm$ (for dsDNA)
c: Concentration of DNA (g/L)	c = Dependent Variable
L: Length of light path (cm)	L = 0.05 cm

- For all Human Genome DNA, the value for ϵ is 50 $mL/\mu g\ cm$. This function applies to any double-stranded DNA (dsDNA) in the human genome.

3 Rearranging the law,

$$a = \epsilon cl$$
$$c = \frac{a}{\epsilon l}$$

4 Substituting values,

$$c = \frac{a}{(50\frac{mL}{\mu g\ cm})\ (0.05\ cm)}$$

$$c = \frac{a}{2.5\frac{mL}{\mu g}}$$

$$c = \frac{a}{2.5\frac{0.001\ L}{0.000001\ g}}$$

$$c = \frac{a}{2500}\frac{g}{L}$$

Volumetric conversion

5 Let $m = cV$

m: mass of solute
c: mass concentration of solution
V_w: Volume of water (assuming negligible volume of dsDNA)

Thus,
$V_w = m/c$

Utilizing the value c from **Beer-Lambert substitution**,

$$V_w = \frac{m}{\frac{a}{2500}}\frac{L}{g}$$

Recall that the mass in the numerator will cancel units with the grams in the denominator.

Electrostatic interaction - Coulomb's law

6 The volume of the DNA solution is now defined as a function of absorbance and mass. This model can be extended to encompass electrostatic interactions that govern the precipitation of DNA by ethanol. The dielectric constant, characterized by Columb's law (Jackson, 1999), is used to quantify the effect of our solvent on the strength of interactions between ions.

$$F = \frac{1}{4\pi\epsilon_0 \epsilon_r} \bullet \frac{q_1 q_2}{r^2}$$

ϵ_0: Vacuum permittivity

ϵ_r: Dielectric constant of the solvent

$q_1 q_2$: Charges

F: Force between two charges

Electrostatic interaction - Precipitation onset

7 The threshold dielectric constant of a water-ethanol mixture before precipitation can be estimated through an approximation based on the volume fractions of ethanol and water. Based on experimental data, this occurs when ethanol constitutes more than 64% of the solution volume.

By forming a weighted average, $\epsilon_{solution} = \phi_e \epsilon_e + \phi_w \epsilon_w$

ϕ_e : Volume fraction of ethanol	ϕ_e : 0.64
ϕ_w : Volume fraction of water	ϕ_w : 1 - 0.64 = 0.36
ϵ_e : Dielectric constant of ethanol	ϵ_e : 24.5 (UW Dalton Research Group)
ϵ_w : Dielectric constant of water	ϵ_w : 80.1 (UW Dalton Research Group)

$\epsilon_{solution} = \phi_e \epsilon_e + \phi_w \epsilon_w$

$\epsilon_{solution} = (0.64)(24.5) + (0.36)(80.1)$

$\epsilon_{solution} = 15.68 + 28.836$

$\epsilon_{solution} = 44.5$

However, this weighting rule is only a rough approximation and is intended as a general guideline. In practice, the dielectric constant ranges between 30 and 40. For the sake of simplicity, this value will not be included in the equation and will instead be left as a constant ($\epsilon_{solution}$).

Electrostatic interaction - Volumetric calculation

8 The dielectric constant is directly correlated with the solubility of ionic compounds. If it is low, so is the interference with electrostatic forces. Thus, a lower dielectric constant means ions can attract each other and precipitate. The dielectric constants are related to volume by their weighted averages.

$\epsilon_{solution} = V_e * \epsilon_e + V_w * \epsilon_w / V_e + V_w$

V_e : Volume of ethanol	V_e = Variable
V_w : Volume of water	V_w = Variable
$\epsilon_{solution}$: Dielectric constant of the solution	$\epsilon_{solution}$ = Constant
ϵ_e : Dielectric constant of EtOH	ϵ_e = 24.5 (Dalton Research Group)
ϵ_w : Dielectric constant of H_2O	ϵ_w = 80.1 (Dalton Research Group)

9 The effective dielectric constant can be determined using a linear, volume-weighted average, targeting a dielectric constant of 40.0.

$$\epsilon_{solution} = \frac{V_e * \epsilon_e + V_w * \epsilon_w}{V_e + V_w}$$

$$\epsilon_{solution} = \frac{24.5\,V_e + 80.1\,V_w}{V_e + V_w}$$

$$\epsilon_{solution}(V_e + V_w) = 24.5V_e + 80.1V_w$$

$$(\epsilon_{solution} - 24.5)V_e = (80.1 - \epsilon_{solution})V_w$$

$$V_e = \frac{80.1 - \epsilon_{solution}}{\epsilon_{solution} - 24.5}V_w$$

10 Utilizing the value for V_w from **Section: Volumetric conversion**,

$$V_e = \frac{80.1 - \epsilon_{solution}}{\epsilon_{solution} - 24.5} \frac{m}{\frac{a}{2500}} \frac{L}{g}$$

$$V_e = \frac{80.1 - \epsilon_{solution}}{\epsilon_{solution} - 24.5} \frac{2500\ m}{a} \frac{L}{g}$$

$$V_e = \frac{2500\ (80.1 - \epsilon_{solution})\ m}{(\epsilon_{solution} - 24.5)\ a} \frac{L}{g}$$

Conclusion and Limitations

11 Therefore, the volume of ethanol required to precipitate DNA in a solution of water is a function of the dsDNA mass and solution absorbance, as given by the following equation:

$$V_e = \frac{2500\ (80.1 - \epsilon_{solution})\ m}{(\epsilon_{solution} - 24.5)\ a} \frac{L}{g}$$

12 Limitations:

- Only works with dsDNA

$\epsilon_{solution}$ is left as a constant, as described by **Section: Precipitation onset**.

The rest of the methodology outlines a two-part approach: first, determining the dielectric constant, and second, validating our theoretical model for ethanol-based DNA precipitation.

Determination of Dielectric Threshold for DNA precipitation

2h 35m

13 Extract DNA samples from cell cultures

- The samples are more concentrated than our target concentration values, allowing us to dilute the samples with nuclease-free water to the target concentration.

14 Prepare Water/DNA/Sodium Acetate samples

	A	B	C	D	E
	Solution X				
		1	**2**	**3**	**4**
	Water (µL)	100µL	100µL	100µL	100µL
	DNA (ng)	1000ng	1300ng	1600ng	2000ng
	Concentration (m_{DNA}/V)	10ng/µL	13ng/µL	16ng/µL	20ng/µL
	Sodium Acetate (mg)	2.46mg	2.46mg	2.46mg	2.46mg
	Glycogen (taken from 20 mg/mL sample)	100µL	100µL	100µL	100µL

14.1 Arrange 240 tubes in a test tube rack, each filled with 100 µL of nuclease-free water (60 per concentration).

14.2 Dissolve mass of DNA corresponding to the target values listed in Table X in 100 µL of water. Prepare = 20 samples for each concentration, three iterations. All solutions were determined with the spectrophotometer strength in mind.

- The concentration stock given was 33.2 µL for a pure DNA dissolved sample.

15 Mix all samples of each concentration with various volumes of ethanol around the previously empirically determined concentration of 70% (Sambrook & Russell, 2001).

	A	B	C	D	E	F	G	H	I	J	K	L	M
	Solution Y1												
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**

	A	B	C	D	E	F	G	H	I	J	K	L	M
	Solution X (V_x)	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL
	Ethanol (µL) (95% stock)	0	131.7	143.6	156.8	171.4	187.9	206.5	227.6	251.9	280	313	352.4
	Concentration (% EtOH)	0%	54 %	56 %	58 %	60 %	62 %	64 %	66 %	68 %	70 %	72 %	74 %
	Volume (V_y (µL))	100	231.7	243.6	256.8	271.4	287.9	306.5	327.6	351.9	380	413	452.4

	A	B	C	D	E	F	G	H	I	J
	Solution Y2									
		13	**14**	**15**	**16**	**17**	**18**	**19**	**20**	
	Solution X (V_x)	100µL	100µL	100µL	100µL	100µL	100µL	100µL	75µL	← 75µL due to the micro centrifuge size limit
	Ethanol (µL) (95% stock)	400	458.8	533.3	630.8	763.6	955.6	1257.1	1350	
	Concentra	76%	78%	80%	82%	84%	86%	88%	90%	

	A	B	C	D	E	F	G	H	I	J
	tion (%EtOH)									
	Volume (V_y (µL))	500	558.8	633.3	730.8	863.6	1055.6	1357.1	1425	

16 Calculate the dielectric constants with the weighted formula

$\epsilon_{solution} = V_e * \epsilon_e + V_w * \epsilon_w / V_e + V_w$

At SATP, ϵ_e = 24.5 (Dalton Research Group) ϵ_w = 80.1 (Dalton Research Group)
With this, we obtain the threshold $\epsilon_{solution}$ for precipitation.

17 Incubate at -20 °C for 01:00:00 . 1h

18 Centrifuge at 16000 x g, 4°C, 00:30:00 . 30m

19 Pipette the liquid supernatant that remains above the solid precipitated DNA pellet.

19.1 Run a Qubit assay to quantify the amount of DNA residual in the supernatant, noting this for later.
Total DNA = Spectrophotometer-measured quantity in final sample + DNA residual found in supernatant.

20 Prepare 1000 µL of ethanol wash per tube, comprised of 70% ethanol, to dissolve residual salts and contaminants without redissolving the DNA pellet.

21 Pipette 1 mL of wash into each sample, and invert the tube to rinse gently.

22 Centrifuge at 16000 x g, 4°C, 00:30:00 . 30m

23 Remove the ethanol wash, then air-dry the pellet for 00:02:00 - 00:05:00 in a tube with the cap loosely ajar to allow airflow until the pellet loses its sheen. Once the shine has worn off, the pellet is ready for resuspension. 5m

24 Add 100 µL of nuclease-free water to each precipitated sample to dissolve the purified DNA pellet for usage in the spectrophotometer.

25 Prepare a blank of nuclease-free water for the spectrophotometer.

25.1 Measure A260 absorbance values to determine the DNA concentration.

26 Utilize initial and final DNA concentration to determine the percentage of DNA precipitated. If it is
above 90%, consider it successfully preserved. If no sample meets this criterion, a flaw is determined to
have occurred in the process of the experiment.

27 Prepare samples as done in **Section: Beer-Lambert substitution** (without ethanol). The mass of the DNA in each sample should be given in the table.

28 Measure the A260 values of each sample (blanking for water, glycogen, and sodium acetate).

28.1 Using the $\epsilon_{solution}$, DNA mass and A260 absorbance value, determine, using purely the model, the volume of ethanol required for precipitation.

29 Add ethanol at the same quantities as in **Section: Volumetric conversion**.

30 Incubate at -20 °C for 00:45:00 - 01:00:00 (flexible; generally good for days).

31 Centrifuge at 16000 x g, 4°C, 00:30:00 . 30m

32 Assess the presence of precipitated DNA in each sample, determining which ethanol percentage and volume met the previously determined threshold for precipitation. If a

band of values is observed, it may be a gradual process. The lowest value of the band will be used for this assessment.

33 Observe the validity of predictions and thus prove the model to be valid or invalid (threshold values should match with the model, within an error margin of 2% threshold value).