Creating Density Gradients Using the OptiMATE Gradient Maker & CsCl Reagent

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OptiMATE Gradient Maker

OptiMATE CsCl reagent

Cesium chloride (CsCl) is commonly used as a gradient-forming material in density gradient separations. To generate a CsCl density gradient, powder-form CsCl is typically mixed with a sample solution to achieve a specific homogeneous density. This homogeneous solution is dispensed into ultracentrifuge tubes and centrifuged overnight (16-48 hours) to form a linear gradient (along the radial length of the tube), which separates particles in the sample at high resolution based on their buoyant density.

This process has a few limitations:

1. Weighing powdered CsCl, which is hygroscopic, can be error-prone and messy. The final volume of the mixture, crucial for planning experiments, is difficult to predict and must be measured after mixing the powder.
2. Maintaining sterility of self-prepared stock CsCl solutions can be challenging.
3. The long ultracentrifugation run times limit throughput.

In this application brief, we describe how to use OptiMATE CsCl reagent to overcome these limitations with ease. Specifically, the tools and processes described here are intended to help port your existing CsCl density gradient protocols to use of OptiMATE technologies to create easier and faster density gradients.

Methods

OptiMATE Cesium Chloride Reagent

OptiMATE CsCl reagent is a ready-to-use solution with a density of 1.784 g/mL. This solution has an 18-month shelf life at room temperature without CsCl precipitation. Additionally, it meets cGMP standards for endotoxin levels (≤ 1 EU/mL) and sterility, eliminating the effort and challenges associated with weighing CsCl powder.

To seamlessly port an existing protocol using powdered CsCl to a one using liquid OptiMATE CsCl reagent without altering the outcomes of your existing protocol, our Intellifuge tool offers an OptiMATE Density Gradients tab, which includes calculators for protocols involving powdered CsCl or stock solutions. As shown in Figure 1, the tool first calculates the final homogeneous solution density based on the density of your sample and/or buffer, the amount of solid CsCl added and the volume of sample to which the CsCl is added. In the second step, you can input the desired volume to easily determine the volumes of OptiMATE CsCl reagent and sample/buffer needed to achieve the target density.

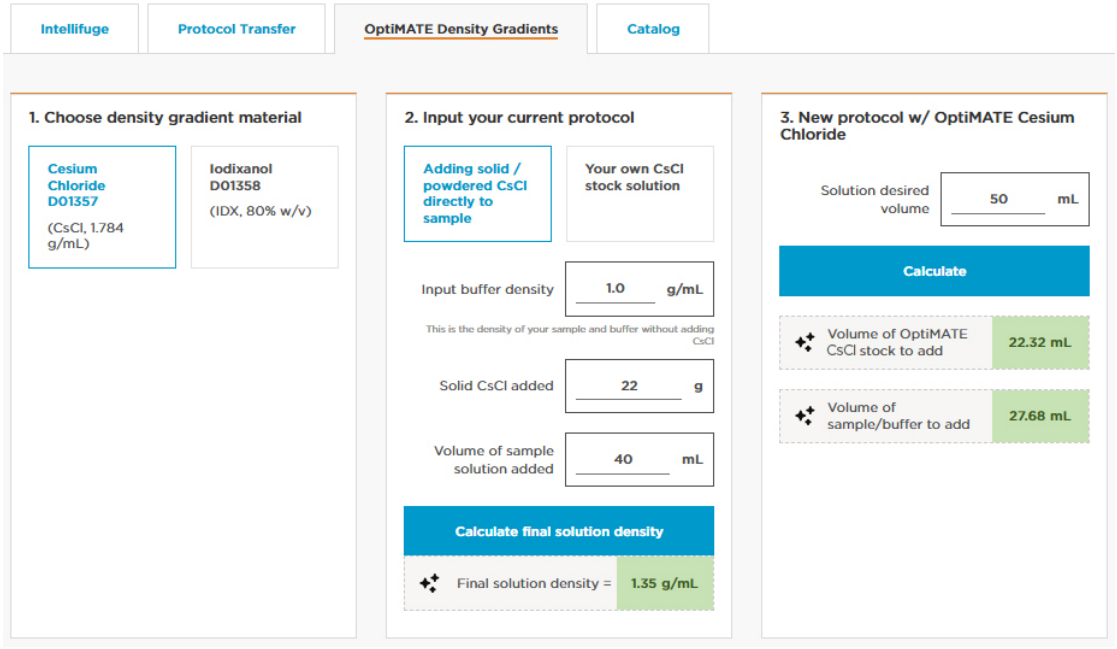

Figure 1: The OptiMATE Density Gradients interface in Intellifuge helps calculate density and the amount of OptiMATE Cesium Chloride reagent to be used.

The calculations used in the Intellifuge tool for CsCl are described below.

It is important to note that the units used to describe concentration (in g/mL) are different from the achieved density (also in g/mL).

The density of CsCl as a function of concentration is derived from international critical tables.[1] This data is used to generate the following relationship between density (d) in g/mL and concentration of CsCl in % weight/weight ([CsCl]):

$$d = 0.0001 \times [CsCl]^2 + 0.0062 \times [CsCl] + 1.005$$

[CsCl] can be calculated based on the weight (in g) or volume (in mL) of buffer or sample used in the homogeneous mixture and amount of CsCl added (in g) using this calculation:

$$[CsCl] = \frac{(amount\ of\ CsCl\ added)}{(amount\ of\ CsCl\ added) + (weight\ of\ buffer\ added)} \times 100$$

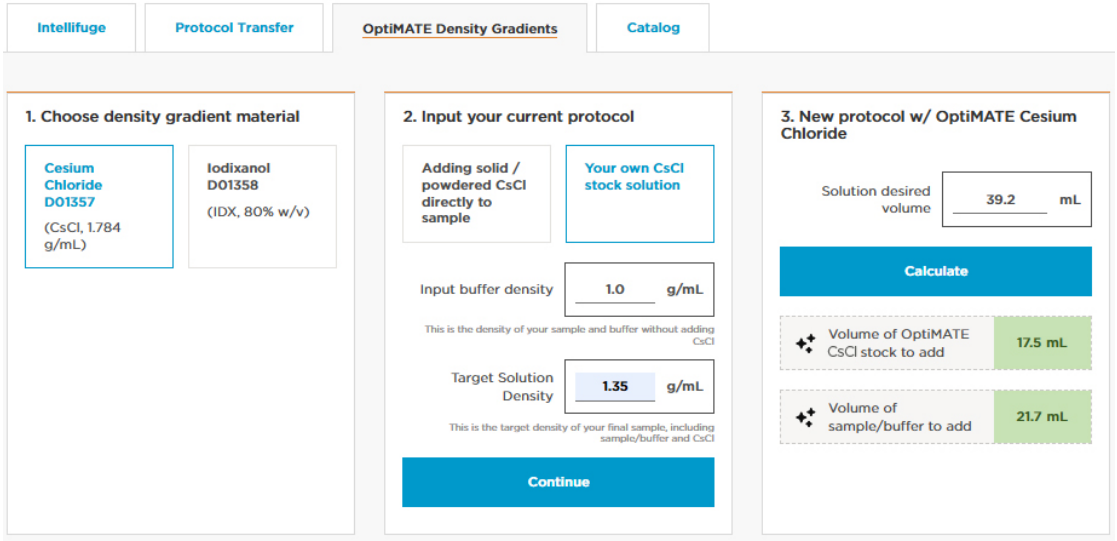

Figure 2: The Intellifuge OptiMATE Density Gradients interface to input a target density to calculate OptiMATE Cesium Chloride reagent to be used.

If the target solution density is already known, the user can employ the Intellifuge tool's Your own CsCl stock solution tab to calculate the volume of OptiMATE CsCl reagent, as well as volume of sample/buffer required to achieve the desired target density (Figure 2).

Calculating required volumes of OptiMATE CsCl reagent and sample/buffer includes these parameters:

Symbol	Parameter	Value
v_t	Total target volume (mL)	To be specified
v_c	Volume of CsCl stock added (mL)	To be calculated
v_s	Volume of sample/buffer (mL)	To be calculated
d_t	Target mixture density (g/mL)	To be specified
d_c	CsCl stock density (g/mL)	1.784
d_s	Sample/buffer density (g/mL)	1 for aqueous solution

Table 1: Parameters to calculate volume (in mL) of OptiMATE CsCl reagent and sample/buffer to be added to get a desired density.

Based on conservation of volume: $v_t = v_c + v_s$

Based on conservation of mass: $v_t \times d_t = v_c \times d_c + v_s \times d_s$

Solving for v_s, the equation becomes:

$$v_s = v_t \times \frac{(d_c - d_t)}{(d_c - d_s)}$$

The target volume, which can be based on the number of tubes to be used, and the density values, can thus be used to calculate v_s, the volume of sample/buffer needed. This in turn can be used to calculate v, the volume of OptiMATE CsCl reagent to be added.

Pre-formed linear density gradients using the OptiMATE Gradient Maker

The OptiMATE CsCl reagent makes it easier to prepare homogeneous CsCl solutions, but these solutions may still require long ultracentrifuge run times (>16 hours) to form a continuous gradient for effective separation of particles. This issue is overcome by generating pre-formed CsCl gradients that incorporate sample using the OptiMATE Gradient Maker. The lengthy run times are primarily due to the time required to form an equilibrium continuous gradient in an ultracentrifuge. By pre-forming the gradient, the ultracentrifugation times are instead dictated by sample migration. Previous studies[2] have demonstrated ultracentrifugation times reduced to just 5 hours with pre-formed gradients, without a compromise in sample purity. A schematic of this is described in Figure 3.

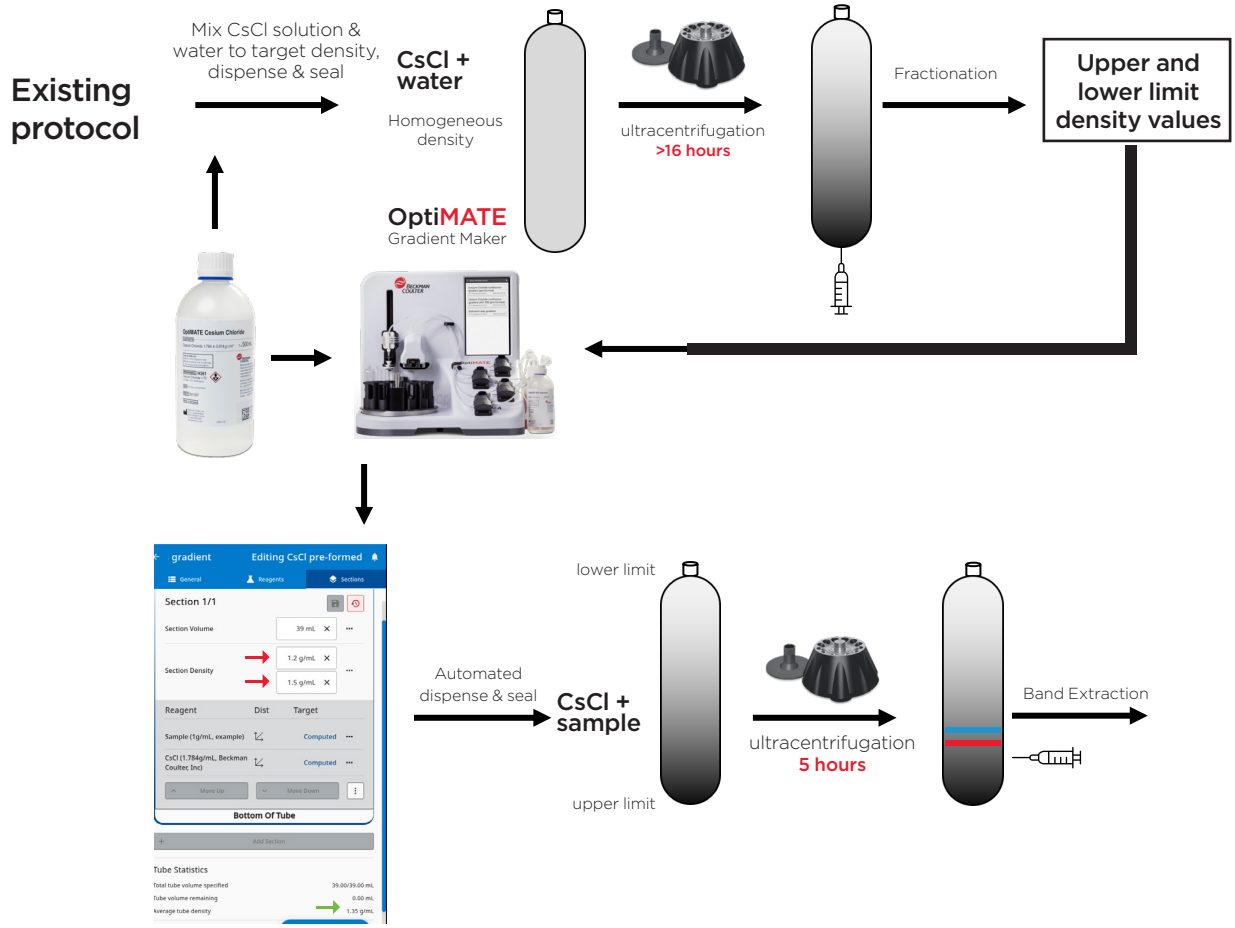

Figure 3: Scheme for porting existing manual CsCl density gradient protocol to use OptiMATE Gradient Maker-generated pre-formed gradients for shorter ultracentrifugation times. The red arrows in the software interface denote input boxes for the target upper and lower limit density values, and the green arrow denotes the average tube density, which should match the homogeneous density from the existing protocol.

How to transfer existing protocols

The user must identify the correct lower and upper limit density values for the pre-formed gradient. This process (described below) requires a solution form of CsCl, which can be the OptiMATE CsCl reagent.

1. Note the density of the homogeneous CsCl solution used in the existing protocol.
2. Run the existing CsCl protocol (the sample may be substituted with buffer) and collect fractions of the contents of the tube after centrifugation
    - Identify the density of the fractions. This can be measured directly or by measuring refractive index (RI) with a refractometer and converting to density using the equation Density = (RI – 1.2388)/0.0946) (derived from International critical tables[1]).
    - Plot the gradient profile using this density data. Fit the linear part of the curve, then extrapolate to determine density at the top and bottom of the tube (your lower and upper limit densities) if the gradient were linear throughout. Notice the fractionated gradient profile tends to curve upwards in a non-linear fashion toward the bottom of the tube.
3. To ensure sample bands appear at a similar location in the tubes as your existing protocol, adjust your lower or upper limit density values in the OptiMATE Gradient Maker user interface to ensure the average density of your solution is equal to your original protocol.
4. When creating the method for the OptiMATE Gradient Maker, select linear distribution and use the lower and upper limit density values picked as the target densities to create the method.
5. If successfully created, this method should generate a pre-formed CsCl gradient that will enable faster centrifugation times (tube type, rotor type, ultracentrifugation speeds and temperatures need not be changed).

References

1. International Critical Tables of Numerical Data, Physics, Chemistry and Technology. National Academies Press, 1930
2. Venkatakrishnan, Jasti and Sternisha. Application note: Rapid, Automated Purification of Adeno-Associated Virus using the OptiMATE Gradient Maker

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