BECKMAN COULTER
Life Sciences

Faster Density Gradient Formation
Purifying viral vector with VTi 90 rotor + CsCl DGUC

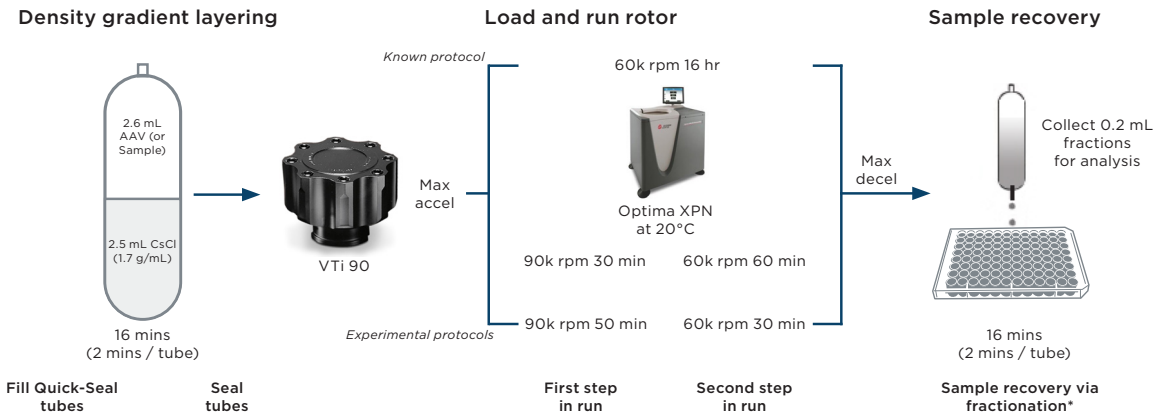
Objective

Optimize traditional run times of sample purification with cesium chloride (CsCl) to facilitate small scale experiment design.

Method

Centrifuge sample with different (1) run speeds, (2) run times, and (3) layering techniques to reach equilibrium as quickly as possible. Fractionate into 0.2 mL fractions. Using a refractometer, measure the fractions to compare density gradient profiles. Experimental protocols whose density gradient profiles are identical to the known protocol's gradient will ensure the sample bands are in the same location.

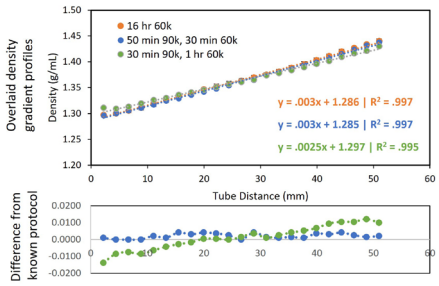
Centrifuge Protocol



*Fractionation was necessary to compare the density gradient curve of the different protocols tested.
** Optima XPN is required for the multi-staged protocol (a protocol that runs multiple speeds in one run).

Results

- Equilibrium was achieved in 80 minutes with an optimized protocol. Other runs either did not achieve the same density profile or were longer than 80 minutes.



Conclusions

1. Starting a separation at a high speed can help form the gradient more quickly.
2. Reducing the run speed for sufficiently long can:
   - Create parity with an existing (slower) protocol, leaving the bands of interest at the exact same position.
   - Flatten the gradient curve, providing a higher resolution.

Multi-stage protocols can significantly reduce purification time