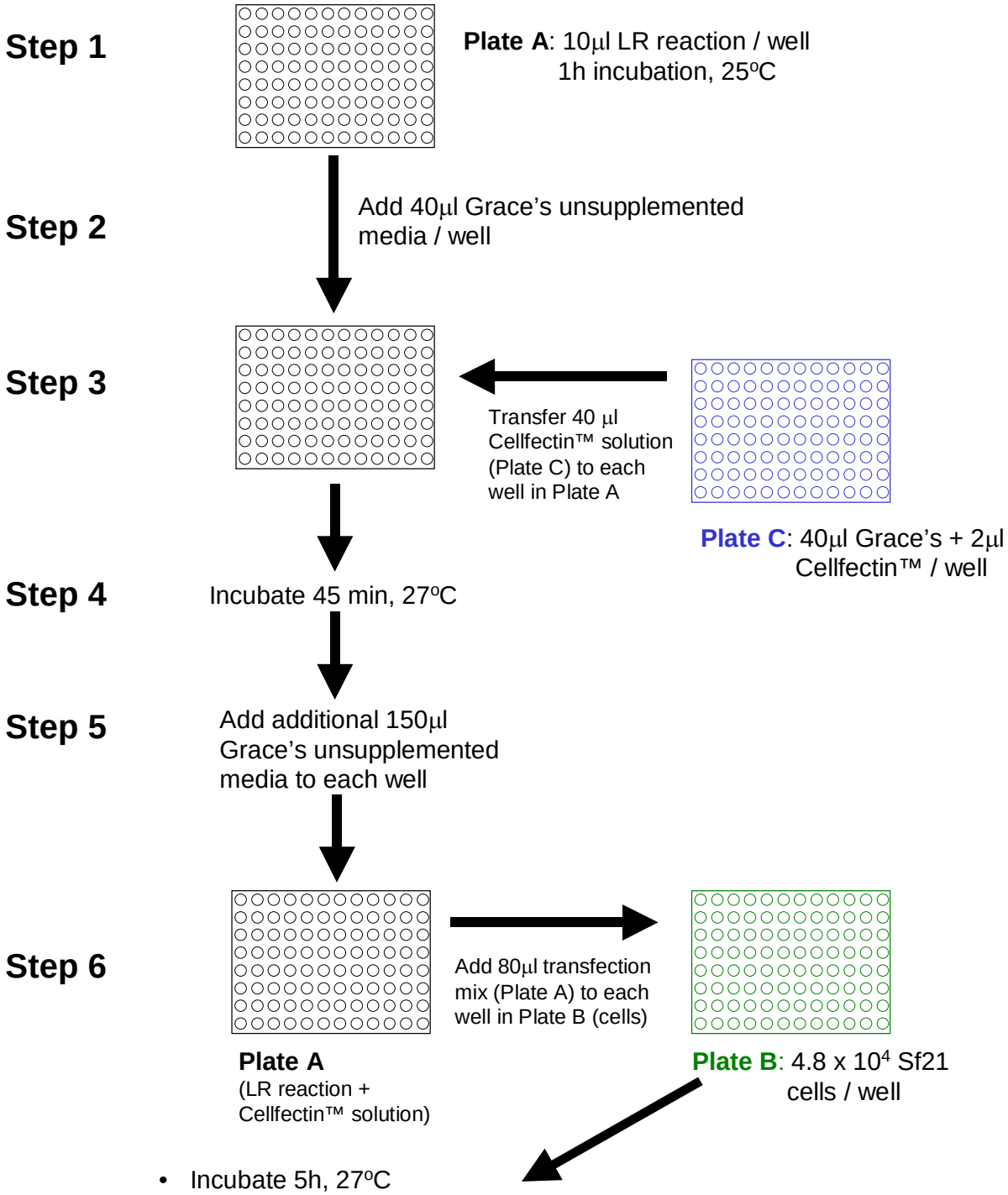
HTP Protocol for use with BaculoDirect™



- Incubate 5h, 27ºC
- Remove transfection mixture from cells, replace with Grace's complete medium + 100µM Ganciclovir
- Grow 3-4 days

High throughput (HTP) screening of expression :

Prepare three 96 well plates. In plate A, assemble 10 μl LR in individual wells. Each 10 μl reaction includes approximately 50 ng entry clone, 150 ng purified linear Baculodirect™ DNA, 2 μl LR clonase buffer, and 2 μl LR clonase. Incubate the LR reactions in the plates for 1 h at RT. During the LR incubation, seed Sf21 cells at 4.8 X 10^4 cells per well in a separate plate and allow cells to attach in plate B. In plate C, dilute 2 μl of Cellfectin to 40 μl per well with Grace's medium. After the 1 h LR reaction, add 40 μl of Grace's unsupplemented media to each well of plate A. Add 40 μl Cellfectin mixture from plate C to the diluted LR reactions and incubate at 27^{o}C for 30-45 min. After this incubation, add 150 μl of Grace's unsupplemented media to the wells of plate A. Wash the cells in plate B twice in Grace's media and then replace the media with 80 μl of the transfection mixture from plate A. Incubate Plate B for 5 h at 27^o C. Then remove the transfection mixture and replace with Grace's complete media with 100 μM ganciclovir. Grow the cells for 3-4 days. Transfer supernatants from each well to a separate plate. Lyse the cells remaining in plate A *in situ* with 100 μl LDS lysis buffer and heat to 80^{o}C for 5 min. Twenty microliters of each protein sample can be separated on NuPAGE gels, transferred to PVDF membranes and visualized by western blot.