Safe Operating Procedure (SOP)	School of Molecular & Biomedical Science
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Procedure: Experimental use of greater than genome length copies of the hepatitis B virus (HBV) genome for establishment of HBV replication in cell culture	
Approved by:	**Signature:**

PPE required: Gown, closed shoes, double glove & eye protection-Centrifuge & Spill Procedures may apply. **The following procedures involve the production of infectious hepatitis B virus (HBV). All personnel working with this material must be vaccinated and trained in the handling of this material.**

Materials

- Plasmid DNA containing greater than genome length HBV genome (e.g. pBlueBac4.5~wt1.3)
- Spectrophotometer.
- Cell culture reagents (media [DMEM and Opti-MEM {Invitrogen}], foetal bovine serum (FBS), L-glutamine, penicillin, streptomycin, trypsin/EDTA, plasticware, haemocytometer, trypan blue, inverted microscope, humidified 37ºC/5% CO_2 incubator, biosafety cabinet).
- Transfection reagents (FuGene6 Transfection Reagent [Roche])
- Benchtop centrifuge (refrigerated).
- 12.5% sodium hypochlorite solution.
- 80% (v/v) ethanol solution.
- Reagents for immunofluorescent staining of HBV antigens and inverted fluorescent microscope.
- Reagents for DNA extraction and Southern blotting.

Methods

HBV DNA transfection

1. Harvest near-confluent Huh-7 or HepG2 cells by trypsinization and re-seed into cell culture trays (e.g 6 well trays) at appropriate density such that cells are approximately 70% confluent the following day (e.g for 6 well trays seed Huh-7 cells at 2×10^5 cells/well).
2. Transfect cells with plasmid DNA containing greater than genome length HBV genome (e.g. pBlueBac4.5~wt1.3) using FuGene6 Transfection Reagent (Roche) according to manufacturer's instructions:
   i.e.: For each transfection of Huh-7 cells in 6 well trays add 100 μl of DMEM (no serum or antibiotics added) and 6 μl of FuGene6 Transfection Reagent. Mix gently by pipetting. Add 2 μg of plasmid DNA and mix gently by pipetting. Incubate tubes at room temperature for 15 minutes. Add transfection complexes to cells 'dropwise', while gently rocking trays.
3. Bund trays and return to culture for 24-96 h. **N.B. *Henceforth treat all samples as infectious; i.e. wear two pairs of latex gloves, full-length laboratory gowns and appropriate eye protection, do not use sharps (including glass pipettes), use only disposable single-use plasticware and treat all used plasticware by passing with 12.5% (w/v) sodium hypochlorite solution and treat unwanted culture fluids with sodium hypochlorite solution to >2.5% (w/v) (final***

concentration). Dispose of all used plasticware and containers of hypochlorite-treated culture media in autoclave bags, seal bags and transfer in locked biohazard 'wheelie' bins to outside containment area (lockable) for collection for incineration. Tubes and cell culture flasks/trays containing infectious material must only be opened in type II biosafetey cabinets unless contents are inactivated by treatment with fresh aqueous hypochlorite solution (≥2.5% w/v final concentration), the detergents Triton X100 (≥1% w/v final concentration), sodium dodecyl sulphate (≥1% w/v final concentration), n octyl glucoside (≥1% w/v final concentration) or DECON (≥10% v/v final concentration), fixed with acetone:methanol (1:1) for 15 minutes (cell monolayers) or inactivated with Trizol (Invitrogen; a monophasic solution of phenol and guanidine isothiocyanate used for isolating total cellular RNA). Culture vessels that do not have liquid-tight seals should be bunded where practical. After use work surfaces within type II biosafety cabinets must be swabbed with 1.25%(w/v) sodium hypochlorite solution and then 80% (v/v) ethanol solution. Closed biosafety cabinets should then further sterilized under UV light (~200 microwatts/cm^2 at 254nm). Hands must be washed before and after handling cell lines or viral preparations.

4. HBV replication and/or antigen expression can be monitored by quantification of intracellular/secreted HBV DNA levels (e.g by Southern blotting) or immunofluorescent labelling, respectively, following appropriate inactivation of samples using any of the above treatments.