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Method Article

Chimeric influenza haemagglutinins: Generation and use in pseudotype neutralization assays

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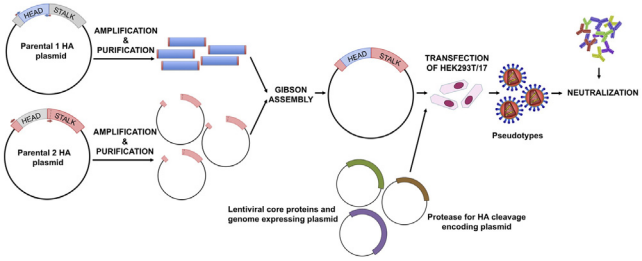
GRAPHICAL ABSTRACT



ABSTRACT

Recently chimeric influenza haemagglutinins (cHAs) have been generated as potential 'universal' vaccination antigens and as tools to identify HA stalk-directed antibodies via their use as antigens in ELISA, and virus or pseudotype-based neutralization assays. The original methods [1,2] used for their generation require the amplification of regions of interest (head and stalk) using primers containing *SapI* sites and subsequent cloning into pDZ plasmid. This requires precise primer design, checking for the absence of *SapI* sites in the sequence of interest, and multi-segment ligation. As an alternative strategy we have developed and optimized a new protocol for assembling the cHA by exploiting Gibson Assembly.

- This method also requires precise primer design, but it is rapid and methodologically simple to perform. We have evaluated that using this method it is possible to construct a cHA encoding DNA in less than a week.
- Additional weeks are however necessary to optimize the production of pseudotyped lentiviral particles and to perform neutralization assays using them as surrogate antigens.

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- In comparison to the original protocols, we have also observed that performing parallel neutralization assays using pseudotypes harbouring the two parental HAs, permits effective delineation between stalk and head antibody responses in the samples tested.

ARTICLE INFO
Method name: Chimeric influenza HA pseudotype production
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Materials and instruments

- Haemagglutinin-expressing plasmids (*parental 1* and *parental 2*)
- Haemagglutinin sequences (nucleotide and amino acid)
- Q5® High-Fidelity DNA Polymerase (New England Biolabs, cat. # M0491S)
- DreamTaq Green PCR Master Mix (Thermo Fisher Scientific, cat. # K1081 or K1082) (OPTIONAL)
- Gibson Assembly Cloning kit (New England Biolabs, cat. # E5510).
- FastDigest *DpnI* (Thermo Fisher Scientific, cat. # FD1703)
- FastDigest or conventional Restriction Enzymes (Thermo Fisher Scientific)
- RNAse/DNase free water
- Agarose
- Nucleic Acid Gel Stain
- Tris base, acetic acid and EDTA (TAE) Buffer
- GeneRuler™ 1 kb DNA Ladder Mix (Thermo Fisher Scientific, cat. # SM0314) or similar DNA Ladder
- Loading dye (Thermo Fisher Scientific, cat. # R0611) or similar loading dye
- QIAquick PCR Purification Kit (QIAGEN, cat. # 28104) or similar kits (OPTIONAL)
- QIAprep Spin Miniprep Kit (QIAGEN, cat. # 27104) or similar kits
- Luria Bertani (LB) agar plates with antibiotics appropriate to the plasmid used
- LB broth with antibiotics appropriate to the plasmid used
- Thermocycler
- Water bath or heating block
- Incubator at 37 °C
- Gel electrophoresis system
- Microwave (to dissolve agarose gel)
- Bioinformatics software for DNA and protein sequence and structure analysis

Cloning the chimeric haemagglutinin

Selection of haemagglutinin parental strains

Before proceeding to clone the chimeric HA (cHA), it is necessary to identify which HA subtypes/strains will be used to generate the cHA. There are different factors to take into consideration. Firstly, it is important to identify the final purpose of the project for which cHA are required. For example if human stalk-directed antibody responses are to be detected it is more appropriate to choose the head region of an HA subtype that is less related to circulating human influenza strains (i.e. H1 and H3) or other to strains that have been shown to infect humans (e.g. H5 and H7), such as H11 or H16. Furthermore, depending on the experimental requirements, selecting for the stalk an HA that is currently, or has previously circulated in humans may also be appropriate. This is extremely important since it permits the minimizing of detection of cross-reactive antibodies against the head, and the maximising of the identification of stalk-directed responses.