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Rapid and sensitive detection of infectious poxvirus particles

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Introduction

- In contrast to clinical samples, the replication competence of a virus is the crucial point for the risk assessment of environmental samples suspected to contain poxviruses.
- Real-time PCR is a sensitive tool, demonstrating the presence of short stretches of viral DNA and permits a rapid virus typing. However, PCR can not prove the replication competence of virus particles.
- This replication competence can only be shown by cell culture propagation of the virus which is time consuming.
- We combined the cell culture approach with rapid real-time PCR. To increase sensitivity and prove replication competence of the virus, we amplified poxvirus genes which are highly expressed during the first few hours of the infection cycle.

The Method

Varying amounts of orthopoxviruses were spinoculated on 15,000 Hep2 cells/well in a 96-well plate for 15 minutes at 1000 rpm. At defined times total RNA was prepared by standard procedures and directly subjected to one-step real-time PCR. Quantification of the gene expression was based on defined numbers of *in vitro* transcribed rpo18 and F1L RNA (figure 1).

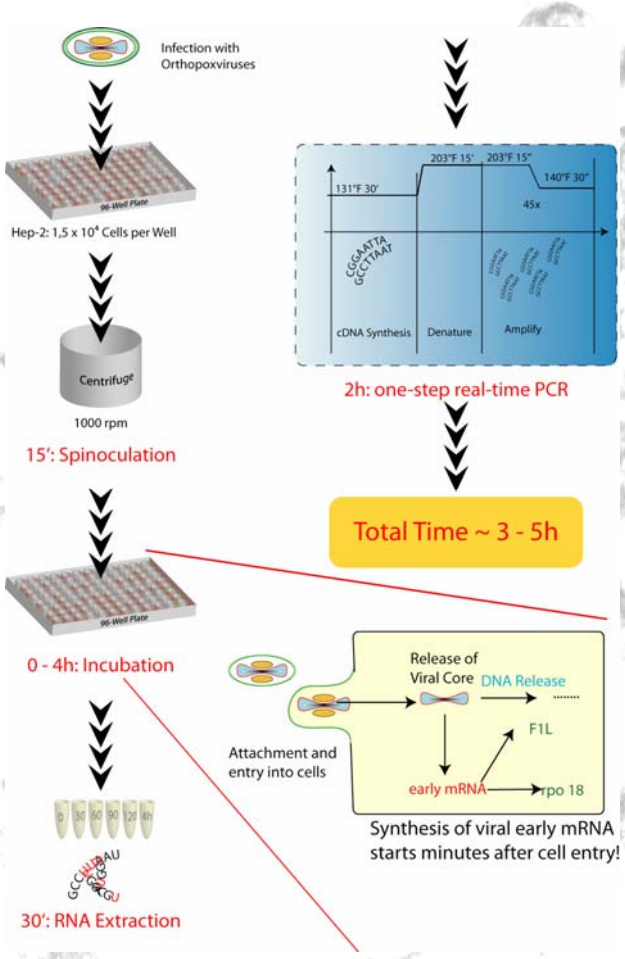

Figure 1: Schematically presentation of the detection procedure

Results

Infection of Hep2 cells with Vaccinia virus strain Lister Elstree (MOI=1) showed a rapid increase of only rpo18/F1L mRNA expression, but not of the corresponding DNA (figure 2).

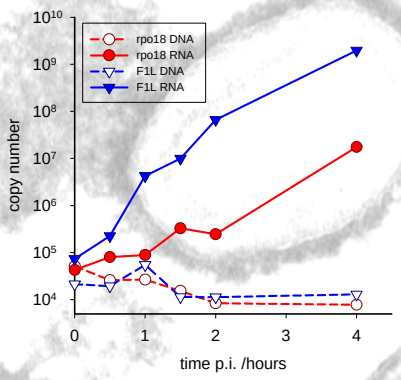

Figure 2: Comparison of the mRNA load and genomic DNA load for the genes rpo18 and F1L during 4 hours post infection.

A rapid increase of mRNA load was also observed after infection with further orthopoxviruses (figure 3).

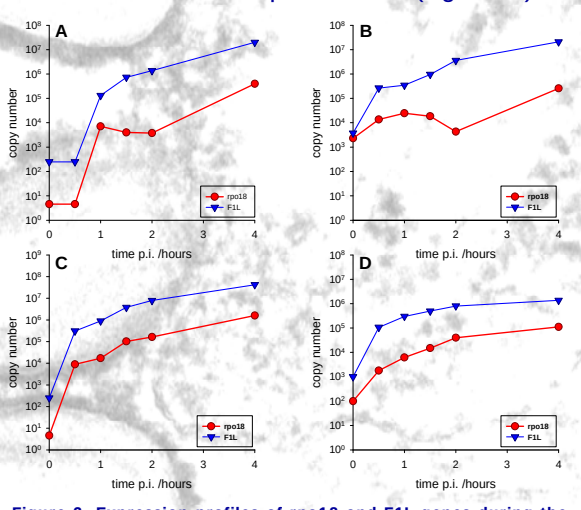

Figure 3: Expression profiles of rpo18 and F1L genes during the first 4 hours post infection. A: Camelpox CP-19, B: Cowpox 81/02, C: Ectromelia Nü, D: Monkexpox.

Probit analysis performed with decreasing MOI of Vaccinia virus revealed a detection limit of 5 PFU for the rpo18 gene and 3 PFU for the F1L gene only 2 hours p.i.

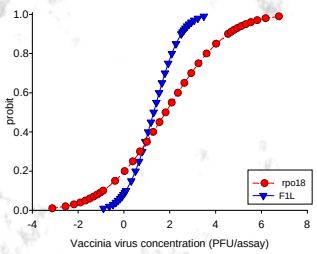

Figure 4: Statistical evaluation of the detection limit.

Conclusions

Benefits of the presented approach

- high sensitivity (3 PFU /assay) within 2h p.i.
  - allows multiple sample dilution
- speed
- detection of replication competent virus
- virus typing by subsequent FMCA or Pyrosequencing

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