dbDNA™ as a rapid and scalable solution to industrial lentiviral vector manufacturing

TOUCHLIGHT

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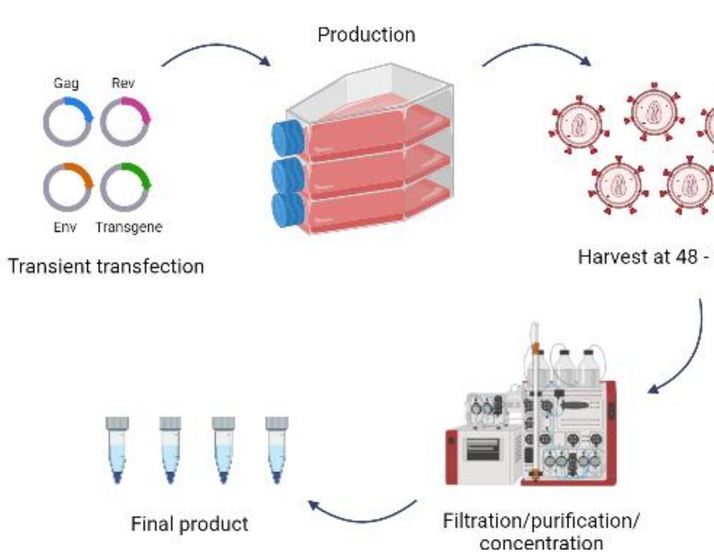
1 Abstract

Traditional bacterial fermentation techniques used to manufacture plasmid are time-consuming, expensive, and inherently unstable. The production of sufficient GMP grade material thus imposes a major bottleneck on industrial scale manufacturing of lentiviral vectors (LVV). Touchlight's linear doggybone DNA (dbDNA™) is an enzymatically amplified DNA vector produced with exceptional speed through an *in vitro* dual enzyme process, enabling industrial scale manufacturing of GMP material in a fraction of the time required for plasmid. Here, we demonstrate that dbDNA™ can be optimised for the manufacture of high titre LVV.

We found that dbDNA™ displayed a unique transfection and expression profile in the context of LVV production, necessitating a reduction in DNA input relative to plasmid of 30 - 50% to maximise titres. Addition of a strong termination signal and spacer sequence improved 3' end processing of viral genomic RNA (vgRNA), leading to more efficient vgRNA packaging and enhanced infectious titres. Using these improved vector architectures along with optimised transfection conditions, we produced a CAR19h28z LVV with equivalent infectious titres as achieved using plasmid, demonstrating that dbDNA technology can provide a highly effective solution to the plasmid bottleneck.

2 Introduction

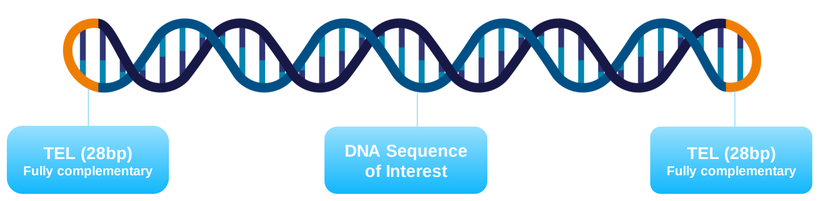
Lentiviral vector manufacturing



The predominant method for producing LVV is based on transient transfection of 3 core packaging plasmids and 1 plasmid encoding the gene of interest. Over a period of 48 – 72h, packaging cells produce functional viral particles which are then harvested from the cell culture medium.

One of the major challenges in LVV manufacturing is achieving high titres, due in part to the fragility of viral particles and the difficulty in maintaining high particle functionality through downstream processing. Producing the required titres of LVV to support clinical programs thus requires substantial starting material, and sourcing sufficient GMP grade plasmid to support industrial scale manufacturing represents a major bottleneck.

dbDNA™ as a solution to plasmid DNA

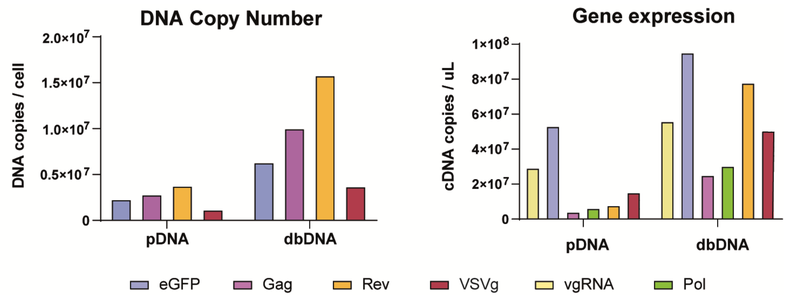


- Linear, double-stranded, and covalently closed DNA vector
- Enzymatically amplified with exceptional speed and small footprint
- No extraneous sequence or bacterial propagation elements

Plasmid DNA	dbDNA™
Long lead times • Lack of fermenter capacity • Long GMP timelines	**Rapid production** • Simple equipment and small footprint • Gram scale batches in days
DNA instability • Complex sequences unstable • Low yields, heterogeneity or batch failure	**High fidelity** • No instability with even highly complex sequences • Linear, reproducible yield
Antibiotic resistance • Bacterial propagation elements • Increased regulatory scrutiny	**Bacterial sequence free** • Smoother regulatory path • Improved safety

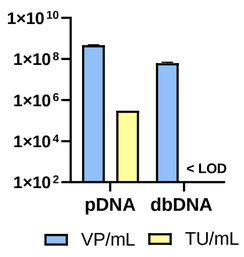
3 Results

Unique dbDNA transfection and expression profile



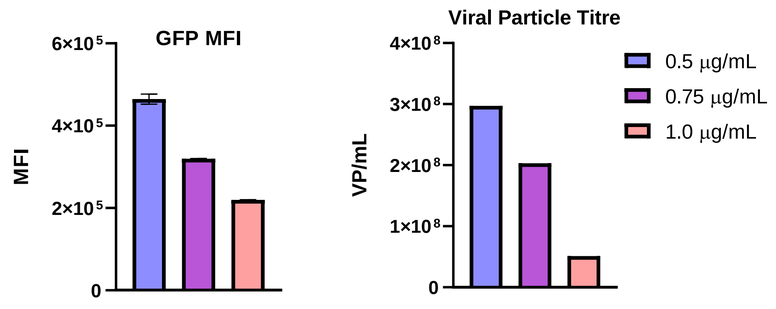
Cells were transfected with a 2:1:1:1 mass ratio (TG:GP:R:V) of pDNA, or the molar equivalent of dbDNA. DNA copy number and gene expression were analysed 72 hours post transfection by RT/qPCR

- Cells transfected with dbDNA contained 3-4-fold more DNA copies per cell than plasmid transfections, and transcript abundance was 2-10-fold higher.



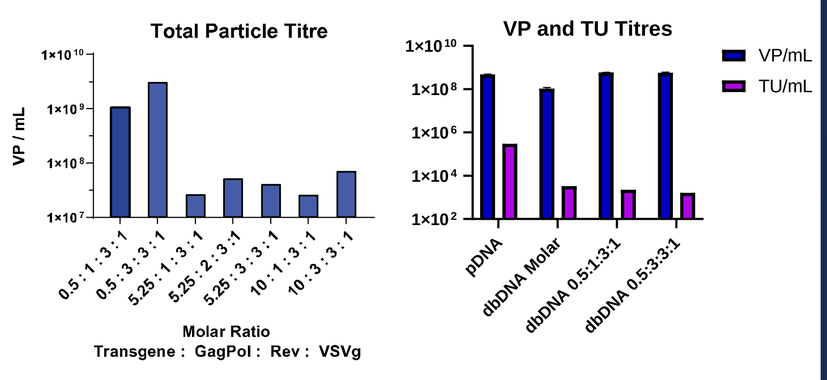
- Despite higher transfection efficiency, VP and TU titres were 10- and 1000-fold lower for dbDNA
- **DNA input and construct ratios require optimisation to accommodate unique transfection and expression profile**

Optimising transfection yields equivalent VP titres



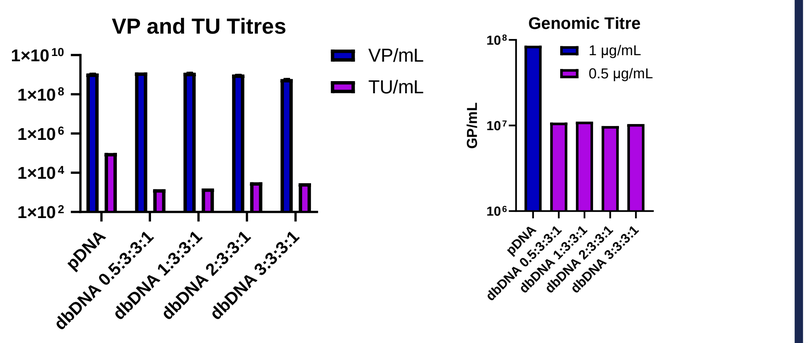
Cells were transfected to produce LVV, as described above, using a lentiviral transfect vector encoding EF1a-eGFP. Decreasing amounts of total input dbDNA were evaluated to determine the effect on transfer vector gene expression (MFI) in producer cells and total viral particle titre (VP/mL) 72 hours post transfection.

- Dose-dependent increase in MFI was observed upon reduction of total input dbDNA
- Correlated with a 6-fold increase in VP titres in samples transfected with 0.5 µg/mL versus 1.0 µg/mL



DoE analysis and high throughput optimisation of construct ratios was carried out in an automated bioreactor Ambr15 system to identify conditions for maximal VP titres. A subset of conditions are shown in the left panel. The 2 best conditions (0.5:1:3:1 and 0.5:3:3:1) were repeated in 50 mL shake flasks and evaluated for VP and TU titres relative to control conditions (pDNA 2:1:1:1 and dbDNA molar equivalent).

- Decreasing ratio of transfer vector correlated with increase in VP titre, irrespective of GagPol ratio
- Top 2 conditions (0.5:3:3:1 and 0.5:1:3:1) transfected using 0.5 µg/mL dbDNA yielded equivalent VP titres as pDNA transfected using 1.0 µg/mL
- However, TU titres still 100-fold lower than pDNA

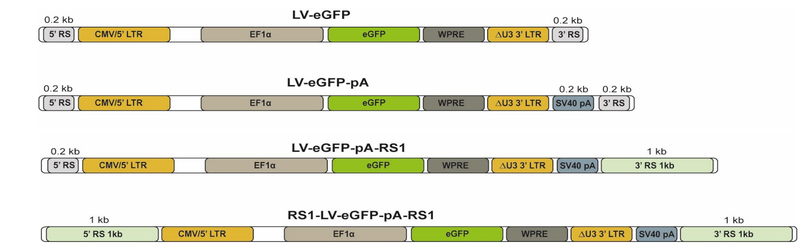


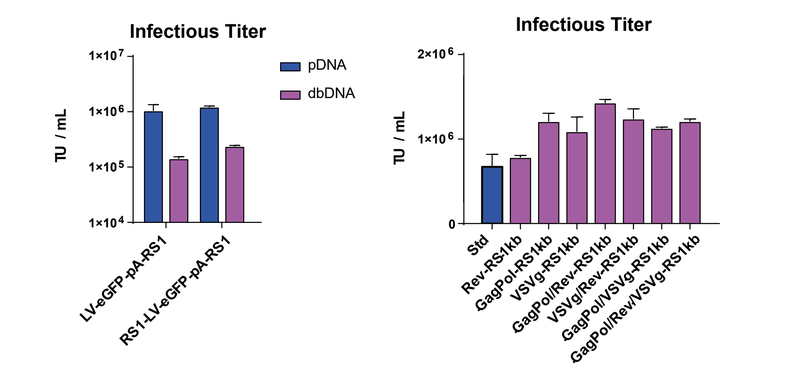
Cells were transfected using 1 ug/mL pDNA (2:1:1:1 mass ratio) or 0.5 ug/mL dbDNA using the shown molar ratios. Viral particle (VP), infectious (TU), and genome-containing particle (GP) titres were performed on raw lysates 72 hours post transfection.

- Increasing ratio of transfer vector has no effect on infectivity of dbDNA LVV
- Low infectivity correlated with 10-fold lower genome containing particles

3 Results

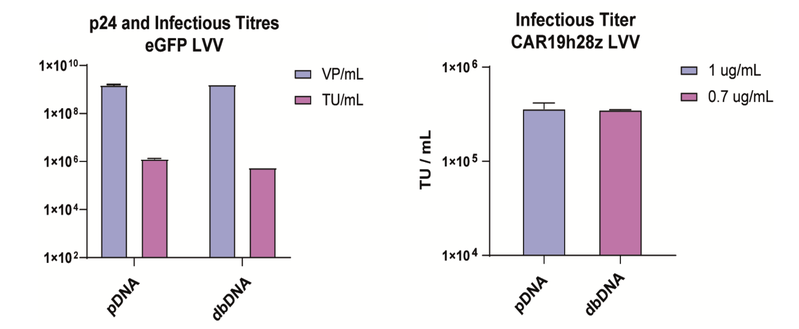
Optimising vector architecture rescues infectivity





In the left panel, productions were carried out using new transfer vector architectures and standard packaging constructs. In the right panel, productions were carried out with dbDNA only, using the RS1-LV-eGFP-RS1 transfer vector and different combination of accessory constructs containing the 3' RS1kb element.

- Strong poly(A) signal and 1 kb spacer sequence added downstream of 3' LTR to improve 3' end processing of vgRNA
- **RS1-LV-eGFP-pA-RS1 combined with GagPol-RS1kb and Rev-RS1kb yields up to 1.4 x 10^6 TU/mL**



- Further optimisation of total input DNA and construct ratios was carried out using optimised architectures

Productions performed using optimised transfection conditions and optimised dbDNA vector architectures yielded equivalent VP and TU titres for an eGFP LVV and CAR19h28z LVV using 70% of the DNA input required for plasmid

4 Conclusions

- **dbDNA is an enzymatic DNA vector produced through a rapid and linearly scalable process**
- **Short lead times, enhanced sequence fidelity, and elimination of bacterial elements make dbDNA an ideal vector for LVV manufacturing**
- **LVV production using dbDNA requires 50 – 70% of DNA input required for pDNA**
- **Transfection conditions need to be optimised for dbDNA. Recommended starting point:**
  - **0.7 µg/mL input dbDNA**
  - **PEIpro:DNA ratio of 3:1**
  - **Molar construct ratio of 4:1:2:1 (TG:GP:R:V)**
- **dbDNA lentiviral transfer vectors with 3' termination element and spacer sequences yield equivalent titres to plasmid**

5 Methods

Enzymatically amplified linear dbDNA™ as a rapid and scalable solution to industrial lentiviral vector manufacturing

Maria Barreira[1,3], Claire Kerridge[1], Sara Jorda[1,4], Didrik Olofsson[2], Alexander Neumann[2], Helen Horton[1] and Sarah Smith-Moore[1]✉

https://www.nature.com/articles/s41434-022-00343-4