Imaging Fluorescent Proteins *in vivo*
expressed from DNA vaccine constructs

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DNA vaccines have huge potential in clinical applications since they are cheap, easy to manufacture, and in animal studies have proved immunogenic and safe. However, clinical trials in the past have so far been disappointing. The optimisation of DNA delivery and expression may improve the immunogenicity in clinical trials. To improve DNA delivery it is necessary to improve the technology used to monitor its expression *in vivo*. One way of doing this is to use fluorescent proteins\(^\text{1}\). Green fluorescent protein is the most widely used, however it is ineffective *in vivo* due to absorbance characteristics of the skin. Far red fluorescent proteins could be used to circumvent this problem, allowing better imaging\(^\text{2}\). However, many of the studies reporting the use of these proteins have either injected cells expressing the proteins\(^\text{3}\), used recombinant virus\(^\text{4}\) or recombinant protein in an artificial mouse\(^\text{5}\). We were interested in optimising the conditions for visualising protein after DNA vaccination.
Material and Methods:
A plasmid expressing the red fluorescent protein tdTomato under a CMV promoter was obtained from Addgene (plasmid 30530 - pCSCMV:tdTomato)\(^6\). It was prepared for *in vivo* use with an EndoFree Plasmid Giga prep kit (Qiagen).

Female BALB/c mice were obtained from Harlan Scientific and used at 6-8 weeks of age. All procedures undertaken had been approved by the local ethics review board and performed by personal licensees under the appropriate project license. Experiments carried out were in accordance with Animals (Scientific Procedures) Act 1986. Prior to immunization mice were shaved and treated with depilatory cream in the area of interest.

50 μg DNA was delivered into mice either intramuscularly (I.M.) or subcutaneously (S.C.), with or without electroporation, which has been shown to increase the expression of DNA vaccines\(^7\). Where used electroporation was performed with two lots of 5 pulses of 150 V with switched polarity between pulses were delivered using a CUY21 EDIT system (BEX, Japan). Electroporation was delivered using a fixed gap needle electrode (LF560S5) inserted into the muscle after the injection.

Mice were imaged at excitation 550 nm and emission 600 nm using the Bruker In-Vivo MS FX PRO system (Bruker BioSpin, MA, USA). Images were obtained with 1x1 binning, f-stop 2.8 and 60 second acquisition time. To avoid autofluorescence at the imaging site, mice were initially shaved and depilated (Immac Cream, Veet). Image analysis was carried out using the Bruker Molecular Imaging Software Suite.

Results and Discussion
Initially we wished to determine the effect of electroporation on signal intensity after immunisation. We discovered a number of sources of noise that can detract from signal, these include the animal faeces and urine, the fur and the indelible marker pen we use to differentiate animals (Figure 1). We used a number of approaches to reduce this background noise, including shaving/depilating the mouse and the use of ear punch to identify mice.

Having reduced the noise signal we then compared expression of tdTomato from plasmid DNA delivered with and without electroporation. It has been shown that electroporation can significantly enhance the expression of DNA vaccines and we wished to determine whether it could affect expression of fluorescent proteins *in vivo*.

![Image](image-url)

Figure 1. Electroporation (EP) significantly enhances intensity of fluorescent protein expression *in vivo*. (A) Mice injected subcutaneously on their backs with 50 μg tdTomato with or without electroporation (EP) and imaged over 72 hours (B) Mice injected subcutaneously in the central epigastric region with 50 or 100 μg tdTomato with or without electroporation. Red circle indicates the region of fluorescent protein expression.
Electroporation significantly enhanced the signal observed and there appeared to be no difference in expression between the 50 and 100 μg groups. So for future studies we used 50 μg with electroporation.

The other aspect of DNA vaccination we were interested in was the route of immunisation. We compared subcutaneous immunisation with intramuscular immunisation and observed that when successful, intramuscular immunisation gave a stronger signal, but was less reliable overall than subcutaneous immunisation (Figure 2).

**Conclusion**

We showed that tdTomato-encoding DNA could be efficiently delivered via electroporation, and thus provide a tool for *in vivo* vaccination with DNA. However, the use of red fluorescent proteins *for in vivo* imaging of delivered DNA is challenging. There are a number of sources of auto-fluorescent noise that can occur within the experiment even within the red spectrum. Careful preparation of the animals and strongly expressing/fluorescing proteins can reduce this problem, but not completely mitigate it. The recent availability of further red emitting fluorescent proteins like the IFP or mKate2 might further improve *in vivo* imaging capabilities.

![Figure 2. Comparison of routes of immunisation. 5 BALB/c mice were intramuscularly (I.M.) (A) and subcutaneously (S.C.) (B) immunised with electroporation with 50 μg of tdTomato plasmid. Imaging was carried out at 48 hours and 72 hours at excitation= 550 nm and emission= 600 nm.](image-url)
References