Barts and The London School of Medicine and Dentistry
GenomeCentre

Technical Note

Title; The effect of Template and Primer Concentration on Sanger Sequence Quality- Guide to maximizing sequence quality

Tech note version no.: 1.0

Importance of template and primer concentration for sequencing success

Successful Sanger sequencing like all reactions, depends on the correct concentration of reactants – crucially that of the template and primers. Using the wrong concentration of template will result in substandard data.

The Genome Centre has optimised the sequencing reaction so that it works well for most templates. However, the reaction is still sensitive to template concentration, especially with plasmids (PCR concentration is somewhat more forgiving as required read lengths are typically shorter). We ask for your plasmid preparations at 100ng/µl which is the optimum concentration for full read length of your insert. Primers should be submitted at 10pmol/µl (10µM).

Plasmids and/or primers that are well above these concentrations can have reduced sequencing quality – "top-heavy sequencing", where peaks begin strong but are followed by a rapid decrease in intensity, resulting in short reads, or high background intensities resulting in mixed bases. Contrast Fig1 and Fig2 for examples.

Fig 3 shows the quality score distribution for three plasmids sequenced at 100ng and 200ng template. While all three samples sequenced from 100ng show phred scores >30 (probability of a mis-called base 1 in 1000) out to 600+bp the same samples sequenced with 200ng show high quality reads only up to 200bp.

Plasmid samples for Sanger sequencing should be quantified before submission and diluted to 100ng/µl. This information should be included on a fully completed submission form. It is important that all sample information fields on the submission form are completed accurately. Sequencing fails that occur due to misinformation will <u>not</u> be repeated free of charge. *Note: Plasmids of a lower concentration may still be sequenced with enough read length, but this is at users' own risk, and full sequencing is not guaranteed.*

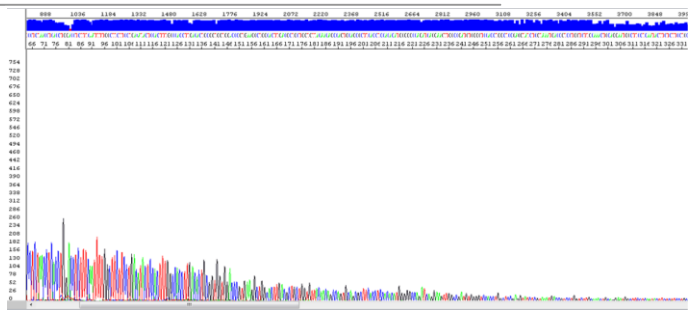

Fig 1 – Too much template -high signal intensity followed by rapid decrease and eventual stop – low sequence quality.

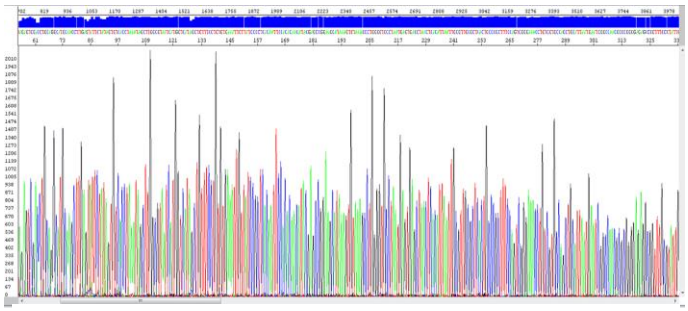

Fig 2 – Optimal template – high, even, signal intensity, no mixed bases, no signal drift

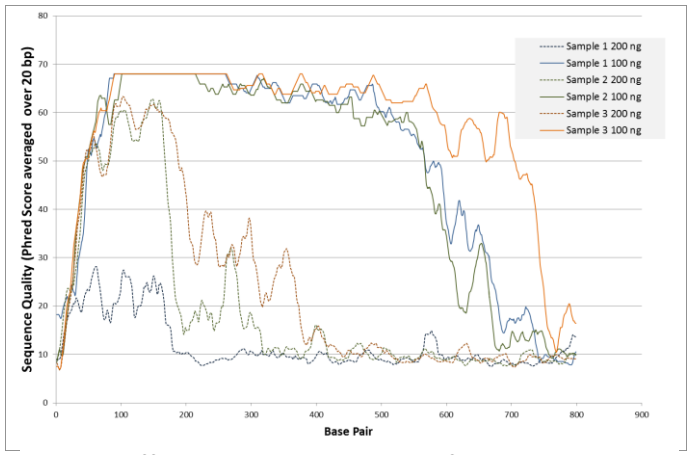

Fig 3 – effect on sequence quality for three plasmid templates

Created;	E Wozniak	Created on	30/11/2012 15:37
Reviewed and Authorised;	C. Mein	Issued on	13/03/2013
		Review on	01/03/2013