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Size Selective Precipitation of DNA using PEG & Salt

In 1 collection

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External link: https://www.longreadclub.org/mountain-protocol/

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Protocol status: Working
We use this protocol and it's working

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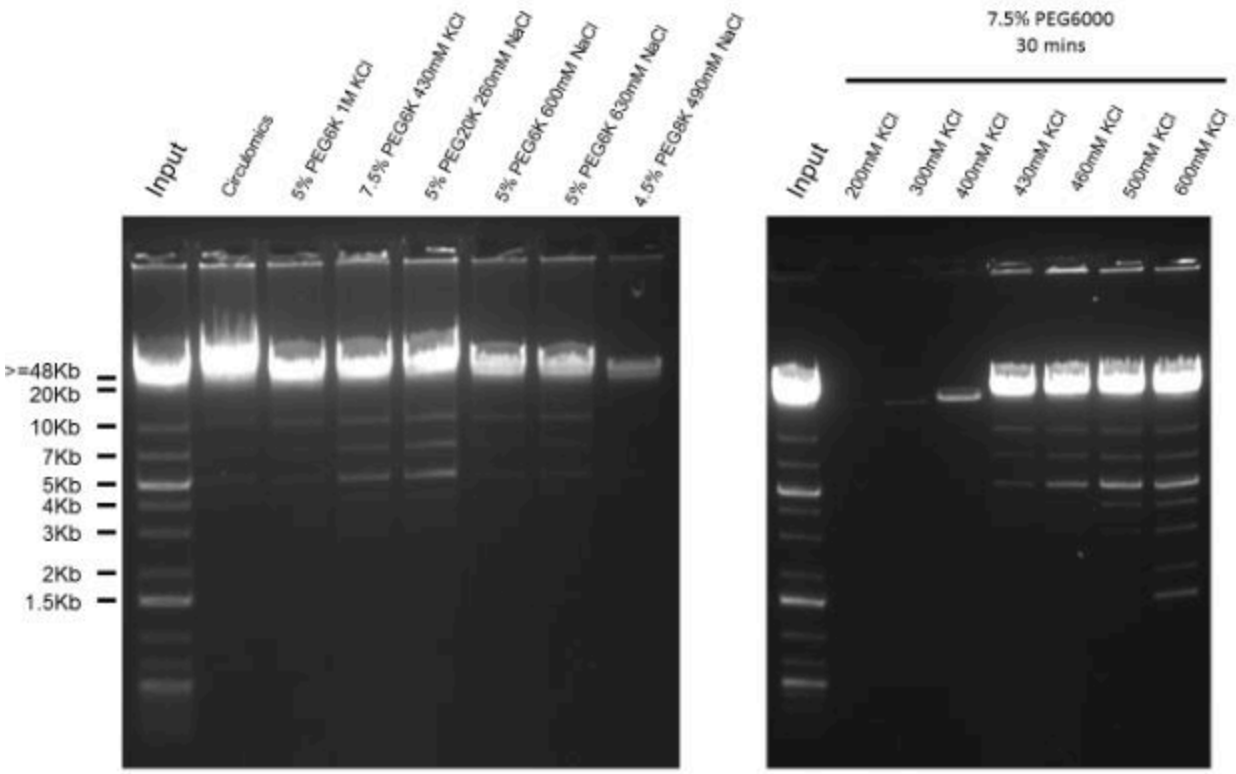
Abstract

Size Selective Precipitation of DNA using PEG & Salt

We and others have been thinking about the possibility of providing DNA size selection / clean up / and removal of expensive reagents in the library preparation process for a little while now. There are a number of possible routes but we have focused more recently on Polyethylene Glycol (PEG) and salt precipitation of DNA as it is cheap, non-toxic and known to be compatible with the existing library preparation process. One of the early demonstrations of combining PEG and salt to selectively size fractionate DNA by selective precipitation was by Lis & Schleif in 1975 (Size fractionation of double-stranded DNA by precipitation with polyethylene glycol. NAR 2:(3) p383). We have been screening different sizes and concentrations of PEG combined with different salt amounts to find suitable combinations for removal of short “contaminating” DNA fragments. This is useful for situations where you are not starting with a DNA sample of HMW and want to target the longest DNA strands you have or want to try and remove shorter fragments produced during a preparation.

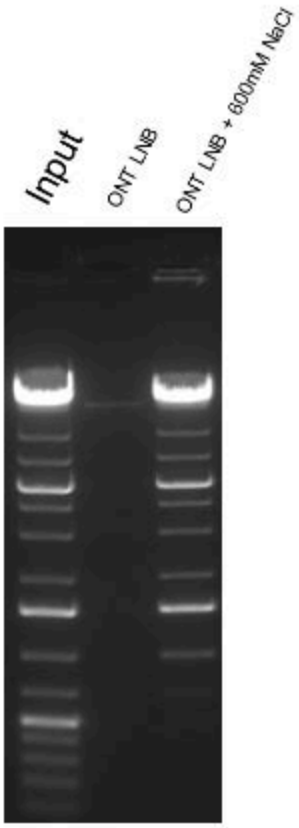
Shown below are some of our current best combinations but the hunt goes on, with changes to type and concentration of PEG, and different cations with different charge densities. The commercially available Circulomics buffers perform a similar task, and are performing a little better at this stage. We do not know what exactly has been used in their solutions.



Mix of Rat 26G (5 pass) sheared genomic, Lambda & Ladder (89ng/ul) 30ul + 30ul PEG buffer, 30min RT and 30 min spin @13.5Krpm, 2x 70% EtOH wash, then DNA into 30ul EB

Lambda & Ladder (100ng/ul) 30ul + 30ul PEG buffer, 30min RT and 30 min spin, 2x 70% EtOH wash, into 30ul EB

One advantage of knowing that PEG/NaCl can be used in this fashion came with the realisation that given ligation buffers often use high PEG concentrations to crowd the DNA fragments we could probably use straight NaCl addition into the ligation reaction once complete to precipitate our adapted DNA. This is shown below with testing on a Lambda+DNA ladder sample and the ONT LNB buffer +/- 600 mM NaCl (There is also the hint there that some level of HMW DNA is even coming out of solution without the salt addition.....). This idea together with PEG/NaCl precipitation when coming out of the LSK109 library End-Prep reaction was taken and used to develop the Bead-free LSK109 ligation prep for ultra-long DNA detailed below in the next section.



NaCl addition to ONT LNB buffer allows
Precipitation of DNA by spinning down

If people feel so inclined I can provide further details on the current matrix of PEG size / concentrations and salt type / concentrations we have looked at so far and then we can perhaps speed up the search. There are obviously other possibilities that can be explored around size selection using charged polymers and higher charge density ion induced precipitation. Only so many hours in the day :o)).

Safety warnings

Please see SDS (Safety Data Sheet) for hazards and safety warnings.