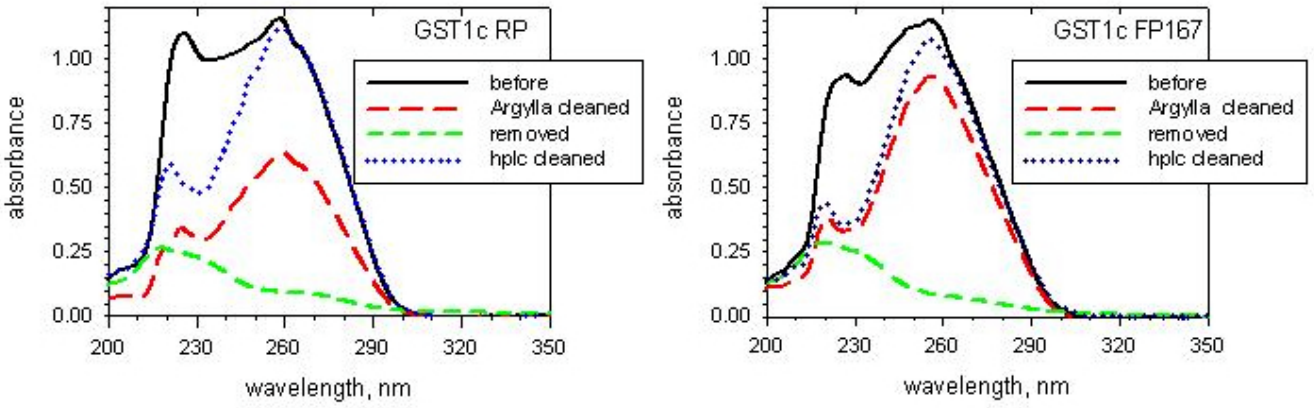
Argylla PrepParticles Clean up of PCR Primer Oligonucleotides

Many laboratories, including the Argylla R&D laboratory, use PCR primer oligonucleotides as supplied by the vendor. Although minor contaminations generally do not affect PCR, sometimes we require the extra purity and are willing to pay for HPLC purification. In other cases it is just convenient to have Argylla PrepParticles® handy on an "as needed basis"

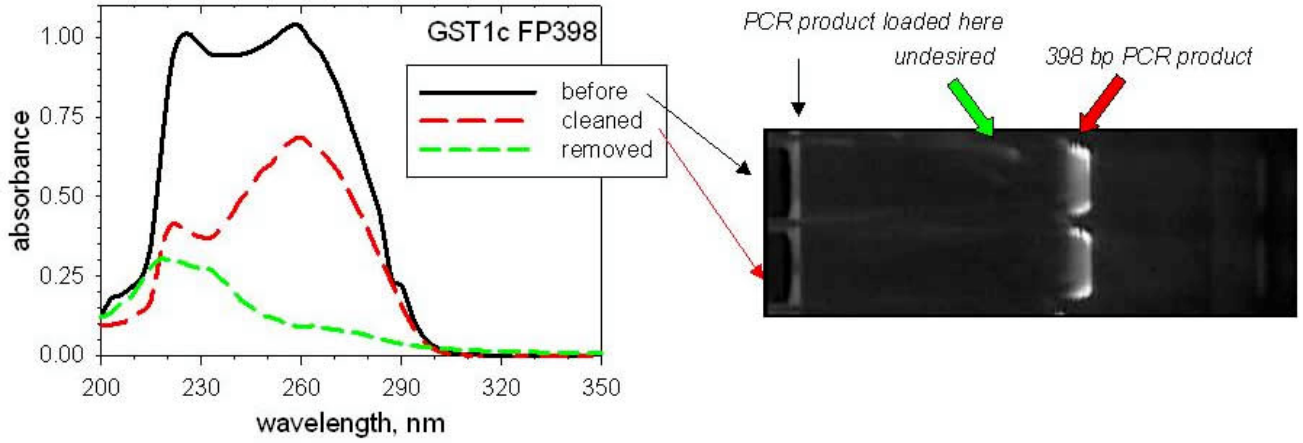
These are some of the glutathione S transferase 1p primers we use in R&D.

GST1c REV 5' - ctc aaa agg ctt cag ttg cc - 3'
GST1c FOR 167 5' - gga gca agc aga gga gaa tc - 3'
GST1c FOR 330 5' - ggc tgt gtc tga atg tga gg - 3'
GST1c FOR 398 5' - cga agg cct tga acc cac t - 3'

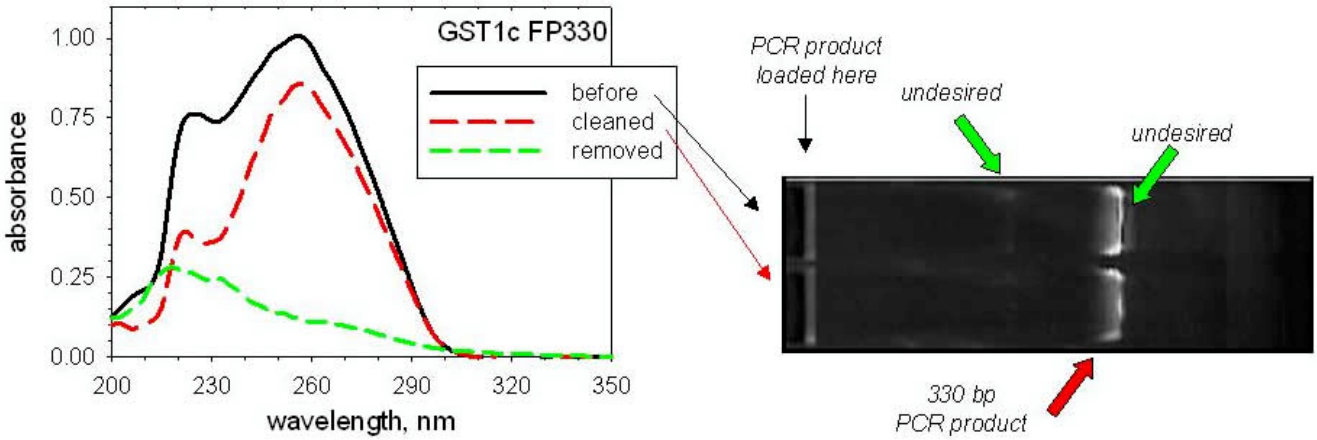


The starting "unclean" oligonucleotides (thick black line) were reconstituted such that they had about the same 260nm absorbance as the HPLC purified oligonucleotides (dark blue dotted line). The supernatants from the initial isopropanol precipitation and the subsequent ethanol/saline rinse were pooled and evaporated to the same volume as the samples (green short dashed line). The identity of the removed material was not determined. It is tempting to speculate that we removed unincorporated nucleotides (260nm). In spite of the rather alarming 260/230nm ratio, the "standard desalted" GST1c FP167 and GST1c RP pair delivered a single 167 bp amplicon (not shown). In this particular case, neither the HPLC purification (blue dotted line) nor the Argylla PrepParticle purification (medium red dashed line) were necessary. In other PCR reactions with "unclean" oligonucleotide primers, aberrant PCR products were observed.

For GST1c FP398 of our GST1c multiplex system, "cleanliness" did have a slight influence on the PCR product. Argylla PrepParticles ® were used to clean a 25μL aliquot of the reconstituted "standard desalted" 398 bp primer. Arrows point to aberrant products seen with the "standard desalted" forward primer.



The spectra demonstrate that standard desalted GST1c forward primer (FP) still contains a large amount of impure material (black trace) that can be removed using Argylla PrepParticles (green short dashed trace) yielding more pure primers (red medium dashed trace). More pure primer resulted in a purer 398 bp PCR product (red arrow) and less undesired material (green arrow) Line arrows link the legend on the UV absorption spectrum graph to lanes on the 2.2% agarose gel that was used to resolve the PCR products from contaminating material.



The spectra demonstrate that standard desalted GST1c forward primer (FP) still contains a large amount of impure material (black trace) that can be removed using Argylla PrepParticles (green short dashed trace) yielding more pure primers (red medium dashed trace). More pure primer resulted in a purer 398 bp PCR product (red block arrow) and less undesired material (green block arrow). Line arrows link the legend on the UV absorption spectrum graph to lanes on the 2.2% agarose gel that was used to resolve the PCR products from contaminating material.

When analyzing RP and FP 330 bp pair of our GST1c multiplex system we discovered that a relatively “clean” appearing GST1c FP330 primer yielded two aberrant products marked by the green block arrows. Cleanup of a small aliquot of the reconstituted “standard desalted” GST1c FP330 primer with our PrepParticles® improved this situation.

For more information, see Oligonucleotide Protocol at www.argylla.com/protocols