KLD Mutagenesis Fragment Prep

1) Prepare PCR with:
   - Highly accurate polymerase (Q5 or equivalent)
   - Long plasmids? GC Enhancer Buffer VERY useful
   - LOW template amount (0.1 to 0.5 ng plasmid/25 uL PCR reaction) -> Make it easier for the DpnI

2) Thermocycling settings:

Initial Denaturation:
95°C for 90 seconds

30 Cycles of:
95°C for 30 seconds
50–67°C for 30 seconds
72°C for 30 seconds/kb

Weak band?
Bump up to 35-40 cycles
More cycles -> More non-specific bands

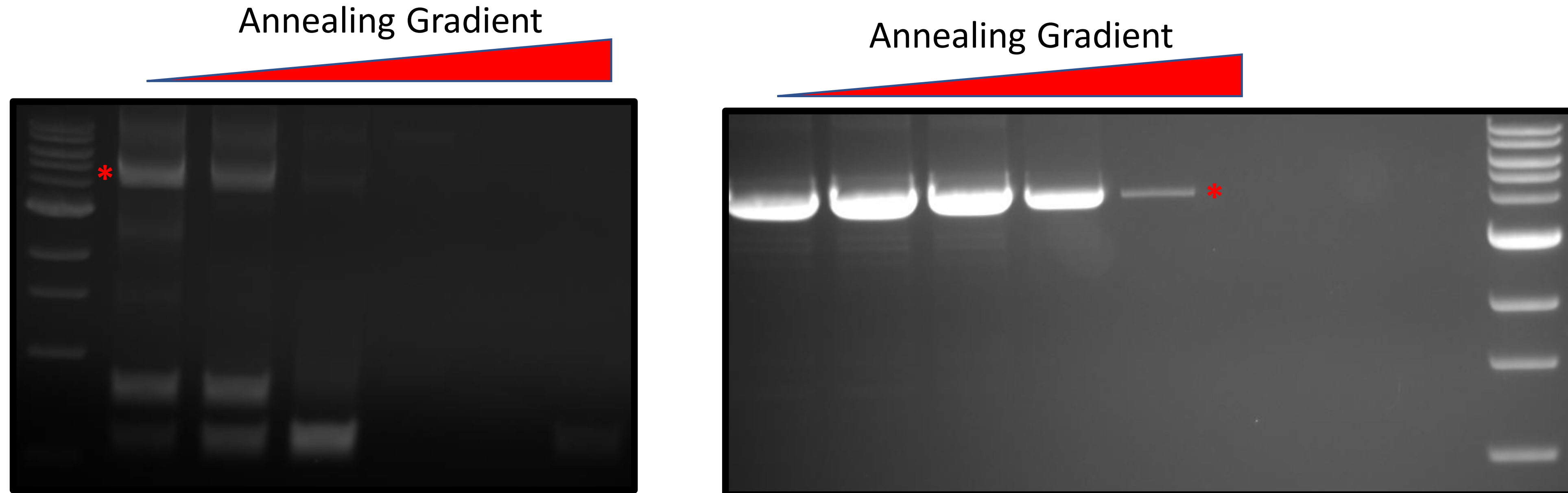
Have a gradient PCR machine?
4-6 reactions from 55-67 °C
No gradient PCR?
Start at 59 °C and pray

Final Extension:
72°C for 2X Cycling Extension Time
END

LET THE PCR MACHINE COAST TO ROOM TEMP!
PCR products can survive for DAYS at RT
Your thermocycler is not a refrigerator!

KLD Mutagenesis QC

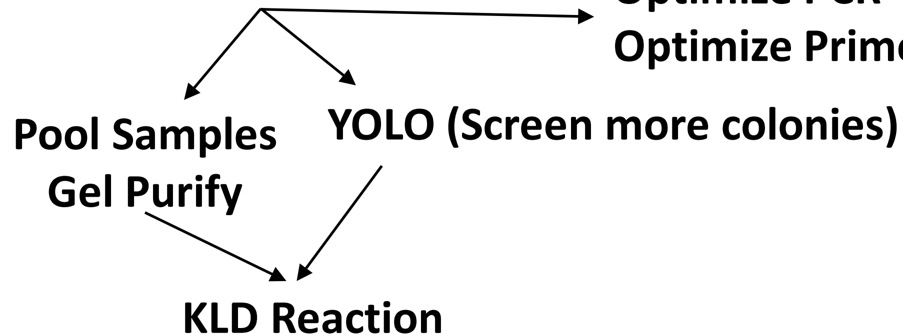
1) Run 5-8uL of PCR product on a 1% agarose
2) Do I have a product of expected size? (*)
3) Is the PCR product pretty clean?

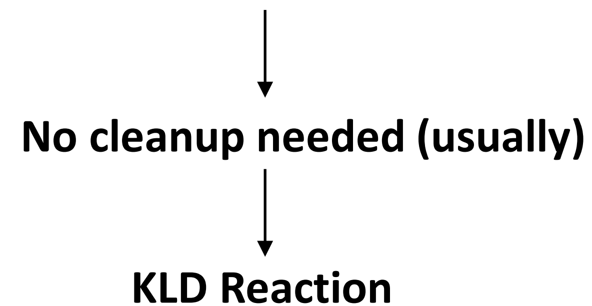


Expected product + other bands?

Optimize PCR
Optimize Primers

Noice and clean?





KLD Reaction

- NEB’s KLD mix -> Nice, but $$$
- Homemade KLD mix -> Works great, assemble yourself (Thank you msr2009/reddit for recipe)

1ul PCR product or gel purified band (5-10ng total should do it)
1ul 10X T4 DNA ligase buffer
1ul T4 PNK
1ul T4 DNA ligase
1ul DpnI
5ul H2O

10uL total
Incubate at RT for 1 hour, transform 1uL of reaction/100uL comp. cells

- That’s…it? If you’ve got a decent PCR product KLD reactions are pretty smooth.