# Team3ispA/ispA: PCR

**protocol:** PrimeStar **program:** PG2K55 **thermocycler**:

|  |  |  |  |
| --- | --- | --- | --- |
| **source:**  *label* | *construct* | *concentration* | *location* |
| sFispA-F dil | sFlexneri | 10uM | Box\_Team3ispA/A3 |
| sFispA-R dil | sFlexneri | 10uM | Box\_Team3ispA/A4 |

|  |  |  |  |
| --- | --- | --- | --- |
| Sf\*  yPispA-F dil  yPispA-R dil  Yp\*  sMispA-F dil  sMispA-R dil  Sm  citrispA-F dil  citrispA-R dil  Cb\*  pAispA-F dil  pAispA-R dil  Pa\* | sFlexneri  yPseudo  yPseudo  yPseudo  sMarc  sMarc  sMarc  citrob  citrob  citrob  pAerug  pAerug  pAerug | miniprep  10uM  10uM  miniprep  10uM  10uM  miniprep  10uM  10uM  miniprep  10uM  10uM  miniprep | genomic\_dnas1/C3  Box\_Team3ispA/B3  Box\_Team3ispA/B4  genomic\_dnas1/F2  Box\_Team3ispA/C3  Box\_Team3ispA/C4  genomic\_dnas1/H5  Box\_Team3ispA/D3  Box\_Team3ispA/D4  genomic\_dnas1/D1  Box\_Team3ispA/E3  Box\_Team3ispA/E4  genomic\_dnas1/E4 |

Tubes marked with \* are actually unlabeled but they should be in the location given on the LabSheet

|  |  |  |  |
| --- | --- | --- | --- |
| **samples:** |  |  |  |
| *label* | *primer1* | *primer2* | *template product* |

|  |  |  |  |
| --- | --- | --- | --- |
| ispA1  ispA2  ispA3  ispA4  ispA5 | sFispA-F dil yPispA-F dil  sMispA-F dil  citrispA-F dil  pAispA-F dil | sFispA-R dil  yPispA-R dil  sMispA-R dil  citrispA-R dil  pAispA-R dil | Sf sFlexneri\_ispA  Yp yPseudo\_ispA  Sm sMarc\_ispA  Cb citrob\_ispA  Pa pAerug\_ispA |

|  |  |  |  |
| --- | --- | --- | --- |
| **Reaction:** |  |  |  |
|  | 32 uL | ddH2O | W |
|  | 10 uL | 5X PrimeSTAR GXL Buffer | B |
|  | 4 uL | PrimeSTAR dNTP Mixture (2.5 mM each) | D |
|  | 1 uL | 10uM primer 1 | F |
|  | 1 uL | 10uM primer 2 | R |
|  | 1 uL | dil20x plasmid template | T |
|  | 1 uL | PrimeSTAR GXL DNA Polymerase | P |
| **Notes:** |  |  |  |

* Do only one thermocycler run for your section
* Never let enzymes warm up! Only take the enzyme cooler out of the freezer when you are actively using it, and only take the tubes out of it when actively dispensing. Hold the enzyme tube by the top of the tube while dispensing and do not place it in a rack.

# Team3ispA/back: PCR

**protocol:** PrimeStar **program:** PGXL4 **thermocycler**:

## source:

*label construct concentration*

*location*

o1 dil o1 10uM Box\_Team3ispA/F3

o2 dil o2 10uM Box\_Team3ispA/F4

T1 pLYC73S dil20x Terp1 /C2

## samples:

*label primer1 primer2 template product*

back1o1 dil o2 dil T1 pLYC73S backbone

|  |  |  |  |
| --- | --- | --- | --- |
| **Reaction:** |  | | |
|  | 32 uL | ddH2O | W |
|  | 10 uL | 5X PrimeSTAR GXL Buffer | B |
|  | 4 uL | PrimeSTAR dNTP Mixture (2.5 mM each) | D |
|  | 1 uL | 10uM primer 1 | F |
|  | 1 uL | 10uM primer 2 | R |
|  | 1 uL | dil20x plasmid template | T |
| **Notes:** | 1 uL | PrimeSTAR GXL DNA Polymerase | P |

* Do only one thermocycler run for your section
* Never let enzymes warm up! Only take the enzyme cooler out of the freezer when you are actively using it, and only take the tubes out of it when actively dispensing. Hold the enzyme tube by the top of the tube while dispensing and do not place it in a rack.