# 1 $\lambda$ DNA

## 1.1 Materials

1. N6 Methyladenine Free $\lambda$ DNA (NEB N3013)
2. 5' biotinylated oligonucleotide: 5'-AGG TCG CCG CCC-3'-[biotin] (IDT)
3. 3' digoxigenin labeled oligonucleotide: 5'-GGG CGG C-3'-[digoxigenin] (IDT)

**Caution:** To prevent shearing $\lambda$ DNA, always cut pipette tip to wider bore and pipette slowly. Never pipette up and down to mix reagents. Mix reagents by tapping and gently stirring with pipette tip.

## 1.2 Procedure

1. Phosphorylate each type of functionalized oligonucleotides by mixing the following and incubate at 37°C for 1 hr:

| **Reagent** | **Concentration** | **Volume ($\mu$L)** |
|---|---|---|
| MiliQ water | | 15 |
| T4 DNA ligase buffer | 10x | 2 |
| T4 PNK | 10 U/$\mu$L | 1 |
| Functionalized oligo | 100 $\mu$M | 2 |
| **Total ($\mu$L)** | | **20** |

2. Anneal biotinylated oligo to $\lambda$ DNA by mixing the following in a 1.6 mL eppendorf tube. Slowly cool the mixture from 65°C to room temperature.

| **Reagent** | **Concentration** | **Volume ($\mu$L)** |
|---|---|---|
| MiliQ water | | 416 |
| T4 DNA ligase buffer | 10x | 50 |
| Phosphorylated biotinylated oligo | 10 pmol | 1 |
| $\lambda$ DNA | 0.5 pmol | 33 |
| **Total ($\mu$L)** | | **500** |

3. Ligate biotinylated oligo to $\lambda$ DNA by mixing the following and incubate at room temperature for 2 hrs:

| **Reagent** | **Concentration** | **Volume ($\mu$L)** |
|---|---|---|
| Annealed $\lambda$ DNA from previous step | | 500 |
| T4 DNA ligase | 400 U/$\mu$L | 2 |
| ATP | 0.2 M | 2.5 |
| **Total ($\mu$L)** | | **504.5** |

4. Anneal digoxigenin labeled oligo to biotinylated $\lambda$ DNA by mixing the following and slowly cool the mixture from 65°C to room temperature.

| **Reagent** | **Concentration** | **Volume ($\mu$L)** |
|---|---|---|
| Biotinylated $\lambda$ DNA from previous step | | 504.5 |
| Phosphorylated digoxigenin labeled oligo | 100 pmol | 10 |
| **Total ($\mu$L)** | | **514.5** |

5. Ligate digoxigenin labeled oligo to biotinylated $\lambda$ DNA by mixing the following and incubate at room temperature for 2 hrs:

| **Reagent** | **Concentration** | **Volume ($\mu$L)** |
|---|---|---|
| Annealed $\lambda$ DNA from previous step | | 514.5 |
| T4 DNA ligase | 400 U/$\mu$L | 2 |
| ATP | 0.2 M | 2.5 |
| **Total ($\mu$L)** | | **504.5** |

6. Store labeled $\lambda$ DNA at 4°C.

# 2 1 kb DNA

## 2.1 Materials

1. M13mp18 RF I DNA (NEB N4018)
2. Forward primer: [amino]-5'-TAT TGC GTT TCC TCG GTT TCC-3'
3. Reverse primer: [biotin]-5'-TTG AAA TAC CGA CCG TGT GAT A-3'

## 2.2 Procedure

1. Set up 10 - 20 of the following 50 $\mu$L reactions in thin-walled PCR tubes:

| **Reagent** | **Concentration** | **Volume ($\mu$L)** |
|---|---|---|
| MiliQ water | | 34.5 |
| Taq buffer | 10x | 5 |
| M13mp18 DNA | 10 ng/$\mu$L | 0.25 |
| Taq polymerase | 5 U/$\mu$L | 1.25 |
| dNTP mix | 2.5 mM | 4 |
| Forward primer | 10 $\mu$M | 0.5 |
| Reverse primer | 10 $\mu$M | 0.5 |
| $MgSO_4$ | 100 mM | 4 |
| **Total ($\mu$L)** | | **50** |

2. Set up the following sequence in a thermocycler:

| Step | Description | Time (min) | Temp ∘C |
|---|---|---|---|
| 1 | Initial denaturation | 1:00 | 95 |
| 2 | Denaturation | 0:45 | 95 |
| 3 | Annealing | 0:45 | 57 |
| 4 | Elongation | 2:00 | 72 |
| 5 | Go to step 2, repeat for 24 more cycles | | |
| 6 | Final elongation | 10:00 | 72 |
| 7 | Hold | $\infty$ | 4 |

3. Purify PCR product on 4-5 purification columns with Qiagen PCR purification kit according to manufacturer instructions. Elute in 50 $\mu$L of 10 mM Hepes pH 7.4 without any Tris. To concentrate DNA, elute 2 of the columns in 50 $\mu$L each, then using elutions to elute rest of columns.

4. Label DNA with digoxigenin through a digoxigenin-NHS ester reaction by mixing the following and incubate at room temperature for 2 hrs:

| Reagent | Concentration | Volume ($\mu$L) |
|---|---|---|
| Sodium bicarbonate, pH 9.0 | 1 M | 10 |
| Digoxigenin-NHS in DMSO | 1 mM | 1 |
| Purified PCR product | | 89 |
| **Total ($\mu$L)** | | **100** |

5. Quench the digoxigenin labeling reaction with Tris.

6. Remove excess digoxigenin from labeled product with either another PCR purification or a desalting column.

7. Store labeled 1 kb DNA at 4°C.

# 3 10 kb DNA

## 3.1 Materials

1. *Bacillus subtilis* genomic DNA isolated by a standard alkaline lysis procedure

2. Forward adapter primer: 5'-TGA GGG ATA TCG AAT TCC TGC AGG CGC CCT GAG ACA CAT GAG ATT ATT GAC A-3'

3. Reverse adapter primer: 5'-GAC GCG AAT TAT TTT TGA TGG CGC CGG CAC CTC TAC AAT TCC CA-3'

4. Biotinylated forward primer: [biotin]-5'-TGA GGG ATA TCG AAT TCC TGC AGG C-3'

5. Digoxigenin labeled reverse primer: [digoxigenin]-5'-GAC GCG AAT TAT TTT TGA TGG CG-3'

## 3.2 Procedure

1. PCR with Q5 polymerase 2x Master Mix (NEB M0492) using *Bacillus subtilis* genomic DNA as template and the adapter primers.

2. Gel purify the PCR product by running a 1x TAE 0.8% low-melting agarose gel with ethidium bromide and excised band with desired length. Melt the band at 65°C and cool it to 42°C. Add $\frac{1}{10}$ volume of 10x $\beta$-Agarase I Reaction Buffer (NEB B0392) and $\frac{1}{100}$ volume of 1 U/$\mu$L $\beta$-Agarase I (NEB M0392). Incubate the mixture for several hours at 42°C to digest the agarose. Digested product can be stored at 4°C before proceeding to following steps.

3. PCR with OneTaq 2x Master Mix (NEB M0482) using previous PCR product as template and the functionalized primers.

4. Gel purify the second PCR product as described previously.

5. Store labeled 10 kb DNA at 4°C.