Wet lab protocols

Designing Life with AI

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Contents

1 General lab tips

1.1 Abbreviations

- Tm : Melting Temperature

1.2 Basic lab knwoledge

When you take something from the freezer put it on ice. When you take something from the fridge usually you can work with it for several hours at room temperature.

Purification protocols are usually printed in the labs.

Long term storage is for when you have to store for two months and more. Short term storage is for less than two months.

2 Restocking some consumables

Some consumables can directly be prepared in the lab. When making those you should either be sure of the concentrations you add to your solution or make a small amount of the solution and keep it for yourself (put it in a dedicated compartment of the fridge/freezer where you're sure no one else will use it).

2.1 Lb+Amp

If you don't find any Lb+Amp in the lab you can make some yourself using Lb medium and ampicillin. Usually Ampicillin is at a 1000X concentration so for a given volume of Lb+Amp you'll mix 1/1000Th of said volume of ampicillin to 999/1000Th of Lb.

2.2 TAE+Agarose

To restock TAE+agarose you need to add the wished percentage of agarose in mass to TAE considering that TAE has roughly the same density as water. Let's say we want an 0.8% agarose solution we can add 8 grams of agarose to 992 ml of TAE.

3 Gel electrophoresis

Materials :

- 1X TAE buffer
- Agarose powder
- 6X loading buffer
- 1kb ladder
- GelRed

Preparing the gel

1. Prepare a mix of 1X TAE buffer and agarose powder, containing 0.6-1% of agarose (0.6-1% of the mass, TAE buffer has roughly the same volumetric mass as water). Depending on the size of the chamber you'll need 80-300 ml of the solution.
2. Add 1/10000 of GelRed (for 120ml you'll add 12μl).
3. Prepare the gel-casting chamber with the size and number of wells that suits your experiment into the frame and pour into the melted agarose. Let it cool for 1 hour.
4. Cover the gel with 1X TAE buffer and load each of the sample into the wells.
5. Run the gel at 120V for 30-60 minutes.
6. Remove the gel and take a picture.

Samples to load

Prepare your samples on a parafilm with a ratio of 1:6 of loading buffer (for a sample of 5μl you'll add 1μl of loading buffer, this is the smallest quantity of sample you should use and sufficient form analysing your samples).

Percentages between 0.5% and 1% of agarose are usually good for most fragments size but if you have issues you can adapt the percentage of your gel (the smaller the fragment the bigger the percentage of agarose).

3.1 DNA Purification from Agarose Gels

Materials :

- Purifications
- HiBind DNA Mini Columns
- 2ml collection tube
- XP2 Binding buffer
- SPW Buffer
- Elution Buffer

Centrifugation Protocol :

1. Perform agarose gel/ethidium bromide electrophoresis to analyze PCR product. Excise the fragment of interest.
2. Determine the volume of your gel slice by weighing it in a clean 1.5 mL microcentrifuge tube. Assuming a density of 1 g/mL, a 0.3 g gel slice will have a volume of 0.3 mL.
3. Add 1 volume XP2 Binding Buffer. Incubate at 60°C for 7 minutes or until the gel has completely melted. Vortex or shake the tube every 2-3 minutes.

   > **Important**: Monitor the pH of the Gel/Binding Buffer mixture after the gel has completely dissolved. DNA yields will significantly decrease when the pH > 8.0. If the color of the mixture becomes orange or red, add 5 µL 5M sodium acetate (pH 5.2) to bring the pH down. After this adjustment, the color of the Gel/Binding Buffer mixture should be light yellow.

4. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube (provided).
5. Add no more than 700 µL DNA/agarose solution from Step 3 to the HiBind® DNA Mini Column. Centrifuge at 10,000 x g for 1 minute at room temperature. Discard the filtrate and reuse the collection tube.
6. Repeat Step 5 until all of the sample has been transferred to the column.
7. Add 300 µL XP2 Binding Buffer. Centrifuge at maximum speed ($\geq$13,000 x g) for 1 minute at room temperature. Discard the filtrate and reuse the collection tube.
8. Add 700 µL SPW Buffer diluted with 100% ethanol (see the bottle for instructions). Centrifuge at maximum speed for 1 minute. Discard the filtrate and reuse the collection tube.

Optional: Repeat Step 8 for a second SPW Buffer wash step.

9. Centrifuge the empty column at maximum speed for 2 minutes to dry the column. This step is critical for removal of trace ethanol that may interfere with downstream applications. Transfer the HiBind® DNA Mini Column into a clean 1.5 mL microcentrifuge tube (not provided).

10. Add 30-50 µL Elution Buffer or sterile deionized water directly to the center of column matrix. Let sit at room temperature for 2 minutes. Centrifuge at maximum speed for 1 minute.

11. Store DNA at -20°C.

Vacuum Protocol :

1. Perform agarose gel/ethidium bromide electrophoresis to analyze PCR product.

2. Excise the fragment of interest.

3. Determine the volume of your gel slice by weighing it in a clean 1.5 mL microcentrifuge tube. Assuming a density of 1 g/mL, a 0.3 g gel slice will have a volume of 0.3 mL.

4. Add 1 volume XP2 Binding Buffer. Incubate at 60°C for 7 minutes or until the gel has completely melted. Vortex or shake the tube every 2-3 minutes.

   Important: Monitor the pH of the Gel/Binding Buffer mixture after the gel has completely dissolved. DNA yields will significantly decrease when the pH > 8.0. If the color of the mixture becomes orange or red, add 5 µL 5M sodium acetate (pH 5.2) to bring the pH down. After this adjustment, the color of the Gel/Binding Buffer mixture should be light yellow.

5. Prepare the vacuum manifold according to manufacturer's instructions and connect the HiBind® DNA Mini Column to the manifold.

6. Add no more than 700 µL DNA/agarose solution from Step 4 to the HiBind® DNA Mini Column. Switch on the vacuum source to draw the sample through the column. Turn off the vacuum.

7. Repeat Step 6 until all of the sample has been transferred to the column.

8. Add 300 µL XP2 Binding Buffer. Switch on the vacuum source to draw the XP2 Binding Buffer through the column. Turn off the vacuum.

9. Add 700 µL SPW Buffer diluted with 100% ethanol (see the bottle for instructions). Switch on the vacuum source to draw the SPW Buffer through the column. Turn off the vacuum.

Optional: Repeat Step 9 for a second SPW Buffer wash step.

10. Transfer the HiBind® DNA Mini Column to a clean 1.5 mL microcentrifuge tube (not provided).

11. Centrifuge the empty HiBind® DNA Mini Column at maximum speed for 2 minutes to dry the column. This step is critical for removal of trace ethanol that may interfere with downstream applications.

12. Transfer the HiBind® DNA Mini Column into a clean 1.5 mL microcentrifuge tube (not provided).

13. Add 30-50 µL Elution Buffer or sterile deionized water directly to the center of column matrix. Let sit at room temperature for 2 minutes. Centrifuge at maximum speed (≥13,000 x g) for 1 minute.

14. Store DNA at -20°C.

4 PCR

Materials :

- Forward primer 100mM
- Reverse primer 100mM
- Phusion or another PCR polymerase (for example Taq polymerase)
- Nuclease-free H_2O
- dNTP mix
- Phusion buffer (Taq buffer)
- $MgCl_2$ *Optional*
- DNA *In various forms, for example a plasmid or bacterial DNA (the bacteria is lysed in the denaturiation phase and it's DNA can be amplified)*

PCR reaction

1. Mix all the following reagents together and adjust the amounts to the number of PCR tubes you're going to use. The following table indicates the amount for a single 50μl PCR reaction.

Component	Final concentration/amount
Phusion buffer 5X (Standard Taq buffer 10X)	10μl (5μl)
Phusion (Taq polymerase)	0.5μl (0.25μl)
dNTP mix	10 mM 1μl
Forward Primer	0.25μl
Reverse Primer	0.25μl
$MgCl_2$ *Optional*	1.5μl
Plasmid DNA *Optional*	12.5-50 ng
Water	to 50μl

2. If you want to do the PCR directly on bacteria take a sterile tip and brush it against a colony of bacteria then dip the tip in each of the PCR tubes.

The content of this table depends on the polymerase (except for the plasmid DNA quantity) if you use a polymerase other than the Phusion (or Taq) you'll have to check the quantites on the manufacturers website.

Program the thermocycler using the following guidelines (which are standard but not absolute, some of these parameters depend on the polymerase used especially the extension time. Additional informations can usually be found on the website of the manufacturer):

The following are the PCR steps for the Phusion polymerase:

Steps	Temp	Time	nb of cycles
Initial denaturation	98°C	30 sec	
Primer annealing	$[T_m\text{-}5,T_m]$°C	45 sec	30-35
Extension	72°C	30s/kb	30-35
Final extension	72°C	5 min	

And these are the PCR steps for the Taq polymerase:

Steps	Temp	Time	nb of cycles
Initial denaturation	95°C	30 sec	
Primer annealing	$[T_m\text{-}5,T_m]$°C	45 sec	30-35
Extension	68°C	1min/kb	30-35
Final extension	68°C	5 min	

To conserve the amplified DNA, PCR products are stored at -20°C for long-term storage or 4°C for short-term storage.

4.1 DNA Purification of PCR Products

Materials :

- Purifications
- HiBind DNA Mini Columns
- 2ml collection tube
- CP buffer
- DNA Wash Buffer
- Elution Buffer

Centrifugation Protocol :

1. Determine the volume of your PCR reaction.
2. Transfer the sample into a clean 1.5 ml microcentrifuge tube.
3. Add 4-5 volumes of CP Buffer. (Volume refers to the size of your PCR reaction. For example, if your PCR reaction is 100μl, you would use 400-500μl of CP Buffer.)

4. Vortex to mix thoroughly. Briefly centrifuge to collect any drops from inside the lid.

5. Insert HiBind DNA Mini Column into a 2ml collection tube.

6. Add the sample from step 5 to the HiBind DNA Mini Column.

7. Centrifuge at maximum speed for 60 seconds at room temperature. Discard the filtrate and reuse the collection tube.

8. Add 700μl of DNA Wash Buffer diluted with 100% ethanol. Centrifuge at maximum speed for 60 seconds. Discard the filtrate and reuse the collection tube.

9. Repeat step 9 for a second DNA Wash Buffer step.

10. Centrifuge the empty HiBind Mini Column at maximum speed for 2 minutes to fry the column. This step is critical for the removal of trace ethanol that may interfere with downstream applications.

11. Transfer the HiBind DNA Mini Column into a clean 1.5ml microcentrifuge tube.

12. Add 30-50μl elution buffer, TE buffer, or sterile deionized water directly in the center of the column matrix. Let sit at room temperature for 2 minutes. Centrifuge at maximum speed for 60 seconds.

13. Store DNA at -20°C.

    In the LPBS this protocol is in the wetlab and there is a version of it where the vacuum manifold is used instead of a centrifuge.

Vacuum Protocol :

1. Perform agarose gel/ethidium bromide electrophoresis to analyze PCR product.

2. Determine the volume of your PCR reaction.

3. Transfer the sample into a clean 1.5 mL microcentrifuge tube (not provided).

4. Add 4-5 volumes CP Buffer. For PCR products smaller than 200 bp, add 5 volumes CP Buffer and 0.4 volumes 100% isopropanol.

   > **Note**: Volume refers to the size of your PCR reaction. For example, if your PCR reaction is 100 µL and is smaller than 200 bp, you would use 500 µL CP Buffer and 40 µL isopropanol.

5. Vortex to mix thoroughly. Briefly centrifuge to collect any drops from the inside of the lid.

6. Prepare the vacuum manifold according to manufacturer's instructions and connect the HiBind® DNA Mini Column to the manifold.

7. Transfer the entire sample to the HiBind® DNA Mini Column. Switch on the vacuum source to draw the sample through the column. Turn off the vacuum.

8. Add 700 µL DNA Wash Buffer diluted with 100% ethanol (see the bottle for instructions). Switch on the vacuum source to draw the DNA Wash Buffer through the column. Turn off the vacuum.

9. Repeat Step 8 for a second DNA Wash Buffer Step.

10. Transfer the HiBind® DNA Mini Column into a 2 mL Collection Tube.

11. Centrifuge the empty HiBind® DNA Mini Column at maximum speed (≥13,000g) for 2 minutes to dry the column. This step is critical for removal of trace ethanol that may interfere with downstream applications.

12. Transfer the HiBind® DNA Mini Column into a clean 1.5 mL microcentrifuge tube.

13. Add 30-50 µL Elution Buffer, TE Buffer, or sterile deionized water directly to the center of column matrix. Let sit at room temperature for 2 minutes. Centrifuge at maximum speed for 60 seconds.

14. Store DNA at -20°C.

5 Gibson assembly

Materials :

1. PCR cleanup product
2. Nuclease-free H_2O
3. Digested vector
4. Gibson assembly mastermix (2X)

Computation of doses

This website provides a convenient tool for calculating gibson doses (which are the same as ligation doses).
Neb gibson calculator

Note that for the vector mass it is standard to put 50ng and the standard ratio of insert to vector is 3:1 but the bigger the ratio the more likely the ligation is to work so if your first Gibson assembly doesnt work you should increase the ratio.

Component	Gene A
Nuclease free water	to 10 μl
Gibson MM	5μl
Vector	varies
Insert	varies

Then incubate the samples at 50°C for 15 minutes.
Source:NEB gibson assembly protocol

6 Yeast DNA Extraction

Materials :

- DNA extraction buffer:
  - 2% Triton X-100
  - 1% SDS
  - 100 mM NaCl (from 5M stock)
  - 10 mM Tris-HCl, pH 8.0
  - 1 mM EDTA, pH 8.0
  - *Ref.: Harju et al., Rapid isolation of yeast genomic DNA: Bust n' Grab, BMC Biotechnology 2004*
- Glass beads for DNA extraction
- 1.5 mL screw-cap tubes
- 1x Tris-EDTA (TE) buffer (pH 7.4-8)
- Phenol-Chloroform-Isoamyl alcohol (25:24:1, stored at 4°C)
- Ethanol 100%
- Ethanol 70%
- **Recommended:** RNase (stock should be 10 mg/mL)

Protocol :

1. Prepare 1.5 mL screw-cap tubes (1 per sample):
   - Add 0.4 g glass beads (corresponding to a volume of roughly 150 µL)
   - Add 200 µL DNA extraction buffer
   - Add 200 µL Phenol:Chloroform:Isoamyl alcohol
   - Add yeast cells (using a pipette tip, take a big chunk of cells; it is best if the cells are fresh)
2. Label tubes.
3. Close screw caps tightly.
4. Load onto FastPrep (let someone show you how).
5. Run the *S. cerevisiae* extraction protocol.
6. Take out tubes.

7. Spin down briefly in a small tabletop centrifuge for a few seconds.
8. Add 200 µL of 1x TE.
9. Centrifuge at top speed for 5 min at room temperature.
10. After centrifugation, samples should contain:
    - an aqueous phase (top, containing the DNA),
    - an interphase (white),
    - and an organic phase (lipids, proteins, etc.).
11. Take a clean 1.5 mL tube (no need for screw caps) for each sample, label, and add 1 mL 100% ethanol.
12. Transfer the aqueous phase of each sample (from step 8) to the new tubes containing 100% ethanol.
13. Invert samples a few times (you should see nucleic acid precipitation, i.e., cloudy).
14. Spin at full speed for 5 min (you should see a nucleic acid pellet).
15. Discard the supernatant, keeping the pellet.
16. Add 500 µL of 70% ethanol to wash the DNA pellet.
17. Centrifuge at top speed for 5 min.
18. Remove as much of the supernatant as possible, leaving as little water-ethanol as possible.
19. Add 50 µL of 1x TE.
20. **Recommended:** Add 10 µg/mL RNase (a 1/1000 dilution of 10 mg/mL stock RNase).
21. **Recommended (continued):** Incubate at 37°C for 5 min.

7 Yeast Transformation

Carry out 1 transformation with DNA and 1 without DNA (no-DNA control is used to count the number of revertants).

Materials :

- Fresh stationary-phase yeast
  - 5 mL suffices for both 1 transformation and 1 control.
- Linearized DNA (either PCR product or linearized plasmid)
  - 1 µg for 1 transformation.
- Drop-out plates (-Leu, -His, -Trp, -Ura, +G418, or +ClonNat)
- 1x Lithium acetate-TE solution (LiOAc/TE)
  - Need: 200 µL suffices for both 1 transformation and 1 control.
  - 5x LiOAc/TE stock:
    - 0.5 M LiOAc (51 g of 99% LiOAc•2H20 per 1 L)
    - 5x TE (50 mM Tris and 5 mM EDTA)
- 1x PEG-LiOAc/TE solution (make fresh)
  - Need: 2 mL suffices for both 1 transformation and 1 control.
  - 40% PEG (we and others use: 3350 MW) in 1x LiOAc/TE
  - Example: to make 2.5 mL, mix 0.5 mL of 5x LiOAc/TE with 2 mL 50% PEG solution.
- 1x Carrier DNA
  - Need: 60 µL suffices for both 1 transformation and 1 control.
  - 10 mg/mL Herring sperm.
- Water bath at 42°C.
- Optional: Pasteur pipettes for plating.

Protocol :

1. Pellet yeast cells at ∼700 g for 2 min.
2. Check for bacterial contamination (supernatant should not be cloudy).
3. Pour supernatant off carefully.
4. Re-suspend yeast cells in 200 µL of 1x LiOAc/TE.

5. Label 1.5 mL microcentrifuge tubes for transformations and control.
6. Heat carrier DNA to 70°C for 5 min.
7. Add 30 µL of carrier DNA to microcentrifuge tubes.
8. Add ∼1 µg of DNA for transformation, no DNA for control.
9. Add 100 µL of yeast cells to microcentrifuge tubes (with and without DNA).
10. Vortex.
11. Add 1 mL of 1x PEG-LiOAc/TE solution to each tube.
12. Vortex.
13. Incubate at 42°C for 30 min.
14. Pellet at ∼700 g for 2 min.
15. Resuspend in 1 mL DI water.
16. Pellet at ∼700 g for 2 min.
17. Plate or let grow overnight and then plate:
    - For all selectable markers (LEU2, HIS3, TRP1, URA3) except KanMX and NatMX:
        a) Add 200 µL DI water.
        b) Pipette up and down to mix.
        c) Streak onto dropout plates.
    - For KanMX and NatMX plasmids:
        a) Add 1 mL non-selective medium (e.g., D-Met).
        b) **Important**: Lock tubes shut (often tubes pop open).
        c) Put on nutator overnight at 30°C.
        d) Pellet at ∼700 g for 2 min.
        e) Add 200 µL DI water.
        f) Pipette up and down to mix.
        g) Streak onto G418 or ClonNat plates.

8 Bacteria transformation

The goal of this part is to transform bacteria with our plasmids in order to obtain a larger quantity of plasmids after incubation.

Materials :

- Plasmid DNA
- suspended competent bacteria
- SOC media
- LB+ Ampicillin plate

Protocol:

1. Pipet up and down slowly 10ng of plasmid DNA into 50 μl of suspended bacteria (If you cant isolate 10ng because your concentration of plasmid DNA is too high just pipet up and down 1μl of you plasmid DNA solution no matter the concentration).
2. Put the tube 30 min on ice.
3. Put the tube at 42°C for 30 sec (hot water bath).
4. Put the tube 2-3 min on ice.
5. Transfer in 1 ml of SOC media in a 15 ml falcon tube and put at 37°C for 30-60 min.
6. Transfer 1 ml into a 1.5 ml eppendorf tube.
7. Centrifuge for 20 sec at max speed (shortspin).

   After this step you should see a little brown stain at the bottom of the tube.
8. Remove the supernatant and leave 50μl - 100μl.
9. Pipet up and down.
10. Plate on LB+ Ampicillin plate with a pipette pasteur.
11. Also plate a negative control to compare the number cell. (In the context of ligation plate the control of the ligation.)
12. Put the plates at 37°C overnight.
13. The day after there should be colonies.

8.1 Plasmid DNA Extraction and Purification from 1-5 mL E. coli culture

Materials :

- Purifications
- HiBind DNA Mini Columns
- 2ml collection tube
- Solution I
- Solution II
- Solution III
- HBC buffer
- DNA Wash Buffer
- Elution Buffer
- RNase A (Pre-Added)

Protocol :

1. Grow 1-5 mL culture overnight in a 10-20 mL culture tube.
2. Centrifuge at 10,000 x g for 1 minute at room temperature. Decant or aspirate and discard the culture media.
3. Add 250 µL Solution I mixed with RNase A (see the bottle for instructions). Vortex to mix thoroughly. Transfer suspension into a new 1.5 mL microcentrifuge tube.
4. Add 250 µL Solution II. Invert and gently rotate the tube several times to obtain a clear lysate. A 2-3 minute incubation may be necessary. Avoid vigorous mixing and do not exceed a 5 minute incubation.
5. Add 350 µL Solution III. Immediately invert several times until a flocculent white precipitate forms. Centrifuge at maximum speed ($\geq$13,000 x g) for 10 minutes. A compact white pellet will form. Promptly proceed to the next step.
6. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube.

   Optional Protocol for Column Equilibration:

   a) Add 100 µL 3M NaOH to the HiBind® DNA Mini Column.

   b) Centrifuge at maximum speed for 30-60 seconds.

   c) Discard the filtrate and reuse the collection tube.

7. Transfer the cleared supernatant from Step 5 by CAREFULLY aspirating it into the HiBind® DNA Mini Column. Centrifuge at maximum speed for 1 minute. Discard the filtrate and reuse the collection tube.

8. Add 500 µL HBC Buffer diluted with 100% isopropanol (see the bottle for instructions). Centrifuge at maximum speed for 1 minute. Discard the filtrate and reuse the collection tube.

9. Add 700 µL DNA Wash Buffer diluted with 100% ethanol (see the bottle for instructions). Centrifuge at maximum speed for 30 seconds. Discard the filtrate and reuse the collection tube.

Optional: Repeat Step 9 for a second DNA Wash Buffer wash step.

10. Centrifuge the empty HiBind® DNA Mini Column at maximum speed for 2 minutes to dry the column. This step is critical for removal of trace ethanol that may interfere with downstream applications.

11. Transfer the HiBind® DNA Mini Column into a nuclease-free 1.5 mL microcentrifuge tube.

12. Add 30-100 µL Elution Buffer or sterile deionized water. Let sit at room temperature for 1 minute. Centrifuge at maximum speed for 1 minute.

13. Store eluted DNA at -20°C.

9 Incoculation

Materials :

- Bacteria
- LB + Ampicillin media

Protocol:

1. Put 6 ml of LB+ Ampicillin media into a falcon tube.
2. Dip the tip of a pipette into a colony (gently) and dip it into the faclon tube then swirl.
3. Close the tube loosely (air needs to be able to flow in and out of the tube).
4. Put in a shaking incubator at 37°C overnight.

10 Digestion

Materials :

- DNA plasmid
- 10X rCutSmart Buffer
- Restriction enzyme 1
- Restriction enzyme 2
- Nuclease-free water

Protocol :

1. Set up the reaction as follows:

Component	50μl reaction
DNA plasmid	1μg
10X rCutSmart Buffer	5μl (1X)
Restricti]on enzyme 1	1μl
Restriction enzyme 2	1μl
Nuclease-free water	to 50μl

2. Incubate at 37°C for 3 hours.

3. Take out of the incubator and run a gel electrphoresis (50min-1h15 depending on the size of the gel). This step is essential to separate what you want to keep from the other part of the plasmid.
   Here a negative control is necessary to ensure that the plasmid has been digested. So along with the digested plasmid run some of the undigested plasmid and compare their heights in the gel. Linearized DNA migrates at a slightly higher rate than circular DNA so the digested plasmid should be a little below the undigested plasmid.

4. Extract the DNA from the gel (the protocol can be found in section 3.1 of this document).

11 Ligation

Materials :

- T4 DNA ligase buffer 10X
- Vector DNA
- Insert DNA
- Nuclease-free water
- T4 DNA ligase

Computation of doses

This website provides a convenient tool for calculating ligation doses.
Neb ligation calculator

Note that for the vector mass it is standard to put 50ng and the standard ratio of insert to vector is 3:1 but the bigger the ratio the more likely the ligation is to work so if your first ligation doesnt work you should increase the ratio.

Protocol :

1. Set up all the given materials in a micro-centrifuge tube on ice. T4 DNA ligase should be added last. In parallel you should set up a negative control. This control consists of the extact same mix but lacking the insert. After transforming and plating the cells you should have a lower amount of colonies on the negative control plates since they have been transformed with linear plasmid that is not readable by bacteria making them vulnerable to the anti-biotic.

Component	20μl reaction
vector DNA	50ng (can vary)
insert DNA	varies
T4 DNA ligase buffer	2μl (10X)
Nuclease-free water	to 20μl
T4 DNA ligase	1μl

2. Gently mix the reaction by pipetting up and down and microfuge briefly.
3. For cohesive (sticky) ends, incubate at 16°C overnight or room temperature for 10 minutes.
4. For blunt ends or single base overhangs, incubate at 16°C overnight or room temperature for 2 hours
5. Heat inactivate at 65°C for 10 minutes.
6. Chill on ice and transform 1-5μl of the reaction into 50μl competent cells.

12 Sequencing

Materials :

- Nuclease free-water
- DNA (Plasmid or linear)
- Primers (optional)

Computation of doses

When sending a sample to be sequenced (by Microsynth) the sample needs a concentration between 40ng/μL - 100ng/μL. You have the choice to either use standard primers that Microsynth will have in their lab (you can view them on their site when completing the info) or use you own primers in which case the final solution needs to have a concentration of primers of 4mM. In the first case total volume of the sample you're going to send is going to be 12 μL and otherwise it's going to be 15μL. Once all the doses have been computed to yield the correct concentration all the reactants just need to be mixed in a 1.5 ml eppendrof tube.
The tube needs to be labelled with Microsynth stickers.

13 Authors

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