Protocols

Transformation

1. Take competent cells out of -80°C and thaw on ice.
2. Take agar plates (containing the appropriate antibiotic) out of 4°C to warm up to room temperature or place in 37°C incubator.

3. Mix 1 to 5µl of DNA (usually 10pg to 100ng) into 50µL of competent cells in a microcentrifuge. GENTLY mix by flicking the bottom of the tube with your finger a few times.
4. Place the competent cell/DNA mixture on ice for 25 mins
5. Heat shock each transformation tube by placing the bottom 1/2 to 2/3 of the tube into a 42°C water bath for 30-60 seconds.
6. Put the tubes back on ice for 5 min.
7. Add 1000µl LB or SOC media (without antibiotic) and grow in 37°C shaking incubator for 45min.
8. Plate some or all of the transformation (100 ul) onto a 10cm LB agar plate containing the appropriate antibiotic.
9. Incubate plates at 37°C overnight.

Pouring Agar Plates

1. Weigh out the following into a 1L Erlenmeyer flask:
   - 5g NaCl
   - 5g Tryptone
   - 2.5g Yeast Extract
   - 7.5g Agar

   - add dH_2O to 500mL

2. Swirl to mix - the contents do not have to be completely in solution, but any powder left on the sides of the flask will caramelize on the glass during autoclaving.
3. Cover the top of the flask with aluminium foil and label with autoclave tape.
4. Autoclave on the liquid setting for 20 minutes.
5. After removing the solution from the autoclave, allow the agar solution to cool to 55°C.Place in water bath at 55 degrees to do this.
6. When pouring plates, keep your bench area sterile by working near a flame or bunsen burner. Also clean it down with ethanol prior.
7. Add the appropriate amount of desired antibiotic to the solution.
8. Pour ~20mL of LB agar per 10cm polystyrene Petri dish.
9. Place the lids on the plates and allow them to cool for 30-60 minutes (until solidified), then invert the plates. Let sit for several more hours or overnight.
10. Label the bottom of plates with antibiotic and date and store in plastic bags or sealed with parafilm at 4°C.

Ligations

1. Combine the following in a PCR or Eppendorf tube:
   - 25ng Vector DNA
   - 75ng Insert DNA (maintain 3:1)
   - Ligase Buffer (1µL/10µL reaction for 10X buffer, and 2µL/10µL reaction for 5X buffer)
   - 1µL T4 DNA Ligase
   - H20 to a total of 10µL
2. Incubate at room temperature for 2hr, or at 16°C overnight depending on the nature of the DNA being ligated.
3. Proceed with bacterial transformation

Basehunter protocol for Detector Construction by PCR

1. HPV Detector Plasmid (uncut) linearized with KPNI for 4 hours at 37 degrees & heat inactivated at 80 degrees for 5 minutes

Sample	Volume (ul)
DNA (68ng/ul)	1.47 (100ng)
KPNI	0.5
Buffer 4	2
H2O	16.03
Total	20

2. Digestion was diluted 1 in 1000 ul of TE buffer (5pg DNA per ul)
3. PCR reaction was set up as follows:

PCR Reactions :

Following is the protocol for solis biodyne PCR reaction.

	1	2	3 (Control)
DNA (5pg/ul)	4	4	4
Solis Biodyne 5x Hot Start	4	4	4
F Primer	1	1	0

R Primer	1	1	0
H2O	10	10	12
Total	20	20	20

Alternative : PCR High fidelity Protocol (ThermoScientific)

Prepare the PCR reaction as follows.

Reactant	**1**	**2**	**3(Control)**
Taq Polymerase	1uL	1ul	0 uL
Primer R	2uL	2uL	2ul
Primer F	2uL	2uL	2uL
DNTPS	2uL	2uL	2uL
5x Buffer	10uL	10Ul	10uL
dH_2O	33uL	33uL	33uL
Template DNA	1uL	1uL	1uL

Note:

Aspirate to mix the contents of the tube.

Step	Temperature	Time	Number of cycles
Initial Denaturation	95	30 seconds	1
Denaturation	95	10 seconds	30
Annealing	60	30 seconds	30
Elongation	72	60 seconds	30
Final Elongation	72	7 minutes	1

PCR Conditions:

Step	**Temperature (degrees Celsius)**	**Time**	**Number of Cycles**
Initial denaturation	95	15 mins	1
Denaturation	95	30 sec	35
Annealing	56	40 sec	35
Elongation	72	3 min	35
Final Elongation	72	7 min	1

1. Run 3ul of PCR products on gel to check if present
2. DPNI digest carried out by adding 0.5ul of DPNI to reaction tube with PCR product - incubate for 1 hour @ 37 degrees & 80 degrees for 10 mins
3. PCR clean up carried out after digest
4. Run 5 ul of clean up product on gel
5. Quadruple digest carried out on sample by adding 1 ul of NtBsQI + 5ul Buffer 4 and incubating for 2 hours at 50 degrees. The final 3 enzymes (KpnI, HindIII & NtBsI) were then added and incubation continued for 6 hours at 37 degrees

6. Digested reaction was reacted with decoy oligos by adding 1ul of 100mM decoys (R & L) and allowing to sit for 10 mins at room temperature
7. PCR Cleanup was carried out again.
8. Run 5ul of product on gel to check DNA is present

Basehunter protocol for Quadruple Digest

1. Mix the following
   - 5 ul NEB Buffer 4
   - 1ug of the (uncut) Detector plasmid
   - MilliQ $H_2 0$ to bring the volume to 49ul
   - 1ul Nt.BspQI enzyme
   -
2. Mix these by pipetting and incubate at 50 degrees Celsius for two hours.

3. Allow to cool for 5 min on bench and then add 1ul each of Nb.BtsI, Kpn-HF & HindIII-HF. Mix and transfer to a new tube. Incubate at 37 degrees Celsius for 4-6 hours.

4. Add 1ul of each decoy oligo from the 100uM stock tubes (decoy left and right). Mix and incubate for 10 minutes at room temperature.

5. Run on a 1% agarose gel, cut out the vector band and gel purify.

6. Run 3ul of the purified detector on an agarose gel to check the purification was successful

7. If a band is visible then the Detector is ready to use

Phosphorylation and Annealing of Oligonucleotides

Making up 100uM stocks of oligos (primers)

Oligonucleotides are shipped as a freeze dried pellet. Spin the tube briefly, in case the pellet has come loose and is on the lid.

Make a stock at a concentration of 100uM in TE buffer.

> On the tube it will say how many nanomoles (nm) of oligo are in the tube (as well as how many milligram (mg)). (100uM = 100 umoles / L = 100nmoles / ml = 100 pmoles/ul) For example if you have 30nmoles of oligo you dissolve it in 0.3ml of TE to get a 100nmoles/ml (100uM) stock .

Add the required volume of TE to the tube and let it sit for 5min at room temperature. Mix by vortexing briefly and you have your oligo stock. Store it at -20 degrees.

Phosphorylation of Oligonucleotides (Protocol for 50µl Reaction)

Oligo	1µl (of 100µM stock)
T4 DNA Ligase Buffer	5µl
T4 Polynucleotide Kinase (PNK)	0.5µl
MilliQ H_2O	42µl

- Reaction is then incubated at 37°C for 30 minutes
- Heat inactivate T4 PNK by incubating reaction at 65°C for 20 minutes

Annealing of phosphorylated oligonucleotides

Phosphorylated Oligo 1	20µl
Phosphorylated Oligo 2	20µl

- Heat for 5 minutes at 95 °C in a heat block (Use one in which block can be removed)

- Remove the heat block from the heater with tube inside and place on a piece of styrofoam or some paper towels on the bench to cool down slowly to room temperature for a couple of hours. Alternatively, set a program in PCR machine to heat at 95 degrees and cool down over a period of one hour.
- Annealed oligo is now ready to use for ligation or can be stored at -20°C.

BaseHunter protocol for Detector Reaction

1. Mix the following

- 1 ul Tris-HCl Buffer (0.1M stock at pH7.5)
- 1ul "activated" Detector (plasmid / PCR product)
- 1-3ul RFP control Plasmid
- Xul Target DNA (ss or ds Oligo or digested genomic or plasmid DNA)
- MilliQ H_20 to bring the volume to 10ul
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2. Mix these by pipetting and incubate at 70 degrees Celsius for 10 minutes (in PCR machine with heated lid).

3. Allow to cool for 10 min on bench

4. Place on ice

5. Use 5ul for transformation