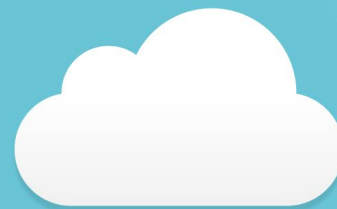
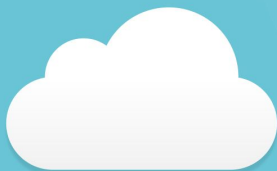


Plasmid Hoopla

Instructions For Assembly



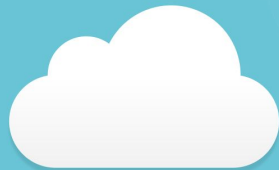
You Will Need:



- A laser cutter
- One board of standard 3 mm thick MDF (with dimensions of at least 3x500x1200 mm)
- One board of 9 mm thick MDF (with dimensions of at least 9x280x525 mm)
- Three 200 mm long wooden poles, each with a 12 mm diameter
- A saw
- A drill
- Some sandpaper
- A pencil
- A 300mm ruler
- A paintbrush for each person who wants to help paint
- A pack of acrylic paints
- A fine point marker pen
- 26 split pin paper fasteners
- 26 short elastic bands
- A pair of scissors
- A roll of velcro adhesive
- A roll of sellotape
- 3 wooden skewers
- A pack of coloured pipe cleaners



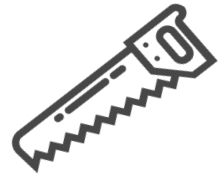
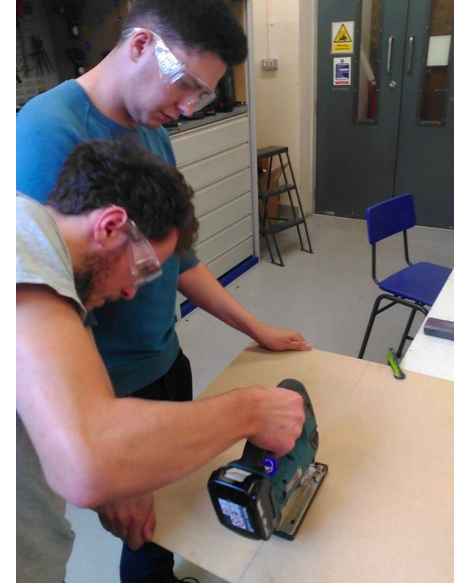
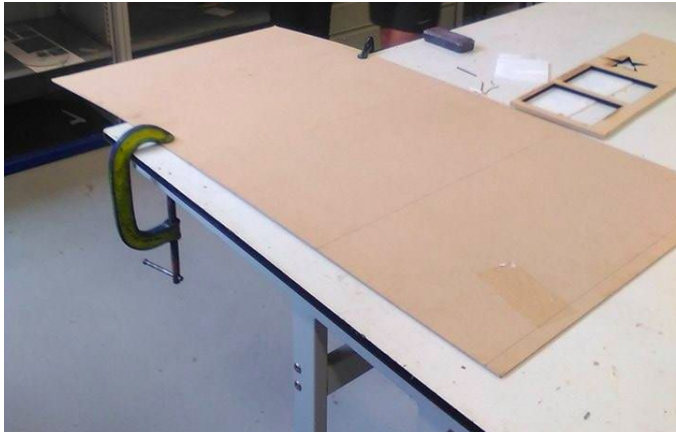
Part One: In the Workshop



Chapter 1 - Prepare your boards



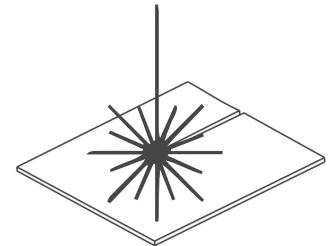
1. From your large 3 mm MDF board, saw 2 smaller boards, measuring 3 x 200 x 600 mm and 3 x 300 x 600 mm



Chapter 2 - printing the plasmid hoop bases



1. Download the 'PuzzleBase.dxf' and 'Puzzle Piece.dxf' files from the 'Plasmid Hoopla' page in the Public Engagement section of our website
2. Transfer these file onto the computer linked to your laser cutter, for example using a USB stick
3. Open the 'PuzzleBase.dxf' file using the design software on your laser cutter's computer, and set the background's size to 200 mm x 600 mm
4. Copy and paste three plasmid hoop bases onto the background, making sure not to change the aspect ratios of any of the pieces and ensuring none of the pieces overlap.
5. Using the 3 x 200 x 600 mm MDF sheet, follow the standard procedure for printing on your laser cutter and print!



Chapter 3 - printing the puzzle pieces



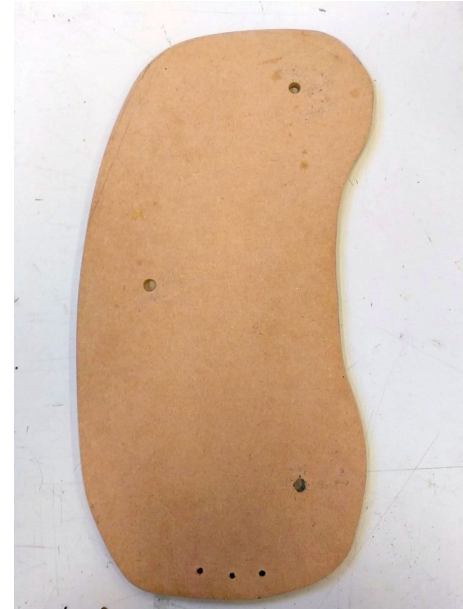
1. Open the 'Puzzle Piece.dxf' using the design software on your laser cutter's computer, and set the background's size to 300 mm x 600 mm
2. Copy and paste as many puzzle pieces onto the background as possible, making sure not to change the aspect ratios of any of the pieces and that none of the pieces overlap. You need at least 12 in total. We're using 13 for good measure.
3. Using the 3 x 600 x 300 mm MDF sheet, follow the standard procedure for printing on your laser cutter and print!



Chapter 4 - the E.coli base



1. Draw the E.coli outline on your 9x280x525 mm board - a roughly rectangular shape with generously curved corners. One of the long sides has a convex curve, and the other has a concave curve - as shown in the picture to the right (don't worry about the holes yet)
2. Saw the edges and sand them until smooth



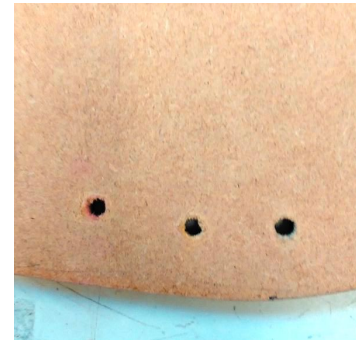
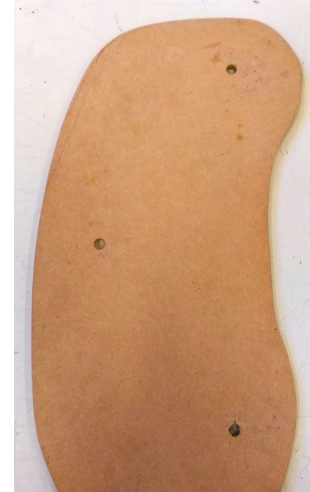
2



Chapter 4 - the E.coli base



3. Drill 3 holes of a 12mm diameter, one 90 mm down from the top of the base and 40 mm from the right-hand side; one 90 mm up from the bottom of the E.coli base and 40 mm from the right-hand side; and a final one 260 mm from the bottom of the base and 60 mm from the left-hand side of the E.coli base. All of the above distance measurements need only be approximate
4. (optional) drill an extra three 6mm diameter holes at the bottom of the E.coli base (later on, once you've finished painting and decorating the rest of the base, you can thread a pipe cleaner through each one, to form decorative flagella)



Chapter 4 - the E.coli base



5. Decorate one side of your E.coli base however you want - you could use the name of your class or school or team
6. Sand one end of each 200 mm-long wooden pole and place one inside each of the three 12 mm holes in the E.coli base

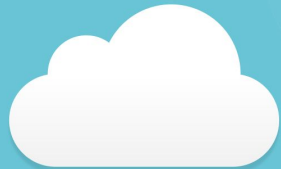


E.Coli base complete!





Finishing the Plasmid puzzle base



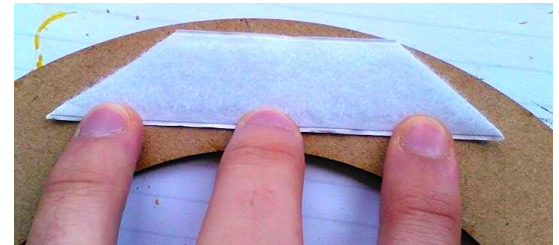
Finishing the plasmid base



1. Measure and cut a strip of approximately 100 mm from the fuzzy side of the velcro. Rather than individually measuring each piece of velcro, you could use the first, measured piece as a guide for cutting the rest
2. Cut diagonal lines into the 100 mm strip, at roughly 45 degree angles, as shown ->
3. Create 15 strips using the instructions in steps 1-2



1



2

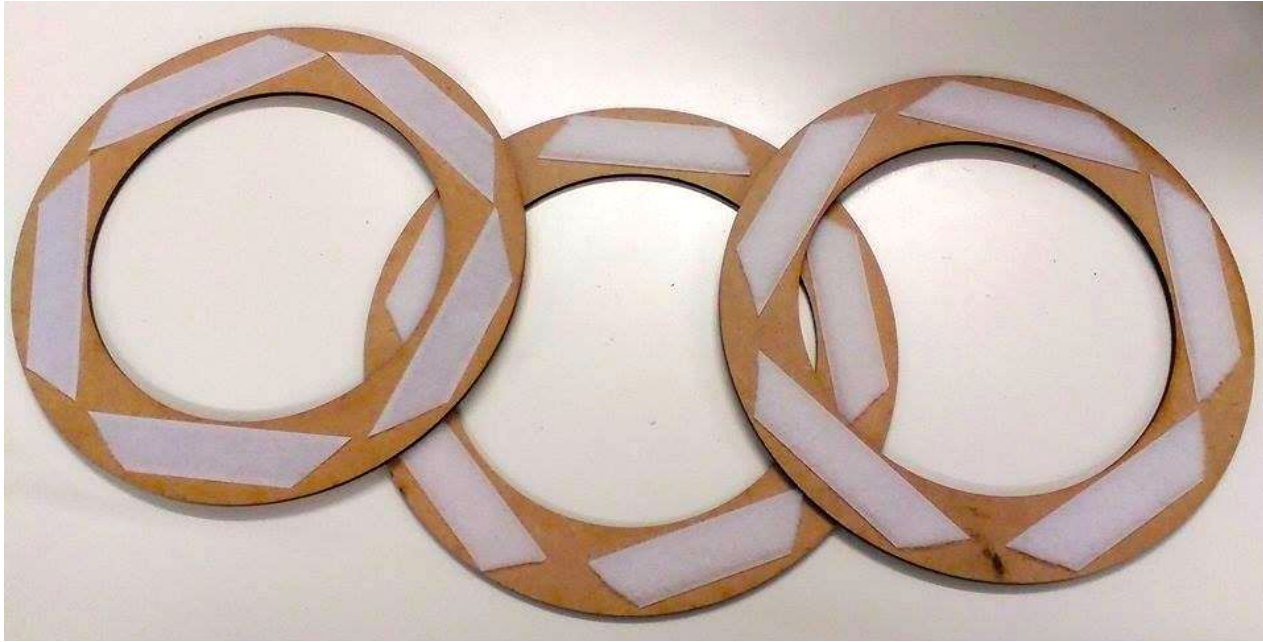
Finishing the plasmid base



1. On each plasmid base, line up 5 of the 100 mm strips corner to corner, as shown below, and stick the velcro down

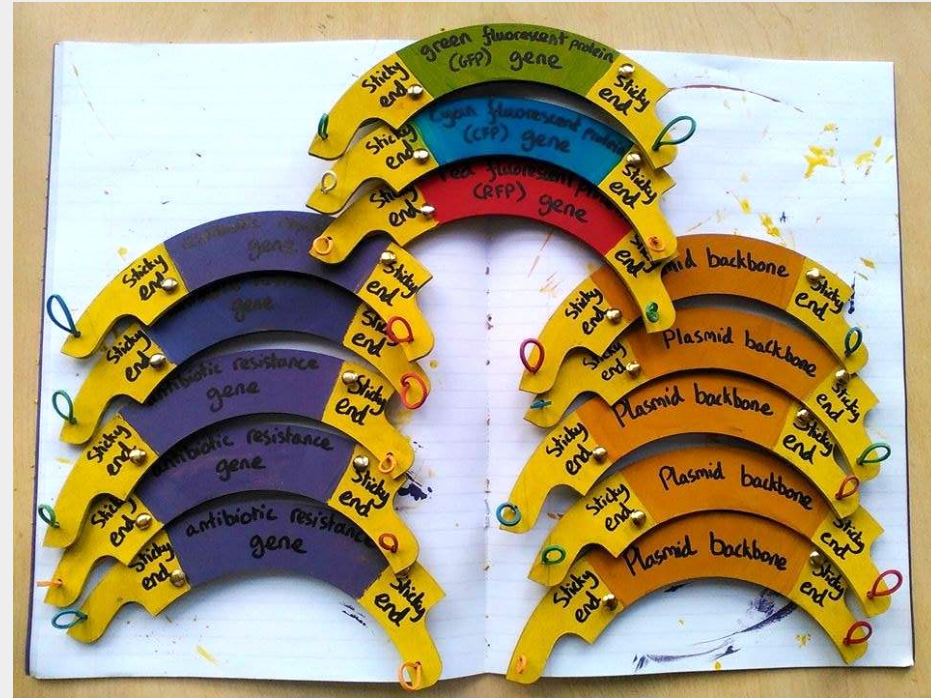
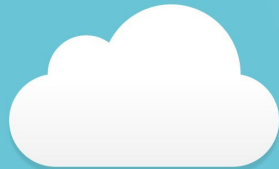


Puzzle bases complete!





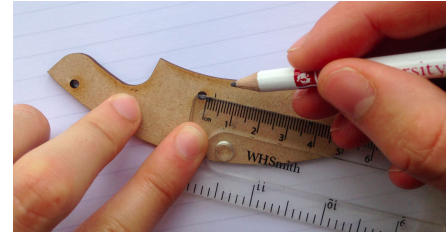
Building the Puzzle Pieces



Chapter One - prepare to paint



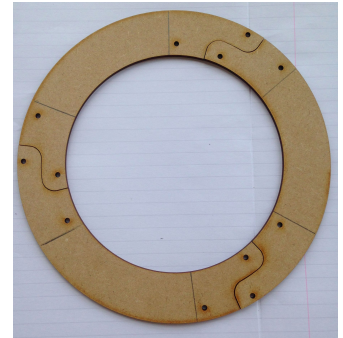
1. Take one puzzle piece and make a mark approximately 3 mm away (in the direction of the centre of the piece) from each of the two holes which are closest to the piece's centrepoint



2. Using the marks' locations as a guide, draw 2 lines connecting the inner and outer edges of the piece



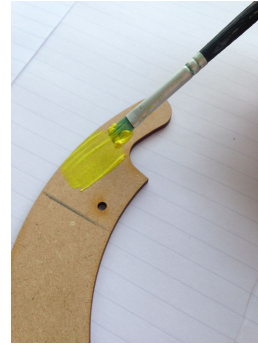
3. Repeat for every puzzle piece



Chapter Two - Paint!



1. Using the lines you've just drawn as a guide, paint the ends of each puzzle piece using a light colour of your choice. We're using yellow. Make sure you paint both sides, so that the ends are covered in yellow the whole way round



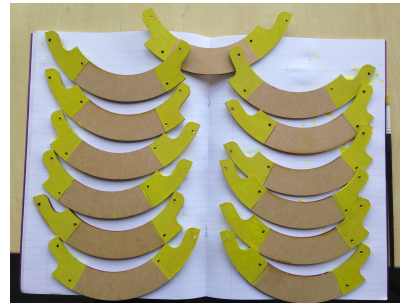
1

2. Use the tip of your paintbrush to get rid of excess paint which has accumulated in the holes



2

3. Repeat for every puzzle piece



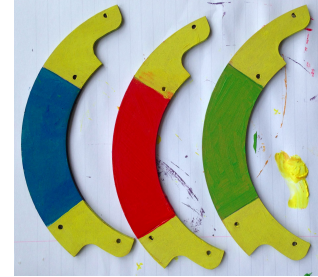
3



Chapter Two - Paint!



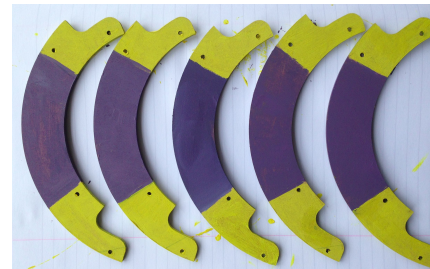
4. Take three puzzle pieces and paint the central segment of each. Paint one of them blue, one red and one green,



5. Divide the remaining puzzle pieces in half. Paint the central segments of one half in a colour not used yet - we chose orange



6. Paint the central segments of the other half in another colour not used yet - we chose



4

5

6

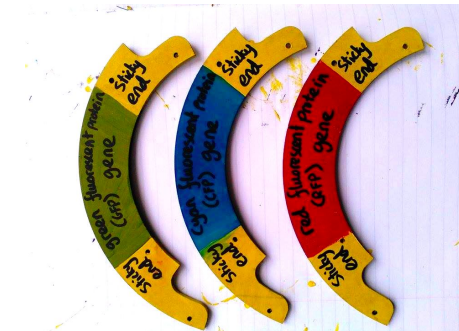
Chapter Three - Label



1. Label each end of all of your puzzle pieces a 'sticky end'. Make sure that all of the pieces are the same way round when you do this. To help you, one of the thin end parts is longer than the other - keep this one on the left. Also, ensure that you don't write anything on the long, thin sections yet - as shown in the picture
2. Take the green, blue and red pieces and label their central segments 'green fluorescent protein (GFP) gene', 'cyan fluorescent protein (CFP) gene', and red fluorescent protein (RFP) gene' respectively



1

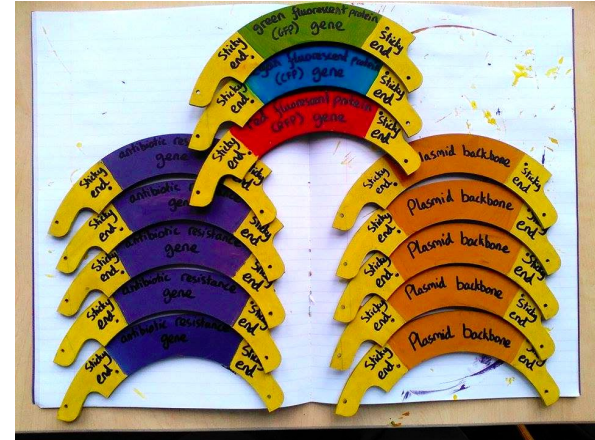
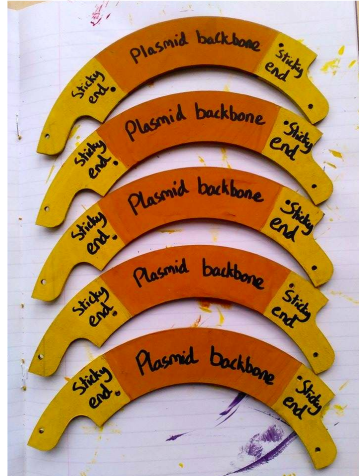


2

Chapter Three - Label



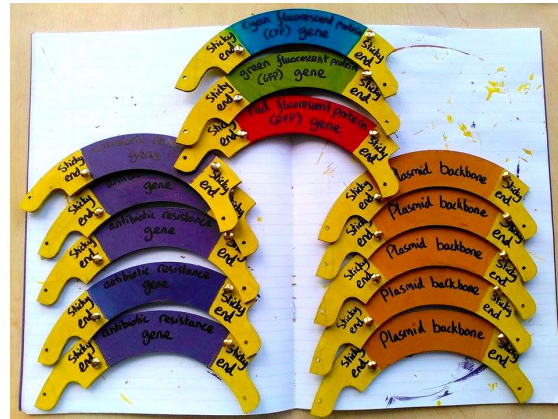
- Of the remaining pieces, label the central segments of one half 'Plasmid backbone', and the central segments of the other half 'antibiotic resistance gene'



Chapter Four - the fiddly bit



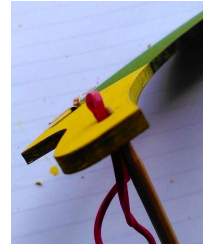
1. For every puzzle piece, place a split pin paper fastener in each of the two holes closest to the piece's centre point and unfold them, like so:



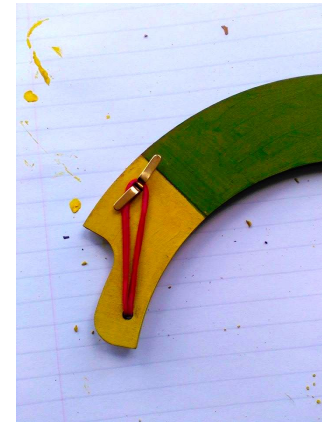
Chapter Four - the fiddly bit



2. For every puzzle piece, push one elastic band through each of the remaining holes - the ones closest to the ends of the pieces - until about half of the elastic band is on either side of the hole. Use one of your wooden skewers to help you with this, as shown ->



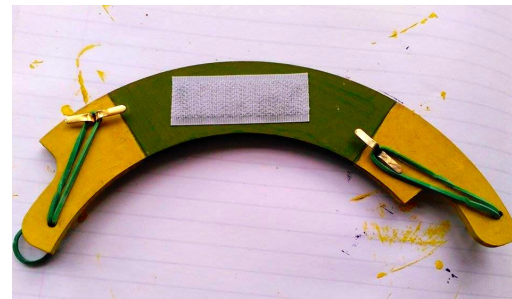
3. Pull every elastic band around its nearest split pin. This will secure the elastic bands and keep them out of the way ->



Chapter Five - Velcro and tape



1. Measure and cut approximately 50 mm of the pointier side of the velcro (as opposed to side with the fuzzier surface). You need to do this for all of the puzzle pieces (in our case, this involved cutting 13 pieces), so rather than individually measuring each piece of velcro, you could use the first, measured piece as a guide for cutting the rest
2. Stick one piece of 50 mm velcro in roughly the centre of the back of each puzzle piece, as shown ->



Chapter Five - Velcro and tape



3. Cut two approximately 35 mm lengths of sellotape and, on one side of a puzzle piece, stick one piece of tape horizontally across the split pin, and the other vertically from the thin edge of the puzzle piece down across the split pin
4. Repeat step 3 for the other end of the puzzle piece
5. Repeat steps 3 and 4 for all of your puzzle pieces



3

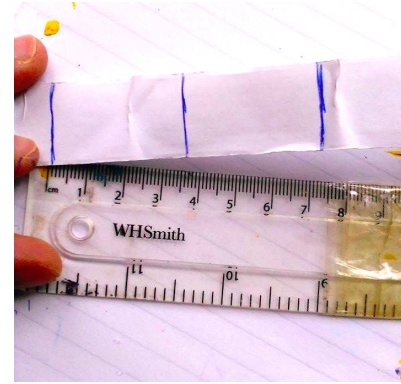


5

Chapter Five - Velcro and tape

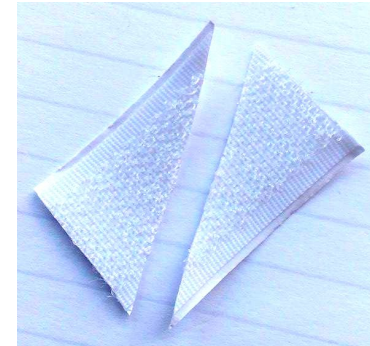


6. Measure and cut two lengths of approximately 35 mm of the pointier side of the velcro (as opposed to side with the fuzzier surface). You will eventually need to do this for all of the puzzle pieces (in our case, this involved cutting 26 pieces), so again, rather than individually measuring each piece of velcro, you could use the first, measured piece as a guide for cutting the rest



6

7. Take each 35 mm piece of velcro and, with the pointy side facing upwards, cut from bottom left to bottom right

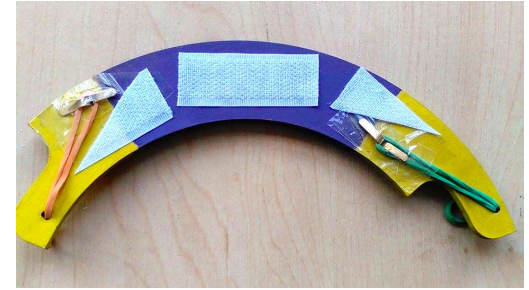


7

Chapter Five - Velcro and tape



8. Take a puzzle piece and stick your triangular pieces of velcro on either side of the central velcro strip, like so, with each triangle's hypotenuse running along beside its respective split pin and elastic band ->



8

9. Repeat steps 6 to 8 for all of your puzzle pieces



9

Chapter Six - the base pairs



- Using the table on the following page, copy the correct three letters of bases onto the left and right long edges of each puzzle piece, trying to keep an even spacing, like so ->



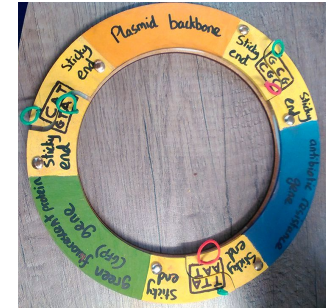
Puzzle Piece	Letters for left edge	Letters for right edge
Green fluorescent protein	TTA	GTA
Red fluorescent proten	TTC	TCG
Cyan fluorescent protein	GGG	TCA
Plasmid backbone 1	AGT	CTA
Plasmid backbone 2	CAT	CGC
Plasmid backbone 3	AGC	CTA
Plasmid backbone 4	GGT	ATC
Plasmid backbone 5	TTT	CTG
Antibiotic resistance gene 1	GAT	AAG
Antibiotic resistance gene 2	GCG	AAT
Antibiotic resistance gene 3	GAT	CCC
Antibiotic resistance gene 4	TGC	TAA
Antibiotic resistance gene 5	AAT	GAT

N.B. It does not matter which plasmid backbone pieces you use as 'plasmid backbone 1', 'plasmid backbone 2', etc - the numbers have just been added to differentiate between the pieces on this table. Don't actually write the numbers after each plasmid backbone label on the puzzle pieces. The same applies for the antibiotic resistance gene pieces

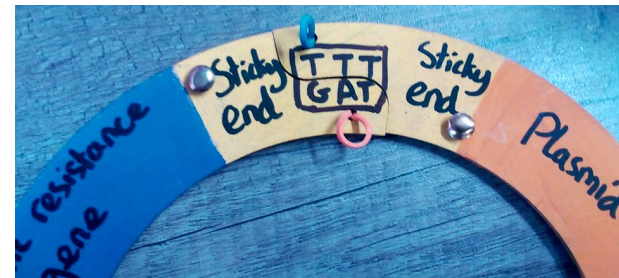
Chapter Six - the base pairs



2. With all the base letters written out, match the correct sets to form the three three-piece plasmids and draw a box around each of the matched letter pairs ->



3. For the pieces left over, place random ends together and, for each end, draw a box around the two sets of non-matching letters, like so ->



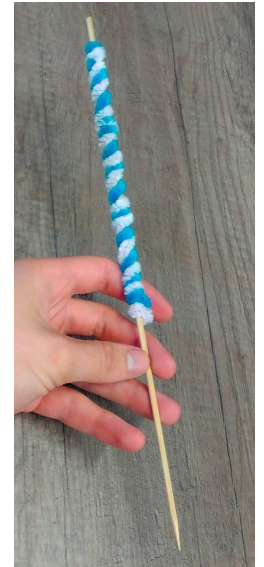
Chapter Seven - the enzymes



1. Take one wooden skewer and wrap a coloured pipe cleaner around it, starting at the non-pointy end and leaving a gap between each turn, as shown



2. Wrap another coloured pipe cleaner around the wooden skewer in the gap between the turns of the other pipe cleaner, and push the pipe cleaners together to ensure no gaps remain



Chapter Seven - the enzymes



3. Repeat steps 1-2 for the other two wooden skewers



You've assembled the game!



Now read the instructions for how to play the game and go for it!

