Technical information

Table of contents

Kit list – required equipment and consumables

As well as the equipment provided as part of the Hands-on DNA project, you will need a few additional items and also consumables. Table 1, below, details the consumables that Nowgen has used in developing and delivering the practical. Similar products are available from a range of suppliers. Please note that a generous discount from the list price can usually be agreed with most suppliers.

Note that all consumables (apart from plastic-ware) can be bought from Carolina, a company in the United States. This approach is convenient, but more expensive than ordering consumables independently. Should you be interested, visit their website at www.carolina.com; their consumables for *A Question of Taste* are at http://bit.ly/HoD-Carolina.

Equipment

Other equipment that is required or useful (*):

- stopwatches
- marker pens
- measuring cylinders
- thermometers
- spatulas
- fridge and freezer
- microwave
- glassware, especially 500ml conical flasks
- rack for microfuge tubes
- rack for 50ml tubes
- ice
- magnetic stirrer*
- paper towels
- containers for safe disposal of pipette tips
- trays to contain students' equipment*
- pipette racks*
- 'floats' for microfuge tubes in waterbath
- laminated dark card*
- thermal printer*

Required consumables

Description	Number required	Supplier and description	Approximate list price	Cost per workshop (25 students)
Gloves	27 pairs	Star Lab	£110.00 for 1000	£5.50
1.5ml microfuge tubes	100 tubes	Scientific Laboratory Supplies Ltd	£6.73 for 500	£0.37
1.5ml screw-cap tubes	25 tubes	Star Lab	£20.60 for 250	£2.06
Pipette tips 1-200µl	10 boxes of 96	Star Lab	£26.80 for 960	£6.70
Pasteur pipettes 1ml	25	Fisher Scientific Cat no. PMK-500-010R	£23.99 for 500	£1.20
PTC tasting strips	25	Anderson Scientific	£5.24 for 100	£1.31
Plastic cups	25	Supermarket	£0.70 for 25	£0.70
Table salt	1g	Supermarket	£1.00 for 1kg	£0.00
500ml drinking water	1 bottle	Supermarket	£0.39 for 500ml	£0.39
Chelex 100	2.5ml of 10% Chelex	Biotech grade 100g Biorad cat no. 14-2832	£142 for 100g	£0.36
Deionised water (used for preparing chelex suspension)		Scientific Laboratory Supplies Ltd	£12.47 for 5L	£0.01
0.2ml PCR tubes*	27 tubes	Applied Biosystems – Microamp fast reaction tubes 0.2ml	£35.00 for 1000	£0.95
PCR beads	27 beads	GE Healthcare cat no. 27-9557-02 (5x96)	£397	£22.30
PTC primers	607.5µl of PTC Primer Mix	Sigma Genosys (see *Advance preparation* document for ordering information)	22.92	£0.11
RNase/ DNase free water (for making primer mix)	607.5µl of primer mix	Qiagen 10 x 50ml cat no.129114	£68	£0.08
HaeIII restriction enzyme	26µl	New England Biolabs Cat no: R0108S (3000 units of 10 units per µl)	£39.00	£3.38
Falcon tubes (50ml) NB: Tubes can be washed and reused	9	Scientific Laboratory Supplies Ltd	£86.68 for 250	£3.12
Agarose (DNase, RNase free)	7.2g	Bioline Cat no. BIO-41025	£130.00 for 500g	£1.87
TAE buffer (50x) NB: running buffer can be re-used 3 times	3L of 1x TAE	Severn Biotech UK Cat no. 20-6001-10	£24.00 for 1L	£1.44
Molecular weight marker: 100bp DNA ladder	45µl per 25 students	New England Biolabs	£43.30 for 100 gels	£3.51
SYBR Safe DNA stain	32µl per 25 students	Invitrogen Cat no. S33102	£44.50 for 400µl	£3.56
Orange G loading buffer	104µl of 6x loading buffer per workshop	New England Biolabs Cat no. B7022S 6ml of 6x loading buffer	£29.00	£0.50
TOTAL per workshop of 25 students				**£59.42**

Table 1 Consumables required to run *A Question of Taste* for 25 students.

* These might not be required, depending on your PCR machine

Advance preparation

This section details the preparation required for the *A Question of Taste* workshop that needs to be done in advance of the day.

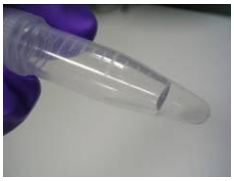
1. Preparing 10% (w/v) Chelex

Weigh out 2g Chelex beads into a small Duran bottle. Add up to 20ml with deionised or distilled water and swirl to mix contents.

20ml of 10% (w/v) Chelex is sufficient for 200 students to do the workshop. The volume made can be scaled up or down as necessary.

10% Chelex that has settled


When aliquoting, use a stirring platform and magnetic stir bar to keep the beads evenly suspended


Check that all aliquots contain approximately the same volume of Chelex beads

2. Primers for PCR

2.1 Ordering primers

Primers (or oligonucleotides/ 'oligos') are ordered from:

Sigma Genosys,
Sigma-Aldrich Company Ltd.,
Dorset, England.

Phone: 01202 712 300 Fax: 01202 715 460

Email: ukcustsv@europe.sial.com Website: www.sigmaaldrich.com/united-kingdom.html

2.1 Ordering primers (continued)

It is very important that you order the correct sequence for the primers. The order should read:

Name	Sequence (5' to 3')	Number of bases	Scale	Purification
PTC-F	CCTTCGTTTTCTTGGTGAATTTTTGGGATGTAGTGAAGAGGCGG	44	0.05	desalted
PTC-R	AGGTTGGCTTGGTTTGCAATCATC	24	0.025	desalted

Table 2 Primers required to amplify the correct region of TAS2R38. Note that there is no need for further purification of the forward primer. At the time of printing, this order costs approximately £23.00.

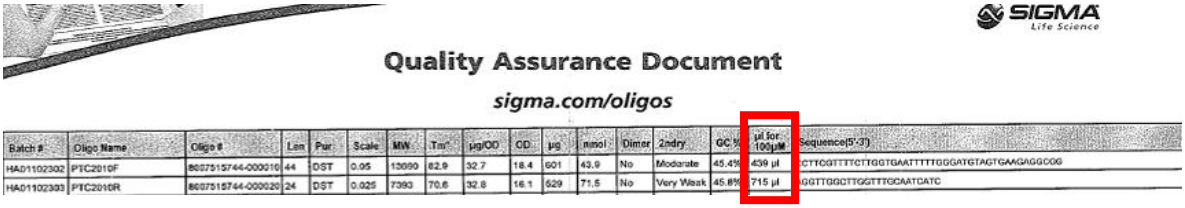
2.2 Making the PTC Primer Mix

Primers are received in lyophilised form, which means that the tube contains dried DNA and looks empty to the naked eye. They come with quality assurance information which is specific to each tube for each delivery. This information describes the chemical properties of the primers.

Figure 1 A lyophilised primer

The first thing to do is to make a 100µM stock solution of each primer:

1. Label the lids of the primers with F, denoting forward, and R, denoting reverse.
2. Centrifuge the tubes at 14,000rpm for 30 seconds.
3. Using a fresh sterile pipette tip, add the required volume of DNase and RNase-free distilled water to each primer to make stock concentrations of 100µM (see picture below).
4. Mix the solution by vortexing each tube for 20 seconds.


SIGMA Life Science

Quality Assurance Document

sigma.com/oligos

Batch #	Oligo Name	Oligo #	Len	Pur	Scale	MW	Tm°	µg/OD	OD	µg	nmol	Dimer	2ndry	GC %	µl for 100µM	Sequence(5'-3')
HA01102302	PTC2010F	8607515744-000010	44	DST	0.05	13690	82.9	32.7	18.4	601	43.9	No	Moderate	45.4%	439 µl	CCTTCGTTTTCTTGGTGAATTTTTGGGATGTAGTGAAGAGGCGG
HA01102303	PTC2010R	8607515744-000020	24	DST	0.025	7393	70.6	32.8	16.1	529	71.5	No	Very Weak	45.8%	715 µl	AGGTTGGCTTGGTTTGCAATCATC

Figure 2 Information provided with primers.
PLEASE NOTE: This is only an example. You **must** use the information provided with the primers you purchase.

2.2 Making the PTC Primer Mix (continued)

You now have 2 stock solutions of 100µM, one containing a forward primer sequence, and one a reverse primer sequence.

To make the **PTC Primer Mix** for the workshop:

1. Label a sterile 1.5ml microfuge tube with 'PTC Primer Mix'.
2. Using a fresh, sterile pipette tip, add 994.8µl of DNase and RNase-free distilled water.
3. Add 2.6µl of forward primer and 2.6µl of reverse primer from the stock solution tubes. **Use a fresh pipette tip to add each primer.**
4. Vortex for a few seconds to mix.

This is the primer mix that you need for the workshop. It is sufficient volume for approximately 44 samples.

The PTC Primer Mix and 100µM stock solutions should both be stored in the freezer. It is good practice to avoid repeatedly freezing and thawing primers, so we recommend making up several tubes of PTC Primer Mix in one sitting. These can be stored in the freezer and defrosted on the day of the workshop. Defrosting 1ml of primer mix will take around 10-15 minutes if left alone.

3. Programming the PCR machine

Program your PCR machine to complete the following program:

Denaturing step	94°C	30 seconds	35 cycles
Annealing step	58°C	45 seconds	
Extension step	72°C	45 seconds	
Finish:	4°C	Hold	

Protocol for setting up and delivering the workshop

This following section details everything you need to deliver the *A Question of Taste* workshop. It is colour coded to show the things you need to do on the day:

Setting up before the workshop (takes 1 – 1.5 hours)

Required consumables and resources

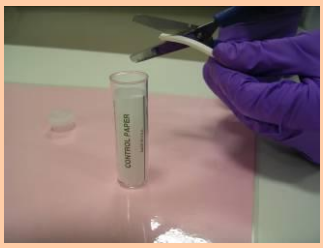
Protocol for delivering the workshop

Part 1: Student taste test

As expectation can affect students' perception of taste, we recommend that the taste test is performed at the beginning of the practical workshop, before students have been given any information about the taste of PTC, and the knowledge that people differ in their ability to taste it. If possible, ask the students to keep their reactions to themselves until all of the participants have recorded their results. This may be difficult as PTC tastes extremely unpleasant to tasters.

Taste test – setting up

Use a clean pair of scissors to cut the corners off from the control paper strips so that they are distinguishable from the PTC papers.



Taste test – provide each student with:

- 1 control taste paper (corner removed)
- 1 PTC taste paper
- a results worksheet and pen

Taste test – protocol

1. Students should wash their hands.
2. Ask students to put the paper strip with the corner removed on to their tongue for 5 seconds. Students should record the intensity of the taste on the results worksheet.
3. Repeat step 2 with the second paper strip.

Part 2: DNA extraction

Students should wear lab coats for this part of the protocol.

DNA extraction – setting up

1. Prepare 0.9% saline solution. Dissolve 4.5g of common table salt in a 500ml bottle of mineral water. Pour approximately 10ml (better to have less than more) into sterile plastic cups (1 per student).
2. Place the bottle of 10% Chelex onto the stirring platform so that the suspension is agitated. Using a 1000µl pipette, transfer 100µl of 10% Chelex into a sterile screw-capped tube (1 per student). Visually inspect the tubes to check there is sufficient Chelex present.
3. Turn on the hot block/ water bath at 99°C.

DNA extraction – provide each student with:

- a plastic cup containing 10ml of 0.9% saline solution
- 1.5ml screw-capped tube containing 100µl 10% Chelex
- a fine permanent marker pen
- 2 x sterile 1.5ml microfuge tubes
- 1ml Pasteur pipette
- three or four paper towels
- 200µl micropipette
- sterile pipette tips (1-200µl)

DNA extraction – protocol

1. Ask students to label all 1.5ml microfuge tubes and the plastic cup with their initials or lab number.
2. Whole class mouthwash – students should rinse their mouths with 10ml of saline solution for one minute (time with stopwatch). Encourage students to chew their cheeks gently to slough some cells off, and to rinse their mouths thoroughly before spitting the solution back into the cup.

DNA extraction – protocol (continued)

3. Ask students to transfer approximately 1ml of saliva/ saline solution to a labelled 1.5ml microfuge tube using a 1ml Pasteur pipette. Students should swirl the contents and pipette from the bottom.

4. Centrifuge tubes at 14,000rpm for 90 seconds.

5. Cell pellets should be checked to ensure all pellets are at least 2mm in diameter.

NOTE: If a pellet is too small, ask the student to gently pour off the supernatant and then to add another 1ml of their saliva/ saline solution to the same tube and spin again.

6. Ask students to gently pour off almost all of the supernatant back into the plastic cup, leaving between 50 and 100µl (the first graduation mark on the microfuge tube). They may need to gently tap tubes upside down on paper towel.

7. Ask students to resuspend their cell pellet by holding the base of two tubes against each other in the vortex mixer.

8. Students should pipette 30µl cell suspension into their screw-capped tube containing 100µl 10% Chelex. Ensure tube lids are closed firmly.

9. Add tubes into hot block at 99°C for 10 minutes.

10. Ask students to vortex their sample for 10 seconds.

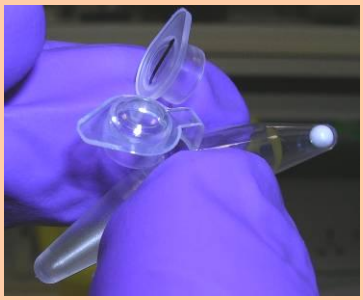
11. Centrifuge samples at 14,000rpm for 90 seconds.

Part 3: Amplifying DNA by PCR

Students should wear lab coats and gloves for this part of the protocol.

PCR – setting up

1. Calculate number of PCR samples (number of participants plus 2 control samples).
2. Transfer the appropriate number of PCR beads into correct PCR tubes.
3. Defrost PTC Primer Mix and pipette 50 or 75µl into sterile microfuge tubes (enough for 2-3 students). Store in fridge.
4. Defrost positive and negative controls



PCR – provide each student with:

- a cup containing ice
- a 0.2ml or 0.5ml PCR tube containing a PCR bead
- aliquot of PTC Primer Mix (one between 2-3 students)
- a fine permanent marker pen
- 20µl and 200µl pipette
- sterile pipette tips (1-200µl)

PCR – protocol

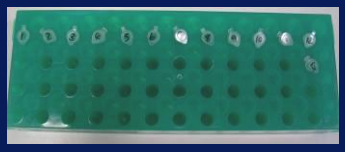
Students should incubate samples on ice at all times when setting up their PCR reaction.

When setting up positive and negative controls:

- **Positive control** – use 2.5µl DNA pre-extracted from a homozygous taster.
- **Negative control** – use 2.5µl DNase and Rnase-free distilled water.

PCR – protocol (continued)

1. Ask students to label the PCR tubes with their initials or lab number.



2. Students should load a fresh pipette tip and transfer 22.5µl of the PTC primer mix into the PCR tubes containing the PCR bead.

3. Students should load a fresh pipette tip and transfer 2.5µl of their cheek cell extract into the PCR tube.

NOTE: It is essential that this 2.5µl is taken from the top of the sample and does not contain any Chelex beads (which inhibit the PCR). If the beads are disturbed, re-spin and repeat step 3.

4. Students should close lids of PCR tubes very tightly to prevent evaporation during PCR.

5. Ask students to place their tubes into the PCR machine.

6. Run the PCR program and make a note of the time the reaction will finish.

Part 4: Pouring agarose gels

Students should wear lab coats and gloves for this part of the practical. The following information is based on using BioRad mini gel tanks with 8 well combs. Volumes of gel, buffer and DNA stain will need to be amended for use with other gel equipment.

Pouring gels – setting up

1. Heat a water bath or oven to 60ºC.
2. Use the following table to calculate:
   a. How many gels you need to run the samples. Each gel will have a molecular ladder and negative control. Therefore there is enough space for 3 people's samples (6 in total) per gel.

   b. The volume of 1x TAE buffer you need. 1x TAE is used to make the gels and as the running buffer for electrophoresis. The following table includes for one positive control, one negative control and the molecular marker lanes.

Number of participants	**1**	**2-4**	**5-7**	**8-10**	**11-13**	**14-16**	**17-19**	**20-22**	**23-25**	**26 - 28**
Number of gels required	1	2	3	4	5	6	7	8	9	10
Total volume of 1x TAE needed (ml)	290	580	870	1160	1450	1740	2030	2320	2610	2900
Making up the gels										
Agarose (g)	0.7	1.3	1.9	2.5	3.1	3.7	4.3	4.9	5.5	6.1
1x TAE (ml)	35	65	95	125	155	185	215	245	275	305

TIP: TAE buffer in the gel tank can be used repeatedly (up to three times). For each workshop, check whether fresh TAE is needed for the gel tanks, if not just prepare enough 1x TAE to make up the gels themselves.

Pouring gels – setting up (continued)

3. Make 1x TAE buffer:
   e.g. For 1 litre, add 20ml of 50x TAE into a measuring cylinder and top up to 1 litre with deionised (preferred) or distilled (acceptable) water.

4. Make up the gel:
   Weigh out the agarose using a top-pan balance and add it to a 500ml conical flask. Use a measuring cylinder to measure the required volume of 1x TAE and add this to the agarose. Swirl contents to mix. Microwave on full power for 30-second intervals. After each interval, remove the flask and swirl the contents to assess the status of the gel. The gel mixture needs to boil and become clear, all powder should be dissolved and no disks should be visible. Depending on the volume of agarose being prepared, this should take 5-10 minutes.

HEALTH AND SAFETY: Molten agarose can superheat and boil over **after it looks settled**. Wear protective eye goggles and heat resistant gloves.

5. Aliquot 30ml of the molten gel into 50ml tubes. Screw on the lid and place in a 60ºC water bath to cool. Spare agarose can be used to make gels that students can practise with.

6. Just before pouring, add 3µl of SYBR Safe DNA stain to each 50ml tube. Screw on the lid and invert slowly 2-3 times to mix.

NOTE: SYBR Safe DNA stain is sensitive to light, so if possible minimise time between pouring and running to no longer than 2-3 hours.

Gel pouring – provide groups of 2 or 3 students with:

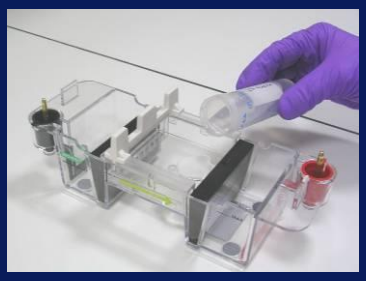
- gel pouring apparatus (tank, tray, comb, casting gates)
- a Falcon tube containing 30ml 2% agarose, containing SYBR Safe, at 60°C
- pipette tips (2-200µl)

HEALTH AND SAFETY: 60°C agarose can cause minor burns. Demonstrators should warn students to take care.

Gel pouring – protocol

1. Ask students to assemble the gel apparatus, ensuring the comb is in the correct position.

2. Ask students to carefully pour the gel into the gel tray. They should pour just enough molten agarose to cover the bottom of the tray. Any bubbles or lumps can be removed using the wide end of a sterile pipette tip.



3. Leave the gels to set for approximately 1 hour.

4. Turn down the water bath to 37ºC in preparation for restriction digest.

Part 5: Digesting PCR products with HaeIII restriction enzyme

Students should wear lab coats for this part of the practical.

Restriction digestion – setting up

1. Turn water bath down to 37ºC.
2. Prepare two 'master' tubes, one containing enzyme and water ('E'), the other containing just water ('H_2O'). There should be sufficient liquid in each tube for each student to take 2.5µl, plus a little extra.
   - For a class of 25 students, each master tube should have 80µl
3. The 'E' tube should contain a 1 in 2.5 dilution of HaeIII in water.
   - Continuing the example above, add 32µl HaeIII to 48µl ultra-pure water
   - 80 ÷ 2.5 = **32**
   - 80 – 32 = **48**
   - Mix well by pipetting up and down.
4. The 'H_2O' tube should contain just water; 70µl in this instance.
5. Split the master tubes into aliquots of 6µl: each pair of students will have 6µl of enzyme in a tube labelled 'E' and a 6µl of water in tube labelled 'H_2O'.

NOTE: **This step should be done just before the enzyme is required. Having the enzyme in water for too long will affect its activity.**

Restriction digestion – provide each student with:

- a cup containing ice
- two sterile 1.5ml microfuge tubes
- a tube containing enzyme (labelled 'E') a tube containing just water (labelled 'H_2O')
- 20µl pipette
- sterile pipette tips 1-200µl

Restriction digestion – protocol

1. Ask students to label two sterile 1.5ml microfuge tubes with their initials and the letter ‘D’ (which denotes ‘digested’) or ‘U’ (which denotes ‘undigested’).

2. Students should load a fresh pipette tip and transfer 10µl of PCR product into each tube. Ensure lids are closed tightly.

3. Students should add 2.5µl of the ‘E’ mixture to their ‘D’ tube and 2.5µl of ‘H_2O’ mixture to their ‘U’ tube. Tubes should be mixed by pipetting or gently tapping.

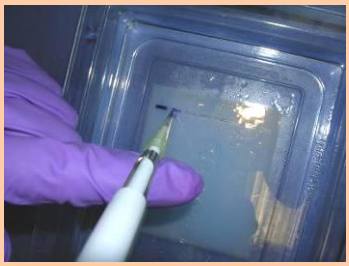
4. Centrifuge samples at 14,000rpm for 10 seconds. Ask students to place their tubes into a 37°C water bath for 30 minutes (use a polystyrene float!). Demonstrators to ensure bottom of tubes are in direct contact with water.

Part 6: Practising gel loading

Students should wear labcoats and gloves for this part of the practical.

Practising gel loading – setting up

Practice gels can be poured in advance, and we recommend reusing practice gels from previous workshops. Gels containing SYBR Safe DNA stain should not be used for practising gel loading. Transfer gels into a shallow vessel (a gel staining tray is ideal) and cover the gels with tap water.



Practising gel loading – provide each pair of students with:

- 20µl pipette
- pipette tips (1-200µl)
- 1.5ml microfuge tube containing loading buffer
- laminated dark-coloured card (loading aid)
- one gel, sat in a shallow tray and immersed in water

Practising gel loading – protocol

1. Demonstrate exemplary gel loading to the students. This should include placing elbows on the bench, using a forefinger to stabilise the end of pipette, slow pipetting action, removal of the pipette from the well before thumb is released etc.

2. Following demonstration, ask students to practice loading 15µl loading buffer into the wells until they feel confident in this technique.

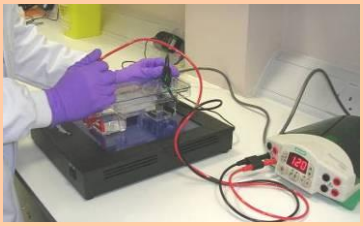
Part 7: Analysing PCR products by gel electrophoresis

Students should wear lab coats and gloves for this part of the practical.

Gel electrophoresis – setting up

1. Assemble gel apparatus (power packs, gel lids, pipettes, tips and tip disposal) in an easily accessible area

2. Decant 1x TAE into 250ml bottles



3. Place a dark coloured A4 laminated sheet underneath each gel tank (this enables students to see gel wells more clearly). Some gels can be run on top of transilluminators so that students can view electrophoresis as it happens.

4. Place a gel logging sheet and pen next to each gel, for students to label which lanes they have used.

Gel electrophoresis – provide groups of 2-3 students with:

- gel apparatus (gel tank and lid, 2% agarose gel, power pack)
- 250ml 1x TAE buffer
- 1 microfuge tube containing loading buffer (Bromophenol blue should not be used)
- 5µl 100bp ladder (between 3 students)
- gel logging sheet and pen

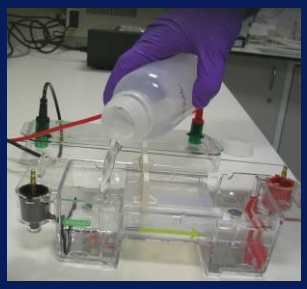
Provide each student with:

- a cup containing ice
- their digested sample (‘D’) in a 1.5ml tube – store on ice
- their undigested sample (‘U’) in a 1.5ml tube – store on ice
- 20µl pipette
- sterile pipette tips (1-200µl)
- laminated dark-coloured card (loading aid)

Gel electrophoresis – protocol

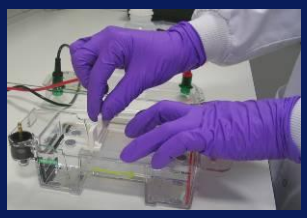
Encourage the students to order their group's tubes in a rack and to move them one by one as they load the samples.

1. Ask students to pour 1x TAE buffer into the gel tank until the gel is covered, and then to remove the comb from the gel.



2. Ask one student from each group to load 5µl molecular ladder into lane 1 on their gel.

3. Students should add 2.5µl of loading buffer to both samples, using a fresh pipette tip each time. Mix well by pipetting up and down.



4. Using fresh pipette tips, students should load 12µl of their undigested sample ('U') into the first available lane on the gel, and 12µl of their digested sample ('D') into the adjacent lane.

5. Students should make a note of which wells they have used on the gel-logging sheet.

6. Set gels running at 120V for 20 minutes.

TIP: Check that small bubbles are being formed on the cathode (black electrode at the top), and larger bubbles on the anode (the red electrode at the bottom).

7. After 20 minutes the bands should be separated enough to analyse. Check by looking at the gels running on top of the transilluminators. If necessary, run the gels for another 5-10 minutes.
8. After gel running, put gels on the transilluminator, cover with the orange filter and switch on the lamp to visualise results.

TIP: Keep the gel logging sheet with the gel at all times.

Part 8: Interpretation of results

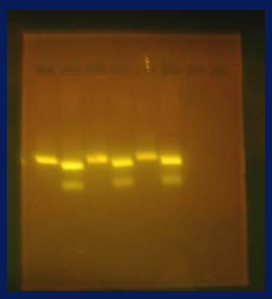
Students should wear lab coats, gloves and orange goggles for this part of the practical.

Interpretation of results – setting up

We recommend that a visual representation of each result is displayed for students to use as a reference.

Interpretation of results – protocol

1. Show students their bands on the gel. Explain molecular ladder, positive and negative controls and ask them to identify their genotype.



2. Ask students to record their results on their worksheet. Students should compare the genotype with the phenotype they observed at the beginning of the workshop.

A Question of Taste expected results

The undigested PCR product is 221 base pairs (bp) in length. This should run just above the 200bp marker in the molecular ladder lane. The digested product produces a 177bp and 44bp product. However, the 44bp product may be difficult to visualise.

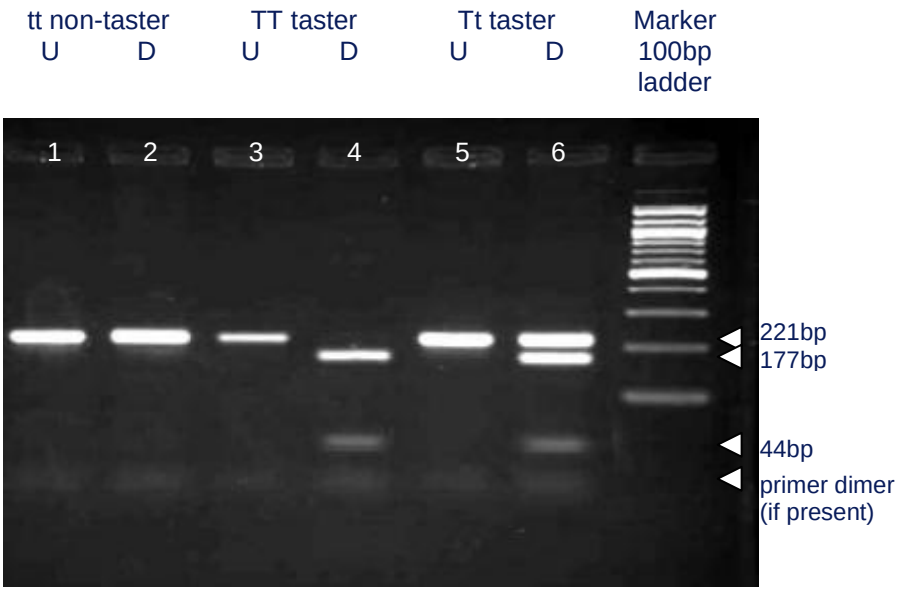


Figure 3 Ideal results from a homozygous non-taster (lanes 1 and 2), a homozygous taster (lanes 3 and 4) and a heterozygous taster (lanes 5 and 6), respectively. Gel picture by Dolan DNA Learning Center.

Genotyping

All undigested samples should have produced a band at 221bp.

TT Digested sample: bands at **177bp (and 44bp)**
People with this genotype can be termed *homozygous tasters*

Tt Digested sample: band at **221bp, 177bp (and 44bp)**
People with this genotype can be termed *heterozygote tasters*

tt Digested sample: band at **221bp**
People with this genotype can be termed *homozygous non-tasters*

At the start of the day...

Protocol	Description	Completed?
Taste test	Paper strips cut and distributed	
	Worksheet photocopied	
DNA extraction	Saline prepared	
	Chelex beads prepared	
	Chelex beads aliquoted	
	Hot block on at 99°C	
PCR	PTC primer stock solutions prepared	
	PTC primer mix prepared	
	PCR beads in correct tubes	
Restriction digestion	HaeIII diluted and aliquoted Hot block/ water bath at 37°C	
Gel electrophoresis	Practice gels ready	
	Water bath on at 60°C	
	2% agarose made and aliquoted	
	1x TAE buffer prepared	
	Loading buffer aliquoted	

Table 3 Tick-list of things that demonstrator(s) must complete

Top tips

Use of equipment

- It is **essential** that centrifuges are balanced correctly. Always ensure centrifuges are properly balanced before starting them.
- When loading microfuge tubes into a centrifuge, always have the hinges facing outwards (that is, towards the rim of the rotor). Pellets will then always form on the edge of the tube directly underneath the hinge, which makes them easier to locate.
- The contents of a microfuge tube should always sit at the base, with no air-bubbles. Droplets of liquid on the side of a microfuge tube can be 'encouraged' to move to the bottom of the tube by tapping the tube gently on a desk, or by holding the tube and sharply flicking your wrist.
- Large air-bubbles, sitting at the bottom of a microfuge tube, underneath the liquid can be removed by sharply flicking the tube with a finger; the tube will probably need to be briefly centrifuged after this, since droplets will almost certainly stick to the side in the process. Small air bubbles on the surface can be removed by centrifuging for a short period (around 10s).
- When using a vortex mixer to resuspend pellets, hold two tubes (one in each hand) such that their bases, near the pellet, are touching. Their hitting each other will speed up resuspension.
- Microfuge tubes with normal, hinged lids *can* pop open when on a hot block; this risk can be removed by using screw cap tubes instead.
- Ensure that caps on PCR tubes are firmly closed before loading into the thermal cycler.
- Thinner agarose gels are easier to visualise, while thicker agarose gels are more robust. However, since students will not be handling gels directly, thin gels should be sufficient. When pouring the gels, stop as soon as the surface of the casting tray is covered.
- After pouring gels or melting the agarose, rinse out the conical flask immediately using hot water to prevent residual agarose building up inside the flask.
- When students are loading gels, encourage them to hold their micropipettes at an angle so they can more clearly see where the end of the tip is. Their free hand should stabilise the barrel of the micropipette.
- When visualising gels, the darker the room, the better. If total darkness is difficult to achieve, a simple viewer can be made by cutting a slit out of a cardboard box that sits over the transilluminator.
- For practice gels, the loading dye in the wells will diffuse out if the gels are left submerged in water overnight. It is best to store practice gels in the fridge, submerged in water, so they can be used repeatedly.

Consumables/ chemicals

- It's often a good idea to centrifuge tubes (for example, containing SYBR Safe and HaeIII) very briefly (around 5-10s) before opening them. This will ensure all liquid is in the base of the tube, not on the sides, or caught in the threading of the lid, which is difficult to see and can easily result in leaks onto gloves. Also, tubes which might appear empty, often have liquid in, which will be spun to the bottom of the tube by centrifugation.
- If making a master mix, or anything that is to be aliquoted (decanted into smaller tubes etc.), make an extra 10% to account for errors in pipetting/ measurement.
- Chelex settles very quickly. When aliquoting it, ensure that the tube is agitated frequently. Better still; if possible, use a magnetic stirrer to keep the whole suspension constantly moving.
- When reconstituting lyophilised primers, centrifuge the tubes briefly (approximately 5s) first before adding water. Once the water is added leave the tubes for between 20 minutes and half an hour before you do anything with them (such as aliquot or store at -20°C); during that time it's a good idea to flick the tubes sharply a few times (for example, immediately after adding water, 5 minutes later, and finally 10 minutes later).
- Avoid exposing HaeIII to room temperature; try and use a cold box when it's out of the freezer and always hold the tube near its top, away from where the enzyme is.

Health and safety

Basic issues

Very few of the chemicals used in A Question of Taste pose any real health risk. All chemicals that are used have a 'Material Safety Data Sheet' (MSDS) that will be available either from the website of the supplier, or it may even be delivered with the chemical itself. Centres should be familiar with the MSDSs of the different chemicals.

Storage

No chemicals have unusual storage requirements; nothing needs to be kept in fire-proof or locked cabinets. All chemicals should all be stored at either room temperature, 4°C or -20°C; they will say on them at what temperature they should be stored.

Because SYBR Safe is light sensitive, it should not be left out for long periods of time. SYBR Safe should be stored at room temperature in a dark cupboard/ drawer.

Disposal

- Students' mouth-wash samples
  - This can be poured directly down the sink by the students; they should be wearing gloves and lab coats when they do this.
  - Samples can be disposed of in bleach or Virkon, but these chemicals are arguably more dangerous than the samples themselves.
  - Plastic cups can be thrown away as normal waste
- Tasting strips
  - These can be disposed of as normal waste.
- Pipette tips
  - Pipette tips are sharp and should be treated with care. Tips should be disposed into a suitably robust container (Nowgen uses plastic tubs), which can then be disposed directly into normal waste.
- Microfuge tubes
  - These *can* go directly into normal waste, but for convenience it's probably easier to dispose of them with pipette tips (also, the hinges are sharp enough to tear waste bags).
- Agarose gels
  - At time of writing, SYBR Safe is considered non-toxic and non-hazardous; gels containing SYBR Safe can therefore be disposed of in normal waste. (See note below on SYBR Safe).
- TAE Buffer can be disposed of directly down the sink.
- Gloves can be thrown away as normal waste

Important additional considerations

Gloves

Centres should be aware that powdered latex gloves **must not** be used under any circumstances. Powdered gloves, in general, should be avoided since they can induce allergies. Nitrile rubber gloves are best since they have least potential to cause any allergic reactions.

SYBR Safe

SYBR Safe is one of a number of fluorescent chemicals that allow DNA to be visualised. Such chemicals work by 'intercalating' within the major or minor groove of DNA. When placed on an appropriate light source (such as a blue-light transilluminator) these chemicals absorb light at one wavelength (colour) and emit it at another.

Most commonly used in laboratories is the chemical ethidium bromide (abbreviated to EtBr or just ethidium). Ethidium is a known mutagen. By binding within the DNA it can cleave it in two, resulting in gross changes to DNA. Ethidium also has low levels of toxicity. It is important to note, however, that ethidium has **not** been conclusively shown to be a carcinogen.

SYBR Safe is a proprietary formulation, created by Invitrogen (part of Life Technologies) to address some of the safety concerns surrounding the use of ethidium in laboratories. To-date, no toxic or mutagenic properties have been described with SYBR Safe. However, we would still recommend caution when using concentrated, liquid SYBR Safe.

SYBR Safe is dissolved in a solvent called dimethyl sulfoxide (DMSO); a non-polar solvent that is capable of easily permeating nitrile gloves. As such, demonstrators and technicians should be aware when handling liquid SYBR Safe, and should any spill onto their gloves, the gloves should be changed as soon as is practical (within minutes). If there is a spillage of liquid SYBR Safe, demonstrators should wear two pairs of gloves (one over the other) while cleaning it up. Spills should be cleaned up using water and 70% ethanol solution.

Once SYBR Safe is in diluted into agarose and the agarose has solidified the likelihood of it being a health risk is negligible. However, demonstrators and technicians should avoid inhaling agarose vapours once SYBR Safe has been added.

More information on SYBR Safe is available on Invitrogen's website: http://bit.ly/SYBRSafe.

PTC

PTC is classified as toxic. However, because of the extremely small amount present in the taster strips, it poses no risk to health. Students are recommended to taste the strip briefly and just once; it is unlikely that students will carry out this practical multiple times, so repeated exposure is not an issue for them. For demonstrators and technicians, we recommend they demonstrate how to do the taste test using a control strip of paper (that is, one not impregnated with PTC) since they will be using it multiple times.

CLEAPSS (an advisory service providing support in science and technology) has recommended that students should not taste more than two PTC strips within this activity. Teachers should ensure that students wash their hands before putting paper strips into their mouths. Paper strips should be disposed of immediately into a normal bin to avoid the spread of germs.

Ethical considerations

It is strongly recommended that children and their parents do not participate together in this activity.

The ability to taste PTC is mainly due to differences at various locations within the *TAS2R38* gene. Therefore, it is a trait which is inherited from parents. Because of this, there are some combinations of results which could cause anxiety to parents and children who participate together in this activity. There are several explanations for unexpected results. These include the number of differences within the *TAS2R38* gene that can affect ability to taste PTC, differences in other genes involved in taste, age, smoking, and the density of a person's taste buds. These complex factors that contribute to the ability to taste PTC suggest that the taste test cannot definitively reveal any sensitive information between family members. However, it is strongly advised that this situation is avoided by preventing parents and children participating in this activity together and by ensuring that students do not have access to PTC strips that they could take home.

This taste test protocol has been considered by a leading UK geneticist who has stated that differences in the ability to taste PTC are highly unlikely to be associated with any other health conditions for the participants.

Nowgen's risk assessment

Visitors will be using the ground floor laboratory at The Nowgen Centre to carry out a practical experiment.

Description of experiment:

1. Students taste two paper strips; a control, and a second strip which contains 3-4 micrograms of phenylthiocarbamide (PTC).
2. Students collect their own cheek cells by swilling 0.9% saline solution around their mouths and spitting the cell solution into a cup
3. DNA is then extracted from the cell solution (using physical and chemical processes)
4. Isolated DNA is added to a tube containing reagents necessary for the polymerase chain reaction (PCR) and PCR carried out using a thermal cycler machine
5. The products of the PCR are cut up, using a specific restriction enzyme
6. The products are loaded and run on an agarose gel
7. Bands of DNA are visualised in an agarose gel (please see explanation in box below about the test that's being done)

In this workshop, students will work with their own DNA. They will use PCR to amplify a region of their *TAS2R38* gene, which is found on chromosome 7. Restriction digest of the PCR product using the HaeIII enzyme will cut some students' DNA at one specific point. This process allows the students to sort themselves into one of three genetic groups. Identifying this genetic difference means that students can understand the process that underlies tasting of the bitter tasting chemical, PTC.

Examining this gene reveals no information about any health related genetic information to the individuals. It is strongly recommended that this practical is not performed by parents and their children, as there are some patterns of results which could lead to doubts about maternity and paternity. Please see separate sheet for further details.

General risks when visiting the Nowgen Centre

Hazard	Possible outcomes	People at risk	Control Measures in place	Likelihood of occurrence
Coats and bags	Trip hazard when visitors move around the centre	Visitors	A coat rail will be used and bags will be kept together in a storage area to ensure walkways are kept clear.	Low
Electrical wires	Trip hazard from wires trailing on the floor	Staff & visitors	Wall mounted projectors will be used. No wires will trail on the floor.	Very low

Risks associated with procedures used in this practical

Hazard	Possible outcomes	People at risk	Control Measures in place	Likelihood of occurrence
Water spillages	Slip hazard from water on the floor	Staff & Visitors	Laboratory has a non-slip floor. We have a large numbers of paper towels and J-cloths ready to deal with any spillages.	Low
Using micropipettes and microtubes	The small plastic pipette tips and tubes could cause injury through stabbing or could be a choking hazard if swallowed	Staff & visitors	Participants will be adequately warned of the possible dangers and trained in how to use a pipette safely and correctly, especially how to dispose of tips carefully.	Low
Tasting PTC paper strips	Ingestion of too much PTC chemical	Visitors	Students will not be permitted to taste more than one paper strip within the workshop. This is below the limit provided by CLEAPSS.	Low
Swilling saline water around mouths	Sharing water could transmit infection	Staff & Visitors	Bottled mineral water and a sealed bag of new plastic cups will be used for every workshop. Each student will be given saline water in a fresh cup that will not be used by any other student. Everyone handling the cups will have washed their hands beforehand.	Low
Cheek cell solution in a cup	Cheek cell solution could transmit infection if spilt or spread unnecessarily	Staff & visitors	Participants will be warned to be careful about spillages and the cell solution will not be left on benches any longer than needed.	Low

Hazard	Possible outcomes	People at risk	Control Measures in place	Likelihood of occurrence
Pouring agarose gels	Molten agarose will be at 60ºC and could cause minor burns to skin.	Staff & visitors	All participants will be warned of heat-hazard and given a measured volume of agarose in a tube ready to pour into the gel apparatus. Agarose will be cooled to 60ºC before use by participants. They will be given the tube wrapped in a paper towel to protect their hands from the heat. All participants will wear gloves, which will also provide some thermal insulation.	Medium
Running gels	Electricity passes through the gel - risks associated with electrical equipment	Staff & visitors	Electricity cannot run through the apparatus unless the lid is secured, so the risk of electrocution is minute. Demonstrators will set up the electrophoresis tanks.	Very low
Visualising DNA	Damage to unprotected eyes from prolonged exposure to intense blue light	Staff & visitors	All participants will only visualise DNA using correct safety equipment. Amber filter unit or viewing glasses will be used at all times.	Low

Equipment used: micropipettes; water bath; heating block; centrifuge; vortex mixer; thermal cycler ('PCR machine'); electrophoresis gel tank; power pack, and transilluminator.

Participants will be supervised at all times in the laboratory. Some of the equipment will not be used by them directly; they will be observing how the equipment is used (for example, a microcentrifuge).

Risks associated with the chemicals used in this PCR practical

Hazard	Possible outcomes	People at risk	Control Measures in place	Likelihood of occurrence
Reagents used in PCR, restriction digestion	Substances could cause minor discomfort if swallowed or if splashed into eyes	Staff & visitors	Participants will be adequately warned of the possible dangers and trained in how to carry out experimental procedures.	Very Low
Agarose gel	Handling the gel may cause mild irritation to the hands	Visitors	Touching the gel should be avoided. The gels are made from agarose, which is a harmless polymer made from seaweed, but the dye and buffer in the gel could be a mild irritant.	Very low

Hazard	Possible outcomes	People at risk	Control Measures in place	Likelihood of occurrence
SYBR Safe™ DNA stain in DMSO (dimethyl sulfoxide)	DMSO may cause irritation to skin. DMSO and SYBR Safe could be absorbed through skin. No known toxicity.	Staff & visitors	Only Nowgen staff will handle stock of SYBR Safe. Students will only handle agarose containing a 1:10,000 dilution of SYBR Safe. Gloves will be worn at all times by people handling SYBR Safe or agarose containing SYBR Safe. Should stock SYBR Safe come into contact with gloves, gloves will be removed and replaced. Gloves will be removed shortly after handling agarose.	Very low

Further notes

- Students will be supervised by Nowgen and/ or trained University demonstrator staff at all times in the laboratory.
- Any spillages or accidents should be reported to Nowgen staff and they will take responsibility for the situation (first aider is on duty if needed).
- In the event of a fire, Nowgen staff will advise visitors of the action needed.
- Lab coats will be worn at all times in the workshop and safety glasses and gloves will be worn at specific points when needed.
- No food and drink is allowed in the lab.
- Hands should be washed before leaving the laboratory.

Risk assessment prepared by Matthew Hickman Signed by: Date: 31.08.1