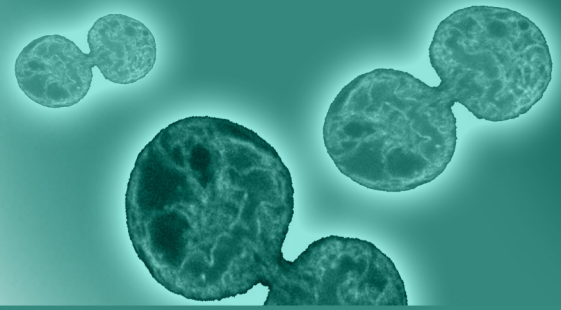# *KRAS* activity
Teachers' notes

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Background to activity

The *KRAS* (pronounced kay - rass) activity enables students to investigate common mutations found in the *KRAS* gene, associated with oncogenesis and the development of colorectal, lung and other cancers.   The *KRAS* gene encodes for the protein KRAS which is an intracellular signalling protein involved in promoting cell growth.

The full activity will stimulate discussion about the causes of cancer, gene mutations, protein structure and protein function.

Note on nomenclature

You will notice variation in the spelling of *KRAS*. This is due to the nomenclature of proteins and genes. When describing genes the name is italicised, so for our purposes the gene is spelt *KRAS*. When describing the protein, KRAS is used.

Estimated duration: 45 – 60 minutes (with presentation and discussion)

To run the *KRAS* session you will need to download the following documents from http://www.yourgenome.org/teachers/kras.shtm:
- "Investigating cancer" presentation.
- *KRAS* gene sequence banner **or** *KRAS* gene sheet*.
- Codon wheel sheet.
- Large arrows for marking mutations on the gene sequence.
- Squares for marking regions which have been checked.
- *KRAS* student worksheet and *KRAS* summary sheets. Alternative worksheets are available for students who are colour blind.

* Two options are available for recording mutations on the *KRAS* gene: the *KRAS* banner or *KRAS* gene sheet. The *KRAS* banner can be printed and presents a larger and more visual means of displaying the class results. The gene sheet (printed on A3 or A4) requires less preparation time and can be used with a larger number of students.

You will also need:
- Blu tack for sticking arrows and squares to the gene sequence banner.
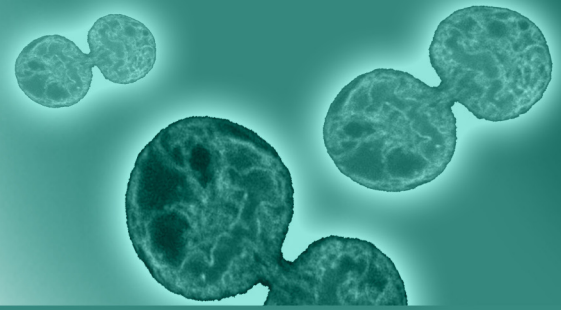- PC and projector.

Optional props/animations
- DNA model.
- Peptide and / or protein model.
- Wellcome Trust Sanger Institute cancer animations (Cancer_rogue_cells and Role_of_cancer_ genes).

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Optional exercise
RasMol software (available from http://rasmol.org/; select latest windows installer) is a molecular modelling software that can be used to highlight amino acids in the KRAS protein that are altered by the mutations in the gene sequence. This can be used either as a demonstration, or by the students, to identify the affected amino acids themselves.   Screen shots from the Rasmol program are provided in the "Investigating Cancer" presentation, so its use is not essential for completing the activity.

Activity preparation

The following activity components need to be assembled before the activity commences:

1. *KRAS* Banner
Print off all 16 sheets of the *KRAS* banner. We recommend laminating the sheets to prevent damage, however this is not essential. Cut along the dashed red lines; these indicate where to trim and overlay the sheets to prevent large gaps forming in the gene sequence. Attach the banner to one wall so it can be seen by the whole class.

2. Arrows and boxes
Print off one set of six arrows and 20 boxes in each colour (blue and red) and cut these out. Once again we recommend that these are laminated so they can be reused. Apply blu tac on the back of each arrow and box so they are ready to be stuck on to the banner.

3. Download RasMol program (optional)
If you wish to use the RasMol program to demonstrate the location of affected amino acids in the protein structure, download the program from: from http://rasmol.org/ and select "Latest windows installer" at the top of the page. Choose save and save to desktop.   Double click on the installer icon and press "I agree". If you have Windows Vista it may ask if you want to allow the installation to take place, select "yes".

4. Save the KRAS protein file
If you plan to use the RasMol program, ensure you have the KRAS protein file saved in an accessible folder or on the desktop.
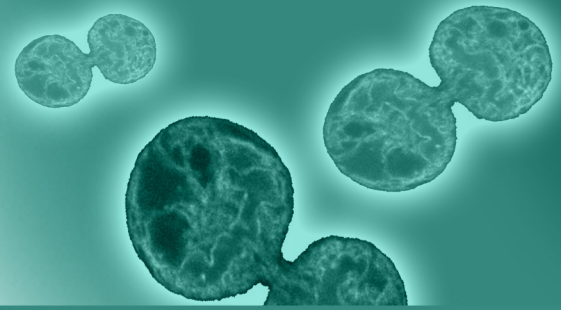
Materials per group of students
- One set of *KRAS* mutation sheets.
- One *KRAS* gene sheet **or** arrows and boxes to mark up mutations on the gene banner.
- Codon wheel(s).
- One summary data sheet to record group results.
- Pens.

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Instructions for running the activity

Stage 1: Introductory presentation

The "Investigating Cancer" presentation provides students with an overview of cancer. It introduces the concept that cancer arises due to abnormalities in DNA sequence and the various causes of these mutations.

There are several sections of the presentation that encourage interaction from the class:
- Slide 5: Tumour karyotype – ask students to count the chromosomes and relate this number back to a diploid human karyotype.
- Slide 12: Ask students to identify and name the mutation types.
- Slide14 – 16: Ask students to name cancers associated with the environmental factors displayed.

Stage 2: *KRAS* mutation finding activity and worksheets

The worksheets and activity can be introduced using the "Investigating cancer" presentation. In the first part of this activity, students identify differences between healthy and tumour cell *KRAS* gene sequences and mark these on the gene sheet or wall-mounted banner of the *KRAS* gene sequence.

The worksheets have raw *KRAS* DNA sequence traces (Sanger sequencing method) from healthy and cancerous cell samples represented as coloured line plots – one for each region of the gene. The four bases are represented on these plots by four different colours, listed below. Each coloured peak represents an individual DNA base.

Students should identify and translate the coloured peaks into base-letter sequences:

- Red   T
- Green   A
- Blue   C
- Black   G (normally these peaks are yellow but this is not easy to read on paper)

There are 11 numbered worksheets in total, each carrying two different regions of the *KRAS* gene. The six mutations present can be found on sheets one to six, so some shuffling is recommended. All sheets must be completed to ensure full coverage of the gene.   It is important to point out to the students that mutations are (relatively) rare, so not everyone will find mutations; this can be used to explore the importance of negative data and comprehensive coverage in scientific studies.

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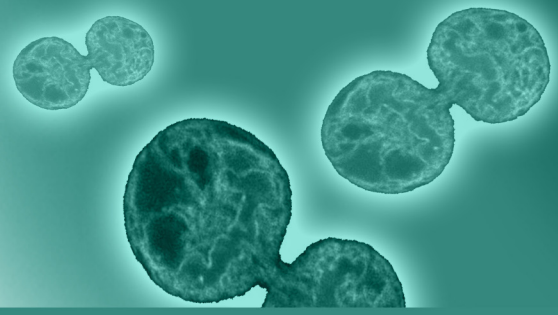
Interpreting the mutations

Students will compare a section of DNA sequence from a healthy cell and a corresponding tumour cell. The easiest way to identify whether a mutation has occurred is to write the DNA sequence below the coloured peaks (there is a key on the sheet to help) and to compare the written sequences.

If one of the letters is different (or a peak has changed colour), this indicates a mutation in the sequence.   See below:
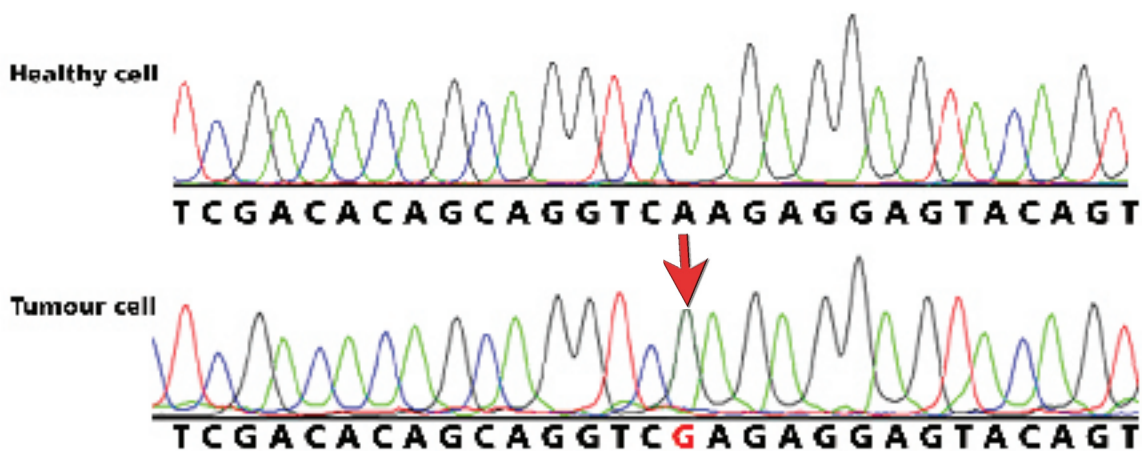
So, healthy cell DNA = A; tumour sequence = G.

If a student finds a double peak at one base position, this should be recorded with the two alternative bases at that position one above the other (as shown below).
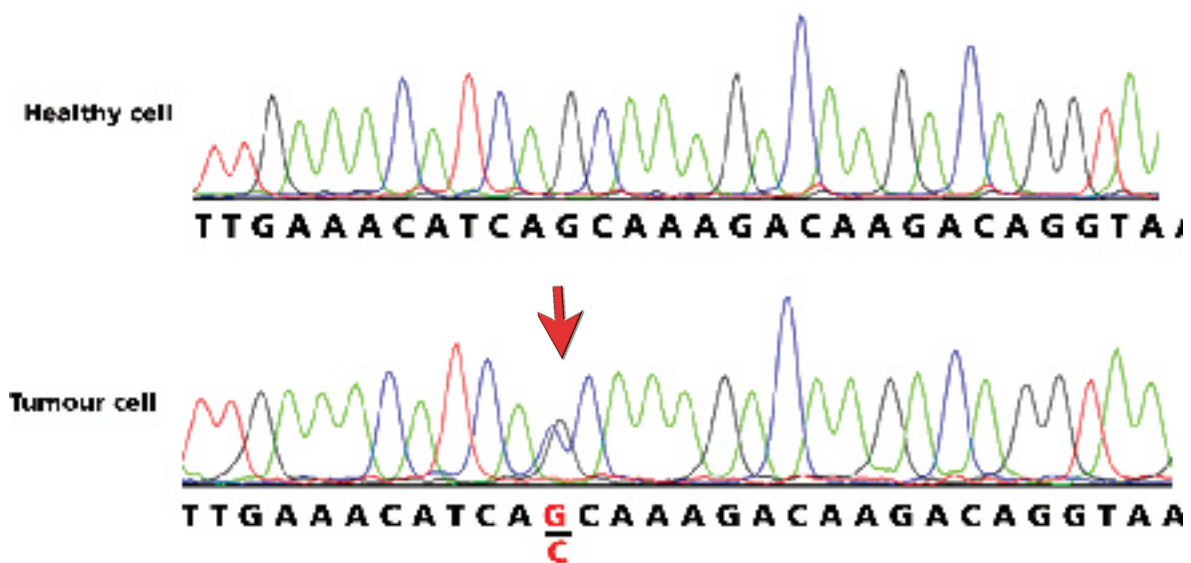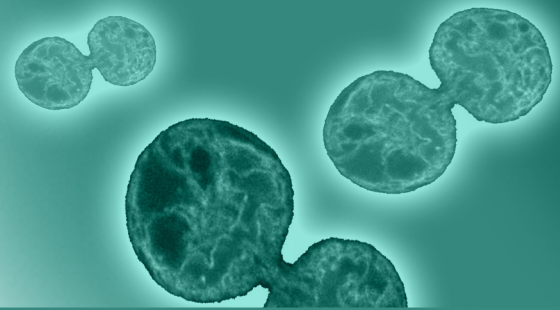

So, healthy DNA sequence = G; tumour sequence = C.

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Using the *KRAS* banner to mark mutations

What happens if students don't find a mutation?

All students should indicate the gene regions they have checked by placing a laminated square marker in the corresponding 'Region Checked' box on the gene banner (see diagram below). This is important as it demonstrates to the rest of the group that the region has been checked.
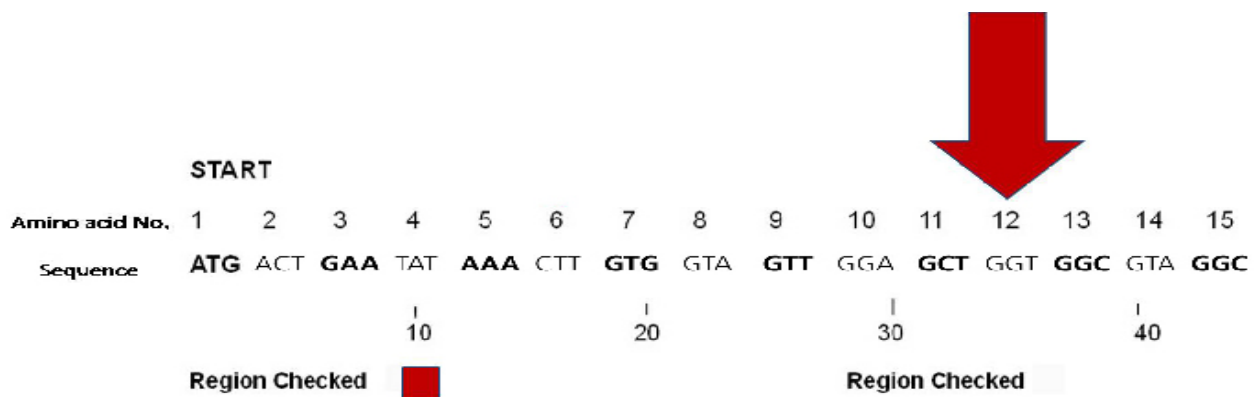
What happens if students find a mutation?

If a student has found a mutation they should indicate the specific base with a laminated arrow on the gene banner (see example above), and make a note of which amino acid codon this lies in (numbers 1 – 189 above the base sequence on the banner). Students who find a mutation fill in the table at the base of the worksheet, as shown below:

Amino Acid Number	Health DNA Sequence	Tumour DNA Sequence	Healthy Amino acid	Tumour Amino acid
12	GGT	GTT	Glycine (G)	Valine (V)

In order to translate the DNA sequence to the amino acid, students should divide the base-letters into three-letter codons; we've indicated these as bold or light text on the banner. They then use the codon wheel to work out whether the mutation has altered the amino acid. An explanation of how to complete the sheet and use the codon wheel is given in the presentation on slides 19 – 20.

When the group has found all the mutations they should record them all on the summary data sheet.
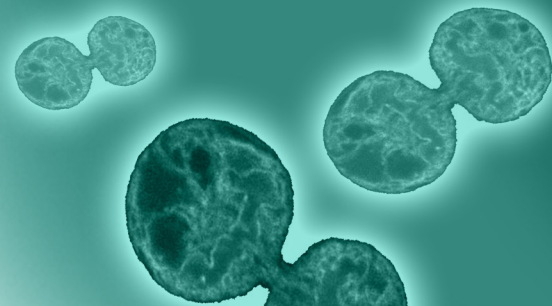
What if I have more than 11 students?

If you have a large class, we recommend that you divide the group into two teams (blue and red arrows/boxes), with both teams checking all 11 sheets and recording their results.

Using the *KRAS* gene sheet to mark mutations

What happens if students don't find a mutation?
All students should indicate the gene regions they have checked by placing a tick under the relevent region on the gene sheet (see diagram below). This is important as it demonstrates to the rest of the group that the region has been checked.

KRAS Gene

What happens if students find a mutation?
If a student has found a mutation they should indicate the specific base by circling it on the gene sheet and make a note of which amino acid codon this lies in (numbers 1–189 above the base sequence on the sheet). An example is shown above. Students who find a mutation fill in the table at the base of the worksheet, as shown below:

Amino acid number	Health DNA sequence	Tumour DNA sequence	Healthy amino acid	Tumour amino acid
12	GGT	GTT	Glycine (G)	Valine (V)

In order to translate the DNA sequence to the amino acid, students should divide the base-letters into three-letter codons; we've indicated these as bold or light text on the banner. They then use the codon wheel to work out whether the mutation has altered the amino acid. An explanation of how to complete the sheet and use the codon wheel is given in the presentation on slides 19 – 20.

When the group has found all the mutations they should record them all on the summary data sheet.

Stage 3: The results

The presentation includes the correct results on slides 24 and 25. Slide 24 is animated so that answers can be revealed and reviewed as the students read them out as a group. Alternatively the group can write their results on a flipchart and compare them to the results on the screen in one step.

Results are shown below. Columns with a * are not shown in the presentation but can be completed on the sheet during the results discussion.

Amino acid	Healthy sequence	Tumour sequence	Healthy amino acid	Tumour amino acid	Type of mutation *	Significant Yes / No*
12	GGT	GTT	G (glycine)	V (valine)	Base substitution (Missense)	yes
13	GGC	GAC	G (glycine)	D (aspartic acid)	Base substitution (Missense)	yes
30	GAC	GAT	D (aspartic acid)	D (aspartic acid)	Base substitution (Silent)	no
61	CAA	CGA	Q (glutamine)	R (arginine)	Base substitution (Missense)	yes
146	GCA	CCA	A (alanine)	P (proline)	Base substitution (Missense)	yes
173	GAT	GAC	D (aspartic acid)	D (aspartic acid)	Base substitution (Silent)	no

Discuss whether the mutations are significant – **will they have an impact on protein function or are they "silent"?**

The results above are all base substitution mutations. Substitution mutations that occur within the protein coding region of a gene may be classified into three kinds, depending upon what the altered codon codes for:

• Silent mutations: which code for the same amino acid.
• Missense mutations: which code for a different amino acid.
• Nonsense mutations: which code for a stop and can truncate (shorten) the protein.
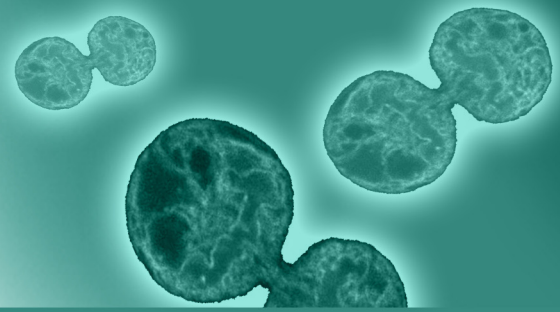
In this case, codons 30 and 173 are silent and therefore do not have a functional impact (the other mutations are missense mutations). Referring back to the mutation locations on the gene banner ask students to remove the arrows above 30 and 173.

Amino acid	Healthy sequence	Tumour sequence	Healthy amino acid	Tumour amino acid
12	GGT	GTT	G (glycine)	V (valine)
13	GGC	GAC	G (glycine)	D (aspartic acid)
61	CAA	CGA	Q (glutamine)	R (arginine)
146	GCA	CCA	A (alanine)	P (proline)

Stage 4: Explore the consequences

The information below will help you discuss the significance of the four mutations with the students.

Does a patient have to acquire all four *KRAS* mutations to develop cancer?
No, the mutations presented in this activity are the four most common mutations on the *KRAS* gene. A patient only needs one of these mutations for the protein to be affected. However this alone will not lead to cancer.   It is thought that mutations in five or more cancer genes are needed for a cell (and its offspring) to become cancerous.

Slide 26 presents a histogram showing all known *KRAS* mutations. This can be used to demonstrate that the mutations occur in key regions of the gene. This pattern of mutations across the gene is consistent with other members of the *Ras* family and has the signature of an oncogene.   There are clusters of mutations that only occur in specific regions of the gene. This means the protein will still function but in this case will encourage inappropriate cell growth as the protein is permanently "switched on" or activated.

Slide 27 shows a histogram of mutations from the tumour suppressor gene *RB1* which provides a comparison to the oncogene *KRAS*.   The key difference between *KRAS* and *RB1* is the range and frequency of mutations.

RB1 has 194 different mutations many of which truncate the protein. Tumour suppressor genes and the proteins they encode normally prevent inappropriate cell growth. However, these mutations "break" the gene, disrupting the sequence so that it can no longer encode a functional protein product; therefore the tumour cell continues to grow unchecked.

Slides 28 – 33 use a 3D space-fill image of the KRAS protein to show where the common mutations occur on the KRAS protein. You will notice they are all in the same region. This suggests that this region of the protein is important in its role as a cell signalling protein.

What is KRAS and what does it do?
KRAS is a protein encoded by the *KRAS* gene. It is a member of the Ras family, a group of proteins called GTPases that are involved in cell signalling. Members of the Ras family include HRAS, KRAS and NRAS.

Ras proteins communicate signals from outside of the cell to the nucleus. They are activated by interactions with GTP molecules and de-activated when the protein hydrolyses a phosphate forming a GDP molecule.   Mutations can permanently activate the protein and lead to inappropriate cell growth.

Slide 34 outlines the function of KRAS and has an animated diagram of a Ras protein to demonstrate this. This final slide allows the student to consider the impact of the mutations on protein function.

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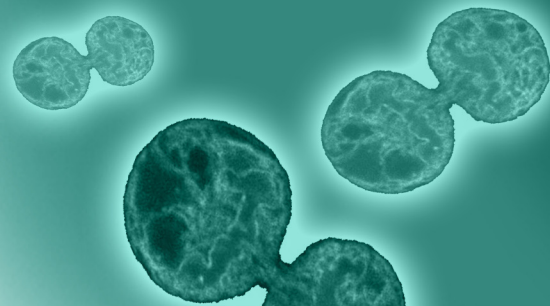
The *KRAS* oncogene has mutations that result in amino acid substitutions in four key regions (AA 12, 13, 61 & 146). The mutated gene encodes proteins with reduced GTPase activity. These proteins are locked into a GTP active state, continuously sending cell growth signals. This, along with other mutations in other cancer genes, can lead to the development of cancer.

Discussion: How does information like this influence our approach to cancer?

Use the information below to stimulate discussion on how genomic information can be used to further our understanding of cancer.

Discussion points for students:
- What experiments or approaches could be used to establish whether *KRAS* mutations are common to many cancer types?
  e.g. Sequencing DNA from multiple cancer types/samples.

- What could be the advantages of knowing this information?
  e.g. Sequence changes in *KRAS* may underlie many different cancer types – any treatment targeted at *KRAS* may have broad applications. Use the information below to stimulate discussion on how genomic information can be used to further our understanding of cancer and develop cancer treatments. *KRAS* is used as an example here.

Identifying and cataloguing key cancer genes:
Sequence changes in various Ras protein genes are found in 30% of all human tumours, with *KRAS* mutations being the most common. The frequency of some common *KRAS* mutations are shown in the table. This data is taken from the Catalogue Of Somatic Mutations In Cancer (COSMIC), a project that catalogues gene sequence changes associated with cancer. COSMIC is free to use from its website (http://www.sanger.ac.uk/genetics/CGP/cosmic/), providing a resource for cancer geneticists and biologists across the world.

COSMIC shows how cancer genetics and genome research has begun to offer important insights into cancer biology.

| Tumour type	Frequency of Mutations
Pancreatic	58%
Large intestine	33%
Biliary tract	31%
Small intestine	20%
Lung	17%
Ovary	14%
Cervix	%

Source: http://www.sanger.ac.uk/genetics/CGP/cosmic/
Data accessed: July 2010

By identifying sequence changes across the genomes of many different cancer samples, tissue types and cell types, we can build up a picture of those mutations common to all cancers and those associated with specific cancer types.   This catalogue or picture of genetic changes in cancer cell genomes helps us to focus cancer research and to better understand the contribution of changes in different genes to cancer development. An example is shown on the next page.
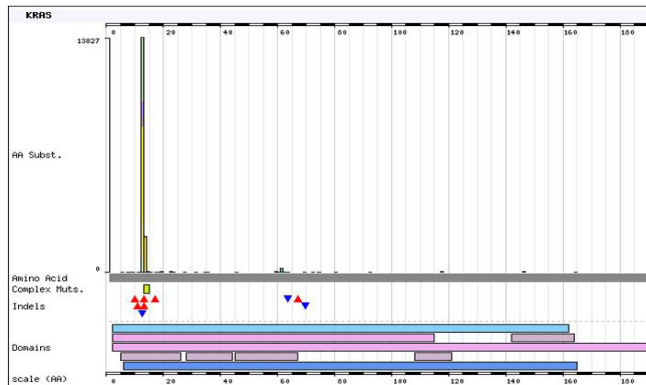
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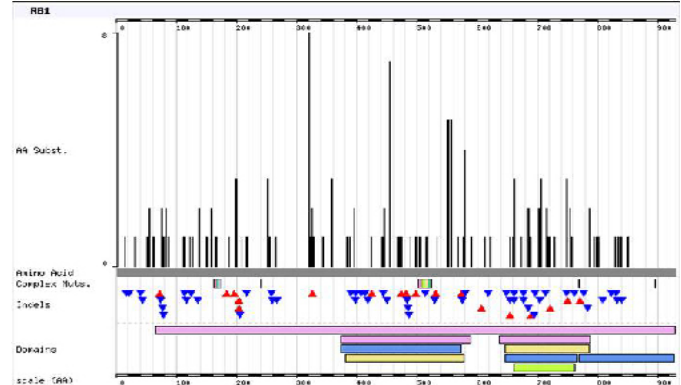# *KRAS* activity
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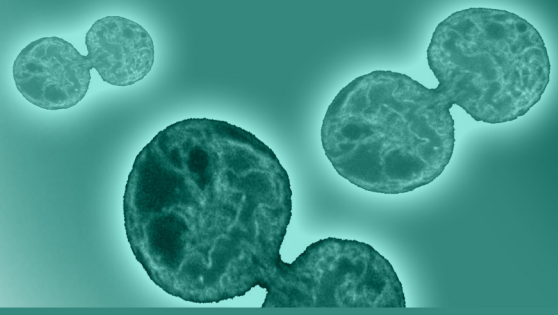
Distribution of somatic mutations in KRAS

Distribution of somatic mutations in RB1

The images above show graphical representations from the COSMIC database of the occurrence of mutations on the oncogene *KRAS* (left) and the tumour suppressor gene *RB1* (right).

The data shows that *KRAS* mutations are restricted to just a few key regions of the gene which results in the protein product being "permanently switched on" leading to inappropriate cell growth. *RB1* mutations in contrast are found across multiple regions of the gene and the majority truncate the protein which results in a non-functional protein product.

Translating research to the clinic
Researchers have only had access to the "finished" human genome sequence since 2004 and the technology to allow large-scale genome sequence comparisons since 2007. This means that whilst some benefits are already being seen we may be some years away from realising the full potential of this work in advancing clinical cancer treatment.

Understanding drug response
Cancer genomics is also helping us to better understand genetic influences on different patients' responses to cancer drugs. Gefitnib is a drug that was originally trialled to treat a broad range of lung cancers and was shown to have only limited effects overall, but was very effective in a small proportion of cases. Analysis of the genetics underlying lung cancers revealed that 4% of patients with a particular form of lung cancer ('non-small cell') have mutations in the *ERBB2* gene. This gene encodes the cell-surface receptor targeted by Gefitnib. By screening patients and targeting Gefitnib only to those with changed *ERBB2*, we may be able to significantly increase survival and recovery in this group.

### Developing new treatments
Data from cancer genome research has the potential to allow cancer researchers to focus biological experiments on key molecules and pathways that could lead to new drugs and treatments. The strong associations between *Ras* and *KRAS* gene mutations and cancer have led to studies aimed at understanding and influencing these pathways.

Research has shown that Ras and KRAS proteins regulate diverse and critical cellular pathways that are important in cell growth, the growth and spread of malignancies, cell cycle regulation and cell migration.

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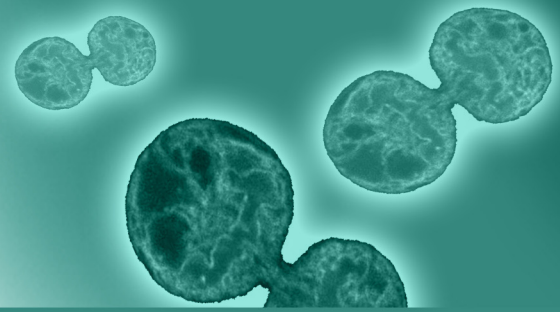
These proteins stimulate cell proliferation to repair tissue and heal wounds in response to epidermal growth factors but can also stimulate angiogenesis (blood vessel growth) through changes in gene expression. Experiments have clearly demonstrated the role of KRAS variants in malignant tumour development and maintenance. Despite looking like a good target for drug development, KRAS has confounded researchers and is at present un-druggable.

Other genes present a better outlook for drug development, for example *BRAF*, a gene mutated in 70% of malignant melanomas.   For more information on a new *BRAF* targeted drug visit the following online article: http://www.nytimes.com/2010/02/23/health/research/23trial.html

Why cancer genomics?

Cancer genomics is already generating information with the potential to prolong cancer patient survival, helping to manage cancer risk and progression through lifestyle choices and targeted medical interventions. However, cancer genome data is first and foremost an information resource to improve understanding of and direct research into, cancer biology. This means that the true potential of cancer genomics may not be realised immediately.

Something that was surprising from initial cancer genome studies was the sheer variety of genetic alterations present in apparently similar cancer samples. When the Cancer Genome Project team at the Wellcome Trust Sanger Institute investigated mutations in kinase protein genes (such as *KRAS*) in kidney cancer samples from 96 different patients they found that each patient sample was different in the number and nature of the mutations present. Findings like this indicate the complexity of cancer genetics, and just how much we have to learn before we can begin to build a coherent understanding of how different genes and pathways contribute to cancer.

Discussion point:

Cancer is a genetic disease, which is why many people believe that significant funding of research into cancer genetics is the best way of developing new cancer treatments and so deal with the disease. Cancer treatment and care for patients also requires large amounts of money (the NHS spent over £2 billion on cancer care alone in 2000). Where and how do students think money should be spent?
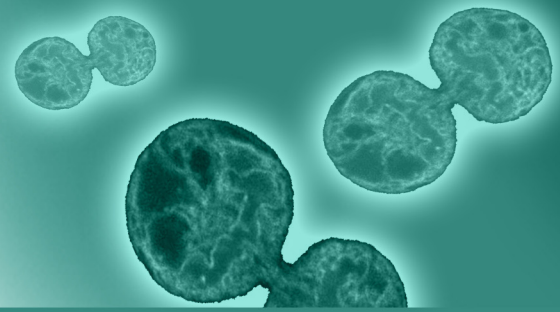
For example:

- The government provides funds for cancer care via the NHS and cancer research via the Medical Research Council.

- Some charities raise and spend funds to improve the lives of cancer patients and their care, e.g. Macmillan Cancer Support.

- Other charities aim to advance our understanding of the genetics and biology of cancer to help the development of new treatments, e.g. Cancer Research UK.

- The Wellcome Trust, the UK's largest biomedical charity, doesn't specifically fund cancer treatment research but does fund cancer genome research, with the view that this will provide the information and resources to drive forward the field as a whole in the years to come.

Optional extra: Model the protein using RasMol

Use of the RasMol software provides another way of exploring the effect of mutations on amino acid sequence and protein structure. This molecular modelling software can be used to highlight amino acids in the KRAS protein that are altered by the mutations in the gene sequence. RasMol can be used either as a demonstration, or by the students, to identify the affected amino acids themselves.

Viewing the KRAS protein in RasMol

If used as demonstration tool only, it is recommended that all of these steps are completed prior to the session:

1. Open RasMol.
2. Click 'File' 'Open' and select the K-Ras PDB file 2PMX.
3. The best way to view the molecule for this activity is space-filled. To do this, click 'Display' and 'Spacefill'.
4. As certain residues are to be coloured during the activity, it is best to make the molecule white. To do this click 'Colours' and 'Monochrome'.
5. This structure contains six chains (A to F) of which only chain A is required. To remove the other five chains from view, pull up the RasMol Command Line window (found in the task bar at bottom of screen) and type the following command:

    restrict :a

    This will hide chains B to F.
    The chains have not been deleted, just hidden.

6. To fix the point of rotation around the central point of chain A (instead of the entire molecule) type the following command:

    centre :a

Highlighting the mutations

To highlight each altered amino acid, the amino acid is first selected and then coloured. For example, to colour amino acid 12 red type the following:

    select 12 (then press enter)
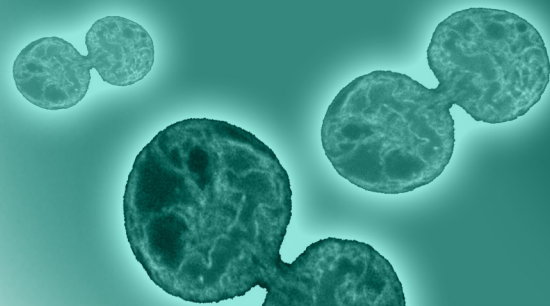   colour red (then press enter)

Repeat this for codons 13, 61 and 146.

In this activity, amino acids 12, 13 and 61 are located on the surface of the protein, adjacent to one another. Amino acid 146 is partially buried and located slightly further away from the other group of three.

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Pre set colours for RasMol

Only a few pre-set colours can be used to display molecules on RasMol, these are shown below:

red	yellow	blue	green	bluegreen	cyan
magenta	violet	orange	redorange	purple	black
white					

Note: Black and white should not be used in this activity. Yellow does not show up well. Also be aware of people who are red-green colour blind.

If you make a mistake by colouring the wrong molecule simply colour the molecule white (see below).
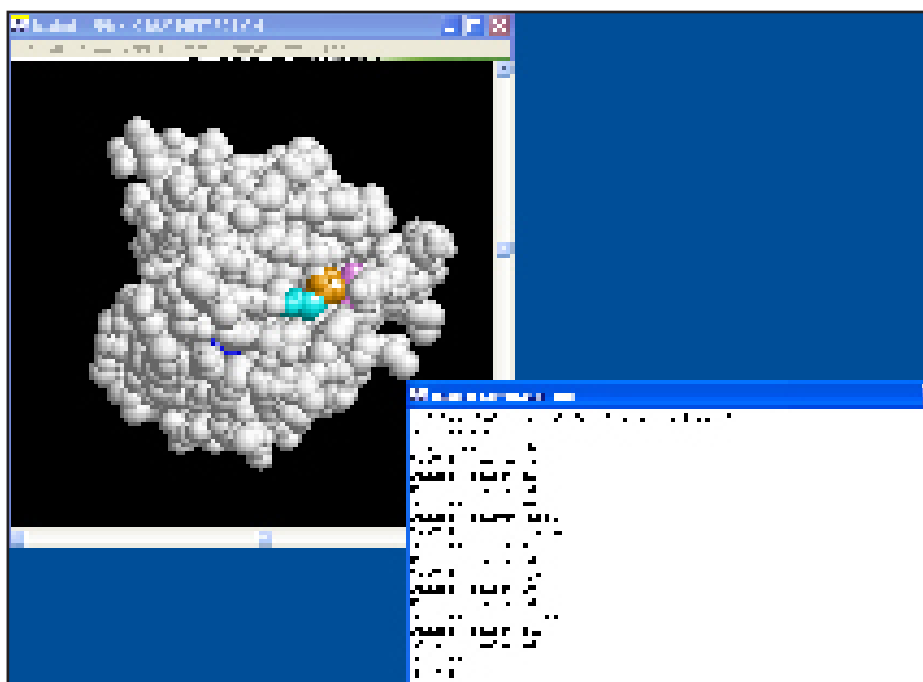
Useful RasMol controls

Zoom in and out	Hold Shift & Alt & the left mouse button. Slide the mouse forwards and back.
To centre the structure	Hold down the right mouse button and move the mouse.
To rotate the structure	Hold down the left mouse button and move the mouse.

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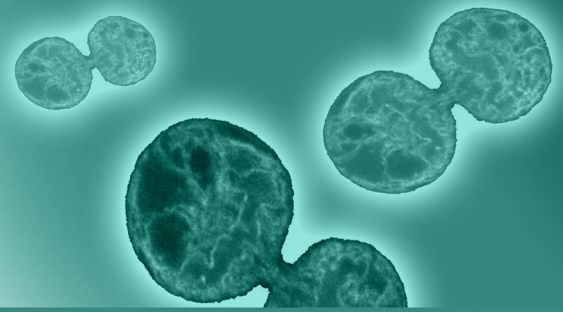
Recommended websites for student reference and discussion

Cancer Research UK
http://info.cancerresearchuk.org/cancerandresearch/
This website offers accessible information on all the major cancers and current research.

New Scientist Website
http://www.newscientist.com/topic/cancer
This website has a topic area focusing on cancer featuring latest articles on cancer research developments and interactive animations demonstrating targeted cancer drug functions.

Nature Milestones: Cancer
http://www.nature.com/milestones/milecancer/index.html
Milestones in Cancer offers a collection of selected review-type articles and an online library of recent research papers from the Nature Publishing Group, available for download as PDFs. It also has a cancer timeline showing major milestones in cancer research.

Inside Cancer
http://www.insidecancer.org/
This multimedia website created by the DNA Learning Center offers a multimedia guide to cancer biology, diagnosis and treatment.

Recent News articles

Bowel cancer gene hotspots find will tailor treatment.
http://news.bbc.co.uk/1/hi/health/8506404.stm   10 February 2010

First cancer genome sequences reveal how mutations lead to disease.
http://www.guardian.co.uk/science/2009/dec/16/cancer-genome-sequences-genetic-mutations.
16 December 2009

Scientists crack 'entire genetic code' of cancer.
http://news.bbc.co.uk/1/hi/health/8414124.stm (includes video interview with Professor Mike Stratton, Leader of the Cancer Genome Project). 16 December 2009

Further reading references

Stratton MR, Campbell PJ and Futreal AP. The cancer genome. *Nature* 2009; 458: 719-724.

Futreal A et al. A census of Human cancer genes. *Nature Reviews* 2004; 4: 177-183 (For a full catalogue of somatic cancer genes described in the above paper and created by the WTSI Cancer Genome Project go to: http://www.sanger.ac.uk/genetics/CGP/cosmic/)

Friday B B and Adjei A A. KRAS as a target for cancer therapy. Biochimica et Biophysica Acta 2005; 1756: 127 – 144

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