

TEACHING THE GENOME GENERATION

Identifying Hereditary Cancer Using BLAST



Introduction

Vacations at the beach, sometimes forgetting to wear sunscreen, nursing sunburns with soothing aloe vera—Patrice’s brain spun with memories of all the events over the last sixty-eight years that could have contributed to her melanoma diagnosis. She immersed herself in the literature, reading articles such as “*What is melanoma?*” and “*Surviving skin cancer.*” One figure stuck with her: *5-10% of melanomas are hereditary.* Patrice remembers her mother never leaving the house without her large sunhat after Patrice’s grandfather had passed away from skin cancer. Patrice wondered if she inherited a gene variant associated with hereditary melanoma, such as *MC1R*, *CDKN2A*, or *BRCA2* as the literature detailed. Nervously awaiting her genetic testing results, Patrice remembered her two daughters received genomic sequencing at birth. Maybe their newborn reports would be telling. She immediately called them and asked them to look at their NGRs. Neither had gene variants associated with melanoma. Maybe it was a coincidence that she and her grandfather both developed melanoma. *Maybe.*

Quick Knowledge Check:

1. How old is Patrice?
2. What type of cancer does Patrice have?
3. Who else in Patrice’s family had this same type of cancer?
4. What do you think the word “hereditary” means when it is used to describe cancer?

Activity 2: Identifying Hereditary Cancer using BLAST

To determine if the variants identified in a tumor are inherited, it is important to investigate the same genes in other body tissues. Why?

In this activity, you will analyze DNA sequences for a gene associated with increased risk for skin cancer, *CDKN2A*. For each section of the activity below, genomic sequencing revealed that none of the individuals has two copies of variant *CDKN2A*; all individuals with *CDKN2A* variants only have one copy variant copy. Therefore, all sequence comparisons will consider only one allele for each individual.

See BLAST Tutorial Series: [Comparing two or more DNA sequences](#)

Patrice

Patrice, age 68, received genetic testing on both her tumor tissue as well as the DNA isolated from a cheek swab. You will use BLAST to compare DNA sequences, specifically sequences of the *CDKN2A* gene. Variants in *CDKN2A* are associated with hereditary melanoma.

1. Locate the [DNA sequences for Patrice](#).
2. Then navigate to the [Nucleotide BLAST](#) website and select the option to “align two or more sequences.”
3. In the top box labeled, “Enter Query Sequence” paste the “CDKN2A Reference Sequence.” Copy the entire text of the sequence as it is written in the sequences file including the description line beginning with “>.”
4. In the box under “Enter Subject Sequence” paste the two sequences: Patrice’s tumor tissue and Patrice’s cheek swab.
5. Run the BLAST alignment and once the results load, use the “Alignments” tab and “Pairwise with dots for identities” view to compare the two sequences to the reference sequence.
 - a) Did you detect a variant in the tumor compared to the reference sequence?
 - b) If yes, describe the sequence difference.

- c) *Did you detect a variant in the cheek swab compared to the reference sequence?*
 - d) *If yes, describe the sequence difference.*
-
- e) *Does Patrice likely have an inherited variant in CDKN2A? Explain your reasoning.*

To confirm your idea, let's compare the *CDKN2A* genes sequenced from cheek swabs of Patrice's living relatives, none of whom have melanoma. Edit your BLAST search to align the [different sequences provided for Patrice's relatives](#) which include her three sisters and one brother.

6. To do this, clear the "Enter Subject Sequence" box and copy and paste each relative's cheek swab sequence into the box. These sequences can be pasted in succession with a return between them. Remember to include the description line starting with ">" for each sequence.
 - a) *What did you discover from comparing the relatives' sequences? Do any of them have a variant in CDKN2A?*
 - b) *If yes, is the variant the same as the one identified in Patrice?*
 - c) *Is this consistent with your answer to question 5e? Explain.*

Patrice reflects on the results of the genetic testing for herself and her family members. Her extended family is not overly close and her only memory of cancer in the family is her maternal grandfather who died of skin cancer when Patrice was very young. Neither of her parents had cancer before they both passed away over 10 years ago.

7. Consider all the information you have about Patrice and her family members:
- a) *Draw a pedigree or family tree indicating the inheritance pattern of heredity melanoma and CDKN2A variants in this family.*

- b) *Patrice's daughter is worried about her children potentially getting melanoma, are they at greater risk for melanoma?*

Monica

Patrice meets Monica in a skin cancer support group that she attends every week. Like Patrice, Monica also has melanoma, but is very young, only twenty-five-year-old. As a much younger individual, Monica received genetic testing at birth, which revealed a variant in the gene *CDKN2A*. Despite this information being included in her report, she was caught off guard with her diagnosis. She lives in a northern US state with long winters and has had very few sunburns in her life. She just did not expect to develop cancer at age 25. Concerned for her family members, Monica alerts her relatives about this variant and suggests that they consider genetic testing as several of them did not receive newborn genomic sequencing. A few of her relatives decide to get tested. Let's compare the *CDKN2A* genes sequenced from cheek swabs of her relatives, including her mother, brother, and paternal aunt, to the *CDKN2A* reference sequence.

1. Locate the [DNA sequences for Monica](#).
2. Then navigate to the [Nucleotide BLAST](#) website and select the option to "align two or more sequences."
3. In the top box labeled, "Enter Query Sequence" paste the "CDKN2A Reference Sequence." Copy the entire text of the sequence as it is written in the sequences file including the description line beginning with ">."
4. To identify which variant Monica has, copy and paste her tumor tissue and cheek swab sequences into the "Enter Subject Sequence." These sequences can be pasted in succession with a return between them. Remember to include the description line starting with ">" for each sequence. Run BLAST.

a) *Describe the variant you observe in Monica's cells.*

5. Now edit your BLAST search to align the [different sequences provided for Monica's relatives](#). To do this, clear the "Enter Subject Sequence" box and copy and paste each relative's cheek swab sequence into the box. These sequences can be pasted in succession with a return between them. Remember to include the description line starting with ">" for each sequence.

a) *What did you discover from comparing the relatives' sequences? Do any of them have a variant in CDKN2A?*

b) *What other information would you like to have to increase your confidence that Monica inherited the CDKN2A variant? Are there any individuals or data points that are missing?*

c) *Assuming that Monica has an inherited variant in CDKN2A, describe how it is possible that her aunt does not have the variant.*

Carlo

Another active member of the skin cancer support group is Carlo. Like Patrice, he did not receive genetic testing at birth seventy-six years ago and therefore, does not know if he has any gene variants associated with increased risk for melanoma. Carlo undergoes genetic testing on his tumor tissue as well as on a cheek swab.

1. Locate the [DNA sequences for Carlo](#).
2. Then navigate to the [Nucleotide BLAST](#) website and select the option to "align two or more sequences."

3. In the top box labeled, “Enter Query Sequence” paste the “CDKN2A Reference Sequence.” Copy the entire text of the sequence as it is written in the sequences file including the description line beginning with “>.”
4. In the box under “Enter Subject Sequence” paste the two sequences: Carlo’s tumor tissue and his cheek swab.
5. Run the BLAST alignment and once the results load, use the “Alignments” tab and “Pairwise with dots for identities” view to compare the two sequences to the reference sequence.
 - a) *Did you detect a variant in the tumor compared to the reference sequence?*

 - b) *If yes, describe the sequence difference.*

 - c) *Did you detect a variant in the cheek swab compared to the reference sequence?*

 - d) *If yes, describe the sequence difference.*

 - e) *Does Carlo likely have an inherited variant in CDKN2A? Explain your reasoning.*

 - f) *Carlo has three sons all who have not had genetic testing. Should Carlo tell his sons to get tested?*

After informing his relatives about the results of his genetic testing, one of Carlo’s sons mentions that his ten-year old son (Carlo’s grandson) has a *CDKN2A* variant on his newborn genomic report.

6. Edit your BLAST search to align the cheek swab from [Carlo’s grandson](#) to the reference sequence. To do this, clear the “Enter Subject Sequence” box and copy and paste the

cheek swab sequence into the box. Remember to include the description line starting with ">."

- a) *Describe the variant present in the CDKN2A sequence from the cheek tissue of Pat110's grandson.*

- b) *Describe how it is possible that Carlo's grandson has a CDKN2A variant in his cheek tissue.*

Resources

1. [BLAST Written Tutorials](#)
2. [BLAST Video Tutorial YouTube Playlist](#)

DNA Sequences for Patrice

CDKN2A Reference Sequence

>Reference Sequence

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Atggagccggcggcggggagcagcatggagccttcggctgactggctggccacggccgcccggggtcgggtagaggaggtgcgggcgc
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```

Patrice Tumor Tissue

>Patrice Tumor Tissue

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 gattga

Patrice Cheek Swab

>Patrice Cheek Swab

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 gattga

Patrice's Sister 1 Check Swab

>Sister 1

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 gattga

Patrice's Sister 2 Check Swab

>Sister 2

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Patrice's Sister 3 Check Swab

>Sister 3

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 gattga

Patrice's Brother Check Swab

>Brother

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gattga

DNA Sequences for Monica

CDKN2A Reference Sequence

>Reference Sequence

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gattga

Monica Tumor Tissue

>Monica Tumor Tissue

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gattga

Monica Cheek Swab

>Monica Cheek Swab

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gattga

Monica's Mother Cheek Swab

>Mother

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gattga

Monica's Brother Cheek Swab

>Brother

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gattga

Monica's Aunt (paternal) Cheek Swab

>Aunt

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```

DNA Sequences for Carlo**CDKN2A Reference Sequence**

>Reference Sequence

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gattga
```

Carlo Tumor Tissue

>Carlo Tumor Tissue

```
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```

Carlo Cheek Swab

>Carlo Cheek Swab

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gattga
```

Carlo's Grandson Cheek Swab

>Grandson

```
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