Steps taken to analyze Blast-Parser results within Excel
Results from TBlastX

Initial preparations

- Read output of Blast-Parser into Excel (make sure all files are visible, including text files; choose delimited file type (click on **Next**); choose comma as a delimiter (click on **Next**); click on **Finish**)
- Insert row before line 1 (put cursor at line 1; click on **Insert**; click on **Rows**)
- Add to top row titles for each column. If you don't know what the columns should be, visit the program that generated the output, particularly the subroutine that printed the output.
- Set the top row apart from the rest by underlining it. To do this, click on the row to select it and then click on the **Borders icon** in the tool bar. Select **single underline**. (Warning: you'll get less than satisfactory results if you click on **U**. That will just underline the individual words of the title line).
- Continue setting the top row apart by changing its color. To do this, click on arrow next to the **Fill color** icon (a tipping bucket) and click on your favorite color.
- Scan the data to see if the values are reasonable. If not, then back to the program!

> *Interlude: What are we trying to do here? We need to pick out those lines in which a match of one end is near the end of another and the two are oriented properly with respect to one another. Deal with the first criterion first.*

Redundency of data

> *Interlude: One thing you notice right away is that there are multiple hits between each contig end and the same region of the chromosome. Upon reflection, you'll see that this must be the case, since TblastX checks each of the six possible reading frames and can't possibly know which of the six (if any) are biologically relevant. So you can get up to six matches per area. Then there are matches that are close by, so the total number may be greater than six.*
> *What to do about this? One strategy is to do nothing. The multiple hits won't interfere with the analysis. On the other hand, it is irritating having to deal with a file six times bigger than it should be, so if you like, you can rid the file of the redundancy.*

Calculate Distances

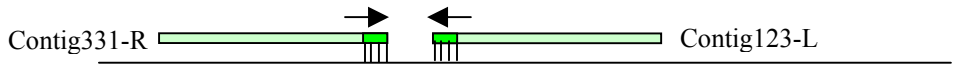
- Sort the output so that the lines are arranged in the order that they appear in the chromosome. To do this select the entire table (click on the upper left gray corner, above **row 1**; click on **Data**; click on **Sort**; make sure the **My list has Header row** is clicked; sort by the beginning of the hit in the chromosome/target sequence – whatever you called that column; click on **OK**).
- Make a new column header in the first available space (probably **M1**) saying something like *distance.*

- Enter a formula in **M3** that will subtract the end of the previous chromosomal location (in **J2**?) from the beginning of the chromosomal location on the current line (in **I3**?). =I3-J2 Problem: Are these locations correct? Are these locations correct for all orientations?
- Whoops! In my output at least, the locations are correct for the some orientations but reversed for the complementary orientations. This isn't a mistake in the Blast Parser, just a feature. One could reasonably list the beginning and end of a hit backwards or forwards for a backward looking hit. Unfortunately, listing it backwards (higher coordinate first) is inconvenient for my calculation of distance.
- Make all beginnings low number first, in the following way: **Insert** two columns before the column labeled distance. Do this by clicking the letter at the top of the column, then click on **Insert** and **Columns**. Do this again for the second column.
- Label the columns something appropriate (e.g. TargetLowEnd, TargetHighEnd).
- Enter a formula in the second row of the column labeled TargetLowEnd (or whatever):
  =MIN(I2,J2)
- Enter a formula in the second row of the column labeled TargetHighEnd (or whatever):
  =MAX(I2,J2)
- Block out the two cells you just entered and extend them to the bottom of the table (grab the little black box at the lower right of the blocked out cells).
- Check to make sure that the formula worked for all orientations of hits.
- OK, back to calculating distance. In the column labeled *Distance*, third row, enter the formula:
  =M3-N2
- Extend the formula to the bottom of the table
- Check to make sure that the subtraction worked properly

Check orientation

Interlude: What geometry are we looking for? This will take some thought. Certainly we don't want hits of the following variety:

The ends of these two contigs are close, but they are not nearby contigs at the opposing termini of a gap. What we DO want is:



*If you found ends in the orientation shown, it would be worthwhile making primers and trying to bridge the gap by PCR. So it is important which **types** of ends (L or R) of contigs are near each other. Consider also that what is labeled Contig331-R might*

well be Contig-447-L but flipped over. In other words, a complementary hit of a left-hand end will be oriented the same way as a direct hit of a right-hand end. So we are left with the following possibilities:

	Second hit			
First hit	**Left, direct**	**Right, direct**	**Left, complement**	**Right, complement**
Left, direct				
Right, direct	*good*	*ignore*		
Left, complement	*good*			
Right,complement				

You can fill in the rest of the table.

We thus need two pieces of information: the end of the contig that was matched and the orientation of the hit with respect to the chromosome. The end of the contig can be found in the first column, i.e., in the name of the contig.

- Label a new column (maybe "EndType"). In row 2, enter the following formula:

  =RIGHT(A2,1)

  This formula extracts the right-most character of cell A2, which happens to contain the letter L or R.

- Extend this formula to the bottom of the table.

  The orientation of a hit can be found from the signs of the open reading frames. "+" means the sequence was considered left-to-right. "-" means it was considered "right-to-left". An orientation of an end is "direct" if it's the same orientation as the chromosome (in other words, flip both DNA fragments if the chromosome is given in the "-" orientation). An orientation is "complementary" if it is opposite to the chromosome. It will simplify matters if we define a column "orientation" and determine its value from the given orientations of the queries (ends) and targets (chromosome).

- Label a new column (maybe "Orientation"). In row 2, enter the following formula:

  =IF(SIGN(G2)=SIGN(H2),"d","c")RIGHT(A2,1)

  This formula defines the orientation as **d**irect if the signs of the reading frames are the same for the contig and the chromosome (they matched in the same orientation); otherwise the orientation is defined as **c**omplementary.

- Extend this formula to the bottom of the table.

- Label a new column (maybe "OrientationCombo") and in row 3 insert the formula:

  =IF(AND(P2="R",Q2="d",P3="L",Q3="d"),"good","bad")

  This means that the cell is set to "good" if the previous hit is from a righthand end in the direct orientation and the current hit is from a lefthand end in direct orientation; otherwise

the cell is set to “bad” (all of the redundant hits except the first will always be set to “bad” since they have the same orientation as others of their set).

- If the formula seems to work properly (extend the formula to many cells to check), modify it to consider all the other combinations in the table above that you want to consider good. Do this using the OR function:

  =IF(OR(AND(..),AND(..),AND(..),AND(..)),”good”,”bad”)

  where AND(..) contains one of the conditions you determined to contain an appropriate set of orientations. If ***any*** of the conditions are met, then the cell will be set to “good”, otherwise it will be set to “bad”.

  Interlude: Programming in Excel is clumsy. It’s difficult to see what the formulas mean as you enter them and far more so later. For that reason, it’s important to go slowly, checking each step to make sure it does what you want.

Combine distance and orientation criteria and identify good PCR candidates

- You want to consider only those instances where the orientations are right and the distance between the ends are within range of PCR (let’s say within 8000 bp). Label a new column (maybe “PCRcandidate”), and in row 3 insert the formula below and extend it to the bottom of the table.

  =IF(AND(R3=”good”,O3<8000),”yes”,”no”)

- The lines marked “yes” are the ***second*** element of a two-end pair. To include the first element as well, label a new column (maybe “PCRpair”), and in row 2 insert the formula below and extend it to the bottom of the table.

  =IF(S3="yes","left",IF(S2="yes","right",""))

  This identifies the left element of a pair as one in which the ***next*** row is marked as a PCR candidate and identifies the right element of a pair as one in which the ***current*** row is so marked. The syntax reads: IF the next row is marked, then put “left” in the cell; otherwise IF the current row is marked, then put “right” in the cell; otherwise leave the cell blank.

- To examine only those hits that are PCR candidates, use Excel’s filtering capability. Click on the column labeled PCRpair (or whatever you labeled it), then click on **Data** in the tool bar, then **Filter**, then **Autofilter**. Click on the **down-arrow** that should have appeared at the side of the PCRpair column and click on **(NonBlanks)**.