





- **1=** Insert your sequence in any format
- 2= Calculate physical parameters of your sequence (Use Oligo design as a manual calculator for nucleic acid sequences or for rapid designing of primers)
- **3=** Obtain the reverse and complementary sequence from your input
- 4=SWAP sequences between your insert window and Reverse complementary window
- **5=** Select an optimum and desired length of your primers (Not less than 15 bp)
- 6= Find primers will show you possible standard primers at position no. 1 both sides (check the annealing position of your primer written just below each P)
- 7= Select an acceptable difference in melting temp. on which you need to get best match
- 8=Best match button will scan your sequence for primers that meet the difference you previously selected or allowed for your primers
- **9=** Buttons that change sequence of the primer from Forward to Reverse, sometimes the best matching pairs are selected at wrong position e.g. Forward primer at the very end of your sequence and RV primer at the very beginning (check the Annealing position for each) if you click these buttons you can simply use the Forward primer as reverse and vice versa.
- **10=** Now you can copy a single line report that contains your sequence, reverse and complement, physical parameters, and selected primers + their physical parameters Select the report field –simply click: cntrl+A >Cntrl+C> and past the report (Cntrl+V) in a document file.
- 11 + 12= Spanning feature is unique function for this application, it allows you to rapidly design primers for a long stretch of DNA sequence on an interrupted pattern defined by the user.

For instance if you select 100 at the scale button no. **12** – this means you design primers along (covering) your DNA sequence but each 100 bp – if your sequence is 1000 bp, then you should get 10 primer pairs covering your sequence and designed each 100 bp – this feature is quite useful for rapid designing of qPCR primers for chromatin immune ppt - ChIP experiments and other similar once like genome walking.

13= Start a new primer design

14=Don't hesitate to report your troubleshooting at adwy@biotech-apps.com

