**Tm score (S_{Tm}) for LHS and RHS:**
if \( Tm < Th + 2.5 \), then set \( S_{Tm} = 0 \)
if \( Tm > Th + 2.5 + 10 \), then set \( S_{Tm} = 1 \)
if \( Th + 2.5 \leq Tm \leq Th + 2.5 + 10 \), then \( S_{Tm} = (Tm – Th – 2.5) / 10 \)
where \( Tm \) is melting temperature of LHS or RHS calculated from UNAFold, \( Th \) is the hybridization temperature.

**ΔG score (S_{ΔG}) for LPO and RPO:**
\[ S_{ΔG} = ΔG – ΔG_{min} \]
if \( S_{ΔG} > 1 \), then set \( S_{ΔG} = 1 \)
if \( S_{ΔG} < 0 \), then set \( S_{ΔG} = 0 \)
where \( ΔG_{min} \) is the minimum \( ΔG \) specified by user.

**GC content score (S_{GC}) for LHS and RHS:**
if \( GC\% < GC\%_{min} \) or \( GC\% > GC\%_{max} \), then set \( S_{GC} = 0 \)
if \( GC\%_{min} \leq GC\% \leq 50 \), then \( S_{GC} = (GC\% - GC\%_{min}) / (50 – GC\%_{min}) \)
if \( 50 < GC\% \leq GC\%_{max} \), then \( S_{GC} = (GC\% - GC\%_{max}) / (50 – GC\%_{max}) \)
where \( GC\% \) is the GC content of LHS or RHS in percentage, \( GC\%_{min} \) and \( GC\%_{max} \) are the minimum and maximum GC content percentage specified by the user.

**Ligation Site score (S_{lig}) for LHS and RHS:**
\( S_{lig} \) is based on the immediate 4 nucleotides next to the ligation site
if all the 4 nucleotides next to ligation site are G or C, \( S_{lig} = 0.25 \)
else if all the 3 nucleotides next to ligation site are G or C, \( S_{lig} = 0.5 \)
else if all the 2 nucleotides next to ligation site are all G or C, \( S_{lig} = 0.75 \)
else \( S_{lig} = 1 \)

**The final score for a pair of LPO and RPO is calculated as:**
\[ S = \left( S_{Tm}(LHS) \times S_{Tm}(RHS) \times S_{ΔG}(LPO) \times S_{ΔG}(RPO) \times S_{GC}(LHS) \times S_{GC}(RHS) \times S_{lig}(LHS) \times S_{lig}(RHS) \right)^{1/8} \]