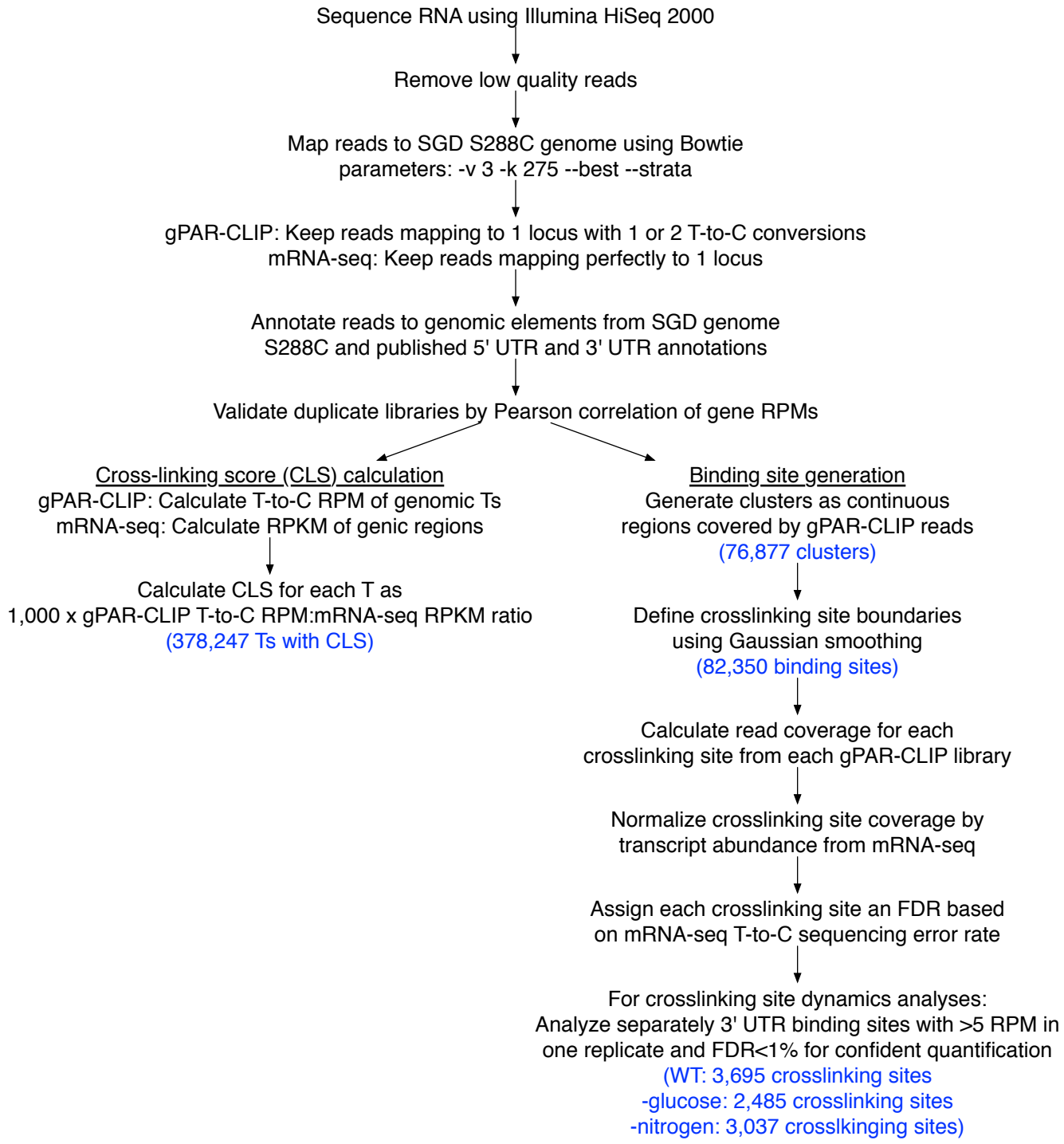Additional File 2