*Type-Common HSV detection via PROXAR / EXPAR*

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| **Figure 12****.** General scheme of PROXAR. Two probes, PT and PX, have “foot” sequences complementary to the chosen target DNA. 3’end of the PX is a random sequence which is complementary to the middle of the PT. When the two probes interact with target DNA they can also interact with each other forming a three way junction (3WJ) and a short “hairpin” region. This ds-DNA serves as target for Bst DNA polymerase which extends the 3’-end of the PX using the single stranded region of the PT as template. The extended PX now contains the recognition sequence for a nicking enzyme (NE), which cuts the synthesized strand after 4 bases on the 3’ side of the recognition sequence and releases the “trigger”, thus clearing the “trigger template” for next round of amplification and nicking. The released “trigger” then serves as a “primer” for exponential amplification of the EXPAR template which can be detected in real time or by lateral flow. |
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| **Figure 13.** Something about the PROXAR computational design tool |

We already discussed this at some length last time… Just brief recap, we designed at PROXimity Amplification Reaction (PROXAR) / EXPAR assay () targeting a conserved regions within the HSV glycoprotein B (gpB) for type common HSV detection.

**Jifeng:** Give an update on the PROXAR assay design tool – we mentioned just the first part last time, now we have the complete thing working. Describe the “twelve step program”…

We developed a computational tool to facilitate our PROXAR design. This tool can be applied to any designated target sequence to generate desired PT/PX combinations automatically.

This tool would first find all possible PT/PX feet (PT feet hybridizes to 5’ side of target, PX feet hybridizes to 3’ side of target, therefore differentiate PT foot, and PX foot), with TM above 65°C at (50nM, 5nM, 0.5 nM), with either 0b, 1b, 2b gap, if gap, preferable A or T. Also make sure the generated PT foot and PX feet did not have too much unwanted hybridize bonds to each other. Any PT/PX foot without certain level of conservation (too much mismatch or gap in the alignments) across different species was excluded from further screening. A few preferable hairpins were attached to PT/PX feet (also make sure the attached hairpins did have too much unwanted hybridization with the target sequence). The tool then hybridized acceptable PX (foot-hairpin) and PT (foot-hairpin) to itself to check if the melting temperature of the hybridization is less than 13°C. If they don’t, those PT/PX combinations were excluded from further screening. For each PX/PX combination remained, each PT(foot-hairpin) was then attached to a suitable X’ - XXXXGACTC (XXXX can be from 256 permutations, but preferably from a “known” template with good performance, X’ is the complementary of the trigger sequence) to generate a “complete PT”. To make sure the desired “complete PT” and PX (foot-hairpin) don’t have too much unwanted hybridization to themselves, we checked the secondary structure of the “complete PT” and PX (foot-hairpin). The “complete PT” and PX (foot-hairpin) are checked for similarity to human genome. For “complete PT” sequence, if one of the high-scoring segment pairs was longer than 19 bp and covers GAGTC, this “complete PT ” were considered to be unwanted. For PX, if one of the high-scoring segment pairs between PX sequence and human genome DNA was longer than 15 bp and there was a 3 mer or even longer match at 3'end of the target, this PX (foot-hairpin) were considered to be unwanted. Finally, we got our desired combinations, which are composed of pairs of acceptable “complete PT” and acceptable PX(foot-hairpin) came with that “complete PT”.