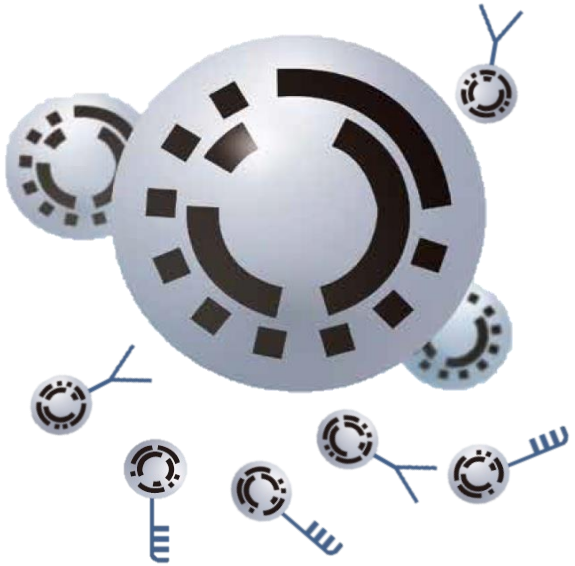
Frequently Asked Questions



1. What happens if I use expired IntelliPlex Assay kits?

Expired kits may lead to wrong or invalid results. Report or results derived from an expired kit will not be generated.

2. Are separate areas required for pre-PCR, sample preparation, and post-PCR operation?

Separate rooms, equipment, and consumables are required for all IntelliPlex assays. Work should be carried out in an unidirectional workflow, and lab equipment including consumables and PPE should not be transferred between rooms. Regular bleaching and cleaning are required to ensure reliable results. Using filtered pipette tips is also required. See ***X-3_Lab Requirement*** for more information.

3. Where should I store IntelliPlex Assay kits after I receive them?

Store all reagents at their respective storage conditions immediately after receiving the kits. The storage instruction can be found on the packaging label or in the user manual. Also be aware that reagents within the same IntelliPlex kits may require different storage conditions. In addition, shipping and storage conditions may be different.

It is also recommended to store reagents in their applicable work places. For example, storing primer mix and reaction mix in the pre-PCR room to reduce contamination. However, ensure only reagents from the same lot of the IntelliPlex kit are used together. Furthermore, storing samples and reagents separately in dedicated freezers is required.

4. How should I extract DNA/RNA from FFPE samples?

Refer to user manual of each kit for accurate information.

5. Can I quantify DNA/RNA samples with instrument other than Qubit™ Fluorometer?

IntelliPlex Assay kits require accurate DNA/RNA input to ensure performance. Excessive or insufficient DNA/RNA input might adversely affect the result. Use only **Qubit™ Fluorometer** to quantify DNA/RNA samples.

6. Does more or less DNA input affect results?

Accurate DNA input is required to ensure performance. Excessive or insufficient DNA input might adversely affect the result. Use input quantities exactly as stated in the User Manual.

7. How long can I store amplicons after PCR amplification?

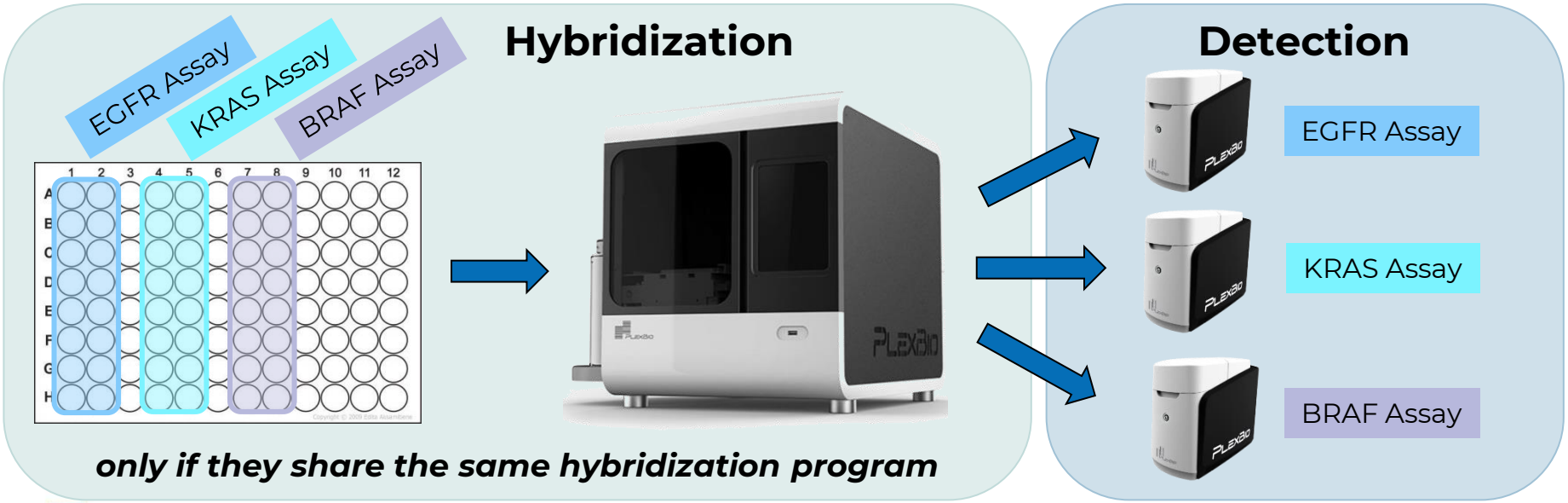
We recommend to perform amplicons denaturation as instructed in User Manual immediately before hybridization.
If needed, amplicons can be stored for up to **1 day at 4˚C**.
Re-denaturation of PCR product is not allowed.
The denatured PCR products should be used in **one hour** for the following hybridization and spin down before adding to wells.

8. After hybridization, how long can I store my plates before fluorescent analysis?

The results should be analyzed as soon as the hybridization process is finished to obtain accurate and valid results. SA-PE fluorescent signal decays from time to time. Besides, as SA-PE is light sensitive, exposure to light should be avoided.

9. Can I do multiple kits at the same time?

Different kits can be hybridized at the same time in the same plate only if they share the same hybridization program. However, DeXipher currently does not support multiple kits reading at once, therefore samples from different kits will need to be analyzed one kit at a time.



10. Does the result “None” in report really mean that there is no target (e.g. mutation) in the samples?

While “None” indicates that target is not detected by IntelliPlex kits, there are several reasons that could affect target detection.

- The quality of sample DNA/RNA
- The absence of sufficient amplifiable DNA/RNA
- The percentage of targets present in the samples is lower than limit of detection (LOD)
- The presence of PCR inhibiting substances

Besides, clinical interpretation of the results should be made in combination with all other clinical findings.

11. Why does πCode count control fail?

A minimum πCode count of each πCode ID is required to ensure the validity of the test. Low πCode count could be caused by several reasons.

- πCodes are not loaded evenly. Make sure to vortex πCodes thoroughly before adding to each well.
- Microbes grow in wash buffer or ddH_2O, which increases πCode loss rate. Use freshly prepared wash buffer and ddH_2O for hybridization.
- Microbes grow in the fluid path tubes. Wash fluid path tubes with 70% ethanol. Please see ***P-3_Long-term Shutdown*** for instruction.
- Plastic tips are dislocated or damaged. Contact PlexBio or local distributor for service**.**
- Follow basic maintenance instruction: empty waste bottle daily, renew U tray monthly, clean bottles and fluid path monthly, and renew V tray for each run, etc. See ***P-2_Basic Maintenance*** for more information.

12. What is Blank control? Why does Blank control fail?

Blank control is an oligonucleotide coupled to πCode, which has no cross reactivity with the targets being tested. Blank control monitors if the assay is performed properly.

- Prepare fresh and sufficient wash buffer connected to the **IntelliPlex 1000 πCode Processor**.
- Check if wash buffer bottle and water bottle are correctly connected to **IntelliPlex 1000 πCode Processor**.
- Make sure the correct hybridization program is selected. Wrong incubation temperature can result in high background signals.
- Make sure amplicons are generated within one day and freshly denatured before hybridization.
- Do not make any marking on **96-well microplate**. Markings may yield fluorescence background.

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- Make sure **PlexBio 100 Fluorescent Analyzer** is calibrated monthly. See ***A-2_Performing Calibration*** for more information.
- Check if the manifolds are clogged. Place a new microplate into **IntelliPlex 1000 πCode Processor** and build a customized program for following hybridization process to see if the volume of buffer in each well is about the same:

  Washing: 1 wash cycles

  ■ 150 µL ☐ ELISA

  ■ Last Dispense Volume

  ☐ Magnetic

  ☐ Clean tip

- If the problem persist, please contact PlexBio or local distributor for service.

13. What is SA-PE control? Why does SA-PE control fail?

SA-PE control is a biotinylated oligonucleotide coupled to πCode. SA-PE directly binds to the biotin on oligonucleotide and results in fluorescent signal. The SA-PE control monitors the addition of SA-PE solution and the fluorescence intensity, which must be above a certain cut-off to ensure proper performance of the assay.

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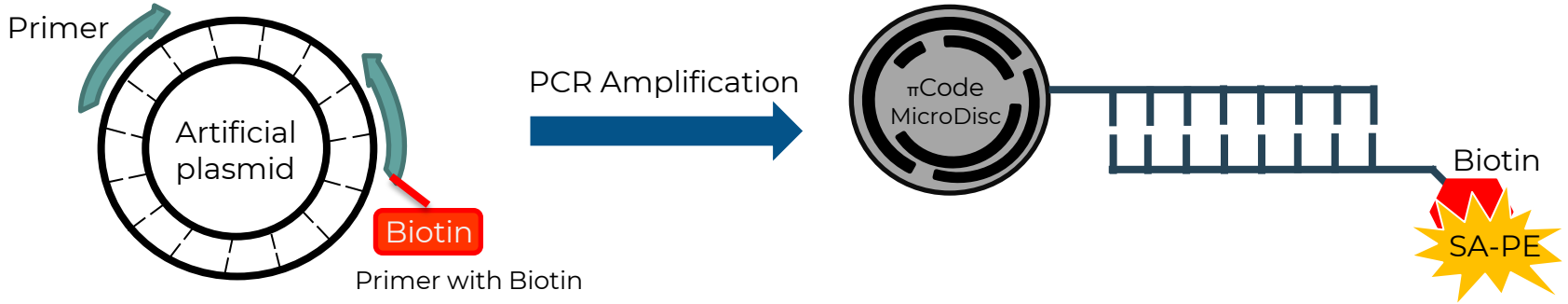
Possible reasons why SA-PE control fails:

- SA-PE solution is not correctly added to **IntelliPlex 1000 πCode Processor**. Make sure sufficient SA-PE solution is loaded in V tray before starting a program. See user manual for SA-PE volume calculation.
- SA-PE solution has lost functionality. Do not use SA-PE solution which is expired or stored improperly.
- Wrong hybridization program is selected.
- Ensure using fresh and correctly formulated wash buffer.
- **IntelliPlex 1000 πCode Processor** fails to dispense SA-PE solution. Contact PlexBio or local distributor for service.

14. What is Internal control? Why does Internal control fail?

Internal control is an artificial plasmid or RNA fragment not naturally presented in human samples, which is amplified simultaneously. The biotin-labeled amplicons bind to specific probes coupled to πCode during hybridization process, and then then labelled fluorescently with SA-PE. Internal control is added to monitor the overall assay performance.



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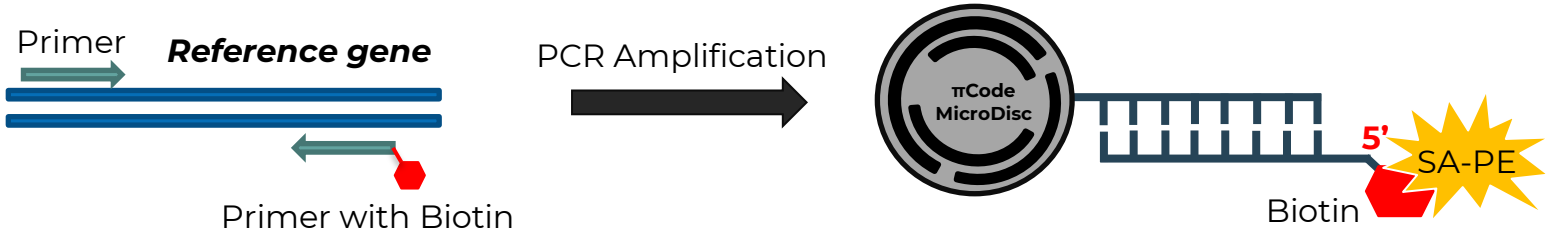
Possible reasons why Internal control fails :

- PCR fail
  - Wrong PCR program is selected
  - Master mix solution is not added, not added in correct quantities or not mixed evenly
  - Expired Kit is used. Make sure not to use reagents from different lots.
  - Kit is no longer functional. Make sure to store reagents in proper storage conditions.
  - PCR machine is not working properly. Ensure annual calibration and validation are performed.
- PCR product is not denatured freshly before hybridization
- PCR product is not added
- The wrong hybridization program is selected.
- Ensure using fresh and correctly formulated wash buffer.
- SA-PE reaction fails. If SA-PE control fails as well, Refer to "**13. What is SA-PE control? Why does SA-PE control fail?**" for instruction.

15. What does reference gene control do? Why does reference gene control fail?

Reference gene is a target naturally present in human samples with similar expression level between different individuals.
Reference gene is co-amplified during the PCR amplification process, and amplicons bind to specific probes coupled to πCode through hybridization and are then labelled fluorescently with SA-PE.
Reference gene control is used to monitor the quality of samples and the process from extraction to hybridization.



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Possible reasons why reference gene control fails:

- PCR fail
  - Wrong PCR program is selected
  - Master mix solution is not added in correct quantities or not mixed evenly
  - Expired Kit is used. Make sure not to use reagents from different lots.
  - Kit is no longer functional. Make sure to store reagents in proper storage conditions.
  - PCR machine is not working properly. Ensure annual calibration is performed
  - Poor sample quality, like severe DNA/RNA fragmentation or presence of PCR inhibitors
  - Samples is not added during PCR reagents preparation.
  - Wrong DNA/RNA quantities is added, or inaccurate quantification is used
- PCR product is not freshly denatured before hybridization
- PCR product is not added to hybridization
- The wrong hybridization program is selected.
- Ensure using fresh and correctly formulated wash buffer.
- SA-PE reaction fails. If SA-PE control fails as well, Refer to "**13. What is SA-PE control? Why does SA-PE control fail?**" for instruction.

16. What is target signal in positive control? Why does target signal in positive control fail?

The positive control is a control that should result in a predefined pattern for the target signals. A failed Positive control indicates a problem with assay performance.
Possible reasons why target signal in positive control fails:

- PCR fail
  - Wrong PCR program is selected
  - Master mix solution is not added in correct quantities or not mixed evenly
  - Expired Kit is used. Make sure not to use reagents from different lots.
  - Kit is no longer functional. Make sure to store reagents in proper storage conditions.
  - PCR machine is not working properly. Ensure annual calibration is performed
  - Poor sample quality, such as severe DNA/RNA fragmentation or presence of PCR inhibitors
  - Samples is not added during PCR reagents preparation.
  - Wrong DNA/RNA quantities is added, or inaccurate quantification is used

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Possible reasons why target signal in positive control fails:

- PCR product is not denatured before hybridization
- PCR product is not added to hybridization or Wrong PCR product is added for hybridization
- Wrong hybridization program is selected.
- Ensure using fresh and correctly formulated wash buffer.
- Wrong well is selected for analysis
- SA-PE reaction fails. If SA-PE control fails as well, Refer to "**13. What is SA-PE control? Why does SA-PE control fail?**" for instruction.

17. What is target signal in negative control? Why does target signal in negative control fail?

The negative control is a control that should **not** be positive for any of the analyzed targets in an assay.
Possible reasons why target signal in negative control fails:

- Contamination during PCR setup including reagents preparation
- Wrong sample is added for PCR amplification
- Wrong PCR product is added for hybridization
- Wrong hybridization program is selected.
- Ensure using fresh and correctly formulated wash buffer.
- Wrong well is selected for analysis

18. Why does DeXipher show “No valid assay assigned”?

All IntelliPlex assays have their own APPs which need to be installed for DeXipher software recognition and analysis, while each ENC file carries lot-specific information.
Possible reasons:

- No plate is inserted or plate is inserted in wrong orientation. Make sure plate is inserted in correct orientation and repeat reading.
- Wrong PC and/or NC is selected for analysis.
- Corresponding kit app is not installed. Contact PlexBio or local distributor for service.
- Corresponding ENC file is not imported. Download the ENC file from PlexBio website, import the ENC file, and repeat reading.
- Two or more lots of reagent are used. Make sure that only one reagent lot is used at a time.
- Some πCode IDs are missing in PC/NC well. Refer to “**11. Why does πCode count control fail?**” for instruction.

19. Why does DeXipher show “Failed to import kit, the file may be corrupted”?

Wrong file is imported (not IntelliPlex kit ENC file), or ENC file is altered or damaged. Check if wrong file is selected, and download the ENC file from website and import it again.

20. Why does DeXipher show “App Name [XXX kit – xxxxxxxx] has more /less than 1 mappings. App Install/Uninstall errors”?

The corresponding Kit App is not installed or is not the latest version. Contact PlexBio or local distributor for service.

Thank You

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