

## RainDance Technologies Primer Library Design FAQs

**Q: What is the average primer design coverage of target regions from RainDance's primer design?**

**A:** The percentage coverage of target regions varies due to different genomic content and complexity of the regions of interest. In most cases, we achieve 99% primer design success rate. We have found that the RainDance coverage is generally higher than hybridization based sequence enrichment methods. For detailed information, please contact [sales@raindancetech.com](mailto:sales@raindancetech.com).

**Q: Can I use any of the next generation sequencing systems with RainDance's Sequence Enrichment Assay?**

**A:** Yes. RainDance has demonstrated the ability to generate high quality results with both short reads (Illumina GAII, Life Technologies SOLiD) and long reads (Roche 454 FLX and Titanium). We believe that RainDance's target-enriched samples are compatible with all next generation sequencing (NGS) systems currently on the market.

**Q: Do different sequencing platforms require different primer design parameters?**

**A:** Yes. The difference in the primer designs for the different sequencing platforms is the allowable amplicon size. For the Roche 454 FLX we limit the amplicon size to match the read length of the 454 FLX platform (220 bases). The other Next Generation Sequencing platforms can handle larger amplicon sizes either due to longer read lengths (454 Titanium) or through their samples preparation (concatenation and fragmentation).

**Q: How much target sequence can be enriched with a single primer library on the RainDance 1000?**

**A:** The RDT 1000 currently supports custom primer libraries containing up to 4,000 unique primer pairs. The total amount of target sequence represented will vary depending on the nature of the specific target sequences and the NGS platform of choice. For Illumina, SOLiD and 454 Titanium, the typical target sequence for exons is 600-700 Kb/4000 member library, and the typical target sequence for contiguous regions is ~1,200 Kb. The number of unique primer pairs supported by the RDT 1000 will increase to 20,000 in mid-2010.

**Q: Do you charge for primer design service?**

**A:** No, RainDance's primer design service is complimentary to our customers.

**Q: How do I submit targets of interest to RainDance?**

**A:** The best way to submit the list of intended targets is via email using the RainDance Primer Design Request Form. Please contact your local RainDance Sales Specialist or email [support@raindancetech.com](mailto:support@raindancetech.com) for more information.

**Q: What kind of gene or genomic region identifiers should I use?**

**A:** If your targets are genes, the preferred identifier is RefSeq ID, CCDS ID, or NCBI GeneID. If your targets are genomic regions, the preferred identifier is genomic coordinates based on NCBI build 36.3. Please avoid using gene name or gene symbols as they are often not unique identifiers.

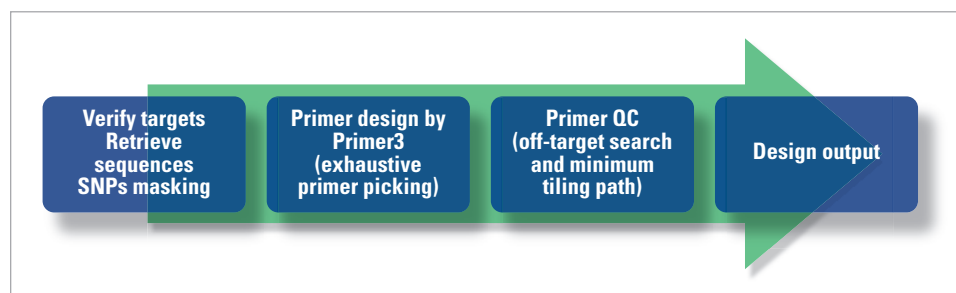
**Q: What other information does RainDance need to design my library?**

**A:**

- Sequencing platform to be used to determine optimum amplicon length
- Your choice of including or excluding putative regulatory region which RainDance defines as 1 Kb upstream from exon 1.

**Q: Which primer design program does RainDance use?**

**A:** RainDance uses Primer3 for primer design and the design team has developed a sophisticated program to automatically evaluate a large number of candidate primers and select the best possible design based on the design parameters. The diagram below briefly illustrates the primer design process.



**Q: Do you perform repeat masking during the design?**

**A:** No. Our primer design software performs exhaustive primer picking, and is usually able to generate satisfactory coverage in most regions of the human genome.

**Q: What information do you provide in primer design report?**

**A:** We provide a summary of overall design performance and design statistics and complete design information on all primers designed including but not limited to primer and amplicon locations and sequences, primer Tm and GC content.

**Q: What do the failure codes mean?**

**A:** RainDance's primer design process generally provides satisfactory coverage for regions of interest; a small percentage of regions might not be covered and for these regions we provide failure codes to indicate technical challenges in the regions:

- **Failure Code #1**
  - Rare: Invalid accession numbers for targets
- **Failure Code #2**
  - Unable to design: Primer design algorithm could not generate primer pairs based on the parameter settings. The most common reasons for this failure are high or low GC content and repetitive sequences.
- **Failure Code #3**
  - Multiple products predicted by in-silicoPCR (isPCR).

**Q: How long does the primer design take?**

**A:** Upon receiving your list of targets, the turn-around time is 5 business days. RainDance will review the design output with you and get your approval before primer library is constructed.

## FREQUENTLY ASKED QUESTIONS

**Q: How long does it take to build the primer library?**

**A:** Upon receiving your approval of the primer library synthesis list and the purchase order, the turn-around time is about 4 weeks.

**Q: Can I send you my own primer sequences?**

**A:** No. To maximize primer design and downstream PCR success we provide primer design service to our customer free of charge.

**Q: Who makes the oligos? What if I already have my own oligos, would you use those?**

**A:** RainDance works with a reputable oligo synthesis provider to make the oligos. We do not accept oligos from customers for primer library construction.

**Q: Can RainDance incorporate sequencer specific adapter sequences as part of the primer design?**

**A:** At this time, we do not incorporate adaptor sequences into the primer design.

**Q: What is the policy for failed amplicons?**

**A:** Our primer design program maximizes the chance for PCR success; however, many factors contribute to the eventual experimental success. We do not guarantee success for every amplicon in the primer library.

**Q: Who do I contact should I have more questions about RainDance's primer design?**

**A:** You may contact RainDance Customer Care by E-mailing [support@raindancetech.com](mailto:support@raindancetech.com)

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