

# GenePrint Light™

## Chemiluminescent Detection

By

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Variable Number Tandem Repeats (VNTRs) were first described in 1980 (1). These are highly polymorphic, multi-allelic DNA markers that contain tandem repeats of 11 to 60 base pair sequences, but represent only a single locus (2). The highly polymorphic nature of VNTR loci makes them a useful tool for human identification. In 1985 Jeffreys *et al.* demonstrated that when DNA probes containing tandem repeats are hybridized with genomic DNA, patterns or genetic “fingerprints” are generated, which can be used to discriminate between individuals (3).

VNTR loci are routinely detected by Restriction Fragment Length Polymorphism (RFLP) analysis. Using this technique, the DNA sample is subjected to restriction enzyme digestion followed by gel electrophoresis. The separated restriction fragments are then transferred to a membrane in a Southern blot procedure (4) and VNTR detection is performed using specific probes that are labeled with radioactive or chemiluminescent tags.

The *GenePrint Light*™ Probes are oligonucleotides comprised of the DNA sequence present in the particular VNTR repeat, conjugated to the enzyme alkaline phosphatase. They allow the detection of VNTRs using a rapid, safe, non-radioactive hybridization procedure and chemiluminescent detection. This method reduces hands-on time with no loss of sensitivity compared to radioactive detection methods (5). All of the *GenePrint Light*™ Probes are formulated for use at a dilution of 1µl of probe per milliliter of hybridization solution.

### Q: How much DNA should be used?

A: The *GenePrint Light*™ Probe, YNH24, is quality controlled to detect 50ng of DNA in a 15-minute exposure after an overnight ramp. All of the other *GenePrint Light*™

Probes are quality controlled to detect at least 100ng of DNA with the same exposure and ramp time.

### Q: How should the DNA be isolated?

A: VNTR analysis requires intact double stranded DNA that is easily digested with restriction endonucleases. Many DNA isolation techniques are suitable for the preparation of sample DNA for VNTR analysis. The Wizard® Genomic DNA Purification Kit can be used to isolate DNA from as little as 300µl of whole blood with no organic extraction. Yields in the range of 5-15µg DNA are consistently isolated from 300µl of whole blood using this system.

### Q: What membranes can be used with the GenePrint Light™ Probes?

A: In general, neutral membranes give lower background than charged membranes. We find that the Pall Biodyne® A membrane (Pall Biosupport) gives a stronger signal than MSI membranes. Pall Biodyne® B membranes give very high background with the chemiluminescent probes and are **not recommended** for use with *GenePrint Light*™ DNA Probes.

### Q: What causes increased background?

A: Increased hybridization time, as well as exposure time, will increase signal but will also increase background.

Cleanliness is very important with chemiluminescent detection. Dirty forceps and powder from gloves will show up as smudges or spots on the film and can contribute to the overall background. These smudges can make interpretation quite difficult if not impossible. Be sure to keep all contact with the membrane to a minimum.

The type of transfer procedure used can also affect background. We recommend a neutral Southern transfer. This gives a lower background than alkaline transfer conditions.

Filtering the wash buffers through a 0.2µm or 0.8µm filter before use can reduce background. This prevents spurious light reaction, which will contribute to the background.

Make sure that the amount of probe used is correct. Using more than 1µl of the *GenePrint Light*™ Probe per milliliter of hybridization solution will give a higher background.

Any variation in the hybridization or wash temperatures will have an effect on the analysis. If the temperatures of one or all of these are lower than recommended, the background will increase.

### Q: What causes a weak signal or no signal?

A: Use of hybridization or wash temperatures higher than those recommended in the protocol can cause reduced signal.

Additionally, if less than 1µl of *GenePrint Light*™ Probe is used per milliliter of hybridization solution, the signal may be too weak to analyze.

A weak signal may also result from insufficient incubation with the Lumi-Phos® Plus detection reagent (Lumigen). Incubate membranes for 5 minutes with Lumi-Phos® Plus to ensure thorough coating and a strong signal.

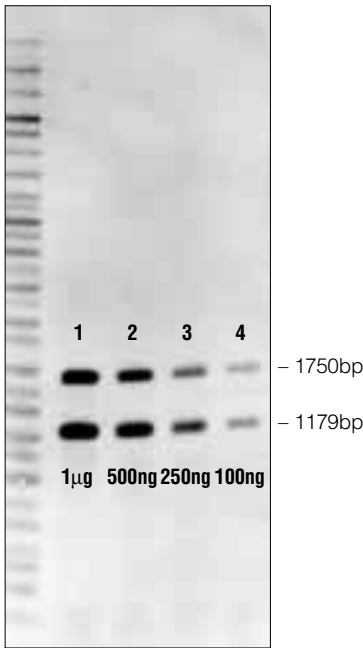
Contamination of the Lumi-Phos® Plus reagent with alkaline phosphatase will also result in loss of signal.

**Q: Should the GenePrint Light™ Probes be heated before use?**

A: Alkaline phosphatase is very sensitive to high temperatures. Heating the probes is not advised, as it will result in a decrease in signal and an increase in background.

**Q: Can roller bottles be used in place of hybridization trays within a shaker?**

A: Roller bottles can be used; however, the use of roller bottles can result in higher background levels.



Detection of D10S28 using the GenePrint Light™ Alkaline Phosphatase-Conjugated Probe – TBQ7 and various amounts of DNA.

**Q: What chemiluminescent detection reagents should be used with the GenePrint Light™ Probes?**

A: All of the GenePrint Light™ Probes are optimized to perform with Lumi-Phos® Plus detection reagent with an overnight ramp.

**Q: Can Southern blots be stripped and reprobed?**

A: Southern blots can be stripped and reprobed (6); however, there will be some loss of sensitivity and an increase in background with each stripping. If stripping and reprobing, we recommend hybridizing the stripped blot with the probe having the lowest sensitivity first and then proceeding to the most sensitive probe. The recommended order of use with the GenePrint Light™ Probes is EFD52 followed by TBQ7, YNH24, D17S79 and the Human Y-Specific Probe.

**Q: Can the GenePrint Light™ Probes be reused?**

A: We do not recommend reusing the GenePrint Light™ Probes. Reuse will decrease the sensitivity of the probe. It is possible to hybridize several membranes at once without changing the amount of probe used per milliliter of hybridization solution; however, an increase in the number of blots within the hybridization may increase background. Always ensure that the blots are in constant motion. It is extremely important to use the recommended amount of solution for the total area of membranes within the block, hybridization, wash and Lumi-Phos® Plus incubations. Any variation from the recommended procedures may result in an increase in background, a decrease in signal or both.

REFERENCES

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